Technical Consultation of the

International Space Station (ISS)

Internal Active Thermal Control System (IATCS)

Cooling Water Chemistry

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F. Toxicological Assessment of IATCS Coolant Biocides (Memo 632)

Signature Page

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1.0 Executive Summary

The on-orbit ISS IATCS consists of a Low Temperature Loop (LTL) and a Moderate Temperature Loop (MTL), which provides coolant to the U.S. Laboratory and airlock modules (Figure 1-1). The nominal circuit volumes and supply temperatures for the LTL are 63 liters (L) and 3.3 to 6.1^{o} Celsius (C), and for the MTL, 200 L and 16.1 to 18.3^{o} C. The LTL and MTL normally operate independently in a dual loop mode, but can be cross-connected (single loop mode) so that a single Pump Package Assembly (PPA) circulates both loops. The water-based IATCS collects heat from sources within the pressurized elements and transfers heat to the External Active Thermal Control Systems (EATCS) via the ammonia-to-water Interface Heat Exchangers (IFHXs) mounted externally to the U.S. Laboratory endcone (Figure 1-2). Future pressurized modules (Node 2, Columbus, etc.) will have independent IATCSs, but the potential exists for fluid from one IATCS to mix with fluid from another IATCS during switching of equipment racks on-orbit.

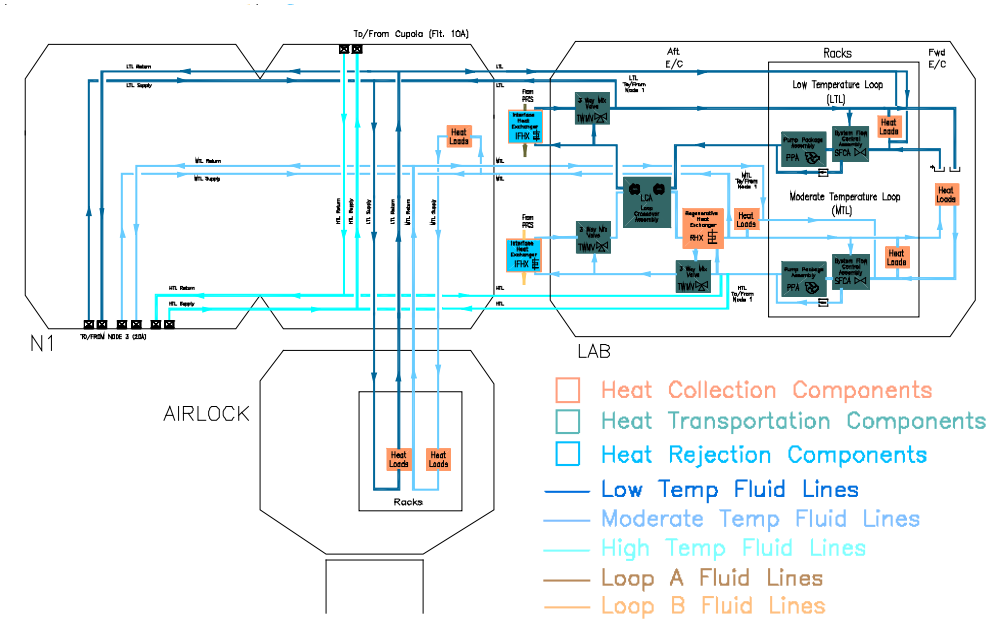

Figure 1-1. U.S. Laboratory, Node 1, and Airlock General IATCS Schematic

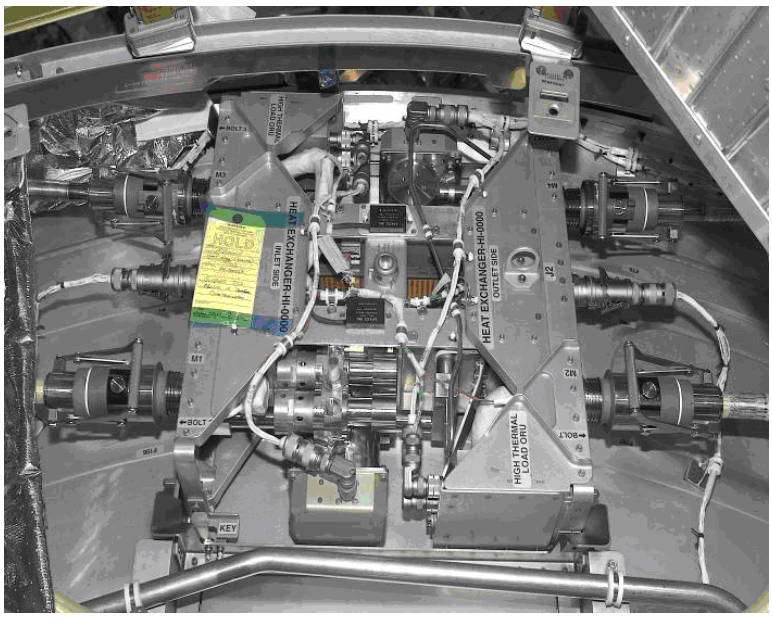

Figure 1-2. Heat Transfers to the EATCS via the Ammonia-to-Water -IFHXs

Since early 2002, the IATCS coolant chemistry has deviated from "as circulated" specification limits identified in SSP 30573, Revision B, ISS Program Fluid Procurement and Use Control Specification (shown in Table 1-1). The chemistry deviation was the result of the normal ISS on-board control range of the partial pressure (2-6 mmHg) of carbon dioxide (CO_2), combined with the use of Teflon flexible hoses for the IATCS coolant. Diffusion of CO_2 from the cabin atmosphere through the flexible hoses and into the coolant loop increased carbonic acid levels in the coolant fluid and lowered the coolant pH (Figure 1-3).

Table 1-1. IATCS Coolant Chemistry Specification Limits

Chlorides	1.0 ppm maximum
Dissolved Oxygen	6.0 ppm minimum
Total Organic Carbon (TOC)	5 ppm maximum
Di or Tri Sodium Phosphate	200 – 250 ppm
Sodium Borate	800 – 1200 ppm
Silver Sulfate	0.1 – 0.3 ppm
pH	9.5 ± 0.5

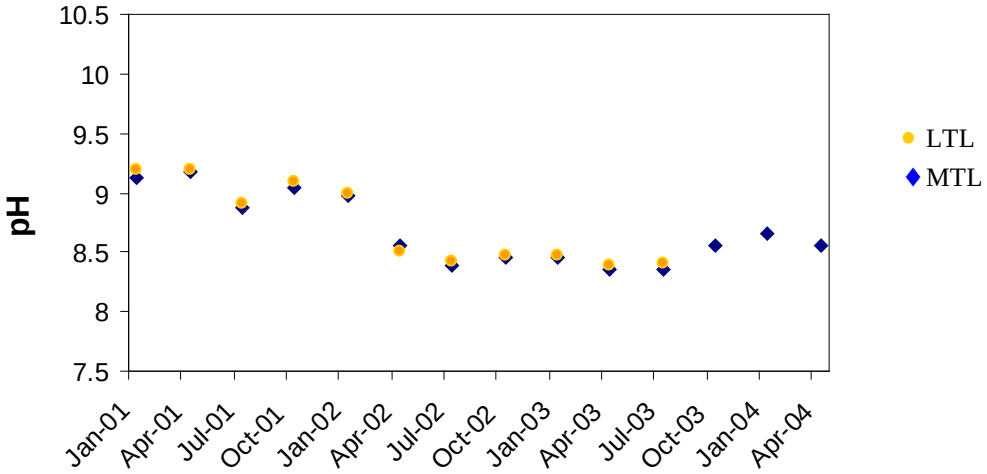

Figure 1-3. pH Decrease caused by Diffusion of Cabin CO_2 into IATCS Coolant (permeated through the IATCS Teflon flexible hoses)

As the pH decreased, the microbe population increased, and the dissolved nickel content increased (shown in Figures 1-4a and 1-4b), as determined from returned ISS IATCS water samples. Subsequently, the phosphate concentration decreased as the nickel phosphate saturation limit was exceeded (shown in Figure 1-4c). Furthermore, nickel precipitates (primarily nickel phosphate) were observed in IATCS filters. A green color was noted on gas traps, which may or may not be due to precipitates. Nickel dissolution and the formation of nickel precipitates were not observed in the ground-based development and certification testing, where IATCS-specified fluid chemistry (especially the pH) remained stable for at least 2 years. Concerns were raised that continued precipitation in the IATCS fluid could lead to other fouling-related issues associated with several system components. Further investigation into the effects of pH reduction increased the area of concern to include increased microbial levels and biofilm development. These latter conditions could lead to galvanic and/or microbial corrosion and reductions in cold plate/HX efficiencies.

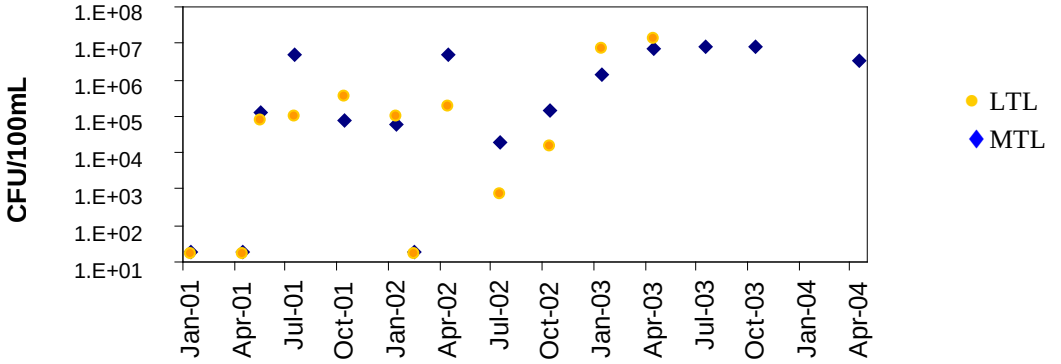

Figure 1-4a. Increase in Microbe Counts in IATCS Loops Coincident with Decreased pH

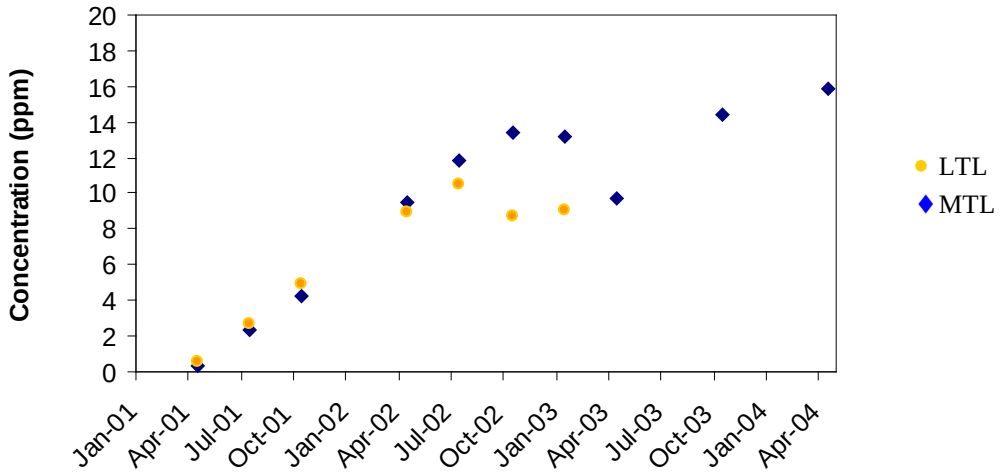

Figure 1-4b. Increase in Dissolved Nickel Ion Content of the IATCS Coolant Loops Coincident with Decreased pH

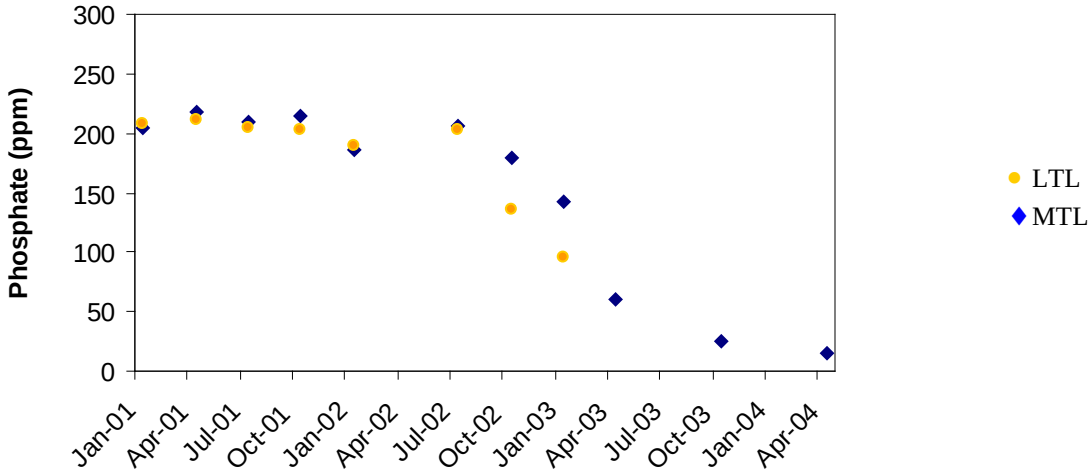

Figure 1-4c. Decrease in Phosphate Concentration in the IATCS Loops following Increases in Dissolved Nickel

The ISS Program requested support in assessing the following:

1. The Program's approach and corrective actions proposed to address the observed chemical changes to the IATCS coolant chemistry.
2. The potential for component life reduction.
3. Possible revisions in the requirements for crew protection and intervention.

The approach is to provide collaborative support in three principal areas:

- Assist in the determination of an antimicrobial selection for Node 2 and U.S. Laboratory.
- Provide an assessment of the likelihood of additional corrosion and its impact on the performance and integrity of the IATCS.
- Provide a proactive assessment of the effect of having quantities of coolant from the different modules intermingling when equipment racks and experiments are moved between laboratories.

This report provides global recommendations on system investigations (Section 12.1), specific recommendations on the principal areas under assessment (Section 12.2), and a number of collateral recommendations on issues integral to the safe operation of the IATCS (Section 11.8). It is recognized that the IATCS is a complex system with chemical and performance responses not readily predictable under the current investigation structure. Therefore, the NESC team has

adopted the governing tenets of *protect the crew, proceed with caution as to do no harm,* and *act only when necessary*.

Seven antimicrobials are recommended for characterization against the reference antimicrobial of glutaraldehyde:

- Iodine
- Iodine/Silver
- Hydrogen Peroxide/Silver
- Silver on a ceramic bed matrix
- Orthophthalaldehyde
- Isothiazolones
- Chlorhexidine

The cleanliness control approaches for rack and equipment transfers between the IATCSs are based on mass/contaminate balance calculations, interface pretreatment regimens, and crew hygiene protocols.

The collateral recommendations (refer to Section 11.8) on synergistic components of the IATCS coolant chemistry address the following issues:

- Glutaraldehyde toxicity assessment.
- Borate/carbonate buffer additions.
- Node 2 antimicrobial implementation.
- Nickel Removal Assembly (NiRA) and Phosphorous Removal Assembly (PhosRA) characterization and implementation.
- Corrosion monitoring equipment for ground-based systems.
- Long-term antimicrobial development.
- Comprehensive ground test roadmap for potential bio- and chemical-fouling, and corrosion damage problems.

2.0 Identification

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Description: Assess the approach and corrective actions proposed to address the observed chemical changes to the IATCS coolant chemistry, the potential for component life reduction, and requirements for crew protection and intervention.	
Date Received: February 2, 2004	**Date ITA/I Initiated:** February 2, 2004
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2.1 Team Members and Consultants

The team was assembled based on recommendations and assistance from the NESC Discipline Experts, Chief Engineers, and the Deputy Director for Safety. The following table indicates the core team of subject matter experts (SME) who will be augmented by a number of additional technical specialists and consultants.

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3.0 ITA/I Plan

The consultation team, formed to support this activity, consisted primarily of personnel from outside the Agency (i.e., The Aerospace Corporation and academia) as available microbiologists and associated expertise were currently engaged by the ISS Program on this issue. Support was provided through:

- Review of ongoing and completed laboratory testing and performance projections.
- Participation in weekly teleconferences with the IATCS System Problem Resolution Team (SPRT).
- Attendance at periodic SPRT and Coolant Working Group (CWG) technical interchange meetings (TIM).
- Contract funding with Mittelman and Associates and Montana State University/Center for Biofilm Engineering on an antimicrobial survey.

The consultation was separated into sections named *Crew Health, System Performance, Component Life, Independent Assessments of Antimicrobials, Hardware Examination, Ground Support Equipment (GSE) Servicing Units, Other ISS Modules Issues, ITACS Equipment Interchangeability, and Collateral Issues.*

4.0 Description of the Problem, Proposed Solutions, and Risk Assessment

4.1 Problem

The IATCS coolant exhibited unexpected chemical changes during the first year of on-orbit operation following the launch and activation in February 2001. The coolant pH dropped from 9.3 to below the minimum specification limit of 9.0, and re-equilibrated between 8.3 and 8.5. This drop in coolant pH was shown to be the result of permeation of CO_2 from the cabin into the coolant via Teflon flexible hoses which created carbonic acid in the fluid. This unexpected diffusion was the result of having a cabin CO_2 partial pressure higher than the ground partial pressure (average 4.0 mmHg vs. <0.2 mmHg). This drop in pH was followed by a concurrent increasing coolant nickel concentration. No other metal ions were observed in the coolant and based on previous tests, the source of nickel ion was thought to be the boron nickel (BNi) braze intermetallics used in the construction of HXs and cold plates (refer to Figure 4-1). Specifically, BNi2 braze alloy was used for the IATCS IFHX and BNi3 braze alloy was used for the IATCS Airlock Servicing and Performance Checkout Unit (SPCU) HX and cold plates (refer to Figures 4-2 through 4-5). Given the failure criticality of the HXs, a Corrosion Team was established by the IATCS CWG to determine the impact of the nickel corrosion on hardware performance life. Previous materials compatibility testing[1] demonstrated that the most active material of all metals used in the IATCS construction was the silicon-rich intermetallic phase, which is found in most nickel-based braze alloys. Corrosion testing, performed by the Corrosion Team, indicated that intermetallic-phase nickel, located mainly in the fillet regions of BNi2 and the fillets and surfaces of BNi3 braze alloys, was corroded by pH conditions lower than 9.5, while corrosion rates of the matrix phase of BNi2 and BNi3 were observed to be significantly slower. Process differences between the hardware manufacturers resulted in different thicknesses of the corrosion resistant matrix phase, yielding significantly different performance life projections for the hardware. Only the single-brazed BNi3 Airlock SPCU HX hardware has a predicted risk of not meeting the required 10-year performance life. Data from the various test conditions indicate that the Airlock SPCU HX has a calculated performance life of 4 years to 21 years, based on varying test factors, degrees of conservatism, and other accelerated test assumptions.

[1] Boeing D950-10389-1, dated December 1998 and D684-12001-02, dated August 2004

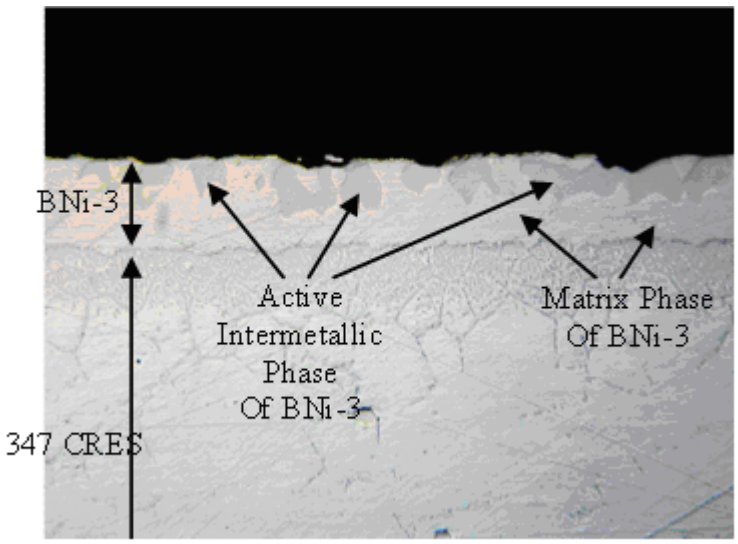

Figure 4-1. BNi3 Braze used in Construction of HXs and Cold Plates

Figure 4-2. Regenerative HX

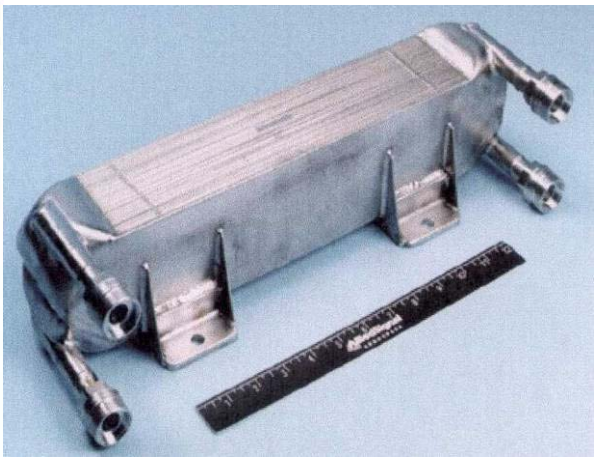

Figure 4-3. SPCU HX

Figure 4-4. Typical Cold Plate

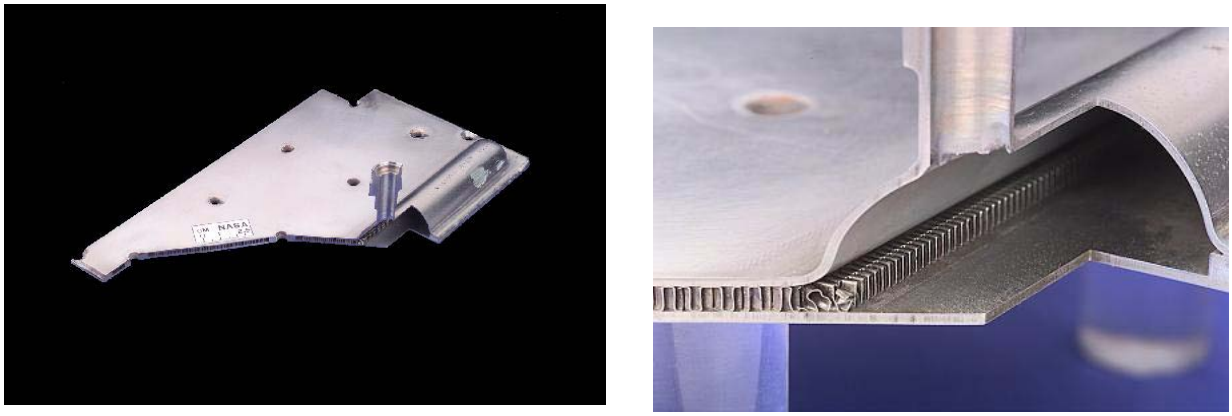

Figure 4-5. Typical Cold Plate Construction Detail

Another factor that could have affected the corrosion of nickel-based braze alloys was the initial IATCS coolant antimicrobial level. Silver sulfate was included in the IATCS coolant as an antimicrobial, but was found to be effective for only short periods in reducing the microbial population because it galvanically plated out as silver onto metallic surfaces, making it unavailable for microbial control. Silver sulfate was added several times to the IATCS coolant during system ground-based testing, resulting in multiple silver deposition episodes. Silver was added to the IATCS on-orbit in the form of silver phosphate in February 2002. Limited qualitative beaker testing conducted at Hamilton Sundstrand (HS) using the original IATCS coolant composition showed that silver nodules would precipitate on the nickel braze alloy used in assembly of the HS-manufactured HX. Galvanic reduction of silver from solution is coupled with the oxidation of nickel from the braze alloy, thereby adding nickel ions into solution. However, the nickel ion concentration has been observed to increase with time in the on-orbit IATCS loop, long after galvanic reactions should be complete. There is concern that the

deposited silver nodules could be inducing galvanic-driven corrosion of the nickel braze surfaces, thereby contributing to the increasing dissolved nickel concentration observed in the on-orbit coolant. The silver-induced galvanic corrosion of the nickel-based braze could also be enhanced with decreasing coolant pH.

The initial increase in nickel ions in the coolant on-orbit is attributed to the rapid dissolution of the braze intermetallics when exposed to the reduced pH and potentially aggravated by the silver phosphate additions. However, as the network of intermetallics is not fully interconnected, the nickel dissolution is limited to those intermetallics that are surface intersecting and exposed to the coolant. General corrosion of the nickel matrix is expected, but at a much lower rate than that of the intermetallics. The intermetallics are a limited reservoir dissolving at a rapid rate whereas the nickel matrix is a much larger reservoir dissolving at a limited rate. Once the nickel level for a given pH exceeds the saturation level for a given nickel precipitate, precipitation occurs until a steady state is established. It should be expected that additional precipitation is occurring at a rate driven by corrosion of the nickel matrix phase of the braze, as the reservoir of intermetallics has essentially been depleted.

Measured nickel in the IATCS seems to be a small percentage of the values calculated as potentially available by HS and Honeywell. The assumption made in these calculations was that the source of nickel was primarily the single braze cold plates. Review of the subject calculations appear to show nickel prediction levels were primarily developed for sizing the proposed nickel removal assembly (NiRA) mechanism and, therefore, are extremely conservative with respect to predicting the mass of nickel available for precipitation.

F-1. *Coolant chemistry is methodically characterized from returned on-orbit samples and from periodic extractions from ground-based flight and development systems. The protocols used are consistent with constituent characterization for other ISS and Space Shuttle water systems.*

4.2 Proposed Solution

The ISS Program's initial action was to modify the coolant chemistry additions to the U.S. Laboratory and Node 2 that included the discontinued use of silver as an antimicrobial and elimination of phosphate additions to minimize further nickel precipitation. In addition, the ISS Program's proposed long-term solution was to use the IATCS SPRT as a forum for coolant issues and proposed actions. It was anticipated there would be a series of tests and analyses performed to quantify risk. Some of these tests included: chemical/pH corrosion testing, nickel precipitation bench test and consultation/analyses, Microbiologically Influenced Corrosion (MIC) testing, and identification of recommended U.S. Laboratory hardware retrieval for destructive examination.

The CWG's projected actions to mitigate the risk involve:

- Development of trigger criteria for the addition of antimicrobial agents to U.S. Laboratory and Node 2.
- Characterization of alternate antimicrobial(s).
- Near-term antimicrobial selection (glutaraldehyde and/or hydrogen peroxide).
- Mid-term antimicrobial selection.
- Development and implementation of NiRA and PhosRA.
- Restoration of the coolant pH to above 9.0 with the addition of borate/carbonate.

These activities and others (as required) form the basis of the Coolant Management Plan.

4.3 Risk Assessment

The ISS Program Risk #4118, entitled *IATCS Coolant Impact to System Integrity* is identified as a 4 x 4 on the standard 5 x 5 risk matrix and is concerned with corrosion damage to the various ITACS components.

The likelihood = 4 was listed as "can not prevent this event, but a different approach or process might". The rationale shown was: *abatement plan offers several options for preventing this corrosion induced leakage event, from adjusting coolant chemistry to retarding the process, to increased frequency of component repair and replacement; and current program of testing and evaluation may conclude that probability is moderate-to-low.*

The Consequences = 4 annotated with "(technical) major reduction, but workarounds available". The rationale includes: *breach in IFHX would not be sudden and catastrophic; ammonia would be detected in the IATCS coolant as part of regular sample analysis and appropriate action taken (Flight Rule exists); leaking IFHX could be isolated, IATCS operated in Single Loop Mode, with some reduction in heat rejection capability, until IFHX replaced; and critical components susceptible to corrosion- induced leaks are replaceable (fluid connections either quick disconnect (QD) or Gamah fittings.*

ISS watch items associated with the ISS Program Risk #4118 includes:

- #5009, entitled *Node 2 IATCS Coolant*
- #5118, entitled *Airlock SPCU HX Failure*

These concerns are segregated from primary ISS Program Risk #4118 to allow emphasis on the ground processing of Node 2 and replacement of the single-brazed BNi3 SPCU HX with a

predicted corrosion life limit.

5.0 Data Analysis

5.1 Crew Health

The determination of microbial risk to crew health is a complex assessment composed of several factors including the astronaut's immune system, special conditions associated with flight that would affect the host-pathogen interaction, and the probability that the astronaut will be exposed to an infectious concentration of a medically significant organism. While studies have demonstrated that, during flight, crew members may be more susceptible to infection and microorganisms may have altered characteristics, the primary characteristic for risk assessment remains the concentration and identity of microorganisms to which the crew are exposed.

Specific to the IATCS, elevated microbial concentration may be occurring based on evaluations of returned flight samples. However, the environmental microorganisms that have been identified, including *Ralstonia paucula*, are not considered medically significant under these conditions and do not pose more than a minimal risk to the crew's health and safety. Organisms with similar characteristics have been isolated in a variety of environmental locations including both the Orbiter and other ISS systems. As with similar fluid systems, proper personal protective equipment, such as gloves and eye shields, are recommended during interactions to minimize potential exposures. However, the decision to add an antimicrobial to the IATCS currently does not have a medical basis.

From a microbial perspective, the IATCS is a dynamic system and microbial monitoring should continue to gain a better understanding of the microbial concentrations and constituents. Also, quantification and identification techniques should be expanded beyond current methodologies. While several more comprehensive techniques, including molecular and fluorescent-based methods, can readily be accomplished at NASA laboratories, some development may be necessary. For example, if data warrants precise enumeration or identification of flight sample organisms, modification or development of collection and/or analysis hardware would be necessary.

If an antimicrobial is determined to be necessary for engineering concerns, or as an attempt to limit microbial levels, the potential toxicity of the disinfectant to the crew should be the initial selection criteria for screening candidates. Leaks are possible either during the operation of the IATCS or during injection of a concentrated disinfectant into the IATCS. Therefore, the use of any antimicrobial with known toxic properties, such as glutaraldehyde, is inadvisable without extensive characterization and remediation protocols, at least in non-emergency conditions. If an emergency situation arises, such as the detection of increased levels of medically significant

microbes, the risk of toxicity versus that of infection on the crew may have to be evaluated to determine if such an antimicrobial is necessary. During the selection of an antimicrobial for the IATCS, several safety factors should be considered including the risk of exposure during storage, exposure during introduction into the system, exposure during filter change out, and long-term exposure from leaks in the system. For these exposure risks, spacecraft maximum allowable concentration (SMAC) values for each antimicrobial candidate should be located, generated, or estimated. Finally, a methodology for antimicrobial detection and remediation should be developed before implementation aboard the ISS.

F-2. *To date, no identified microorganisms in on-orbit and ground-based IATCS coolants are considered medically significant under these conditions and, thus, none pose an increased risk to crew health and safety. Current methodologies for enumeration and identification of microorganisms are adequate, but they could be updated to include fluorescent and molecular techniques if necessary.*

5.2 System Performance

U.S. Laboratory system performance was examined from both the standpoint of the risks of no action and the risks of introducing a new variable (antimicrobial).

The perceived risks of no action are seen as continued elevated microbial levels, the maturation of an established biofilm, and continued nickel precipitation. There is no history of crew health concerns for non-potable water systems. The development of a biofilm can reduce the flow cross-section of passages and develop an insulating layer on the heat transfer surfaces. Along with the deposition of inorganic precipitates, these effects could reduce HX performance through fouling, reduced heat transfer efficiency, and an increase in pressure across HXs, filters, gas traps, and pump assemblies. Clogging of the passive filter and gas traps also could produce increased pressure differentials.

The U.S. Laboratory IATCS performance history began with launch and activation in February 2001. This has been described in detail in Section 4.1, Problem, and summarized as follows:

> The coolant pH decreased from 9.3 to 8.3 due to the diffusion of the cabin atmosphere CO_2 across permeable Teflon flexible hoses into the IATCS coolant. The microbial count was observed to steadily increase approaching 10^6 CFU per 100 milliliter (mL). Silver phosphate was introduced in February 2002 as an antimicrobial, but was effective for only short periods in reducing the CFU counts.

Concurrent with the increase in microbial counts, the nickel ion concentration was observed to increase with decreasing phosphate ion levels. It was determined that the decreased pH caused increased corrosion of a nickel braze intermetallic used in the fabrication of various IATCS hardware. At pH 8.3, free nickel ions combined with the phosphate ions to form crystalline nickel phosphate precipitate (nickel carbonate and hydroxide precipitates would also be expected to form if the pH is raised). Significant quantities of nickel phosphate precipitates were found to have clogged the MTL filter. It is suspected that nickel precipitates are also responsible for observed gas trap pressure fluctuations, QD leakage anomalies, and possibly a PPA malfunction. Most performance issues have been observed in the MTL. These include development of large pressure differentials in the MTL filter and gas trap, resulting in the replacement of the filter in October 2002 and the gas trap in March 2003 (refer to Figure 5-1). The MTL contains the majority of the fluid volume (200 L) in the IATCS, and it would be expected to generate the bulk of the nickel precipitates contributing to performance degradation.

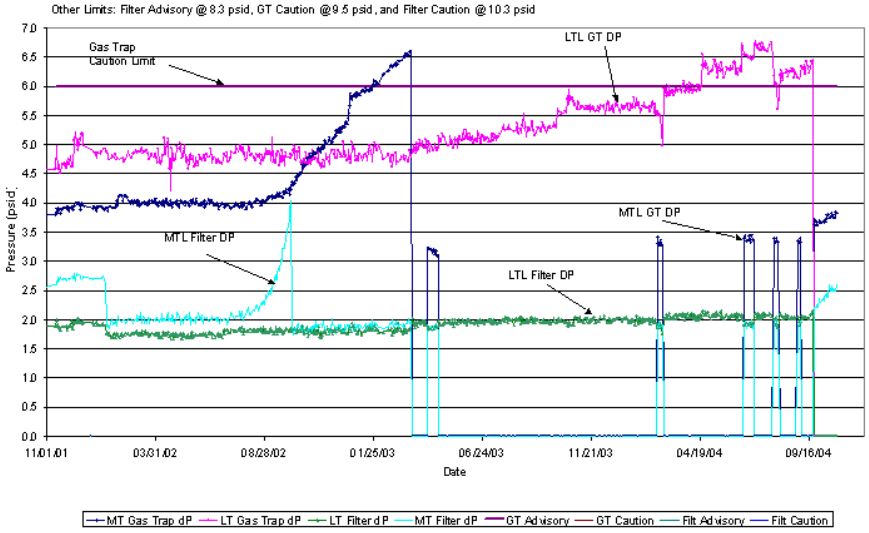

Figure 5-1. IATCS Gas Trap and Filter Pressure History

The status of the U.S. Laboratory IATCS performance appears to show no reduction in operational capacity at the current (minimal) thermal loads. The coolant pH has stabilized at 8.4, and analyses of returned coolant samples have not identified any unique microorganism hazardous to crew health. Only transient pressure changes have been observed in the MTL filter and gas trap, with no significant permanent pressure increases. The LTL has not shown a pressure increase across the filter, but the gas trap has developed a gradual pressure increase over time.

Overall, the U.S. Laboratory coolant and system performance appears to have reached a steady state. The microbial levels have stabilized at 10^6 CFU per 100 mL, and a majority of the precipitation appears to have occurred, as no additional changes of filters or gas traps have been necessary (refer to Figure 5-2).

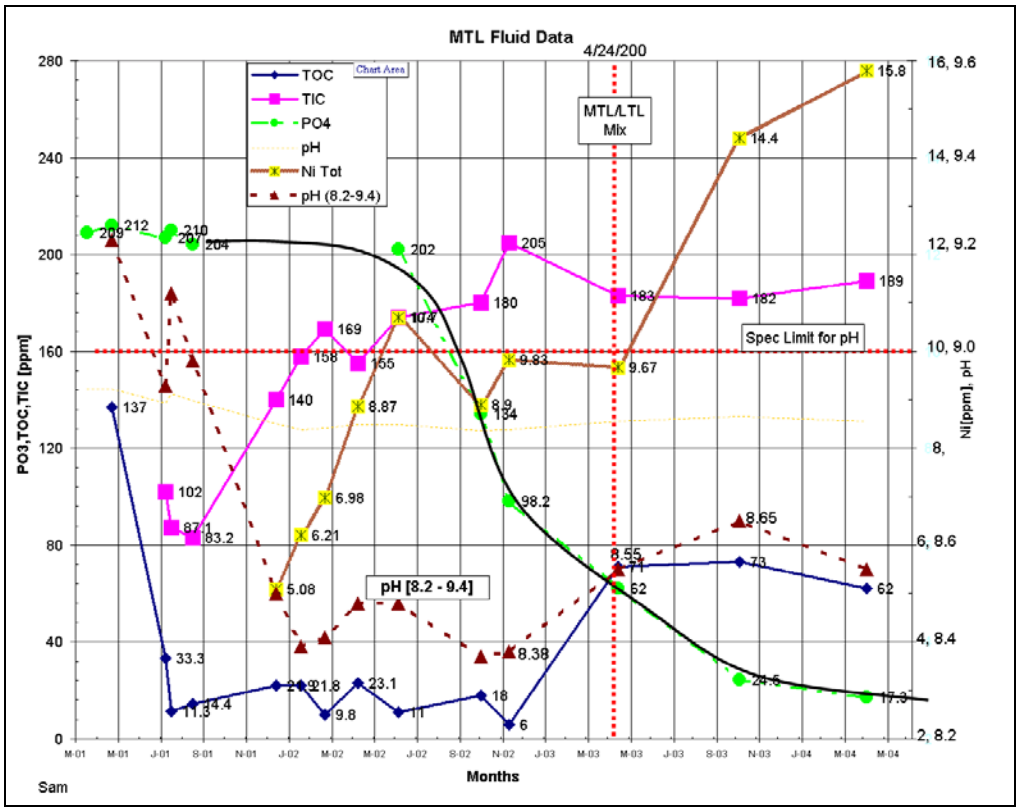

Figure 5-2. U.S. Laboratory IATCS Fluid Chemistry History (up to 7S)

The current risk of no action for the U.S. Laboratory on-orbit appears minimal or unclear. Bio-fouling does not currently appear to be a problem and nickel precipitate formation no longer appears to be a significant performance issue. After initial precipitate formation and filter/gas trap change-out, the system appears to be at a steady state. Reduced heat transfer at HX surfaces cannot be quantitatively ascertained as there is not a method for measuring heat extraction capacity. There is no quantitative or qualitative mechanism for measuring the effects of biofilm or precipitates on HX performance. Current operational load does not require full heat transfer capabilities of HX, so any losses under greater loads are unknown. There is no method for determining current or future margin for HX capacity.

F-3. *The U.S. Laboratory coolant and system performance appears to have reached a steady state. The microbial levels have stabilized and a majority of the precipitation appears to have occurred, as no additional filter or gas trap changes have been necessary.*

F-4. *There is no quantitative or qualitative method for measuring HX performance or assessing the deleterious effects on performance of biofilm or nickel precipitate formation, other than changes in flow pressure.*

The risks of action from the addition of an antimicrobial may include introduction of a crew toxin, disruption of coolant performance, and release of biofilm mass and entrained particulates. Some specific identified concerns with the addition of an antimicrobial are: crew exposures above short- and long-term SMAC levels, decreased coolant surface tension interfering with the gas trap operation, increased assimilable organic carbon (used by some microbes as a nutrient) for microbial growth, increased general or pitting corrosion, and renewed nickel precipitation.

Investigations to date have not identified one antimicrobial that balances all of the desirable crew health characteristics (toxicity), IATCS performance (chemical/physical properties), component life (material compatibility), and effectiveness. Glutaraldehyde has been established as the front-runner from initial characterization, with hydrogen peroxide (as a sole antimicrobial agent not mixed with another species) being considered less favorably for further consideration due to material compatibility concerns.

A review of published data[2] on corrosion evaluation testing of glutaraldehyde and hydrogen peroxide indicates a positive hysteresis in Cyclic Potentiodynamic Polarization (CPP) characteristics for eight different IATCS materials of construction suggesting the potential for pitting corrosion, albeit at potentials well above those expected for the materials in any of the variants of IATCS coolant studied to date. The electrochemical potentials used in that study are more noble (positive) than the range anticipated for any of the construction materials in any of the IATCS coolant chemistries under discussion. The materials compatibility requirement is that the proposed antimicrobial "must not adversely impact" the materials of construction. Further laboratory testing at realistic potentials using the original set of antimicrobials, as well as the new ones identified in this report, will be needed before the suggested potential for pitting corrosion can be directly assessed against the materials compatibility requirement.

[2] SAE International, 2004-01-2472, Selection of an Alternate Biocide for the ISS Internal Thermal Control System Coolant – Phase II

The absence of a set of specific requirements increases the complexity of the antimicrobial characterization and selection. This may negate or complicate scheduling of planned efforts.

The risk of a sudden catastrophic release of biomass from the biofilm in the U.S. Laboratory, based on the application of an antimicrobial agent, is viewed as *not likely*. It is probable that a biofilm exists, but the introduction of an antimicrobial is expected to be gradual and the effectiveness is not expected to be instantaneous. The only scenario under discussion that might be capable of producing such an effect would be the sudden introduction of a massive dose of hydrogen peroxide, which could remove the biofilm from surfaces. Moreover, several potential antimicrobials, including glutaraldehyde, would act to make the biofilm more resistant to removal rather than produce a biomass release.

The risk to action appears indeterminate for antimicrobial addition due to the incomplete listing of measurable requirements for the IATCS containing modules under consideration and for the entire implementation and performance phases.

F-5. *There is no concise quantifiable list of requirements identified for the selection of an IATCS antimicrobial that spans the use range of storage, application, utilization, leakage, exposure, and remediation.*

6.0 Component Life

In order for component life to be limited, corrosion damage must penetrate both the braze layer and the Corrosion Resistant Steel (CRES) 347 parting sheet material. Both the braze layer and the parting sheet are multiphase materials with one phase being more susceptible to dissolution. Thus, selective phase attack in both layers is the likely failure mode. The braze layer consists of a solid solution matrix phase and an intermetallic phase that is susceptible to corrosion at pH less than 9. The CRES 347 consists of a solid solution matrix and grain boundaries that contain what appear to be chromium borides. The CRES 347 matrix is highly corrosion resistant to the conditions of interest, whereas there is some question as to the susceptibility of the grain boundary regions to accelerated attack. The intermetallic phase (identified as Ni_6Si_2B) is mostly observed on and near the outer surface of the braze layer, extending into the thickness of the braze. The decoration of the grain boundaries is most intense near the braze/parting sheet interface because the braze is the source of boron.

Only the components with single-braze BNi3 material are of concern as susceptible to penetration via selective phase corrosion of intermetallics. The processing of the BNi2 material produced a single-phase microstructure except at fillets. Historically the ISS Program's position has been that corrosion rates of the braze matrices of BNi2 and BNi3 in the IATCS (determined electrochemically) are low, but have been considered life-limiting given the thickness of the

braze layers, anticipated exposure times, and the assumption that the CRES 347 material provides no contribution to component life. The airlock SPCU HX was the only component with a predicted life not meeting the required 10-year performance period and it has been replaced on the ISS.

The analyses and the experiments supporting them concerning component life predictions do not take into account several factors that can greatly influence the data collected. These factors and the assumptions used lead to component life predictions that are likely very conservative.

With regard to the supporting experiments performed, corrosion rates of brazes were determined both electrochemically and via Scanning Electron Microscope (SEM) analyses of metallurgical cross-sections of samples exposed for long periods of time (up to over 400 days) to variants of IATCS, often at elevated temperatures. Although both methods can provide useful information, there are limitations that must be taken into account. Electrochemical determination of corrosion rates in low conductivity solutions such as the IATCS coolant (and deionized water (DI)) is difficult, as the high resistance of solution must be taken into account. Such a correction does not appear to have been performed in the electrochemical studies conducted. Nonetheless, electrochemical measurements are the tool of choice, particularly for situations in which low corrosion rates are expected. The use of SEM examination of cross-sections for corrosion rate analysis is not optimal. Lack of a reference for the surface topographies combined with the low corrosion rates make measurements susceptible to large errors, especially in overestimation of attack depths. Artifacts developed during preparation of metallurgical cross-sections are possible and likely the source of some of the observed damage. Long-term exposures show that very little corrosion is seen with corrosion rates dropping with time.

The analyses of expected component life, including the data developed in the corrosion studies supporting the IATCS, do not consider the deceleration of corrosion rates with time normally observed. In addition, the cyclic potentiodynamic polarization (CPP) data are interpreted as indicative of localized corrosion susceptibility of brazes in a wide variety of IATCS variants, including those containing proposed antimicrobials. Interpretation was based on existence of positive hysteresis in CPP and post-CPP examination of surfaces, but the “pitting potentials” are hundreds of millivolt (mV) above the E_{corr} (corrosion potential) expected in the IATCS and many “pitting potentials” are above the oxygen evolution potential. Such potentials are completely irrelevant to practice because it is highly unlikely that the naturally developed E_{corr} of the system will rise that high. Damage observed in surface micrographs taken after the CPP tests was most likely caused by the acid generated at the high potentials due to the oxidation of water at the surface that creates molecular oxygen and hydronium ions. Modern interpretation of localized corrosion susceptibility compares the repassivation potential to the long-term open circuit corrosion potential. If the repassivation potential is far more positive, the likelihood of stabilized localized corrosion is very small. This approach was pioneered during development of corrosion

prediction models for storage of high-level nuclear waste and has been validated out to several years.

Current service-life predictions assume that the CRES 347 is sensitized throughout its thickness and that the IATCS solution is sufficiently aggressive to render the parting sheet ineffective as a barrier. Corrosion-resistant alloys such as CRES 347 can have metallurgical phases that are susceptible to localized dissolution. One example is intergranular corrosion (IGC) in which chromium-containing compounds form at grain boundaries, leaving the surrounding regions devoid of the alloying element that provides the corrosion resistance. When sufficient precipitation occurs (due to sufficient time at a temperature where the chromium-bearing compounds are insoluble), an interconnected network of sensitized grain boundaries is formed that links one side of the material to the other. IGC requires both a susceptible microstructure and appropriate environmental conditions (solution composition and temperature). That is, sensitization is a necessary, but not sufficient, condition for service-life limiting IGC. The degree of sensitization can be determined quantitatively using American Society for Testing and Materials (ASTM) standardized methods. The IATCS solution composition is quite benign with respect to localized corrosion of stainless steel: a very low chloride concentration, a mildly alkaline to alkaline pH, and a large excess of non-chloride ions (which act to inhibit localized corrosion). IGC of sensitized stainless steels typically occurs in oxidizing solutions containing substantial chloride ion concentrations.

The susceptibility of sensitized CRES 347 to IGC has not been verified under the IATCS coolant conditions. The assumption of susceptibility leads to artificially-shortened life predictions as no credit is taken for presence of the stainless steel parting sheet. The microstructures in cross-sections shown in the Boeing Assessment Report (refer to Appendix D of this report), in which grain boundaries are decorated, were created by standard metallographic methods (oxalic acid etching) that do not directly correlate to susceptibility in other environments. ASTM Method G-108 has been shown to quantify the susceptibility of stainless steels to IGC in actual environments (e.g., boiling water nuclear reactors). The actual susceptibility of the CRES 347 should be determined under realistic IATCS coolant conditions.

Penetration of brazed IATCS components, via selective braze area attack and IGC of the CRES 347 parting sheet, would require a through thickness interconnected network of braze intermetallics connected to a sensitized CRES 347 grain boundary that transverses the parting sheet. Microstructure and SEM examination of BNi3 braze samples on the CRES parting sheet material do not show this total through thickness intermetallic/sensitized grain boundary condition. Braze intermetallic corrosion is limited to those locations that are surface-intersecting. The degree of sensitization in the CRES 347 is a gradient with the maximum at the braze/parting sheet interface. Based on the analyses of precipitates formed in the long-term corrosion exposures, it appears that the most likely situation is that the majority of the exposed

intermetallics have corroded out from the braze due to the extended time at the decreased pH. In the analyses of those precipitates, as well as those from the failed gas trap, the ratio of nickel to phosphorus was close to that expected for stoichiometric dissolution of the intermetallics, with no iron or chromium levels detected. If substantial dissolution of the braze matrix had occurred, one would expect levels of nickel in the precipitate above that of the intermetallic stoichiometry. The absence of iron and chromium support the idea that no or little damage has reached sufficiently sensitized grain boundaries of the CRES 347.

MIC is currently perceived as an issue with no direct corrosion testing to support the concern. It persists as a general concern due to the high microbial counts in the U.S. Laboratory IATCS coolant. The commonly theorized initiation sites for MIC are the cavities produced by the corrosion of the intermetallics where sulfate reducing bacteria (SRB) could grow. The risks of MIC to crew safety and system performance are the same as those cited above for general and localized corrosion. However, there is no direct laboratory testing evidence that MIC has occurred to date. The destructive examination of returned U.S. Laboratory hardware may allow more rationalization of the MIC concern. This discussion is presented in Section 11.0, Collateral Issues.

F-6. *The overall corrosion rates used in the component life analyses are based on the braze matrix minimum thickness and its corrosion rate measured from long-term exposures. No contribution to component life is considered from the partially sensitized CRES 347. This approach of utilizing only the braze matrix corrosion rate provides a very conservative estimate of component life even considering the potential for MIC.*

7.0 Independent Assessments of Antimicrobials

In an effort to expand the knowledge-base beyond the SPRT team members, contracts were awarded to Mittelman and Associates (Braintree, MA) and Montana State University (Bozeman, MT) by the ISS Program and the NESC. The objective was to solicit additional independent input to potential antimicrobials for use in the IATCS. The narrative sections of their reports are located in Appendices A and B of this report.

7.1 Mittelman and Associates

In summary, Mittelman and Associates reviewed the IATCS requirements and assessed the potential individual use of fifteen (15) antimicrobials. Their assessment included a numerical scoring system to quantify their choice. Scoring was based on several characteristics including safety, material compatibility, antimicrobial efficacy, IATCS chemistry compatibility, stability, and industrial experience with the compound. Based on total point scores, the antimicrobials were grouped into four tiers, with glutaraldehyde and isothiazolones obtaining the highest scores.

The second tier included chlorhexidine, ozone, orthophthalaldehyde, and peracetic acid. The numerical score differences between the antimicrobials in the first and second tiers were not appreciable and were considered as one grouping. The remaining antimicrobial choices were considered less suitable for IATCS use.

Mittelman and Associates recommended investigation of the following emerging microbial treatment technologies.

- Reduction of Coolant Water Activity
- Application of Titanium-doped Nitrogen Oxides
- Radio Frequency Dosing
- Application of Cationic Polymers
- Gamma Irradiation
- Application of Ethylenediamine Tetraacetic Acid (EDTA)

Mittelman and Associates also stressed the need for an investigation into methods of residual chemical inactivation in the event of an inadvertent release of the antimicrobial chemical aboard the ISS. They recommended that candidate antimicrobials should be evaluated based on their efficacy against bacteria both in solution and in biofilms. An evaluation of dosing schedules and antimicrobials in combination was cited as potentially beneficial, as well as the investigation of non-aqueous cooling fluids.

7.2 Montana State University

In summary, the consulting group at Montana State University provided a qualitative assessment of antimicrobials and their potential for use. They felt they could not quantitatively rank agents for application until more information was gathered concerning the specific characteristics and operation of the IATCS. In order of priority, they felt the criteria for selection of the antimicrobial should be:

- Toxicological Assessment
- Material and Chemical Compatibility
- Antimicrobial Efficiency

The group felt that antimicrobials used in combination could help to lower the overall concentrations needed for antimicrobial efficiency. This could be an important advantage in mitigating toxicological and crew health issues.

The Montana State group stressed the need for ground-based studies before application on the IATCS. They believed the need for remedial action on the IATCS in its present status is balanced by the potential harm that could be caused by the remediation process itself. Potential negative factors include both engineering complications, such as system blockage, and crew health issues from the addition of toxic compounds. They stressed the need to address the root cause(s) of the microbial growth and to try to return coolant chemistry to its original state.

Their assessment focused away from organic antimicrobials as these agents, or their breakdown products, have the potential to add carbon sources to the IATCS fluid, which could promote additional microbial growth. They also advised against antimicrobial compounds containing chlorine or phosphorous, since these may have material incompatibility and additional pH suppression issues. While they feel more data needs to be gathered, their initial recommendations included hydrogen peroxide, iodine, silver, a combination of hydrogen peroxide and silver, or a combination of iodine and silver.

7.3 NESC Assessment

The Mittelman and Associates and Montana State University assessments provided some direction for antimicrobial selection, although differences in their professional opinions resulted in no single antimicrobial being selected by both groups.

After review of the recommended antimicrobials and their evaluations by the two independent organizations, the NESC team identified the following compounds/combinations for investigation in order of preference:

- Iodine
- Iodine/Silver
- Hydrogen Peroxide/Silver
- Silver on a ceramic bed matrix
- Orthophthalaldehyde
- Isothiazolones
- Chlorhexidine

Specific considerations were used in the evaluation of these compounds including a hesitancy to use organic antimicrobials, as these could increase TOC in the IATCS fluid and act as a nutrient source for microorganisms. Inorganic antimicrobials were generally considered more acceptable, although material compatibility issues would need to be resolved before selection.

These evaluation factors are considered qualitative and subjective based on information presented by Mittelman and Associates and Montana State University in combination with IATCS knowledge, associated research, and prior experience. However, no specific and quantifiable requirements were identified to provide a more definitive list of antimicrobials for additional characterization or selection.

Iodine was chosen as a practical alternative for investigation based on prior use in NASA spacecraft, limited toxicological hazards, and established mechanisms for addition into spacecraft fluid systems. Questions remain as to its efficacy above pH 9.0 and material compatibility with all materials in the IATCS.

Silver, in some forms, has been attempted in the IATCS and was used in Mir and in the ISS Service Module potable water systems. The consultation team and one of the independent assessments agree that either in combination with hydrogen peroxide or iodine, or as a solid on a ceramic bed matrix. Silver still has the potential for IATCS disinfection in much lower concentrations than have been used in the past.

Orthophthalaldehyde is organic, but has disinfectant qualities worth investigating. It is an aldehyde, similar to glutaraldehyde, but it is reported as having a lower vapor pressure and less toxicity according to limited toxicological investigations.

Hydrogen peroxide is an excellent disinfectant with the capability of disrupting biofilms that have already formed. This disruptive capability may provide some advantage in cleaning up ground-based systems, such as Node 2 prior to flight or the GSE. For this same reason, however, strong hydrogen peroxide solutions should not be used in the U.S. Laboratory on-orbit where disruption of existing biofilms could cause problems with clogging filters and gas traps. Hydrogen peroxide can be hazardous in a concentrated form, but has the advantage of producing no toxic residual. As a strong oxidant, it suffers from a short life as a disinfectant. For this reason, it may have better use in combination with another disinfectant such as silver. Combinations such as this may provide some synergistic benefit, enabling the use of decreased concentrations of the components.

Both i*sothiazolones* and c*hlorhexidine* are excellent disinfectants that could be used in this application. However, they contain chlorine in their chemical makeup, which would decrease their material compatibility. Finally, glutaraldehyde was omitted from the list of new candidates due to its current level of characterization and its relatively high toxicity.

8.0 Hardware Examination

To date no retrieved hardware has been available for destructive examination due to the limited down mass allocation in the Russian Soyuz vehicle and the interrupted flights of the Space Shuttle after the STS-107 accident. However, the return of STS-114, currently scheduled for July 2005, is manifested with the return of the SPCU HX, a failed PPA, a Teflon flex hose, filters, and a malfunctioning Common Cabin Air Assembly (CCAA) HX. Planning is underway in developing a recommendation for comprehensive dissection and characterization of each component. However, these plans may not be adequate considering data prioritization.

Each IATCS component has unique configuration attributes and limitations for data extraction. As an example, the PPA to be examined will have been isolated from the coolant loop for more than 12 months with a finite volume of stagnant coolant. The microbial characteristics of this component would not be representative of the entire IATCS. Therefore, it is appropriate to establish a set of primary and secondary objectives for each component scheduled for return. The primary objectives would be to establish the minimum data needed to determine future actions. The secondary objectives would be the confirmation of primary objective information from other hardware or for engineering information. Table 8-1 shows suggested primary and secondary objectives for the identified hardware schedule for the return of STS-114.

F-7. *Hardware dissection planning does not appear to be based on clear data extraction objectives.*

Table 8-1. Identified Hardware and Primary/Secondary Objectives for Return of STS-114

Hardware ID	Primary Objectives	Secondary Objectives
SPCU HX	- Braze corrosion characterization in IATCS and two Extravehicular Mobility Unit (EMU) loops evaluating general corrosion, pitting, IGC, and microbial/pitting. - EMU particulate (rust) source identification and characterization. - Biofilm characterization including speciation, thickness, and coverage for potential component life assessment modifications and heat transfer predictions. - Metallurgical characterization of the braze components exposed to the IATCS loop.	- Particulate characterization including chemistry, size, shape, and quantity. - IATCS and EMU coolant chemistry with recognition that the delay from hardware removal to retrieval minimizes conclusions drawn from this information.
PPA	- Pump anomaly determination, including: - Particulate characterization including chemistry, size, shape, and quantity	Depending on pump anomaly root cause: - IATCS and EMU coolant chemistry with recognition that the delay from hardware removal to retrieval minimizes conclusions drawn from this information. - Biofilm characterization including speciation, thickness, and coverage for potential component life assessment modifications and heat transfer predictions. As with the coolant chemistry, the delay from hardware removal to retrieval marginalizes any conclusions obtained.
Flex hose	- Biofilm characterization including speciation, thickness, and coverage for potential component life assessment modifications and heat transfer predictions.	- Material property and configuration to examine Teflon material property loss and surface roughness changes.
Filters	- Particulate characterization including chemistry, size, shape, and quantity.	- Filter physical characteristics.
CCAA HX	- Braze corrosion characterization in the IATCS loop to examine general, pitting, and microbial/pitting. Delay from hardware removal to retrieval and dissection emphasis on air loop minimizes conclusions drawn from information. - Metallurgical characterization of the braze components exposed to the IATCS loop.	None.

9.0 Ground Support Equipment (GSE) Servicing Units

GSE servicing units are used periodically in the servicing of Node 2 hardware to circulate IATCS coolant and to provide functional checkout. The servicing units are maintained and controlled by KSC operations personnel and are required to obtain approval from the SPRT for any configuration changes that may affect the IATCS. However, the coolant used in the servicing units adheres to the chemistry identified in SSP 30573, Revision B, which specifies silver sulfate and di- or tri-sodium phosphate, whereas the Node 2 IATCS coolant chemistry was purposely formulated without silver and phosphorous. Finally, the servicing unit coolant is not routinely checked for microorganism levels. During the most recent coolant extraction of Node 2, the servicing unit was checked for microorganism levels prior to being connected to Node 2. It was determined that prior to servicing, Node 2 had CFU levels lower than the servicing unit. However, as was expected, after servicing, the CFU levels of both Node 2 and the GSE unit were measured at the elevated levels seen in the GSE unit prior to coolant intermingling. Following this exposure, actions have been implemented to minimize future cross-contamination risks. Examples of these procedure changes to the servicing units include recurring microbial monitoring, the installation of 0.2 micron filters, and periodic disinfections. The microbial measurement and disinfectant intervals have not been established and matched to IATCS requirements.

F-8. *The coolant chemistry used in the servicing units is not representative of the coolant selected for Node 2. Periodic servicing has the potential for introducing silver and phosphorous to the Node 2 IATCS.*

F-9. *The microorganism levels are not controlled in the servicing units and, in at least one case, resulted in the introduction of additional microbial counts to the Node 2 IATCS.*

10.0 Other ISS Modules Issues

Although not specifically within the scope of this consultation effort, consideration of the other ISS modules containing IATCSs should be discussed. These include the completed European Space Administration (ESA) Columbus Orbital Facility (COF) and the Japanese Aerospace Exploration Agency (JAXA) Japanese Experimental Module (JEM) Pressurized Module and the under construction Node 3. The COF and JEM have been reported to currently contain DI water and nominal coolant, SSP 30573, Revision B, respectively. The materials of construction for the COF and JEM are not specifically known, but are assumed to be similar with respect to the U.S. Laboratory and Node 2 HX and cold plate construction. It was also reported that their coolant chemistries are not routinely monitored for microorganism or for metal ions levels.

It appears ESA and JAXA are monitoring NASA's investigations and are awaiting the U.S. Laboratory and Node 2 proposed coolant chemistry/IATCS modifications. However, there does not appear to be a clear avenue of technical interchange between the various space Agencies. If ESA and JAXA are maintaining a reactive posture, corrosion and/or microbial growth are likely occurring in their IATCSs.

F-10. *There does not appear to be an integrated exchange of technical information with the other space Agencies on the cause and corrective actions associated with the observed IATCS corrosion and microbial growth.*

10.1 IATCS Equipment Interchangeability

The ISS equipment bays and racks are designed for interchangeability and to accommodate experiment upgrade and replacement. The potential for hardware introduction or migration necessitates consideration of IATCS coolant mingling from both chemical and microbiological points of view. As it is not considered practical to drain and replenish the equipment being transferred on-orbit, consideration needs to be given to development of a protocol for the eventuality of equipment interchanges.

As the coolant volume contained in one experiment cold plate or rack is a limited percentage of the IATCS capacity, chemical issues for coolant mingling can be approached from mass/contaminate balance calculations, interface pretreatment regiments, and/or crew hygiene protocols. The maximum allowable rack coolant quantity is 6.9 L for the MTL and 3.4 L for the LTL pursuant SSP 57001, Revision G. If it is assumed the rack volume is greater than that of a cold plate, then the rack coolant percentages with respect to the MTL and LTL are 3.5 and 5.4%, respectively.

Assuming the coolant chemistry of the migrating hardware and the receiving IATCS is known, simple mass/contaminant balance calculations could be performed to assess the impact. These calculations would be assessed against toxicity, chemistry specification limits, and material compatibility to ensure no exposure or performance issues are identified. Passing this evaluation, the equipment migration could proceed. An extreme test case for this approach is the utilization of the GSE servicing unit to Node 2. Although the volume of the servicing unit is approximately the same as the ITACS (265 L), the effect of combining the two systems could be predicted with mass/contaminate balance calculations.

Microbiological contamination issues cannot be approached by the same type of mass/ contaminate balance calculations because they do not scale according to relative concentrations and volumes. Given favorable growth conditions, unacceptable microbial counts can develop

from a single organism. A separate set of criteria needs to be developed for handling microbial issues related to equipment changes and exchanges. These criteria need to consider compatibility of various antimicrobials as well as dangers to crew health, system performance, and component life.

If chemical or microbiological concerns exist on mingling coolant to or from a lower quality coolant loop, development of an inlet or outlet pre-treatment mechanism would be required. The capacity of an equipment inlet system would need to be larger than that of an outlet system, as it would be treating the entire coolant volume versus just the equipment or rack coolant volume on an outlet system.

In all cases, hygiene protocol would be required to protect the crew from potential toxin exposure as well as minimization of microorganism introduction to the IATCS. This protocol would include crew training on potential risks, personal protective equipment, and remediation equipment. A conceptual evaluation flow for addressing chemical issues is illustrated in Figure 10-1. A similar evaluation flow could be used for microbiological issues, except that the acceptance criteria would be different.

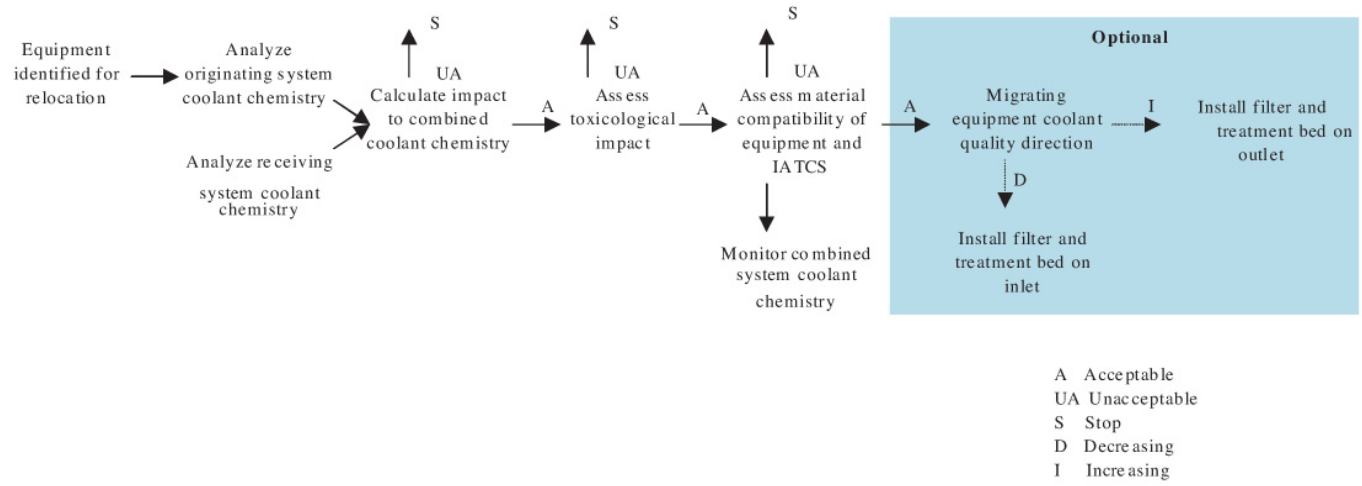

Figure 10-1. Conceptual Coolant Intermingling Evaluation Flow

F-11. *There are no equipment and rack change-out protocols to prevent chemical or microbiological problems arising from intermingling of coolant between different IATCS units.*

11.0 Collateral Issues

This section includes a discussion of issues that were beyond the scope of the current NESC task, but should be addressed. Collateral recommendations are provided in Section 11.8.

11.1 Glutaraldehyde Toxicity Assessment

In June 2004, Jay Perry[3] conducted an engineering assessment to fully understand the Environmental Control and Life Support (ECLS) system-related impacts associated with changing the IATCS antimicrobial additive from silver to glutaraldehyde (refer to Appendix C). This analysis examined the current ability of the active contamination control system on board the ISS trace contaminant control subsystem (TCCS) to control glutaraldehyde contamination levels in the cabin atmosphere to below 180-day spacecraft maximum allowable concentration (SMAC) values. The ability to effectively remove glutaraldehyde from the ISS cabin atmosphere was also determined for the scenario in which the TCCS glutaraldehyde removal was assisted by humidity condensate absorption (via CCAA and SKV heat exchangers), and by contamination control equipment (BMP) in the Russian On-orbit Segment (ROS). For the analyses involving humidity condensate absorption, specified limits for glutaraldehyde contamination of the condensate were taken into consideration. Potential sources of glutaraldehyde contamination in the cabin atmosphere that were considered included spills of stock solutions and coolant leaks within the allowable specification limits.

This analysis concluded that the TCCS on board the ISS alone is not capable of accommodating leaks of glutaraldehyde containing IATCS fluid at the system specified leak limits, regardless of the glutaraldehyde concentration. However, a combination of ECLS trace contaminant control and glutaraldehyde absorption by water processing systems could accommodate IATCS fluid concentrations of <25 mg/liter glutaraldehyde, without exceeding either the SMAC limits or the humidity condensate contamination limits. The analysis also concluded that any decision by the ISS Program to use glutaraldehyde as a antimicrobial additive to the IATCS fluid in the United States On-orbit Segment (USOS) must be reviewed by the International Partners within the Common Environments Team forum, since fugitive glutaraldehyde emissions in the common cabin environment would impact contamination control equipment on board the ROS.

The Boeing analysis of ISS glutaraldehyde scrub capacity (Appendix D) examined the ability of various combinations of the TCCS, BMP, CCAA, and SKV systems to control both specified and nominal leak rates of IATCS fluid containing 50 ppm of glutaraldehyde. Boeing carried out this evaluation for the ISS in its current configuration, as well as for its assembly complete configuration. This analysis found that a 50 ppm glutaraldehyde concentration in the IATCS

[3] Senior Engineer, ISS Air Quality Control Systems, Environmental Control and Life Support Group

fluid produced a cabin atmosphere that could not be controlled using any combination of the TCCS or BMP contamination control systems. The assembly complete configuration was also found to be significantly less forgiving than the current configuration with regards to effective glutaraldehyde removal. The calculations showed that cabin air contamination could not generally be controlled below the 180-day SMAC limits for the assembly complete configuration unless all of the scrubbing hardware was actively functioning and, even then, control was marginal.

A memorandum prepared by Martin E. Coleman[4] to assess the differences between glutaraldehyde and orthophthaldehyde, reported that glutaraldehyde could be considered relatively non-hazardous if spills of coolant (comprised of a highly dilute glutaraldehyde solution) are collected and neutralized by the ISS crew before significant evaporation of water occurs (refer to Appendix F). However, if a delay should occur due to the inability to detect the coolant leak/spill, then the concentration of glutaraldehyde would increase, creating a crew toxicological hazard. Because there is a significant probability that such an undetected leak/spill could occur, and because the on-orbit contamination control systems do not appear to be adequate for controlling glutaraldehyde contamination from IATCS fluid leaks, *it is believed that glutaraldehyde should not be considered for on-orbit use at this time*. Furthermore, additional studies would be necessary before glutaraldehyde could be considered for use as an on-orbit emergency antimicrobial.

11.2 Borate/Carbonate Buffer Additions

The purpose of the borate/carbonate buffer additions is to increase the pH of the IATCS coolant back to the nominal initial value. The increased pH would slow the rate of subsequent corrosion and inhibit microbe growth. The increased pH would result in any free nickel precipitating as nickel hydroxide and/or nickel carbonate, so the borate/carbonate additions would have to be preceded by implementation of NiRA to remove free nickel ions in solution.

Concerns with this approach follow generally from the admonitions in the Global Recommendations (Section 12.1) to *proceed with caution as to do no harm, and act only when necessary*. Insufficient testing has currently been performed to determine the effects of borate/carbonate additions on coolant chemistry and materials compatibility issues. Several years of involvement with IATCS coolant chemistry issues have demonstrated that it is virtually impossible to model what could occur on-orbit when the system is undergoing relatively slow changes, much less if numerous rapid changes are initiated to the apparent current steady state.

[4] Wyle Toxicology Consultant, mecoleman33@sbeglobal.net

11.3 Node 2 Antimicrobial Implementation

The concerns of implementing an antimicrobial agent on Node 2 may be less than implementation in an on-orbit IATCS. This is because an effective, but potentially toxic agent, used to preclude microbial growth and biofilm formation during ground storage/operation, could then be removed or neutralized before launching. As such, using glutaraldehyde with a concentration of >100 ppm (to achieve the minimum lethal concentration) during ground storage is feasible if verification of complete remediation can be assured prior to flight. However, it is not clear that the introduction of an antimicrobial to the Node 2 IATCS is warranted at this time based on the measured CFU levels. The microbial levels are significantly lower than U.S. Laboratory and have not shown a trend towards rapid increases. It should be cautioned that the use of glutaraldehyde (or any organic-based antimicrobial) will impact the ability to interpret measured data on TOC levels and trends.

11.4 Nickel Removal Assembly (NiRA) and Phosphorous Removal Assembly (PhosRA) Characterization and Implementation

While at Alenia, Node 2 experienced a number of DI water flushes. Then, it was shipped to KSC with DI water that was maintained for approximately six months (while at KSC). The amount of nickel leaching during that period is unknown, but may have been substantial since the pH of DI water is 6; whereas the pH of the on-orbit coolant ranges from 9.3 to 8.3. No comprehensive coolant chemistry records are available to assess the amount of nickel removed. Much of the nickel available in the braze intermetallics may have leached out during the period of DI water exposure. The application of a NiRA may not be necessary as the Node 2 IATCS was subsequently flushed, presumably removing the majority of the nickel ions.

With regard to the use of NiRA and PhosRA on-orbit, there are significant concerns. Recent bench test data have shown that even after multiple, extended applications of NiRA, some dissolved nickel remains in the solution. This implies that NiRA would have to be placed in contact with the coolant for periods significantly longer than initially predicted. There has been no data obtained on the effects of exposing the coolant to NiRA for this long and concerns, such as the predilection of the NiRA for acting as a microbial growth bed, have not been addressed. In addition, insufficient testing of the NiRA at higher pH (specifically its ability to remove nickel hydroxide) has not been conducted.

11.5 Corrosion Sensing Equipment for Ground-Based Systems

Corrosion sensing equipment has been tested in the laboratory for its ability to measure general corrosion rates of materials of interest in the IATCS. It should be noted that none of the corrosion sensing equipment being considered measures the corrosion rate of the actual structure. Instead, the equipment measures the corrosivity of the coolant towards the materials of interest

(e.g., the BNi3 matrix material) via measurement of the polarization resistance (R_p) of a suitable probe. R_p has been shown both theoretically and experimentally to be inversely proportional to the uniform corrosion rate of metallic materials via a proportionality constant known as B. The electro-chemical identification and monitoring of localized corrosion (i.e., pitting of the braze or IGC of the CRES 347) is much more difficult, although some progress has been made lately.

The applicability of the corrosivity measurements to the IATCS structure is determined by the ability to produce sensors, or probes, that mimic the material of interest as well as its surface condition. The complex metallurgical microstructure of the braze as well as the unique history of coolant chemistries of U.S. Laboratory and Node 2 represent substantial barriers to achieving the level of replication required to use the data for highly accurate service life prediction. Instead, the best use of this technology is for the determination of how changes in coolant chemical composition affect the corrosion rate of a particular material that is mounted in the sensor. In addition, the effects of surface condition of the materials of interest can be probed by, for example, adding Braycote grease to the coolant, removal of surface-intersecting intermetallics in the BNi3, or encouraging a biofilm to develop. At present, conclusions regarding the effects of such surface conditions and changes are based primarily on insufficient objective evidence. In addition, the corrosion rates of the materials of interest can be determined as a function of time, which will improve life prediction estimates that assume a constant corrosion rate.

The low corrosion rates expected and the low conductivity of the IATCS coolant represent challenges to corrosion rate measurement. There are a number of commercial systems available with equivalent ability to measure the uniform corrosion rate with the R_p technique. In making such measurements it is important to be sure that the measured R_p value has been corrected for the ohmic resistance of the IATCS coolant. Failure to correct for this resistance can lead to substantial underestimation of the corrosion rate. The R_p equipment tested to date by the ISS Program, the SmartCet® system, did not correct for ohmic resistance during the trial, although it purports to be able to do so. The correction of corrosion rates determined via the R_p for variations in the B value (as done by the SmartCet® system) is of little technical consequence. Over the range of technically reasonable values for B, the corrosion rate changes by, at most, a factor of three, and most likely in the range of 10-30%.

As mentioned, the monitoring of localized corrosion is challenging. In addition, it is not possible to determine that corrosion has been caused or influenced by microbes using electrochemical methods alone. It is only possible to directly monitor MIC when cause and effect between corrosion and the microbes has already been independently established using a multitude of electrochemical, metallurgical, and biological techniques.

Two systems are being considered by the ISS Program for monitoring of localized corrosion of the braze – the SmartCet® system by InterCorr and the Multi-electrode Array Sensor System (MASS), developed at Southwest Research Institute. The pitting factor (PF) that SmartCet® purports to use to detect localized corrosion is not proven. The theory behind the PF is not developed sufficiently and the literature presents cases where it fails. For use in any given system, the PF must be corroborated by damage observed on the test electrode at the end of the test. Even with this precaution, the PF should not be overly emphasized as it will not be possible to relate the time course of pitting on the sample to the measured PF given the long exposure time. The MASS has been proven effective in monitoring localized corrosion in some chemical plant applications. Significant development effort will be needed to determine an appropriate design and fabrication of MASS electrodes representing the brazed IATCS hardware. Thus, the two sensors are complementary in that SmartCet® provides an average corrosion rate over a large area, while the MASS provides a better measure of localized corrosion because of the use of multiple, smaller sensors.

Use of these sensors for quantitative monitoring of the corrosion rates of structures in Node 2 or U.S. Laboratory is not recommended due to the challenges in reproducing the surface conditions currently present on those structures due to previous corrosion, biofilm development, and the unique history of Node 2 and U.S. Laboratory (i.e., contamination of surfaces with Braycote grease).

The most useful information gained from the application of corrosion monitors would come from carefully controlled experiments where the conditions of surface preparation and coolant composition have been well-defined. When using Faraday's Law[5] of Electrolysis to extract penetration rates from the measured corrosion current densities, verification of these corrosion rates should be obtained through visual inspection of samples and, if possible, the determination of nickel ion concentration in the coolant.

11.6 Long-Term Antimicrobial Development

Continued long-term antimicrobial development is recommended. Current candidates such as glutaraldehyde and orthophthalaldehyde pose questionable health threats and should be used only in the case of an on-orbit microbial emergency or on the ground where the toxicity levels could be adjusted to acceptable levels prior to launching. The aforementioned list of antimicrobial agents recommended by the independent consulting teams (Mittelman and Associates, Montana State University, and NESC) should be tested for their efficacy in laboratory bench test and simulated IATCS conditions.

[5] http://members.aol.com/logan20/faraday.html

11.7 Comprehensive Ground Test Roadmap for Potential Bio- and Chem-Fouling Problems

Currently, the best IATCS test bed available is the system at MSFC. However, this test bed does not accurately reflect current conditions of on-orbit U.S. Laboratory coolant system or Node 2 from the standpoint of corrosion, precipitates, or microbial growth.

Test bed development must represent an actual operating system with respect to configuration and must contain similar geometries (i.e., surface area, flow shear stresses, filters, pumps, gas traps, and cold plates). The fluid chemical composition must mimic as closely as possible the actual system under investigation in terms of corrosion susceptibility, microbial types and concentrations, and precipitate types, concentrations, and locations. The U.S. Laboratory and Node 2 IATCSs represent distinctly different operating systems because of their unique histories.

Test bed operation should be used to monitor the evolution of the IATCS characteristics and to evaluate any proposed changes to these systems. Any proposed changes to an actual operating system must be tested and verified in an appropriate test bed with the controlled change of only one variable at a time. The required metrics are microbial enumeration and identification, evaluation of system performance, and determination of component life.

11.8 Collateral Recommendations

Based on the issues discussed in Sections 11.1 through 11.7, the following list of collateral recommendations (CR) was compiled.

CR-1. Prohibit the use of glutaraldehyde as an on-orbit antimicrobial due to crew toxicity concerns and the absence of a remediation protocol. (Section 11.1)

CR-2. Use of glutaraldehyde as a ground-based antimicrobial for Node 2 and the GSE acceptable if measured microbial levels are unacceptably high and increasing, and verification of complete remediation can be assured prior to flight. (Section 11.3)

CR-3. Prohibit the use of silver as a near-term emergency antimicrobial unless there is clear trigger action (quantifiable health, performance, or component life concern), recognition of the short-term effectiveness of silver, and an assessment of the potential collateral galvanic corrosion effects.

CR-4. Discontinue implementation plans for on-orbit remediation procedures, including borate/carbonate additions and the use of NiRA and PhosRA based on current and projected on-orbit conditions for U.S. Laboratory. However, continue ground-test

development of remediation procedures in case a quantifiable on-orbit emergency condition develops that would require some or all of these to be implemented in the future. (Sections 11.2 and 11.4)

CR-5. Evaluate commercial corrosion monitoring systems to identify the most reliable and robust system(s) that can detect corrosion rate changes indicative of the onset of localized corrosion in IATCS units. These systems should be used only in laboratory-scale tests of the surface condition and coolant chemistry effects. Use of corrosion monitoring devices in U.S. Laboratory and Node 2 is not recommended at this time. (Section 11.5)

CR-6. Do not base any decisions on the PF (as used by SmartCet®) unless the capability can be demonstrated in a single-blind test, under controlled laboratory conditions with post-test confirmation of prediction. (Section 11.5)

CR-7. Continue specific testing for MIC including the possible effects of preferential colonization by SRB at sites (pits) where the intermetallics have been removed. (Section 11.5)

CR-8. Continue characterization of alternative antimicrobials based on qualitative performance and acceptance requirements to address evolving microbial populations in water-based cooling systems. (Section 11.6)

CR-9. A system level fleet leader test bed, representative of flight configuration and coolant conditions, should be maintained throughout the life of the Program to evaluate unforeseen performance characteristics. (Section 11.7)

12.0 Recommendations

12.1 Global Recommendations

As stated in Section 1.0, the NESC team has adopted the governing tenets of *protect the crew, proceed with caution as to do no harm,* and *act only when necessary*. These tenets will provide invaluable guidance in assessing the issues faced and interjecting possible investigation areas and courses of action.

Protect the crew is self-evident in that no action should be implemented that would create a condition where exposure to a hazardous condition can be anticipated. There has been considerable discussion on antimicrobial toxicity with respect to the coolant parasitic leakage and low-level exposure limits. However, during transportation, storage, and handling,

concentrated antimicrobials also require careful consideration and characterization. The nearly closed-loop nature of the ISS environment poses severe limitations on discrete and long-term exposure limits. However, ground processing also requires appropriate screening for toxicity notification and monitoring.

Proceed with caution as to do no harm is important when dealing with a complex system with operational nuances not readily appreciated. This tenet directs careful problem definition and comprehensive characterization. Controlled experimentation at increasing complexity is warranted.

Act only when necessary is intended to use quantifiable triggering criteria for implementing actions. These should be based on demonstrated actual or imminent threat to crew health, meaningful loss of performance, or meaningful component life reduction.

12.2 Principal Recommendations

R-1. Continue quantitative characterization and trending for IATCS coolants in ground-based and on-orbit systems to compare specification to actual chemistry and to monitor microorganism species and levels. (F-1 and F-2)

R-2. Re-evaluate coolant chemistry and microbial intervention rationale for U.S. Laboratory based on apparent system steady state with regard to microorganism levels and nickel precipitation. Reassess the overall corrosion rate assumptions used in the calculation of component life based on a re-examination of the laboratory corrosion testing data and evaluation of returned hardware. (F-3, F-6, and F-8)

R-3. Evaluate susceptibility of the CRES 347 (sensitized and unsensitized) to IGC in IATCS coolant via long-term exposures and ASTM G 108 Electrochemical Potentiokinetic Reactivation testing on surfaces of CRES 347 after removal of brazes for both BNi2 and BNi3. Susceptibility should be determined as a function of distance from the braze/CRES 347 interface. (F-6)

R-4. Assess the development of a quantitative ground-based and on-orbit test method for the determination of HX performance. Based on experience with the Ocean Thermal Energy Conversion (OTEC) program, a heat transfer monitoring device would be a very sensitive method to monitor biofilm development. (F-4)

R-5. Establish a set of qualitative performance and acceptance requirements for evaluating proposed antimicrobials over the entire application range. These requirements will be used to set characterization priority and final antimicrobial selection. (F-5)

R-6. Develop specific primary and secondary dissection objectives for returned hardware that

includes inspection for the detection of pitting corrosion as well as measurement of biofilm speciation, thickness, and coverage and entrapped nickel precipitates. Planning should include development of a protocol for how hardware is to be removed, handled, packaged, transported and analyzed as well as for how chemical, metallurgical, and biological examinations are to be performed. (F-7)

R-7. Perform laboratory corrosion testing of BNi3 material to determine: (a) the effects of NiRA on corrosion rate, (b) the accurate dissolution rate of braze material, including time dependence, and (c) the effects of antimicrobials on materials of construction. Use electrochemical techniques that measure corrosion rate with time at the open circuit potential. Determine repassivation potential for materials of concern using modern methods. Compare steady state open circuit potential to repassivation potential to assess likelihood of localized corrosion. (F-3 and F-6)

R-8. Match the GSE coolant chemistries to the compositions of the flight systems to avoid the introduction of undesirable constituents. Microbial species and levels in the GSE should be controlled as not to introduce a new species and to a minimum level that does not exceed the CFU count of the flight system being serviced. (F-9 and F-10)

R-9. Develop a coolant intermingling protocol to determine instances where the utilization of GSE and/or the relocation of equipment and racks between IATCS are acceptable. (F-9 and F-11)

R-10. Evaluate the initiation of TIMs with ESA and JAXA on the results of ongoing investigations and the potential for inadvertent corrosion damage and/or uncontrolled growth of microorganisms in the COF and JEM. (F-10)

13.0 Lessons Learned

1. Future closed-loop water-based cooling systems should not use gas permeable tubing that could allow unanticipated changes to coolant stability.
2. GSE servicing units should be governed by the same configuration and coolant controls as flight systems to minimize the introduction of uncontrolled contaminants, microorganisms, and nutrients.
3. A system level fleet leader test bed, representative of flight configuration and coolant condition, should be maintained throughout the life of the Program to evaluate unforeseen performance characteristics.
4. Proposed corrective actions should be comprehensively characterized in the laboratory and validated in a test bed to monitor, remediate, and address unintended consequences before implementation in flight systems.

5. Continued antimicrobial development should be pursued to address evolving microbial populations in water-based cooling systems. These antimicrobials should not be toxic to the crew, produce performance issues, impact other subsystems, or effect component life.

6. With such a complex chemical/materials situation as is present in IATCS, it is virtually impossible to perform modeling that accurately predicts on-orbit behavior.

14.0 List of Acronyms and Symbols

AOX	Assimilable Organics
ASTM	American Society for Testing and Materials
ATP	Acceptance Test Plan
BMP	Contamination Control Equipment a in the Russian On-orbit Segment
BNi	Boron Nickel
C	Celsius
CCAA	Common Cabin Air Assembly
CFU	Colony Forming Unit
COF	Columbus Orbital Facility
CO_2	Carbon Dioxide
CPP	Cyclic Potentiodynamic Polarization
CRES	Corrosion Resistant Steel
CWG	Coolant Working Group
Cxt	Concentration and Time
DI	Deionized
EATCS	External Active Thermal Control System
Ecorr	Corrosion Potential
ECLS	Environmental Control and Life Support
EDTA	Ethylenediamine Tetraacetic Acid
ESA	European Space Administration
EMU	Extravehicular Mobility Unit
EPR	Ethylene Propylene Rubber
GSE	Ground Support Equipment
H_2O	Water
HOI	Hypoidous Acid
HS	Hamilton Sundstrand
HX	Heat Exchanger
IATCS	Internal Active Thermal Control System
IFHX	Interface Heat Exchanger
IGC	Intergranular Corrosion
ISS	International Space Station

ITA	Independent Technical Assessment
JAXA	Japanese Aerospace Exploration Agency
JEM	Japanese Experimental Module
JSC	Johnson Space Center
KSC	Kennedy Space Center
LaRC	Langley Research Center
L	Liter
LTL	Lower Temperature Loop
MASS	Multi-electrode Array Sensor System
MCV	Microbial Check Valve
MIC	Microbiologically Influenced Corrosion
mL	milliliter
MSFC	Marshall Space Flight Center
MTL	Moderate Temperature Loop
mV	millivolt
NASA	National Aeronautics and Space Administration
NESC	NASA Engineering and Safety Center
NiRA	Nickel Removal Assembly
O_2	Oxygen
OTEC	Ocean Thermal Energy Conversion
PF	Pitting Factor
PhosRA	Phosphorous Removal Assembly
PPA	Pump Package Assembly
PPM	Parts Per Million
QD	Quick Disconnect
R_p	Polarization Resistance
SEM	Scanning Electron Microscope
SKV	Heat Exchanger (type)
SMAC	Spacecraft Maximum Allowable Concentration
SPCU	Servicing and Performance Checkout Unit
SPRT	System Problem Resolution Team
SRB	Sulfate Reducing Bacteria
STS	Space Transportation System
TCCS	Trace Contaminant Control Subsystem
TIC	Total Inorganic Carbon
TIM	Technical Interchange Meeting
TOC	Total Organic Carbon
USOS	United States On-orbit Segment

15.0 Definition of Terms

Corrective Actions	Changes to design processes, work instructions, workmanship practices, training, inspections, tests, procedures, specifications, drawings, tools, equipment, facilities, resources, or material that result in preventing, minimizing, or limiting the potential for recurrence of a problem.
Finding	A conclusion based on facts established during the assessment/inspection by the investigating authority.
Lessons Learned	Knowledge or understanding gained by experience. The experience may be positive, as in a successful test or mission, or negative, as in a mishap or failure. A lesson must be significant in that it has real or assumed impact on operations; valid in that it is factually and technically correct; and applicable in that it identifies a specific design, process, or decision that reduces or limits the potential for failures and mishaps, or reinforces a positive result.
Observation	A factor, event, or circumstance identified during the assessment/inspection that did not contribute to the problem, but if left uncorrected has the potential to cause a mishap, injury, or increase the severity should a mishap occur.
Problem	The subject of the independent technical assessment/inspection.
Recommendation	An action identified by the assessment/inspection team to correct a root cause or deficiency identified during the investigation. The recommendations may be used by the responsible C/P/P/O in the preparation of a corrective action plan.
Root Cause	Along a chain of events leading to a mishap or close call, the first causal action or failure to act that could have been controlled systemically either by policy/practice/procedure or individual adherence to policy/practice/procedure.

16.0 Appendices

A. Mittelman and Associates Antimicrobial Survey Technical Report
B. Montana State University Antimicrobial Survey Technical Report: Assessment of ISS IATCS Loop Microbial Control Options
C. MSFC Glutaraldehyde Toxicity Assessment
D. Boeing Glutaraldehyde Toxicity Assessment
E. Altran Report (Technical Report 02660-TR-001)
F. Toxicological Assessment of IATCS Coolant Biocides (Memo 632)

Approval and Document Revision History

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Appendix A. Mittelman and Associates Antimicrobial Survey Technical Report

PROJECT REPORT

Assessment of ISS IATCS Loop Microbial Control Options

NASA RFP PS410-06/09/04

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September 15, 2004

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By: _____
Marc W. Mittelman, Ph.D., Principal Investigator
Co-Investigators: **Dr. Ralph Mitchell and Dr. Harry Ridgway**

I. Executive Summary and Recommendations

The Internal Active Thermal Control System (IATCS) of the International Space Station (ISS) is a closed loop system that provides a relatively constant temperature coolant supply to equipment, payloads, and avionics. The coolant is a water-based fluid that contains borate as a buffer and phosphate as a corrosion inhibitor. Silver has been used for control of biological activity; however, silver has been ineffective as an antimicrobial agent. Additionally, concerns have been raised about the potential for corrosion associated with the use of silver in the IATCS.

Mittelman & Associates have conducted a review of cooling water antimicrobial agents. The suitability of currently available chemical treatment agents along with emerging technologies was assessed for application to the IATCS. Fifteen candidate antimicrobial agents used in the treatment of cooling water systems were considered as part of this assessment. The agents were evaluated on the basis of six weighted criteria: safety/toxicity, material compatibility, antimicrobial efficacy, IATCS chemistry compatibility, stability, and industrial experience. A study matrix was developed, and each of the agents received a numerical score based on these six criteria (Table 3). Glutaraldehyde and isothiazolones received the highest ranking. Chlorhexadine, ozone, orthopthalaldehyde (OPA), and peracetic acid were ranked closely behind these two agents (Figure 2). On the basis of the ranking scores, the remaining candidate agents were considered to be less suitable for use in the IATCS. Selection of the penultimate treatment will be problematic, since there is no currently available treatment that will meet all of the stringent IATCS criteria. The results of this evaluation demonstrated that compromises will need to be made in order balance effective biological control with the other IATCS application criteria.

There are a number of emerging biological treatment technologies that may hold future promise for application to the IATCS and other fluid handling systems. These include reduction of coolant water activity, application of titanium-doped nitrogen oxides, radiofrequency dosing, application of cationic polymers, gamma irradiation, and application of EDTA. Application of some of these emerging technologies may necessitate changes in systems design and/or materials of construction.

Based upon the findings from this investigation, the following recommendations may be considered: 1) Perform additional testing on the primary antimicrobial candidates identified in the study matrix. This testing should include methods of residual chemical inactivation, in the event of an inadvertent release of the antimicrobial chemical. In addition, the candidates should be evaluated on the basis of their efficacy against biofilm bacteria. 2) Evaluate the feasibility of scheduled antimicrobial agent dosing with concentrated agents in order to maintain effective control of biological activity. 4) The testing of candidate antimicrobials should include combination treatments; e.g., hydrogen peroxide and ozone. 4) Evaluate the feasibility of using alternative, non-aqueous cooling fluids to reduce or eliminate biological activity. Depending upon materials and engineering considerations, other alternative treatment modalities described herein should be evaluated.

II. Nature of IATCS Biological Fouling (NASA)

The Internal Active Thermal Control System (IATCS) of the International Space Station (ISS) is a closed loop system that provides a relatively constant temperature coolant supply to equipment, payloads and avionics. The coolant is a water-based fluid that contains borate as a buffer and phosphate as a corrosion inhibitor. Silver has been previously added to control the bacterial population. The IATCS is composed of two loops, the Moderate Temperature Loop (MTL) and the Low Temperature Loop (LTL). The MTL contains approximately 200 liters of fluid and has a supply temperature range of 16.1 to 18.3 ^{o}C. The LTL contains approximately 60 liters of fluid and has a supply temperature range of 3.3 to 6.1 ^{o}C. The two loops can operate independently in a dual-loop mode or in series in single-loop mode.

Since January 2000, the chemical and microbial state of the on-orbit fluid has been monitored by ground analysis. Many chemical parameters have changed over time including a drop in pH from the requirement of 9.5 +/- 0.5 to ~ 8.4, an increase in the level of total inorganic carbon (TIC), total organic carbon (TOC) and nickel in the fluid, and a decrease in the phosphate level. In addition, silver ion levels in the fluid have decreased rapidly as silver is deposited on internal surfaces of the system. The lack of availability of silver ions coupled with changes in the fluid have created a favorable environment for microbial growth. Counts of heterotrophic bacteria have increased from <10 colony forming units (CFU)/ 100 milliliters (mL) to 107 CFU/ 100 mL. The increase of the microbial population is of concern because uncontrolled microbiological growth in the ITCS can deteriorate the performance of critical components within the system and potentially impact human health if opportunistic pathogens become established. Microorganisms are capable of degrading the coolant chemistry, attaching to surfaces and forming biofilm, causing subsequent biofouling of filters, tubing, and pumps, decreasing flow rates, reducing heat transfer, initiation and acceleration of corrosion, and enhanced mineral scale formation

The chemical composition of the IATCS fluid will be changed in hardware to be launched in the future. Phosphate and silver will no longer be added and the pH of the fluid will be lowered using CO2 to a pH ~8.4, the level that the fluid will be in flight due to the permeation of CO2 through Teflon hoses. The microbial control methodology that will be implemented in the ISS will have to be compatible with that chemistry and the materials of construction in the IATCS.

A complicating difference between the ISS and most terrestrial systems is the closed cycle nature of the air environment within the ISS and the effect of coolant additives on that environment. IATCS coolant continually seeps into that environment by permeation and seal leakage. As it does, its constituents enter the environment as vapors and precipitates. These constituents of the air can be removed by means of humidity control systems that remove condensate from the atmosphere or trace contaminant control systems that filter the recirculating air. The leak rate, crew size, and number and type of contaminant removal devices in operation will drive equilibrium atmospheric concentrations of volatile components of the coolant. For the protection of the crew, Spacecraft Maximum Allowable Concentrations (SMACs) must not be exceeded as chemicals that are part of the selected microbial control scheme enter the ISS environment.

III. Introduction: Biological Fouling of Cooling Water Systems

Microorganisms, including bacteria and fungi, are common inhabitants of industrial fluid handling systems. Their proliferation is dependent upon a number of interrelated factors, including the availability of nutrients, temperature, pH, and redox potential. As with other ecosystems, industrial systems can support the growth of a diverse array of microorganisms. It is the combination of a discrete set of physicochemical factors and the establishment of a diverse microbial community that leads to biological fouling activities.

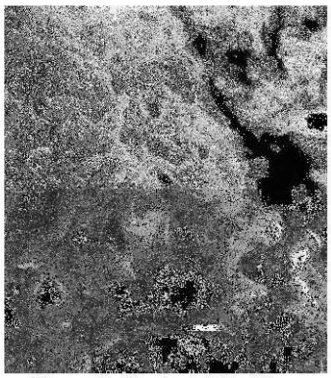


Figure 1. Bacterial biofilm, steel surface. Bar = 1 μm.

Biological fouling ("biofouling"), including microbially influenced corrosion (MIC), is rarely associated with a single corrosion mechanism or individual microbial species. All biofouling events, however, are associated with the development of an attached population—usually bacterial—that forms a fouling biofilm (Figure 1). Biofilms consist of individual microbial cells, extracellular polymeric substances (slime) produced by their activity, immobilized metabolic products, and entrained organic and inorganic matter. It is the growth of microorganisms within biofilms that leads to the initiation and/or propagation of biological fouling, including corrosion activity. Understanding those factors that promote biofilm formation, microbial activity within biofilms, and the interfacial reactions between the biofilm and the underlying surface is key to the management of biofouling.

Perhaps the most significant adaptive mechanism used by bacteria is adhesion to surfaces; indeed, the majority of bacteria in nutrient-limited environments (such as the IATCS) are attached to surfaces. Recognition of this important growth characteristic is a key consideration in developing effective monitoring programs. Sampling of planktonic environments, whether by membrane filtration or the application of evolving electrochemical and acoustic techniques, can only recover a small fraction of the total system bioburden. Several workers have shown that nutrient-limiting environments promote the attachment of bacteria to surfaces (Mittelman, Islander et al. 1987; Marshall 1988). Surface area is a major limiting factor for microbial growth in nearly every environment. The ratio of planktonic (free-floating) bacteria to biofilm bacteria is a function of several interrelated factors, including surface energetics, materials of construction (Lewis and Gilmour 1987; Vanhaecke et al. 1990), topography, hydraulic factors, and biofilm chemistry.

The majority of engineered materials are susceptible to MIC activities (notable exceptions include titanium and those surfaces containing diffusible antimicrobial agents). Table 1 provides an overview of MIC-material interactions. While the range of affected materials is broad, there are significant differences in the rates of corrosion as a function of underlying surface chemistry and bulk-phase physicochemical factors. For example, copper is much more susceptible to MIC activities in soft, low pH, and stagnant water systems; e.g., fire protection pipelines (Chamberlain and Angell 1991).

Table 1. MIC-Material Interactions.

Material	MIC Activity[1]	Responsible Agents[1]	References
Mild steel	Acid- and hydrogen sulfide-mediated pitting	*Hafnia alvei*; *Desulfovibrio desulfuricans*	(Parra et al. 1996; Campaignolle and Crolet 1997)
Stainless steel	Cathodic depolarization of protective hydrogen leading to pitting	*Desulfovibrio desulfuricans*	(Ibars et al. 1992; Service and Freeman 1999)
Copper	Organic acid-mediated pitting	*Acidovorax delafieldii*	(Chamberlain and Angell 1991; Davidson et al. 1996)
Aluminum	Organic acid-mediated pitting	*Cladosporium resinae* (fungus)	(Cabral 1980)
Concrete	Sulfuric acid-mediated deterioration	*Thiobacillus thiooxidans*	(Sand and Bock 1990)
Glass	Oxalic acid-mediated attack	*Aspergillus* spp. (fungus)	(Drewello and Weissmann 1997)
Polymeric and composite materials	Enzymatic hydrolysis	*Aspergillus* spp. (fungus)	(Gu et al. 1997)

[1]Examples of the more common MIC mechanisms and organisms are listed.

Bacteria surviving in cooling water environments must adapt to survival under relatively extreme environmental conditions of low water activity (surfaces; some products), high or low temperatures (water systems; equipment), and extremely low nutrient concentrations (water systems; some products). These same organisms, when cultured under eutrophic and mesothermic conditions, may fail to grow and produce visible colonies. Therefore, recovery and enumeration techniques should take into account the environmental conditions under which the organisms were recovered. The development of the low-carbon/nitrogen containing medium, R2A, was an attempt to address these problems—at least for water environments (Reasoner and Geldreich 1985).

Monitoring of microorganisms associated with biological fouling activities is an important part of an overall control strategy. Sample acquisition and the selection of sampling locations are critical for obtaining accurate, useful data. Many of the biofouling microorganisms are sensitive to oxygen, temperature, and the effects of drying. Whenever possible, microbiological samples should be taken before the start of system maintenance or repair activities. While bulk-phase samples can provide useful information on the overall system condition, surface samples provide the best evidence for microbiological assessments.

On-line instrumentation is available for most of the critical physical and chemical parameters; however testing for microbial contaminants generally requires laboratory analysis. Microorganisms are identified based upon shape and size considerations, biochemical reactions, and observations of characteristic growth patterns on specialized media. For example, sulfate-

reducing bacteria reduce oxidized sulfur compounds under anaerobic conditions to sulfides, which react with iron compounds in microbiological media to produce a black precipitate. A review of some of the techniques for evaluating biological fouling and MIC activity has been appended (Mittelman 1994). On-line instrumentation has also been described for the detection of microbial growth and activity at surfaces (Dowling et al. 1988; Bremer and Geesey 1991; Mittelman et al. 1993; Jacobs et al. 1996).

MIC associated bacteria are grouped on the basis of their mode of attack on ferrous and nonferrous metals. The most common MIC groups include sulfate reducing, iron oxidizing, acid producing, sulfur oxidizing, and nitrate reducing bacteria. Acid production, hydrogen sulfide generation, tubercle formation, and the subsequent development of differential aeration cells can lead to deterioration and failure of mild steel, copper, stainless steel, and other ferrous and nonferrous metals used as materials of construction. The only commonly employed metal found to-date that has been shown to be impervious to MIC attack is titanium. It has been suggested that the thick TiO2 passive film that forms in aqueous environments is protective in this regard. Therefore, given the appropriate environmental conditions and presence of suitable growth factors, MIC can affect nearly all water system ferrous and non ferrous metallic components.

Iron oxidizing bacteria) obtain energy through oxidation of reduced ferrous species to the ferric state. Iron oxidation by bacterial species in this group usually results in the formation of ferric hydroxide, Fe(OH)3, which is precipitated in their slime. *Crenothrix polyspora*, *Sphaerotilus natans*, *Gallionella ferruginea*, and *Siderocapsa treubii* are common types of iron bacteria. Sulfur bacteria obtain energy by reducing or oxidizing inorganic sulfur compounds that are present in feed waters. The bacteria most often associated with MIC in water systems belong to the anaerobic sulfate-reducing (SRB) group, which includes *Desulfovibrio desulfuricans*. Direct attack of ferrous and non-ferrous metals by their hydrogen sulfide metabolic by-product is a significant problem in many industries. Sulfur oxidizing bacteria, such as *Thiobacillus* spp., are aerobic microorganisms that can produce sulfuric acid. This group of organisms often lives in close association with SRB. Nitrate reducing bacteria (NRB) can utilize nitrogen containing organic compounds in feedwaters, producing significant quantities of ammonia. In addition to odor problems, ammonia production is associated with stress corrosion cracking of copper alloys. Nitrite-based corrosion inhibitors may be a source of nitrogen for this group of bacteria.

Differentiation of biological fouling from abiotic mechanisms of fouling and deterioration is an essential first step in designing an effective treatment program. Problem resolution in affected systems requires an integrated approach involving microbiologists, metallurgists, mechanical engineers, electrochemists, and non-destructive testing specialists. These individuals use materials testing, engineering analysis, and on-site inspection resources to develop remedial treatment plans as well as cost-effective programs for replacement and/or design modifications. Where water chemistry, material processing techniques, and/or electrolytic processes have been eliminated as possible causes for fouling events, biological activity should be suspected.

IV. Scope of Work (NASA)

1. Research the antimicrobial chemicals and technologies currently available.
2. Provide a study comparison matrix of the different chemicals/technologies.
3. Recommend a methodology to select the best candidate chemical/technologies to be tested at a later time.
4. Prioritize the list of candidates based on the recommended methodology

V. Commercially Available Cooling Water Treatments

A number of physical and chemical options exist for controlling cooling water system biological fouling and MIC. Several of the listed and appended literature citations describe new treatment approaches for cooling waters. In all cases, application of alternative treatments requires a careful process and materials compatibility analysis. For example, quaternary ammonium compounds (Sweeny and Himpler, 1995) are subject to foaming and can be susceptible to biodegradation in situ. Hydrogen peroxide is highly reactive with organic compounds (e.g., some corrosion inhibitors), and only effective in fairly high concentrations (10% v/v).

The suitability of currently available chemical treatment technologies was assessed for application to the IATCS. Fifteen candidate antimicrobial agents used in the treatment of cooling water systems were considered as part of this assessment. The agents were evaluated on the basis of six weighted criteria: safety/toxicity, material compatibility, antimicrobial efficacy, IATCS chemistry compatibility, stability, and industrial experience. A study matrix was developed, and each of the agents received a numerical score based on these six criteria (Table 2; Figure 2). Glutaraldehyde and isothiazolones received the highest ranking, based on the six criteria. Chlorhexidine, ozone, orthopthalaldehyde (OPA) and peracetic acid were ranked closely behind the first group. The other chemicals were all considered to be less suitable for use in the IATCS.

Definitions:

- *antibiotic*: *in vivo* treatment; bacteriostat
- *antiseptic*: *in vivo* treatment; disinfectant
- *bacteriostat*: prevents bacterial growth, not necessarily spores
- *bactericide*: kills bacteria, not necessarily spores
- *biocide*: kills all living organisms; sterilant
- *biostat*: prevents bacterial growth, including spores
- *disinfectant*: kills disease-causing organisms; inanimate applications
- *germicide*: kills disease-causing organisms; includes animate objects
- *microbicide*: kills all microscopic organisms; not necessarily macroscopic
- *preservative*: prevents deterioration of materials
- *sanitizer*: reduction of disease-causing organisms; inanimate objects
- *sporocide*: destroys microbial spores; sterilant
- *sterilant*: destroys all living organisms

glutaraldehyde

Glutaraldehyde, 1,5-pentanedial, is a mild oxidizing agent that is an effective and broad-spectrum antimicrobial. The antimicrobial mode of action for glutaraldehyde and other aldehydes is cross-linkage of membrane and intracellular proteins. Aldehydes are highly reactive with amino acids and sulfhydryl-containing compounds. When used in sufficient concentrations for adequate contact times, glutaraldehyde is bactericidal and sporocidal. Concentrations in range of 1-2% (v/v) for contact times of 1 h are required for inactivation of spore-forming bacteria and some fungi. Lower concentrations of glutaraldehyde, in the 100-500 mg/L range, are effective in controlling bioburden in industrial water systems. However, the efficacy of this compound—as well as most antimicrobial agents—is dependent on temperature, the presence of reactive organic compounds, and the presence of reducing agents. In addition, glutaraldehyde is most active in the pH range of 7.5-8.5.

Glutaraldehyde is stable in concentrated solutions, but is susceptible to polymerization when present in dilute concentrations. Polymerization of glutaraldehyde can also occur in the presence of significant organic fouling and can be associated with light exposure. Therefore, it is important to maintain solutions as concentrates (>25%) in light-protected containers/environments. Dilute solutions in the 10 mg/L to2500 mg/L concentration range have been found to be stable for at least 3 days at 4 ºC (39 ºF). The stability of glutaraldehyde at higher temperatures and in various suspending solutions must be empirically determined. The activity of glutaraldehyde is potentiated at more alkaline ph levels (ph 8.5-9.5); however, the stability decreases. Conversely, activity is significantly decreased below ph 8, stability is increased.

Residual concentrations of glutaraldehyde may be inactivated by 1) reaction with ammonia, 2) alkalinization to pH 11-12, and 3) neutralization with sodium bisulfite at a 2.2:1 molar ratio of bisulfite:glutaraldehyde. Glycine has also been used as neutralizing agent.

Glutaraldehyde is the most commonly employed cold sterilant for hospital and biomedical applications. As such, the toxicity (human and environmental) of glutaraldehyde has been extensively reviewed. As with other biocides, glutaraldehyde must be handled carefully. Exposure to concentrated and diluted solutions can result in hypersensitivity reactions; glutaraldehyde is a mucosal irritant. In addition, glutaraldehyde solutions possess a distinct odor which is objectionable to some individuals. There are a number of publications describing materials compatibility with glutaraldehyde formulations. Most of these publications, which are primarily from the biomedical literature, suggest that glutaraldehyde is compatible with a broad range of ferrous metals and polymers.

High-performance liquid chromatography (HPLC), iodometry, reaction with amino compounds, and gas chromatography (GC) have been employed for glutaraldehyde analysis. However, only the HPLC method is specific and quantitative for glutaraldehyde.

isothiazolones

Isothiazolones are thiol-oxidizing antimicrobials, 4-isothiazolin-3-one. Their mode of action involves reaction with thiol-containing amino acids (e.g., cysteine), interrupting protein synthesis. These compounds show enhanced activity under alkaline conditions, which are typically employed in closed-loop cooling water systems. Low-levels of isothiazolones (in the range of 1-10 mg/L) have been shown to be effective in inhibiting bacterial growth in cooling

waters (Shaw et al., 1998). Isothiazolones are stable in aqueous solutions, and are oxidized to mercaptoacrylamide

Kathon™ (Rohm and Haas) formulations for water treatment are widely used in the cooling water industry. Kathon WT (Rohm and Haas) for water treatment is 5-chloro-2-methyl-3(2H)-isothiazolone. There are numerous literature reports concerning the suitability of this class of antimicrobial agents for closed-loop cooling water systems.

The toxicity (human and environmental) of isothiazolone has been reviewed. As with other antimicrobials, isothiazolone must be handled carefully. Isothiazolone concentrates are skin and mucosal irritants. However, isothiazolones in dilute form are extensively used in the cosmetic industry as preservatives (e.g., shampoos), and are generally regarded as safe. There are a number of publications describing materials compatibility with isothiazolones formulations. Most of these publications, which are primarily from the industrial literature, suggest that isothiazolones are compatible with a broad range of ferrous metals, non ferrous metals, and polymers.

Rapid, colorimetric tests for isothiazolones are not readily available. An HPLC method has been described for quantitative analysis (Sible, 1996). A rapid and quantitative capillary electrophoresis technique has recently been described (Bartak, et al., 2001)

chlorhexidine

Chlorhexidine is a cationic bisguanide, 1,6-di(4-chlorophenyl-diguanido) hexane. The agent is normally used as the soluble chlorhexidine gluconate. Chlorhexidine shows broad-spectrum antimicrobial activity in the pH range of 5.5-7, with good activity to pH 8 (depending upon the challenge organism). The gluconate preparation is stable when diluted, with a shelf-life of approximately 1 year (when stored at room temperature in the dark). However, reports of microbial growth in diluted solutions with long-term storage suggest that chlorhexidine gluconate should be stored as a concentrate.

The toxicity profile of chlorhexidine has been extensively studied. The compound is used in clinical practice as a whole-body wash, oral disinfectant, and in treating wounds and burns. Hypersensitivity reactions are rare; however, there have been isolated reports of dermal irritation among some Japanese consumers.

The use of chlorhexidine gluconate as a water system treatment in dental water units is increasing. The American Dental Association (ADA) has approved the use of chlorhexidine gluconate to prevent biological growth in dental unit water systems. A few papers have been published on the compatibility of this compound with stainless steels and polymers. There have not been any reports of accelerated corrosion associated with this application. Test kits for chlorhexidine are commercially available from dental supply houses.

ozone

Ozone is the triatomic allotrope of oxygen, O_3. For cooling tower and other industrial applications, ozone is produced by either electrostatic discharge (corona arc) through dry air or oxygen, or using a 185 nm ultraviolet light in water. A membrane-based process (Membrel™) has also been described. Ozone is unstable in ozone-air concentrations exceeding 20%; therefore, it is prepared *in situ* on demand.

Ozone has broad spectrum activity against bacteria and fungi, and is effective in residual concentrations of approximately 0.03-0.1 mg/L. The half-life of ozone is relatively short, approximately 20 min in deionized water maintained at 20 ºC. However, 5-10 min of daily contact time with 0.1 mg/L ozone is reportedly effective for biofouling control in clean systems free of surface deposits (and biofilms). Synergistic combinations of ozone and other oxidizing agents (e.g., hydrogen peroxide) have been successfully employed in cooling water applications.

Ozone is a strong oxidizing agent, which poses exposure risks to both personnel and to carbon-containing materials. However, on-line ozone monitoring systems (air and water) are commercially available and have a good track record for effective monitoring/control. Ozone is readily inactivated via exposure to either 254 nm or 185 nm UV light, and is subject to immediate thermal degradation (to oxygen). The effects of dissolved ozone on heat exchanger corrosion have been reviewed. The passive behavior of stainless steels and titanium were not found to be affected by dissolved ozone in simulated freshwater cooling systems. However, copper alloys were found to be susceptible to attack by dissolved ozone. There is a report of "micro pitting" of titanium in ozonated artificial seawater.

Ozone is used extensively in the bottled water industry, and in pharmaceutical applications. In addition, there have been a number of successful closed loop water system applications in the pharmaceutical (compendial water) and microelectronics industry (high purity waters). Where ozone is used with the appropriate materials of construction and health/safety considerations, biological fouling is well-controlled.

orthopthalaldehyde

Orthopthalaldehyde (OPA), 1,2-benzenedicarboxaldehyde, is an aldehyde biocide. The compound received FDA approval as a cold sterilant in 1999. The mode action of OPA is similar to that of glutaraldehyde and other aldehyde compounds. OPA, like glutaraldehyde, is a biocide, with broad spectrum activity.

Although OPA has not been used as extensively as glutaraldehyde, it exhibits some advantages relative to other aldehydes. Unlike glutaraldehyde, for example, OPA is stable over a very wide pH range (3-9), is not a known irritant to the eyes and other mucosal surfaces, and has little odor. OPA will discolor human skin gray, but is otherwise a non-irritant relative to glutaraldehyde. Toxicological and material compatibility studies, while more limited than those associated with glutaraldehyde, suggest that OPA is as safe or safer than glutaraldehyde.

Monitoring and inactivation of OPA are similar as for glutaraldehyde. Glycine is an effective neutralizing agent.

peracetic acid

Peracetic acid (PAA), $C_2H_4O_3$, is the peroxide of acetic acid. Block (1991) notes, "It would be desirable to have a chemical with the attributes of hydrogen peroxide—effective germicidal and sterilizing capabilities, no harmful decomposition products, and infinite water solubility—but with greater lipid solubility and freedom from deactivation by catalase and peroxidases." PAA is biocide with a broad spectrum of antimicrobial efficacy. PAA is widely used in controlling biological growth in water systems used in renal dialysis centers, the treatment of

reverse osmosis membranes and ultrafilters, microbial control in pharmaceutical water systems, and the disinfection of food processing equipment.

PAA is more effective at lower pH values; however, good antimicrobial efficacy has been reported at pH values up to 8.5. In concentrated form (3% or greater), PAA is stable and effective—even in the presence of organic matter and at very low temperatures (<4 ºC). As was noted above, synergistic combinations of PAA (<0.05% v/v) and hydrogen peroxide (3%) have been found be highly effective in both water systems and membrane treatment applications.

PAA is a strong oxidizing agent, which poses exposure risks to both personnel and to some materials of construction. At recommended use concentrations is a skin and mucosal irritant; however, there are no reports of acute or chronic toxicity associated with PAA when the appropriate chemical handling procedures are employed. PAA has strong and objectionable acetic acid odor. As with hydrogen peroxide, PAA is rapidly degraded by ultraviolet light (either 254 nm or 185 nm)—the degradation products are acetic acid and water. Weak reducing agents, such as sodium thiosulfate, can also be used as neutralizers. There are commercially available test kits for PAA residuals.

triclosan

Triclosan (2, 4, 4' – trichloro – 2' – hydroxydiphenyl ether) is a member of the bis-phenol group of antimicrobial antimicrobials. Like other bis-phenols, triclosan has limited solubility in water (10 mg/L at 20 °C) but is readily soluble in a range of organic solvents as well as dilute alkali. Depending on the concentration, triclosan is both bacteriostatic and bactericidal, and it has limited fungistatic activity as well. Triclosan's activity is reasonably broad, being effective against both Gram-positive and most Gram-negative bacteria. However, triclosan is less effective against *Serratia marcescens*, *Pseudomonas* spp. (particularly *P. aeruginosa*) and *Alcaligenes* spp. The MICs for each of the latter species is usually > 100 mg/L.

Triclosan has found applications in various personal care products, including dentifrices, bar soaps, liquid hand soaps, liquid body soaps, and deodorants. It is also used in textile and plastic applications. In particular, 97% of triclosan finds its way into personal care applications as compared to 2% for textiles and 1% for plastics. The triclosan in personal care products is split between oral care products (40%) and skin care/skin cleansing products (60%). Almost all of the triclosan in skin care/skin cleansing products is found in rinse-off products (90%). The remainder of the triclosan is found in deodorants and other leave-on products, such as lotions.

As with chlorhexidine, triclosan has had limited applications in water systems. Literature reports suggest that *P. aeruginosa* strains may be resistant to triclosan. However, its generally good broad spectrum activity—coupled with low toxicity and good material compatibility—suggest that this compound should be further evaluated for IATCS applications.

hydrogen peroxide

Hydrogen peroxide, H_2O_2 is an oxidizing agent that is frequently employed as a water systems biofouling treatment. Hydrogen peroxide has broad spectrum of activity against bacteria and fungi. At concentrations exceeding 10% (v/v) the compound is sporocidal.

Hydrogen peroxide is stable when stored in dark containers at room temperature (3% and greater concentrations). There is a report that suggests the rate of loss is less than 2%/y in small

containers. Hydrogen peroxide is a strong oxidizing agent, which poses exposure risks to both personnel and to some materials of construction in concentrations exceeding 3% (v/v). Discoloration and evidence of changes in the passive films of stainless steels have been reported for endoscopes exposed to 7.5% concentrations.

A concentration of 10% (v/v) is often used for reducing bioburden (sessile and planktonic) in high purity water systems associated with pharmaceutical and microelectronic manufacturing operations. It is unlikely that concentrations <3% (v/v) are effective in systems containing preexisting surface contamination and/or bulk phase organic constituents. Synergistic combinations of hydrogen peroxide with peracetic acid, ozone, and ultraviolet exposure are often employed in water system treatments.

Hydrogen peroxide is rapidly inactivated by exposure to ultraviolet light. The enzyme catalase is also employed in *in vitro* studies as a neutralizing agent. There are commercially available test kits for hydrogen peroxide residuals.

quaternary ammonium compounds

Many quaternary ammonium compounds (QAC) are detergents with broad spectrum antimicrobial activity. Compounds with aliphatic chains in the range of 12-20 carbon units appear to possess the best antimicrobial efficacy profile. QAC's are used extensively in the healthcare industry, veterinary practice, and food processing as a surface disinfectant. In addition, QAC are often used for cleaning water systems (including cooling water systems) that are contaminated with organics such as oils and greases. Some QAC's, such as cetylpyridinium chloride, are also approved for use as skin antiseptics, eyewashes, and mouthwashes.

QAC's are generally regarded as safe for personnel contact; as with all antimicrobial agents, however, the concentrated forms can be hazardous to unprotected skin and mucosal tissues. These compounds also appear to be safe for use with stainless steels and polymeric materials.

Foaming of QAC's, as with other surface-active agents, is a significant problem. Foaming increases rinsing times and, in a closed loop system, may affect heat transfer efficacy. While the diluted QAC's are chemically stable, there a number of literature reports (including patient case histories) of pseudomonad growth in dilute solutions.

alcohols

Isopropanol and ethanol are commonly used surface disinfectants. Gram negative and Gram positive bacteria are killed with concentrations in the range of 30-70% (v/v); concentrations exceeding 70% are less effective. Isopropanol is slightly more effective than ethanol on a molar basis. Alcohols have little activity against spore-forming bacteria, fungi, and mycobacteria.

Alcohols are flammable and contribute to atmospheric volatile organic carbon (VOC) loading. Coupled with the high concentrations required for antimicrobial efficacy, these properties suggest that alcohols are not suitable treatments for the IATCS.

formaldehyde

Formaldehyde was used for many years as a biocide in medicine and industry. The antimicrobial efficacy, acute human toxicity, and material compatibility profiles are somewhat

similar to those of glutaraldehyde. However, it is now recognized as a mutagenic agent, and its use is more restricted.

Formaldehyde was used for decades in cooling water systems and other industrial fluid handling systems. However, the agent is no longer used in North America. Formaldehyde is not a suitable treatment for the IATCS.

iodine/bromine compounds

Iodine and bromine are halogens with oxidizing properties similar, but inferior to, those of chlorine. Iodine has been used in ISS as a potable water system treatment (in the tri-iodide form). While iodine chemistry in solution is complex relative to that of chlorine or bromine, dilute iodine solutions do appear to retain some efficacy at pH values up to 9. Organo-iodine agents have been used in swimming pool applications: like bromine, iodine reportedly is less irritating to skin and mucosal membranes than is chlorine. Iodine is also used as drinking water treatment in emergency situations.

While iodine is a broad spectrum antimicrobial, some pseudomonads reportedly develop an acquired resistance. Similar resistance has not been reported for bromine compounds, which show much greater efficacy at elevated pH levels (8-9) than chlorine-containing compounds. Iodine and bromine are both degraded by light exposure in concentrated and diluted forms.

The toxicity of iodine and bromine compounds has been evaluated in a number of studies. At use-dilutions (<10 mg/L), both iodine and bromine solutions are generally regarded as safe. In more concentrated solutions, both compounds are skin and mucosal irritants. Iodine and bromine are neutralized with weak reducing agents such as sodium thiosulfate and sodium bisulfite.

As with chlorine, iodine and bromine are corrosive to ferrous and non ferrous metals. While chlorides are most often associated with stress corrosion cracking, it is likely that iodine and bromine also pose a corrosion risk. Their use in the IATCS should therefore be carefully evaluated for material compatibility.

phenolic compounds

Halogenated phenols and other substituted phenol compounds are commonly used as surface disinfectants in healthcare, veterinary, and food production environments. Many of these derivatives exhibit broad spectrum antimicrobial efficacy; however, as weak acids most of these agents are relatively ineffective at pH values >8.

Phenolic compounds are rarely employed as cooling water treatments for biofouling control. However, as was discussed above, the hydroxydiphenyl ether compound, Triclosan, may be worthy of further study.

silver salts

The use of silver salts in the IATCS has been found to be ineffective in controlling microbial populations. In addition, there is evidence that silver can initiate or propagate localized corrosion on heat transfer surfaces.

sodium hypochlorite

While sodium hypochlorite and other chlorine-based oxidizers are commonly used in water disinfection, their activity is more limited at the pH levels employed in closed loop cooling systems; i.e., >8-8.5. While more pH-stable chlorine-containing compounds are available, the potential for chloride stress corrosion crack is sufficiently high as to preclude their use in the IATCS.

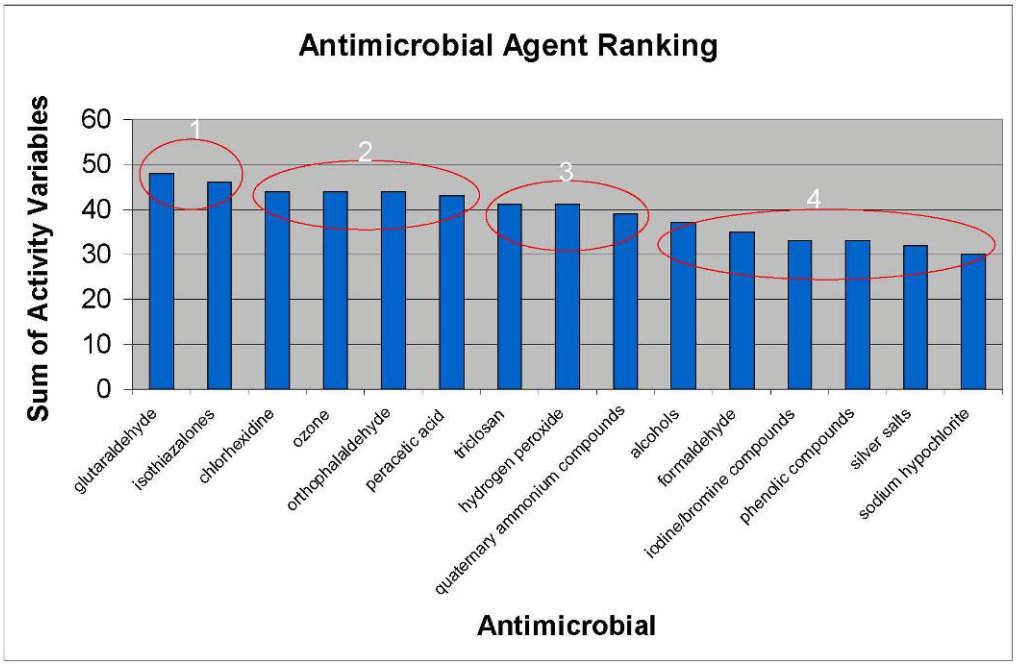
Table 2. Antimicrobial agent evaluation matrix.

Agent	Manufacturer	Mode of Action	Safety/ Toxicity, 1-15[1]	Material Compatibility, 1-15	Antimicrobial Efficacy, 1-10	ITCS Chemistry Compatibility, 1-10	Stability[2], 1-5	Industry Experience[3], 1-5	Total, of 60
glutaraldehyde	Union Carbide	alkylation of amino acids and nucleic acids	10	13	10	7	3	5	48
isothiazolones	Rohm & Haas	interactions thiol-containing amino acids	10	12	8	7	4	5	46
chlorhexidine	Zeneca	cell membrane disruption	12	13	7	5	4	3	44
ozone	generic	cellular component oxidation	10	10	8	8	3	5	44
orthopthalaldehyde	ASP/J&J	alkylation of amino acids and nucleic acids	10	12	9	7	4	2	44
peracetic acid	J&J	cellular component oxidation	10	12	10	5	2	4	43
triclosan	Ciba-Geigy	disruption of cellular lipid synthesis	13	13	4	5	4	2	41
hydrogen peroxide	generic	cell membrane lipid oxidation	12	10	5	8	1	5	41
quaternary ammonium compounds	Lonza	cell membrane disruption	12	12	6	2	2	5	39
alcohols	generic	cell wall protein denaturation	12	12	1	5	2	5	37

Agent	Manufacturer	Mode of Action	Safety/ Toxicity, 1-15[1]	Material Compatibility, 1-15	Antimicrobial Efficacy, 1-10	ITCS Chemistry Compatibility, 1-10	Stability[2], 1-5	Industry Experience[3], 1-5	Total, of 60
formaldehyde	generic	alkylation of amino acids and nucleic acids	2	12	9	6	2	4	35
iodine/bromine compounds	Lonza	cellular component oxidation	12	6	4	5	3	3	33
phenolic compounds	generic	cell wall disruption	8	8	6	5	3	3	33
silver salts	generic	interactions with amino acid sulfhydryl groups	12	3	4	9	2	2	32
sodium hypochlorite	generic	cellular component oxidation	10	2	6	5	2	5	30

[1]Lower numbers indicate unsuitable conditions. [2]Stability of diluted agent. [3]Industry experience relative to water treatment and other related applications

Figure 2. Antimicrobial agent ranking (extracted from Table 3).



VI. Emerging Technologies for Water Treatment

While the focus of this project is on antimicrobial agents and technologies that are currently available and have some history of use by industry, there are number of emerging technologies that should be of interest to NASA. Implementation of the technologies described herein may necessitate significant design and materials modifications to the ISS IATCS. However, future modifications to the existing systems or new designs might enable the application of one or more of these novel biological control strategies.

Reduction of Coolant Water Activity (A_w).

Microorganisms require water to carry out their metabolic processes. The concentration of water that is needed to sustain microbial life varies, but it is well known that lowering the water activity** (a_w) in food products below some threshold level effectively precludes all microbial growth. Examples of food products that are preserved due to low a_w include honey, maple syrup, salted or dried fish and meats, and fruit preserves. Seiler (1976) established an empirical relationship between a_w and the time to the observed onset of fungal growth; i.e., the "mold-free shelf life" (MFSL):

$$\mathrm{Log}_{10}(MFSL_{days}) = 7.91 - (8.1 \times a_w), @\ 21°C \qquad \text{Equation 1}$$

It is apparent from Equation 1 that products with water activities below ~0.6 would be effectively preserved and free from microbial growth for >1000 days (~3 years) at room temperature. In fact, the theoretical and demonstrated absolute limit of microbial growth is about aw = 0.6. However, in practice, growth of most bacteria is inhibited well above this value, or below about aw = 0.91 (equivalent to about 57 % w/w sucrose). Similarly most yeasts cease growing below aw = 0.87 (equivalent to about 65 % w/w sucrose) and most molds cease growing below aw > 0.80 (equivalent to about 73 % w/w sucrose).

Table 3 (http://www.martin.chaplin.btinternet.co.uk/index.html) lists the water activities for a number of salt solutions and foodstuffs. Salts such as LiCl and LiBr are especially effective at decreasing the hydrogen-bonding structure of water and are known as kosmotropes. Such strongly hydrated ions considerably increase the difference between the hydrogen bond donating and accepting capacity of the linked water molecules resulting in the breakdown of the normal tetrahedral network. Because of their strong water interactions, kosmotropes can effectively lower the water activity below that required to sustain microbial life. In particular, high concentrations of substances such as LiBr, LiCl, urea, and $MgSO_4$ can reduce water activity dramatically, and at still reasonable viscosities, to produce a strongly antimicrobial environment.

Table 3. Indicative values of water activities

Substance	λ_w	x_w	a_w
Saturated LiCl	0.19	0.57	0.11
Saturated $MgCl_2$	0.83	0.40	0.33
Saturated $SrCl_2$	1.03	0.69	0.71
Saturated $BaCl_2$	1.18	0.76	0.90
Bread	-	35	0.96
Cheese	-	37	0.97
Dried fruit (*e.g.* sultanas)	-	18	0.76
Raw meat	-	60	0.98
Dry pasta	-	12	0.50
Cooked pasta	-	72	0.97
Preserves (*e.g.* jam)	-	28	0.88

Exploration of the use of pH-neutral solutions of 2-6 Molar LiBr or LiCl as potential replacements for the existing coolant solution aboard the ISS should be considered. Parameters that would need to be experimentally evaluated would include the heat capacity of the lithium solutions, the potential for corrosivity and materials compatibility, and antimicrobial activity. If this strategy is demonstrated on earth to produce a successful coolant and also remain free of microbial growth, it could potentially be implemented aboard the ISS by adding dry lithium salts to the existing coolant system.

**Water activity is defined as equal to the ratio of the fugacity (the real gas equivalent of an ideal gas's partial pressure) of the water to its fugacity under reference conditions, but it approximates well to the more easily determined ratio of partial pressures under normal working conditions. The activity coefficient (λ_w) has dependence on the partial molar volume and hydrogen bond strength (which includes dependence on the temperature and dielectric constant) of the water and only in dilute solutions (*i.e.* $a_w > 0.95$) can it be approximated by unity. The water activity (a_w) is related to the chemical potential (λ_w; at equilibrium, λ_w of liquid water and its vapor phase are identical) by $\lambda_w = \lambda_w° + RTLn(a_w)$ where $\lambda_w°$ is the standard chemical potential of water. Prediction equations for the water activity of multicomponent systems have been developed (Sereno et al, 2001), based on the Gibbs-Duhem equation $VdP - SdT - \sum_{i=1}^{N} n_i d\mu_i = 0$, which at constant temperature and pressure simplifies to $\sum_{i=1}^{N} n_i d\mu_i = 0$ and therefore $\sum_{i=1}^{N} n_i d(\ln a_i) = 0$, where the terms n_i are the relative proportions of components n of chemical potential μ and activity a. The resultant equations, although starting on this firm theoretical base, require empirical simplifications due to the problems involving the interactions between the components and the paucity in our knowledge of the molecular interactions of the components with water. Water

activity prediction may also be achieved by combining the effects of the chemical groups (rather than molecules) present, where suitable parameters are available (Tavares, 1988). In conclusion, prediction of the water activity of mixed components presents difficulty and, except in cases of simple interpolation, is best determined experimentally.

Ultrasonic Disinfection.

Ultrasonic energy has been widely used for the cleaning and disinfection of medical products and for cleaning tools, jewelry, and other materials. Recent research indicates that ultrasonic irradiation may also be used to help disinfect water and wastewater (see reference below). Used in combination with more conventional chemical antimicrobials or ultraviolet irradiation, sonolysis can be an especially effective antimicrobial technology. Ultrasonic treatment has the advantage of being safe and non-invasive and it can destroy surface biofilms. However, ultrasonic treatment alone does nothing to prevent or impede microbial colonization in distant reaches of the coolant recirculating system that are not directly exposed to the sonic energy.

The feasibility of engineering a miniaturized ultrasonic disinfection system for the ISS coolant system should be considered. Cavitation, induced by ultrasound at low frequencies, is an effective means for the disintegration of bacterial cells. Two effects can be observed: At low ultrasound doses bacterial flocs can be declumped by mechanical shear stresses, and at increased doses ultrasound cavitation has an impact on the cell walls such that they are broken. In lab scale experiments (Neiss and Blume, 2002) a horn sonotrode operated at 20 kHz was run in combination with a low-pressure mercury arc lamp to treat wastewater samples taken from the effluent of a municipal treatment plant. At low ultrasound intensities a drastic change in samples' particle size distribution was observed. Consequently, subsequent UV irradiation was far more efficient as the number of large particles which impede disinfection processes was minimized by the sonication. Hence, applied UV doses could be reduced notably to obtain the same or even better disinfection effects.

Nitrogen-Doped Titanium Oxide Treatment.

Photocatalytic destruction of organic molecules and microorganisms by visible-light activation of nitrogen-doped titanium oxides (TiON) has been recently demonstrated in several laboratories. While still under development, research progress in this relatively new technology has been rapid over the last two years. Multi-gram quantities of TiON-coated materials are now available from non-commercial sources for experimentation and product development research. Of particular interest is the recent demonstration that visible-light activated TiON was able to inactivate suspensions of *Escherichia coli* and other microorganisms (Reference 2). The TiON technology is non-invasive and can function with normal room fluorescent lighting. Adaptation of the TiON technology to the ISS could involve the use of compact and light-weight optical-fiber reactors embedded in optically-clear sections of the recirculating coolant system. The technology would still require the application of one or more soluble antimicrobials to ensure anti-microbial treatment in remote areas of the coolant system. While the TiON technology appears highly promising at this time, fouling issues could become problematic over time.

A miniaturized, light-weight, and compact TiON water treatment device that could be installed in line with the ISS coolant system should be developed The following abstracts describe potential applications:

Reference 1.

http://www.photonics.com/spectra/tech/XQ/ASP/techid.1256/QX/read.htm

Light-Reactive Coating Cleans Surfaces. If you shine ultraviolet light on a surface coated with titanium dioxide, a common white pigment in paint, it self-sterilizes and defogs. Until now, however, if you used visible light, only approximately 5 percent of which is in the UV region, it would not. The addition of nitrogen to titanium oxide extends the optical absorption of the film into the visible range, enabling its use on self-cleaning and -sterilizing surfaces. After one minute of exposure to a tungsten lamp, the organic constituents of fingerprints -- perspiration and oil -- are breaking down on the surface coated with the photocatalyst. Courtesy of Ryoji Asahi, Toyota Central R&D Laboratories Inc. Researchers at Toyota Central R&D Laboratories Inc. in Nagakute, Japan, have discovered that adding nitrogen ions to titanium dioxide yields a photocatalytic coating that displays these effects under visible light. The possible applications include antifogging mirrors and glass, self-sterilizing bathroom tiles and self-cleaning computer touch-displays. To produce the coatings, the researchers sputter titanium dioxide in a mixture of nitrogen and argon gas. They perform postannealing in nitrogen at 550 °C for four hours. The resulting 300-nm-thick films are yellowish, transparent and crystalline. Nitrogen-doped titanium dioxide is active up to approximately 500 nm. Upon exposure to this range of visible light, the photocatalytic coating destroys any organic molecules it contacts by exciting electron/hole pairs in the conduction band at the surface. If oxygen or water is present, superoxides and hydroxyl radicals form, which also attack any organic molecules. Moreover, these hydroxyl radicals strongly attract any other water on the surface, breaking its surface tension and flattening the contact angle to 6°. (A typical contact angle for water on a glass surface is 20° to 50°.) Because the water thus cannot form droplets, the surfaces treated with the film do not cloud or fog. The researchers have determined that the doped films work in everyday lighting situations. They tested the photodegradation of acetaldehyde gas on a table coated with nitrogen-doped titanium oxide at an illumination of approximately 300 lx across the visible spectrum, which is typical of fluorescent lighting in a Japanese living room. They found that the decomposition rate of the gas by a nitrogen-doped coating was 10 times faster than that by titanium dioxide alone. Combining films with InGaN LEDs. The scientists are studying the use of the doped coating with InGaN LEDs, which have a peak emission of 390 to 420 nm. The coating can use the full range of the LEDs' output, and they believe that this combination can be developed into compact and inexpensive photocatalysis units, such as air or water purifiers. Another possible application is self-cleaning computer touch-displays, on which the organic constituents of fingerprints -- perspiration and oil -- can be broken down. They also are looking at other deposition techniques to optimize the photocatalytic performance for the desired application according to cost, coating area and any temperature restrictions of the substrate. Although the researchers hope to market the technique, the breakdown of organic molecules is dependent on other factors, including surface defects and the matching of the conduction/valence band to the reduction-oxidation level of the targeted molecules. "It is safe to say no photocatalyst can decompose all organic molecules," said Ryoji Asahi, a researcher at the lab and member of the team. "We must test each molecule to be decomposed for each application."

Reference 2.

http://www.watercampws.uiuc.edu/research/summary.php?project_id=3X3

Photo-Oxidative Treatment with Nitrogen-Doped Titanium Oxides

Investigators: Jian-Ku Shang (), James Economy (MatSE). Project Objective: To develop new water treatment methods based on nitrogen-doped titanium oxides that are strongly oxidative in water under the visible light. Background: Photo-oxidative treatment is intensely investigated in many countries as a potential replacement of the advanced oxidative processes (AOPs) currently based on UV/ozone and UV/peroxide. Essential to the photo-oxidative process is the photocatalyst, which, upon irradiation, produces electrons and holes needed for reduction and oxidation reactions. So far, the primary choice of the hotocatalyst has been TiO2. TiO2 is a semiconductor with an energy bandgap of 3.05-3.8 eV at room temperature. When exposed to UV radiation below 390 nm, TiO2 is elevated to an excited electronic state in which it becomes strongly photocatalytic, promoting formation of oxygen and hydroxyl radicals. The free radicals can cause rapid breakdown of organic molecules, thus making TiO2 an extremely strong oxidizer (second only to fluorine) for removing organic and biological impurities in water. However, the energy bandgap of titanium oxide is so wide that a special UV system must be installed for adequate photo-oxidation efficiency, seriously limiting its use in water purification. Research Plan: To be photocatalytic under the visible light, a semiconductor must have a small bandgap that will broaden the photo-absorption range, e.g., below 390 nm for TiO2, to the visible range, preferably beyond the maximum in the intensity of the solar system at 460 nm. To reduce the energy bandgap of titanium oxide, we are investigating the synthesis, structure, chemistry and photoelectronic properties of N-doped titanium oxides. Ti-O-N with N concentrations up to 100% will be synthesized by ion-beam mixing and thermochemical processes. The structure and composition of Ti-O-N compounds will be characterized by X-ray diffraction, X-ray photoelectron spectroscopy, and electron microscopy. The optical properties of the Ti-O-N coatings will be determined by cathodoluminescence and photospectroscopy. By designing viable synthetic pathways and examining the relationship of photoelectronic properties with chemistry and structure of Ti-O-N compounds, our work will lead to development of nitrogen-doped titanium-oxide surface coatings, which will have a much enhanced photo-oxidative efficiency under the visible light. The new photo-oxidative TiO2-xNx surface coatings will be built onto porous and solid substrates to be formed into laminated, corrugated, and tubular structures for efficient removal of organic and biological impurities in water.

Reference 3.

http://www.arofe.army.mil/Conferences/Recent_Abstract/200th_Meeting/symposia/i1/1092.pdf

Visible-light Photocatalysis in Nitrogen-doped Titanium Oxides. R. Asahi, T. Morikawa, T. Ohwaki, K. Aoki, and Y. Taga. Toyota Central R&D Laboratories Inc. Nagakute, Aichi 480-1192, Japan. While TiO2, on which most of the photocatalytic studies have been focused, shows relatively high reactivity and chemical stability under ultraviolet (UV) light (larger than the band gap of 3.2 eV in the anatase crystalline phase or the wavelength l<387 nm), new development of photocatalysts that can yield high reactivity under visible light (l>380 nm) has been desired to make use of the main part of the solar power, and to extend their applications to living space

under relatively poor illumination of interior lighting. Here we present properties in N doped TiO2, showing a significant improvement over TiO2 in photocatalytic activity under visible light. TiO2-xNx films, which were prepared by sputtering the TiO2 target in N2 (40%)/Ar gas mixture, have noticeably absorbed visible light (l<500 nm) where the TiO2 films have not. Photocatalytic activity has been evaluated by measuring decomposition rates of methylene blue as a function of the cutoff wavelength of the optical high-path filters under fluorescent light (Fig. 1). We have also evaluated photodecompositions of gaseous acetaldehyde and contact angles of water on the sample films under interior lighting. These results clearly show that the photocatalytic activity of the TiO2-xNx samples are superior to that of the TiO2 samples in the visible range of irradiation, while the both samples yield similar activity in the UV range of irradiation. We have also calculated densities of states for the doping of (C, N, F, P, or S) in the anatase TiO2 crystal, by the full-potential linearized augmented plane wave formalism in the framework of the local density approximation. Among these materials, the substitutional doping of N for O has proven effective for the band-gap narrowing and the visible-light photocatalytic activity as N 2p states mix with O 2p states in the valence bands. On the other hand, molecularly chemisorbed states along with the interstitial doping of N hardly amalgamate with the band states of TiO2, and are thus unlikely effective for photocatalysis. These insights are indeed consistent with experimental results including X-ray photoemission spectroscopy studies.

Radio Frequency Disinfection of ISS Coolant.

The U.S. Agricultural Research Service (ARS) has recently developed a food (liquid beverage) disinfection method involving radio frequency electric fields (RFEF). The method utilizes high-energy (15-20 kV/cm @ 21-40 kHz) RFEF to obtain several log reductions in *Escherichia coli* and other bacteria (reference 1, below). It may be feasible to modify and extend the use of the RFEF technology to the ISS coolant system. The advantage of this technology is that it is non-invasive and does not involve the use of organic or inorganic antimicrobials, which could negatively impact ISS cabin air quality. The method should also be generally compatible with existing coolant system materials of construction, although this would need to be confirmed experimentally. A possible disadvantage of the RFEF technology is that it may require too much energy to be practical aboard the ISS. However, depending on the specific ISS requirements, it might be possible to employ a more streamlined and less energy-intensive RFEF system. The effectiveness of the RFEF technology for the removal or discouragement of biofilm growth remains unknown.

Determination of the energy and spatial requirements of the RFEF technology should be considered. Determine the feasibility of adapting this technology for controlling microbial growth and biofilms in the ISS cooling system water.

Reference 1

http://www.ars.usda.gov/research/publications/Publications.htm?seq_no_115=157814

Research Project: Development of Gentle Intervention Processes to Enhance the Safety of Heat Sensitive Foods. Title: Radio Frequency Electric Fields Inactivation of Escherichia Coli in Orange Juice. Geveke, David and Brunkhorst, Christopher - PRINCETON UNIVERSITY.

Technical Abstract: The nonthermal process of radio frequency electric fields (RFEF) has been shown to inactivate bacteria in apple juice at moderately low temperatures, but has not been applied to other juices. The objective of this study was to extend the RFEF process to inactivate bacteria in orange juice. An 80 kW RFEF system was used to process pulp free orange juice at a flow rate of 1.4 l/min. Escherichia coli K12 in the juice was exposed to electric field strengths of 15 and 20 kV/cm at frequencies ranging from 21 to 40 kHz. Following treatment at an outlet temperature of 65 C, the population of E. coli was reduced by 3.3 log, relative to the control. Increasing the electric field strength and temperature and decreasing the frequency enhanced the inactivation. The results of the present study provide the first evidence that the RFEF process inactivates bacteria in orange juice at moderately low temperatures.

Use of Chitosan and Synthetic Polycationic Antimicrobials for Microbial Growth Control.

Amino-functionalized polymers derived from natural materials (e.g., chitosan from chitin; poly-L-lysine from bacteria) and synthetic routes (e.g., amino-derivatized polyvinyl) have been demonstrated to exhibit strong anti-microbial activity. One advantage of such polymeric antimicrobials is that they can be more conveniently incorporated into tubing surfaces, paints, or flow-through packed columns for water disinfection purposes (Park et al, 2002)). Polyamine antimicrobials appear to inhibit porin-mediated ion fluxes in the cell walls of bacteria such as *E. coli* (Vega and Delcour, 1996)). Several natural-product polyamines have been discovered and their antimicrobial properties studied, including chitosan (Ikinci et al, 2002; Park et al., 2002; Shin et al., 2001; Tsai and Hwang, 2004), ε-poly-L-lysine (Ikinci et al, 2002), and poly(arginyl-histidine) (Nishikawa and Ogawa, 2004). While an effective antimicrobial with a fairly broad spectrum of activity, chitosan is water insoluble unless the pH is lowered below about 4 or the molecular weight is dramatically reduced. However, the lower molecular weight forms of chitosan may exhibit reduced anti-microbial activity (Shin et al, 2001). The carboxymethylated derivatives of chitosan are water soluble but also exhibit somewhat reduced antimicrobial activity. Because different laboratories have used different techniques to quantify antimicrobial activity, it is unclear whether the synthetic polyamine anti-microbial agents are any more or less inhibitory than chitosan or its derivatives. Some examples of synthetic polyamine antimicrobials include: quaternary ammonium functionalized poly(propyleneimine) dendrimers (Chen et al, 2000), polymethacrylate containing pendant biguanide units (Ikeda et al, 1986), poly(vinylbenzyl ammonium chloride) (Ikeda et al, 1986), and polymeric phosphonium salts (Kanazawa et al, 2003). In addition, El-Masry et al (2004) recently reported the synthesis and characterization of a novel class of microbicidal halamine polymers. There are few if any reports about the ability of biofilms (or organic conditioning films) to develop on antimicrobial polymer surfaces. Similarly, there is little or no information about the biodegradability of antimicrobial polymers. Thus, their long-term efficacy in an environment such as the ISS could be called into question.

The application of antimicrobial polymers in the ISS recirculating cooling fluid might include their incorporation into the coolant channel walls, tubing and other surfaces should be considered. Alternatively, antimicrobial polymers could be engineered into a light-weight portable and miniaturized reactor unit through which coolant passed on a continuous or semi-continuous basis. As the coolant passed across the high-surface-area polymer, suspended microorganisms would be inactivated. As stated above, eventual degradation and bio-organic

fouling of the polymer-treated surfaces could be of concern. Thus, research into the antimicrobial efficacy of polyamine anti-microbial films and their potential employment in the ISS must necessarily address long-term stability and fouling issues.

ABSTRACTS OF CITED PAPERS

Halamine polymers: 2. Preparation of new triazine-diones biocidal polymers by grafting polymerization.
Pigment & Resin Technology 1 April 2004, vol. 33, no. 4, pp. 211-219(9)
A.M. El-Masry; H.Y. Moustafa; A.I. Ahmed; A.F. Shaaban
Abstract: New N-halamine polymeric compounds were prepared by reacting cyanuric acid and polyacrylonitrile. Grafting of acrylonitrile monomer onto cotton linters was carried out and the product was reacted with cyanuric acid and finally was chlorinated. Cyanoethylation of polyvinyl alcohol was performed using acrylonitrile monomer to give polyvinylcyanoethyl ether, which, in turn, was reacted with cyanuric acid. The biological activity of the various chlorinated compounds obtained was examined against Gram (+) and Gram (-) bacteria using columns and dishes methods. A high disinfecting power of the chlorinated compounds obtained was observed. Thus, the bacteria was deactivated after the first cycle without contact with the product. All of the compounds prepared were insoluble in water and most of inorganic solvents. These compounds were also found to be very stable and did not decompose to give any toxic compounds. Thus, the chlorinated compounds prepared had no harmful effects on humans.

http://ift.confex.com/ift/2002/techprogram/session_1650.htm

Antimicrobial activity of chitosan incorporated polyethylene films

S. I. PARK1, K. S. Marsh2, P. L. Dawson1, J. C. Acton1, and I. Han1. (1) Department of Food Science and Human Nutrition, Clemson University, 224 P&A Bldg., Clemson, SC 29634, (2) Kenneth S. Marsh & Assoc, Ltd., 102B Ole Towne Square, Central, SC 29630 It is well known that chitosan has an antimicrobial activity against various microorganisms. Even though chitosan is still not approved as a food additive, its advantageous properties still generate attentions for food applications. The usage of chitosan as an antimicrobial additive in food packaging films could be one of these advantageous properties The objective of this study was to investigate the feasibility of developing a chitosan based antimicrobial packaging film through the incorporation of chitosan into low density polyethylene (LDPE) film. LDPE powder was coated with chitosan dissolved in lactic acid solution with different ratios and antimicrobial films were formed by heat pressing method. The antimicrobial effectiveness of these films was examined against three pathogenic bacteria. Also, the physical (tensile strength and elongation at break) and barrier (water vapor permeability and oxygen permeability) properties of these films were evaluated. The results showed chitosan could be released from the film matrix and inhibit microbial growth. The antimicrobial activity increased with increasing concentration of chitosan in the film matrix. Both gram positive and gram negative bacteria were inhibited by those antimicrobial films when the incorporation of chitosan was above 1.43%. The antimicrobial effectiveness of films tested was decreased with increasing storage time. The oxygen permeability was not affected by the incorporation of chitosan, while the water vapor permeability was dramatically changed with the addition of chitosan with high concentration

(2.14%). The tensile strength was slightly decreased with increasing the chitosan concentration in films. The elongation values were more critically reduced by the addition of chitosan than that of tensile strength. These experiments indicated that chitosan has some attractive properties as food additives and as food packaging materials. The controlled release of chitosan from film matrix seems to effectively inhibit bacterial growth if the amount of chitosan in film matrix is sufficient to inhibit the recovery of microbial growth. Session 100B, Food Packaging 8:30 AM - 12:00 PM, 2002-06-19 2002 Annual Meeting and Food Expo - Anaheim, California

http://www.ingenta.com/isis/searching/ExpandTOC/ingenta;jsessionid=bmdb4kj326fps.circus?issue=pubinfobike://bsc/fis/2004/00000070/00000004&index=20

Fisheries ScienceVolume 70 Issue 4 Page 675 - August 2004

In vitro and in vivo antibacterial activity of shrimp chitosan against some intestinal bacteria. GUO-JANE TSAI1* AND SAN-PIN HWANG1. ABSTRACT: The effects of shrimp chitosan with deacetylation degrees (DD) of 50%, 70% and 95% (DD50, DD70, DD95) on the growth of the intestinal bacteria were investigated in vitro in the laboratory media, and in vivo by an oral feeding test using hamsters as the animal model. The antibacterial activities of these chitosan products against one strain of pathogenic *Clostridium perfringens* and 13 strains of probiotics, including seven strains of Lactobacillus, and six strains of *Bifidobacterium* were evaluated. In vitro, the antibacterial activities of DD95 and DD70 were much higher than that of DD50. The strains of probiotics were more resistant to chitosan than the pathogen of C. perfringens. The minimal lethal concentration for DD95 against C. perfringens was 250 ppm; whereas, the survival percentages for most probiotics tested were above 90% for DD95 at 500 ppm. The animals were fed on either a control diet, or diets containing powdered chitosan instead of 5% cellulose in the control diet for 4 weeks. The cecal bacterial counts of total aerobes, total anaerobes, lactobacilli, bifidobacteria and clostridia were similar for the control and experimental groups. The reasons for the differences in the antibacterial activity in vitro and in vivo are discussed.

http://www3.interscience.wiley.com/cgi-bin/abstract/78504379/ABSTRACT

Molecular weight effect on antimicrobial activity of chitosan treated cotton fabrics

Y. Shin 1 *, D.I. Yoo 2, J. Jang 1. 1Department of Clothing & Textiles, Chonnam National University, Kwangju 500-757, Korea; 2Department of Textile Engineering, Chonnam National University, Kwangju 500-757, Korea; email: Y. Shin (yshin@chonnam.chonnam.ac.kr). *Correspondence to Y. Shin, Department of Clothing & Textiles, Chonnam National University, Kwangju 500-757, Korea. Abstract: The effect of the molecular weight of chitosan on antimicrobial activity was investigated using three chitosans of different molecular weights [1800 (water soluble), 100,000, and 210,000] and similar degrees of deacetylation (86-89%). Cotton fabrics were treated with chitosan by the pad-dry-cure method. The molecular weight dependence of the antimicrobial activity of chitosan was more pronounced at a low treatment concentration. Chitosans with molecular weight of 100,000 and 210,000 effectively inhibited *Staphylococcus aureus* at a 0.5% treatment concentration. Chitosan with a molecular weight of 1800 was effective against S. aureus at a 1.0% treatment concentration. *Escherichia coli* was effectively inhibited by chitosan with a molecular weight of 210,000 at a 0.3% treatment

concentration and by chitosans with a molecular weight of 1800 and 100,000 at a 1.0% treatment concentration. Proteus *vulgaris* was effectively inhibited by chitosans with molecular weight of 100,000 and 210,000 at a 0.3% treatment concentration and by chitosan with a molecular weight of 1800 at a 0.5% treatment concentration. None of the chitosans significantly inhibited *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* below a 1.0% treatment concentration. Chitosans with high molecular weights were more effective in inhibiting bacterial growth than chitosans with low molecular weights. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 80: 2495-2501, 2001

Applied and Environmental Microbiology, July 2002, p. 3575-3581, Vol. 68, No. 7
0099-2240/02/$04.00+0 DOI: 10.1128/AEM.68.7.3575-3581.2002
Copyright © 2002, American Society for Microbiology. Distribution of Microbes Producing Antimicrobial E-Poly-L-Lysine Polymers in Soil Microflora Determined by a Novel Method. Masanobu Nishikawa* and Ken'ichi Ogawa

http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=232627&rendertype=abstract

J. Bacteriol., Jul 1996, 3715-3721, Vol 178, No. 13
Polyamines decrease *Escherichia coli* outer membrane permeability. AL Dela Vega and AH Delcour, Department of Biology, University of Houston, Texas 77204, USA. Abstract: The permeability of the outer membranes of gram-negative bacteria to hydrophilic compounds is mostly due to the presence of porin channels. We tested the effects of four polyamines (putrescine, cadaverine, spermidine, and spermine) on two processes known to depend on intact porin function: fluxes of beta-lactam antibiotics in live cells and chemotaxis. In both cases, inhibition was observed. Measurements of the rate of permeation of cephaloridine and of chemotaxis in swarm plates and capillary assays were used to determine the concentration dependence of this modulation. The effective concentration ranges depended on the nature of the polyamine and varied from submillimolar for spermine to tens of millimolar for cadaverine. Both OmpC and OmpF porins were inhibited, although the effects on OmpC appeared to be milder. These results are in agreement with our observations that polyamines inhibit porin-mediated ion fluxes in electrophysiological experiments, and they suggest that a low-affinity polyamine binding site might exist in these porins. These results reveal the potential use of porins as targets for blocking agents and suggest that polyamines may act as endogenous modulators of outer membrane permeability.

http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=176450

Polycationic biocides with pendant active groups: molecular weight dependence of antibacterial activity. T Ikeda, H Hirayama, H Yamaguchi, S Tazuke, and M Watanabe

Abstract: Two types of polycations with pendant active groups were synthesized: one is polymethacrylate containing pendant biguanide units, and the other is poly(vinylbenzyl ammonium chloride). The two polycations were found to exhibit higher bactericidal activity against Staphylococcus aureus than the corresponding monomers. Fractionation of the polycations was successfully performed on gel filtration chromatography, and examination of the antibacterial activity against S. aureus of the well-characterized polymer samples with various

molecular weights (MW) revealed that the activity was strongly dependent on the MW of the polycations and that there existed an optimal MW range for the cidal action of the polymeric biocides. Experiments on the lysis of protoplasts of Bacillus subtilis in contact with the polycations have shown that target sites of the polycationic biocides are cytoplasmic membranes of bacteria.

http://www3.interscience.wiley.com/cgi-bin/abstract/104048395/ABSTRACT

Novel polycationic biocides: Synthesis and antibacterial activity of polymeric phosphonium salts. Akihiko Kanazawa, Tomiki Ikeda, Takeshi Endo*; Research Laboratory of Resources Utilization, Tokyo Institute of Technology, 4259, Nagatsuta, Midori-ku, Yokohama 227, Japan; *Correspondence to Takeshi Endo, Research Laboratory of Resources Utilization, Tokyo Institute of Technology, 4259, Nagatsuta, Midori-ku, Yokohama 227, Japan. Abstract:

Various polymeric phosphonium salts and the corresponding low-molecular-weight model compounds were prepared and their antibacterial activities against Staphylococcus aureus and Escherichia coli were explored by the viable cell counting method in sterile distilled water. Antibacterial activity of the polymers was found to be higher than that of the corresponding model compounds, particularly against S. aureus. Furthermore, the polymeric phosphonium salt exhibited a higher activity by 2 orders of magnitude than the polymeric quaternary ammonium salt with the same structure except the cationic part. Compounds with the longest alkyl chain (octyl) studied were found to exhibit particularly high activity, and this finding may be ascribed to the contribution of the increased hydrophobicity of the compounds to the cidal activity. © 1993 John Wiley & Sons, Inc.

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=11710139&dopt=Citation

Biomacromolecules. 2000 Fall;1(3):473-80. Related Articles, Links

Quaternary ammonium functionalized poly(propylene imine) dendrimers as effective antimicrobials: structure-activity studies. Chen CZ, Beck-Tan NC, Dhurjati P, van Dyk TK, LaRossa RA, Cooper SL. Department of Chemical Engineering, University of Delaware, Newark, Delaware 19716, USA. Abstract: Quaternary ammonium functionalized poly(propyleneimine) dendrimers were synthesized and their antibacterial properties were evaluated using a bioluminescence method. These quaternary ammonium dendrimers are very potent biocides. The antibacterial properties depend on the size of the dendrimer, the length of hydrophobic chains in the quaternary ammonium groups, and the counteranion. Since these dendrimers are well characterized and monodisperse, they also serve as an effective system to study the structure-activity relationship. The antimicrobial properties of these dendrimer biocides have a parabolic dependence on molecular weight, which is different from the bell-shaped molecular weight dependence of conventional polymer biocides. The dependence on the hydrophobic chain of the quaternary ammonium structure is similar to conventional polymer biocides, and shows a parabolic relationship with dendrimer biocides carrying C10 hydrophobes the most potent. The antimicrobial properties of these novel biocides with bromide anions are more potent than those with chloride anions. Biocides derived from hyperbranched polymers were also synthesized and found to possess somewhat lower effectiveness.

Gamma Irradiation for Microbial Control.

Gamma irradiation is an effective antimicrobial treatment strategy that can be physically designed to satisfy a variety of engineering constraints, including miniaturization. Gamma irradiation via cobalt-60 is used extensively in food and medical products sterilization. It has also been used as a means to preserve biologically-fouled reverse osmosis membranes for long-term storage. In most situations, the gamma dosage can be adjusted to inactivate target microbial populations (including biofilms) without damaging surrounding materials (see reference); however, finding the proper dosage usually requires research and development. The advantage of gamma irradiation is that it is non-invasive and therefore does not result in the accumulation of chemical agents in the coolant water. Key disadvantages include the unlikely possibility of radiation exposure to humans and the lack of a soluble antimicrobial to control biofilm formation in remote areas of the recirculating coolant system not directly irradiated. The half-life of cobalt-60 is about 5.3 years. Another significant disadvantage of gamma source disinfection is that substantial radiation shielding (e.g., lead) may be required to protect ISS personnel from unwanted exposures. The cost of transporting heavy shielding materials to the ISS from Earth might preclude the use of gamma irradiation aboard the ISS.

The feasibility of engineering a miniaturized light-weight gamma irradiation system for the ISS coolant system should be determined.

Reference 1.

Biologicals (2002) 30, 207–216

http://www.idealibrary.com

Effective use of Gamma Irradiation for Pathogen Inactivation of Monoclonal Antibody Preparations. Teri Grieb, Ren-Yo Forng, Robert Brown, Timothy Owolabi, Ewa Maddox, Anna McBain, William N. Drohan, David M. Mann and Wilson H. Burgess*; Clearant, Inc., Rockville, MD, U.S.A. Abstract. Gamma irradiation has been used for decades as an effective method of pathogen inactivation of relatively inert materials. Until recently, its application to biologicals has resulted in unacceptable losses in functional activity. In this report we demonstrate that the damaging secondary effects of gamma irradiation can be controlled while maintaining the pathogen inactivation properties due to damage by primary effects. Control is achieved by a combination of protection from free radical damage to a monoclonal antibody through the use of the antioxidant ascorbate and by freeze-drying to minimize the potential for generating free radicals. The data demonstrate a synergy of these two approaches that results in quantitative recovery of functional activity while maintaining the ability to inactivate greater than 5 logs of porcine parvovirus infectivity.

Chelation of Multi-Valent Cations to Suppress Microbial Growth.

Microorganisms typically require the presence of trace quantities of divalent cations such as Ca^{++} or Mg^{++} to maintain the structural and functional integrity of their cell walls or cell membranes. Divalent cations are also required for the stability and activity of many enzymes,

some of which are involved in intermediary metabolism and DNA replication. Concentrations of divalent cations required for these purposes generally range from about 10^{-3} to 10^{-5} Molar. If divalent cations are removed from the environment or their concentrations reduced below physiological requirements, most microbes will cease to grow or become irreversibly inactivated. Divalent cation chelating agents such as diethylenetriaminepentaacetic acid (DTPA), tri(carboxymethyl)amine (NTA), and ethylenediaminetetracetic acid (EDTA) have been shown to be effective broad-spectrum bacteriostats or antimicrobials. Because EDTA exhibits a very low toxicity for humans, animals and the environment, the U.S. Food and Drug Administration has approved its use in food preservation. EDTA has been demonstrated to work effectively and synergistically with other antimicrobial agents, such as benzoic acid, methyl/propyl/butyl parabens and antibiotics (Reference 1). EDTA is also reasonably stable and can persist for months or years under most circumstances where food preservation is the primary application. However, as is the case for most organic compounds, EDTA may eventually biodegrade if a suitable microbial consortium develops (Reference 2). The time elapsed before the onset of biodegradation can vary greatly depending on the specific chemical and microbiological environment in which the compound is placed. A number of chelating agents are commercially available, some of which may have properties better suited than EDTA for application in the ISS coolant recirculation system.

The efficacy of EDTA and related chemical chelating agents as microbiostats and microbicides in simulated ISS coolant water should be determined. Determine the chemical and biological stability and duration of effectiveness of the compounds as antimicrobials. Determine EDTA effectiveness alone and in combination with other potential antimicrobials. Determine materials compatibility.

Reference 1:

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=14723685

The synergistic effect of EDTA/antimicrobial combinations on Pseudomonas aeruginosa

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Abstract: Aims: To demonstrate that the nonlinear concentration-dependent inhibition of Pseudomonas aeruginosa to EDTA can be used to successfully model and predict the potentiation of antimicrobials by EDTA. Methods and Results: A model used successfully to describe the concentration-dependent inhibition of bacterial growth caused by many antimicrobials was unable to describe the inhibition of P. aeruginosa by EDTA. Examination of the inhibition profiles for EDTA against P. aeruginosa revealed a biphasic inhibitory pattern suggesting different mechanisms of action at different concentrations. A modelled, two-stage inhibitory process was shown to fit the observations. This model was then used to examine the effect of combining EDTA with other antimicrobials. The apparent synergy of mixtures of EDTA with quaternary ammonium surfactants (QAC) and specific antibiotics was successfully

modelled. Minimum inhibitory concentrations (MIC) of the QAC and that of oxacillin and cefamandole were reduced by a factor of 3–10, whereas ampicillin was reduced by a factor of 70 from an MIC of 1524 to 21 mg l-1 in the presence of 500 mg l-1 of EDTA. Conclusions: A nonlinear concentration-dependent inhibition of P. aeruginosa by EDTA gives rise to apparent observation of synergy with other antimicrobials. Significance and Impact of the Study: This is a further example where the current methodology for the examination of antimicrobial synergy (the summed fractional inhibitory concentrations) leads to false conclusions.

Reference 2: http://aem.asm.org/cgi/content/full/64/4/1319

Appl Environ Microbiol, April 1998, p. 1319-1322, Vol. 64, No. 4
Biodegradation of Metal-EDTA Complexes by an Enriched Microbial Population. Russell A. P. Thomas,1 Kirsten Lawlor,2 Mark Bailey,2 and Lynne E. Macaskie1. School of Biological Sciences, The University of Birmingham, Birmingham B15 2TT,1 and NERC Institute of Virology and Environmental Microbiology, Oxford OX1 3SR,2 United Kingdom. Abstract: A mixed culture utilizing EDTA as the sole carbon source was isolated from a mixed inoculum of water from the River Mersey (United Kingdom) and sludge from an industrial effluent treatment plant. Fourteen component organisms were isolated from the culture, including representatives of the genera *Methylobacterium*, *Variovorax*, *Enterobacter*, *Aureobacterium*, and *Bacillus*. The mixed culture biodegraded metal-EDTA complexes slowly; the biodegradability was in the order Fe>Cu>Co>Ni>Cd. By incorporation of inorganic phosphate into the medium as a precipitant ligand, heavy metals were removed in parallel to EDTA degradation. The mixed culture also utilized a number of possible EDTA degradation intermediates as carbon sources.

VII. Comments on Wilson *et al.* Reviews

Review of a selection of an alternate biocide for the International Space Station. Internal active thermal control system coolant loops. M. Wilson et al. SAE paper 2003-01-2568(2003).

The paper describes the coolant for the internal active thermal control system (IATES) as a water-based fluid containing borate and phosphate. The moderate temperature loop (MTL) contains approximately 200 liters of fluid. The low temperature loop (LTL) contains approximately 63 liters of fluid. Between January 2000 and 2003 the pH has changed from 9.5 to 8.4. There was also an increase in both organic and inorganic compounds, and a decrease in phosphate. Silver was added to the original system as an antimicrobial. The silver had deposited by 2003 and was undetectable in 2003.

These changed conditions were favorable for microbial growth. Bacterial counts had increased from less than 10 colony forming units (CFU) per ml. in the original system to 106 CFU/ml in 2003. This increase in the bacterial population in the IATCS has the potential to from biofilms. The biofilms would impede the systems performance and may accelerate corrosion.

The authors tested antimicrobials that may replace silver in the ISS-ISTES coolant fluid. The requirements are quite stringent, i.e. effectiveness at low concentration, stability, solubility, homogeneity and ease of application. Their objective was to reduce the number of planktonic (circulatory) microorganisms and to control formation and development of biofilms.

The authors considered only those chemicals that would be acceptable under the strict NASA guidelines. For example, antimicrobials containing chlorine, bromine or heavy metals were eliminated. Aldehydes, thiols and non-halogen oxidizers were considered to be good candidates. They ranked these antimicrobials in descending order. They were hydrogen peroxide, bismuth thiols, Bellaire 375, enzymes, glutaraldehyde, and quaternary ammoniums.

Tests were carried out to determine the minimum inhibitory concentration (MIC) of a range of these chemicals. Enzymes were not included. Bismuth thiols containing formed emulsions and were excluded. The quaternary ammonium compounds had the potential to release chlorine and were excluded. The anti-corrosion agent 2,5 dimencapto-thiazole failed to inhibit bacterial growth at 1000 ppm and was excluded.

The article describes the selection of a number of candidate antimicrobials for testing that appear to be compatible with the IATCS. These include hydrogen peroxide, glutaraldehyde, and Baquacid.

The elimination of antimicrobial chemicals in the initial assessment seems reasonable. Biocides were excluded either because they were unstable or toxic to humans, making them unacceptable for use in the IATCS. However, there may be other forms of some of the chemicals that are more compatible with the NASA guidelines. For example there are non-toxic thiazalone compounds that are excellent antimicrobials.

Selection of an alternative biocide for the ISS internal control system coolant - Phase II. M. Wilson et al. SAE papers 041 CES-238 (2004). This paper describes the testing phase of the selection of an alternative antimicrobial to silver for use in the internal active thermal control system. (IATCS) for the ISS. The selection of antimicrobials to be tested was described in SAE paper # 2003-01-2568 (2003).

In this 2004 paper, Wilson and his colleagues report on the current status of their efforts to select an alternative antimicrobial for the ISS-ITCS. They chose for testing, hydrogen peroxide, with a minimum inhibitory concentration (MIC) of 50 ppm; glutaraldehyde, with a MIC of 25 ppm; and bismuth: 2,3 –dimercapto-1-propanol: 2-mencaptopyridine-N-oxide, (Bis:Bal:pyr) MIC = 1.7 mg bismuth.. Peroxide was chosen because of its broad spectrum oxidizing antimicrobial activity. The highly reactive hydroxyl molecule acts against most cellular material. Glutaraldehyde cross-links proteins and nucleic acids and has well known very wide biocidal activity. Bis: Bal: Pyr has a broad spectrum of biocidal activity, and is active at low concentrations.

The authors carried out comparative tests to determine the minimum time for the candidate antimicrobials to be effective at approximately five and ten times the MIC. They were challenged with six different bacteria. The results showed that glutaraldehyde killed all bacteria at a concentration of 190 ppm in two hours. Complete kill for hydrogen peroxide at 492 ppm, was longer than twelve hours. Bis: Bal: Pyr at 16 ppm took approximately 48 hours to kill all the bacteria.

The three antimicrobials were compared for stability in the fluid over a three month period. Both glutaraldehyde and Bis: Bal: Pyr remained stable throughout the three month period. However the hydrogen peroxide concentration declined significantly after 30 days.

Compatibility tests were also carried out to evaluate the antimicrobial's compatibility with the IATCS System. None of the three antimicrobials tested caused a significant change in surface tension. No significant corrosion was detected in the presence of glutaraldehyde. Both hydrogen peroxide and Bis: Bal: Pyr: displayed relatively high corrosion rates when metals used in the ITCS were exposed to them.

Tests were also conducted to assess the compatibility of the test antimicrobials with non-metallic material used in the construction of the ITCS. Two epoxies, two nylons and polypropylenes were exposed to the antimicrobials for 63 days. No incompatibility was detected with any of the three antimicrobials being tested.

Glutaraldehyde was identified as the leading candidate to protect the ITCS coolant from microbial activity, based on its stability and materials compatibility. There was a concern that, if ammonia was released into the coolant fluid, it may react with the glutaraldehyde. Tests showed that the interaction was minimal over a 76 day test period. This slight decline, if it occurs could be compensated during antimicrobial makeup.

Our detailed review of these two papers by Wilson et al. indicated that the procedures used were appropriate and addressed all of issues related to the use of these antimicrobials in the IATCS fluid. The conclusion of the authors that glutaraldehyde was the best candidate tested makes good sense based on efficiency, stability, and materials compatibility.

We would like to have information about the capacity of glutaraldehyde at these concentrations to control biofilms growing on the surface of the IATCS. It also would be useful to show the ability of the antimicrobials to control fungal growth.

The issue of other alternatives to glutaraldehyde needs to be addressed. There is a good literature on thiazolones. They are safe and stable and should be considered.

The issue of the NASA requirement for low concentrations of glutaraldehyde also needs to be addressed. This biocide is extremely safe. However, if it is released to the ISS environment, there is a wide range of safe inactivating chemicals available. For example, sodium bisulfite is used extensively as an inactivating agent. The combination glutaraldehyde-bisulfite complex is totally non-toxic. (See Susan Jordan et al (1996).)

VIII Discussion

The Internal Active Thermal Control System (IATCS) of the International Space Station (ISS) is an essential component of the station. It maintains coolant to be a broad range of equipment, including payloads and avionics. Because the coolant in the IATCS is water-based, it is susceptible to biological activity. Silver has been added as an antimicrobial. Tests have shown that the addition of silver was ineffective as a means of controlling biological activity. The lack of availability of the silver, caused by deposition of the silver, has resulted in environmental conditions in the IATCS that would support the growth of microorganisms. These conditions also have the potential to result in corrosion of critical components of the IATCS. The potential release of IATCS coolant into the atmosphere of the ISS poses a significant health risk to the crew.

It is particularly important to focus on the formation of microbial biofilms in the assessment of measures to control microbial growth in the coolant system. Measurement of biological

activity in the liquid would not provide an assessment of the risk of potential problems in the IATCS caused by microbial activity. Control measures need to be sufficient to prevent biofilm formation. Both biofouling and corrosion of the system would be caused by biofilms growing on the surfaces of the system materials. There is a large body of literature demonstrating the ability of biofilm bacteria to cause MIC and biofouling. Key publications showing these relationships are described in section IX of this report.

There are a number of commercially available chemicals suitable for protection of cooling water systems against microbial activity. The microorganisms have the potential to cause biofouling or MIC in the coolant system. However, compatibility with NASA requirements and the ISS placed severe restraints on the use of many of these chemicals. In particular, safety considerations are severe in the closed environment of the ISS. For example, chlorine-based chemicals, with the potential to release free chlorine, could not be considered for use in the IATCS.

We reviewed fifteen candidate chemicals for possible further testing. We evaluated the chemicals on the basis of six criteria. The most important two criteria were safety and materials compatibility. We conferred these two parameters with the greatest weight. Chemicals that were not considered safe or that caused damage to the materials in the system clearly could not be seen as viable for use in the IATCS. Antimicrobial activity and chemical compatibility were weighted slightly less than the first two parameters. The least weight was conferred to stability and industrial experience. We considered that some instability could be compensated by later additions of the chemical. Newer chemicals may be very suitable, yet have limited industrial experience.

Our analysis yielded numerical scores for each chemical based on each parameter. We separated the fifteen chemicals into four groups on the basis of the final scores. The two chemicals in the first group, glutaraldehyde and the thiazolones appear to be the most ideal candidates for further testing. They scored well on all six criteria. The concentrations required would provide good protection against microbial activity, be acceptable under the NASA safety criteria and they should be compatible with the materials of construction. The second group of four chemicals, chlorhexidine, ozone, orthopthalaldehyde, and peracetic acid scored slightly below the first group. All four of these candidates have a reasonable potential for use in the IATCS, and should be considered for further testing. We did not consider the remaining chemicals in group three and four to be sufficiently strong candidates, compared to the first two groups. We do not recommend that they be tested further.

It is important to understand that our analysis did not yield one candidate that is ideal for use in the IATCS. It may be that a combination of two chemicals would be best suited for use in the coolant system. Tests of combinations of the chemicals in our matrix should be initiated.

We investigated the possibility of using emerging technologies for control of biological activity in the IATCS. We concluded that none of the emerging technologies that appear to be suitable for use in the cooling system could be utilized without extensive further testing. However, some of the emerging technologies would be very beneficial, if further tests were successful. In particular the addition of water reduction molecules, such as lithium salts, to the coolant, would have the effect of reducing the water concentration and therefore microbial activity. However, the effect on heat transfer capacity and corrosivity of the solution would need to be evaluated.

The use of chelating agents such as EDTA has the potential to control microbial growth. They are particularly effective when used in combination with other antimicrobials. It may be possible to increase the effectiveness of glutaraldehyde or one of the other chemicals in our matrix by the addition of a chelating agent. Combined use of one of these agents would permit application of a lower concentration of the glutaraldehyde or other chemicals in our matrix.

We considered a number of radiation-based technologies in our analysis of emerging technologies. Some of these systems may be applicable to the IATCS. Of particular interest is the visible light photoactivation of nitrogen-doped titanium oxides. This technology functions at normal light levels and is highly effective as an antimicrobial agent. However, it would require the use of either fiber optics or visible-light transparent cooling systems. Use of this technology would require extensive tests and modification of the IATCS.

In conclusion we are of the opinion that there are effective off-the-shelf antimicrobial agents available for use in the IATCS. None of these chemicals is totally compatible with the NASA requirements for the ISS. Further tests are therefore required to determine the most appropriate chemical for use in the IATCS. It may be necessary to use a combination of two chemicals at low concentrations in order to meet the safety and materials compatibility criteria set by NASA.

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X Appendices
Appendix I, Telephone Conference Notes

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(781) 930-3003

September 15, 2004

National Aeronautics and
Space Administration
George C. Marshall Space Flight Center
Marshall Space Flight Center, AL 35812
ATTN: Robert L. Martin, Contracting Officer PS41O/2004; cc: Ms. Monsi Roman

SUBJECT: September 15, 2004 Conference Call Notes (Req. #4200074193)

Dear Mr. Martin,

On September 15, 2004, a 45 min conference call was held between me, Dr. Harry Ridgway, Ms. Monsi Roman, Dr. Steven Dexter (Univ. Delaware), and other NASA staff members. The purpose of this conference call was to provide NASA with an update on the status of our research program. This conference call was the last call scheduled for this project. The following issues were discussed:

1. Dr. Mittelman reviewed the antimicrobial selection matrix and scoring system. Glutaraldehyde and isothiazolone received the highest rankings among the 15 cooling water system treatments evaluated.
2. Ms. Roman noted that peracetic acid was reported to demonstrate decreased efficacy at higher pH levels. Dr. Mittelman suggested that peracetic acid was effective up to a pH of 8 or 8.5.
3. Dr. Mittelman noted that chlorhexidine and triclosan were two agents not previously used in cooling systems, but demonstrated good safety, compatibility, and efficacy profiles in the selection matrix.
4. Ms. Roman discussed the previous findings from Boeing suggesting that isothiazolones were not effective. The group generally agreed that these findings were contrary to industry experience and reports from a number of publications. Dr. Mittelman suggested that IATCS water chemistry compatibility may be an important factor in this regard. In addition, neither the methodology nor the specific compound evaluated were disclosed.
5. Dr. Dexter noted the importance of conducting antimicrobial efficacy studies using biofilm populations in addition to planktonic populations.
6. Dr. Ridgway discussed EDTA applications, and the potential for EDTA combination treatments with the cooling water antimicrobials.
7. Dr. Mittelman provided an update on the project report draft, which has been completed and is currently under review. The report will be sent to Ms. Roman on or about September 20. An electronic version will be provided to Ms. Roman, along with hard copies of relevant publications.

8. Ms. Roman discussed the Huntsville meetings, scheduled for September 29, 2004. Drs. Mittelman and Pyle (MSU) will present their findings; discussions will follow the presentations.

Sincerely,

Marc W. Mittelman, Ph.D.
Principal

Mittelman & Associates
140 Wood Road Ste. 200
Braintree, MA 02184
(781) 930-3003

September 3, 2004

National Aeronautics and
Space Administration
George C. Marshall Space Flight Center
Marshall Space Flight Center, AL 35812
ATTN: Robert L. Martin, Contracting Officer PS41O/2004; cc: Ms. Monsi Roman

SUBJECT: September 3, 2004 Conference Call Notes (Req. #4200074193)

Dear Mr. Martin,

On September 3, 2004, a 45 min conference call was held between me, Dr. Ralph Mitchell, Dr. Harry Ridgway, Ms. Monsi Roman, Dr. Steven Dexter (Univ. Delaware), and other NASA staff members. The purpose of this conference call was to provide NASA with an update on the status of our research program. The following issues were discussed:

1. Dr. Ridgway introduced the concept of decreasing water activity in the coolant fluid as means for controlling planktonic and sessile bioburden. He noted that below a water activity of 0.60, microbial growth (bacteria, fungi) ceased. Dr. Ridgway suggested consideration of alternate cooling fluids, including LiBr and organic-based heat-transfer solutions. The group discussed the importance of material compatibility and heat transfer efficiency.
2. Drs. Mittelman and Mitchell discussed hydrogen peroxide applications. Dr. Mittelman relayed information from Ontario Hydro (Toronto) that suggested 50-100 mg/L hydrogen peroxide treatments twice/year were effective in a high purity water fuel storage system. The half-life in this application was approximately 72 h. The issue of organic compounds in the ITCS fluid reacting with low levels of the hydrogen peroxide was discussed by the group.
3. Ms. Roman requested that information describing hydrogen peroxide-silver interactions be included in our report.
4. Dr. Ridgway described an electrolytic system for generating free radicals in situ. The system can be miniaturized and may be useful in a closed loop cooling system. He will include information on this process in our report.
5. Dr. Mitchell and Ms. Roman discussed potential foaming and wettability issues associated with the application of isothiazolones. Dr. Mitchell will address these concerns in our report.
6. Dr. Dexter discussed microbially-mediated hydrogen peroxide generation in marine systems, and suggested that low levels may be detectable in cooling loops as a result of biological activity (in biofilms).
7. Potential meeting dates for the Mittelman/Mitchell and Ford/Pyle team briefings in Huntsville were discussed. Ms. Roman will prepare an e-mail to poll participants for their

availability during late September or early October. Alternate venues may be considered in order to facilitate travel by Dr. Dexter, who cannot travel by air.

Our next conference call is scheduled for September 15 at 1:30 PM CDT.

Sincerely,

Marc W. Mittelman, Ph.D.
Principal

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August 20, 2004

National Aeronautics and
Space Administration
George C. Marshall Space Flight Center
Marshall Space Flight Center, AL 35812
ATTN: Robert L. Martin, Contracting Officer PS41O/2004; cc: Ms. Monsi Roman
SUBJECT: August 20, 2004 Conference Call Notes (Req. #4200074193)

Dear Mr. Martin,

On August 20, 2004, a 1 h conference call was held between me, Dr. Ralph Mitchell, Dr. Harry Ridgway, Ms. Monsi Roman, Mr. Mike Holt, Dr. Steven Dexter (Univ. Delaware), and Mr. Jeff Linns. The purpose of this conference call was to provide NASA with an update on the status of our research program. The following issues were discussed:

1. Dr. Mittelman reviewed his discussions with Advanced Sterilization Products (a Johnson & Johnson company). ASP is a leading manufacturer of antimicrobial solutions for medicine and industry. Mr. Charles Roberts is director of biocide development; Dr. Martin Favero (ex of CDC) is VP, R&D. ASP is providing information on novel antimicrobials with a broad spectrum of activity and good material compatibility properties. Some of their newly emerging compounds include performic acid, ortho-phthalaldehyde, and new glutaraldehyde formulations. Mr. Holt and Ms. Roman agreed Mr. Roberts and/or Dr. Favero would make a positive addition to the project team meeting (in Sept-Oct.). Mr. Holt will explore the feasibility of inviting their participation.
2. Dr. Mitchell discussed his preliminary findings on the efficacy and safety of glutaraldehyde. The issue of biocide neutralization was discussed, and will be included as part of the study compound matrix.
3. Dr. Ridgway discussed applications of N-doped titanium dioxide, which can be light activated (visible light). EDTA and sodium benzoate were described as potential synergistic agents for combination antimicrobial treatments. Dr. Ridgway will also consider the application of cilantro (per a Boeing request); however, the group agreed that significant time should not be devoted to natural products in general.
4. Ms. Roman and Mr. Holt indicated that they are currently arranging potential meeting times and venues for the group presentations/discussion in September or October, 2004.

Our next conference call is scheduled for September 3 at 12:00 noon EST.

Sincerely,

Marc W. Mittelman, Ph.D.
Principal

Mittelman & Associates
140 Wood Road Ste. 200
Braintree, MA 02184
(781) 930-3003

August 20, 2004

National Aeronautics and
Space Administration
George C. Marshall Space Flight Center
Marshall Space Flight Center, AL 35812
ATTN: Robert L. Martin, Contracting Officer PS41O/2004; cc: Ms. Monsi Roman

SUBJECT: August 13, 2004 Conference Call Notes (Req. #4200074193)

Dear Mr. Martin,

On August 13, 2004, a 1 h conference call was held between Dr. Ralph Mitchell, Ms. Monsi Roman, and Mr. Mike Holt. The purpose of this conference call was to provide NASA with an update on the status of our research program. The following issues were discussed:

1. Dr. Mitchell discussed his review of the two Boeing-NASA review papers, which addressed antimicrobials for the cooling systems. Dr. Mitchell indicated that he generally supported the conclusions of the Wilson et al. papers.
2. Dr. Mitchell discussed some alternative antimicrobials, including EDTA. These compounds will be included in the study test matrix.
3. NASA staff requested that we evaluate the suitability/feasibility of slow-release glutaraldehyde formulations.
4. Dr. Mitchell will contact Rohm and Haas to discuss recent information on the efficacy and materials compatibility of isothiazolones.
5. The group discussed the importance of biocide activity against fungi.
6. Dr. Ridgway will address combination treatments, including the use of synergistic EDTA dosages.

Our next conference call is scheduled for August 20 at 12:00 noon EST.

Sincerely,

Marc W. Mittelman, Ph.D.
Principal

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140 Wood Road Ste. 200
Braintree, MA 02184
(781) 930-3003

August 9, 2004

National Aeronautics and
Space Administration
George C. Marshall Space Flight Center
Marshall Space Flight Center, AL 35812
ATTN: Robert L. Martin, Contracting Officer PS41O/2004; cc: Ms. Monsi Roman

SUBJECT: August 3, 2004 Conference Call Notes (Req. #4200074193)

Dear Mr. Martin,

On August 3, 2004, a 1 h conference call was held between me, Dr. Ralph Mitchell, Ms. Monsi Roman, and Mr. Mike Holt. (An earlier 1h conference call had established the study parameters; this call was held in late July). The purpose of this conference call was to provide NASA with an update on the status of our research program. The following issues were discussed:

1. Most of the literature search work has been completed; a total of approximately 200 pertinent reference citations have been obtained.
2. Dr. Ridgway is researching the use of EDTA and other atypical compounds (e.g., TiON) for their applicability to the system.
3. Dr. Mittelman is evaluating glutaraldehyde and isothiazolone applications; problems with glutaraldehyde toxicity were noted by Ms. Roman. Dr. Mittelman will contact Union Carbide (Dr. Roman provided a contact) for further information.
4. Dr. Mittelman will contact Advanced Sterilization Product regarding their new cold sterilants under development; e.g., OPA, performic acid).
5. Dr. Mitchell is evaluating the literature describing other commonly used antimicrobials in closed-loop water/cooling water systems for efficacy, stability, and safety.
6. Ms. Roman discussed the importance of antimicrobial compatibility with recycling system chemistry and materials of construction.
7. Dr. Ronald Latanision has been contacted by Drs. Mittelman and Mitchell, and has agreed to review the materials compatibility information developed as part of this study.

Our next conference call is scheduled for August 13 at 12:00 noon EST.

Sincerely,

Marc W. Mittelman, Ph.D.
Principal

Appendix II, Selected Relevant Publications
Appended.

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Appendix B. Montana State University Antimicrobial Survey Technical Report

ASSESSMENT OF ISS IATCS LOOP MICROBIAL CONTROL OPTIONS

Final Report
By

Barry H. Pyle[1], Ph.D., Susan C. Broadaway[1], B.S., Elinor deL. Pulcini[1,2], Ph.D., Tim E. Ford[1], Ph.D., Anne K. Camper[2], Ph.D. and Paul Sturman[2], Ph.D.
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Montana State University – Bozeman

October, 2004

INTRODUCTION AND BACKGROUND

The Internal Active Thermal Control System (IATCS) of the International Space Station (ISS) is a closed loop system that provides a relatively constant temperature coolant supply to equipment, payloads and avionics. The coolant is a water-based fluid that contains borate as a buffer and phosphate as a corrosion inhibitor. Silver has been previously added to control the bacterial population. The IATCS is composed of two loops, the Moderate Temperature Loop (MTL) and the Low Temperature Loop (LTL). The MTL contains approximately 200 liters of fluid and has a supply temperature range of 16.1 to 18.3^{o} C. The LTL contains approximately 60 liters of fluid and has a supply temperature range of 3.3 to 6.1^{o} C. The two loops can operate independently in a dual-loop mode or in series in single-loop mode.

Since January 2000, the chemical and microbial state of the on-orbit fluid has been monitored by analysis of samples returned to earth. Many chemical parameters have changed over time including a drop in pH from the requirement of 9.5 +/- 0.5 to ~ 8.4, an increase in the level of total inorganic carbon (TIC), total organic carbon (TOC) and nickel in the fluid, and a decrease in the phosphate level. In addition, silver ion levels in the fluid have decreased rapidly as silver is deposited on internal surfaces of the system. It has been suggested that the lack of availability of silver ions coupled with changes in the fluid have created a favorable environment for

microbial growth. Counts of heterotrophic bacteria have increased from <10 colony forming units (CFU)/100 milliliters (mL) to 10^6 - 10^7 CFU/100 mL. The increase of the microbial population is of concern because uncontrolled microbiological growth in the IATCS can cause deterioration in the performance of critical components within the system and potentially impact human health if opportunistic pathogens become established. Microorganisms are capable of degrading the coolant chemistry, attaching to surfaces and forming biofilm, causing subsequent biofouling of filters, tubing, and pumps, decreasing flow rates, reducing heat transfer, initiation and acceleration of corrosion, and enhanced mineral scale formation.

The system was dosed with silver early in 2002 (Dickey, 2002). On-orbit fluid samples indicated silver biocide filters were working, which was confirmed by water samples that were analyzed upon return to earth. There was a concern that silver was likely damaging the hardware. The report noted that CO_2 exchange between the cabin atmosphere and coolant had apparently reached equilibrium resulting in a pH of 8.4, and that the coolant working group would consider whether pH 8.4 is acceptable (Dickey, 2002).

Alternative biocides were selected (Wilson et al, 2003) based on the need for safe, non-intrusive implementation and operation in a functioning system; the ability to control existing planktonic and biofilm residing microorganisms; negligible impact on system-wetted components; and, negligible reactivity with existing coolant additives. The specific assessment criteria were weighted according to their perceived significance (Table 1).

Table 1: Biocide Assessment Criteria and Weighting Factors (from Wilson et al, 2003)

Assessment Criteria	Weight
Material Compatibility	4x
Chemical Compatibility	3x
Safety/Toxicity	3x
Disinfection Effectiveness	2x
Stability	2x
Byproduct Acceptability	2x
On-orbit Implementation	1x
Cost	1x
In-flight Monitoring	1x
Technology Readiness	1x

Subsequently, the stability of the following biocides in the IATCS fluid was examined (Wilson et al, 2003): hydrogen peroxide, glutaraldehyde, bismuth thiols, and a polymeric guanide hydrochloride (Baquacil). Later, the biocidal efficacies of hydrogen peroxide, glutaraldehyde and

bismuth thiols were also determined (Wilson et al, 2004), in addition to chemical and metal compatibility. The antimicrobial tests were done in multiwell microtiter plates. Glutaraldehyde was selected as the optimum “technical solution" based on material compatibility, stability, and long duration effectiveness. It was reported that glutaraldehyde reacts slowly with trace levels of ammonia in the coolant, and that this might interfere with on-orbit measurements of ammonia unless the test procedure is adjusted for use in the presence of glutaraldehyde (Wilson et al, 2004).

The present evaluation of alternate antimicrobial agents was based on 20 criteria:

1. Economy (cost)
2. Application (application schemes)
3. Storage stability (shelf life for concentrate); stability after application
4. Odor (not harmful/offensive)
5. Toxicity (fluid, off-gassing, effects on ISS trace contaminant control equipment)
6. Cleaning power (removal of biofilm and/or organic/inorganic films)
7. Penetrative power (reduction of bacteria/material in biofilm)
8. Residual activity
9. By-product/degradation products
10. Reduction of planktonic counts
11. Activity before and after dilution
12. Degradation by organics
13. Material compatibility (corrosion of metals, compatibility w/ non-metals)
14. Broad spectrum activity
15. Speed of action
16. Compatibility with other chemicals in the system
17. Industry experience
18. Monitoring (of the biocide or its effectiveness)
19. Effectiveness
20. Removal method/difficulty

To these 20 criteria, the following 17 fluid- and materials-related constraints were added:

- Coolant specification for chlorides less than 1 ppm
- Limit exposure to compounds that will increase general and pitting corrosion rates
- Limit the use of heavy metals including silver, copper, etc. that will increase the corrosion potential of nickel braze coatings on cold plates and heat exchangers

- Maintain surface tension of coolant from 70 – 78 dynes/cm for optimal gas trap performance
- Limit adverse impacts on wetted metallic materials including but not limited to the following materials:

  1. CRES 15-5 PH and 17-7 PH.
  2. Titanium 6-4.
  3. CRES 302.
  4. Hastelloy W weld material deposited on CRES 347.
  5. BNi2 braze material deposited on CRES 347 and Ni-201 to simulate a parting sheet – fin heat exchanger configuration. Processing includes a diffusion cycle to reduce the amount of lower melting intermetallics within the braze layer.
  6. BNi3 braze material deposited on CRES 347 and Ni-201 to simulate a parting sheet – fin heat exchanger, and cold plate configuration. Processing does not include a diffusion cycle to reduce the amount of lower melting intermetallic phases within the braze layer.
  7. BNi3 braze material deposited on CRES 347 with a Nioro (AMS 4787, BAu-4) repair to simulate a cold plate repair process.

- Limit adverse impacts on wetted nonmetallic materials including but not limited to the following materials:

  1. Nylon 11
  2. Nylon 66
  3. Polypropylene
  4. Valox® (polybutyleneterephthalate)
  5. Ethylene propylene rubber (EPR)
  6. Epoxy resins, amine cured

The objective was to utilize a matrix which would allow comparison of potential agents to select the 3 top candidate chemical/technologies to be tested at a later time. Data was gathered from manufacturers brochures, published literature, and websites to complete as much of the matrix as possible. We found that, for many agents, there was such a paucity of information available that there were many blank fields in the matrix. In addition, having a total 37 criteria made it impossible to gather information on every criterion for almost any agent. ***This led to the conclusion that selection could not be based on a ranking or scoring of alternative antimicrobial agents, as had been done previously (Wilson et al, 2003) because there was no way of assigning values for missing data.*** Instead, it was decided to evaluate potential agents in relation to the effects and effectiveness of candidate agents in the system, based on current

knowledge of bacterial growth and biofilm dynamics in relation to significant characteristics of the coolant system.

In recommending remedial actions, we believe that it is critical that the unique constraints of the IATCS aboard the ISS are taken into consideration. This system is located in a remote and isolated facility. Shipment of parts and materials is both difficult and costly, especially while the NASA Shuttle Orbiters are unavailable. The physical location and connections aboard ISS prevent major changes in the existing configuration. The system is affected by other crucial life support systems on board, including the air purification system which may, for example, affect levels of carbon dioxide. Leakage or spillage of antimicrobial agents or their products may lead to an extremely hazardous situation, even at concentrations much lower than those normally considered to be hazardous on earth. It would be difficult if not impossible to drain and discharge the present fluid in the system. Effects on system materials may cause irreparable or even catastrophic failure in relation to life support on board the ISS. Of particular concern is the toxicology of any particular agent in relation to crew health and safety. While an antimicrobial may be effective at concentrations higher than those permitted by NASA toxicological assessment, it would be imprudent to either exceed the toxicological recommendations to achieve a nominally effective dose rate, or to apply a suboptimal dose. With a lower than optimal dose of antimicrobial, there would be a significant potential for microorganisms to survive the treatment which could result in microbial growth and degradation of the agent, in addition to the possibility of selecting for strains that may resist the antimicrobial.

EVALUATION CRITERIA

The effects of both the microbes and the chemistry of the system, including additives, must be considered. Thus, the prime factors our group has focused on are:

- Significance of microorganisms now in the system.
- Antimicrobial effectiveness in relation to the bacteria likely to be present for both planktonic and attached as biofilms.
- Effects on pH of the coolant and microbes.
- Effects on assimilable organics (AOX) in the system.

Significance of Microorganisms in the System

There is little consensus on the significance of the numbers of microorganisms in the system at present. While the IATCS microbial populations fluctuate around 10^5 CFU/ml (equivalent to 10^7 CFU/100ml), similar systems on earth with 10^3-10^4 CFU/ml would most likely be treated. Clearly, the extreme consequences of biocorrosion or pitting, which may lead to breakthrough of the refrigerant and subsequent system failure requires a conservative approach. However, if

appropriate constituents and variables are monitored and remain stable, we recommend no antimicrobial treatment until the most solution has been identified following complete assessment. It is possible that if microbial numbers do not increase from existing levels or if they can be lowered by the least invasive treatments, there may be no further increase in long-term risks. Recommendations on monitoring and microbial control are provided below. While planktonic population numbers may not directly reflect surface colonization by biofilms, if there is no clear evidence of consequences typical of biofouling, e.g. lower heat transfer efficiency, increased flow resistance, or excessive release of corrosion products, it is likely that biofilm growth is limited.

The identification of microorganisms in the two cooling loops suggest that there is a large proportion of hydrogen oxidizers, including some *Acidovorax* spp., *Comamonas* sp., and *Ralstonia* spp., which in total comprise 63% in the low temperature loop and 60% in the moderate temperature loop. The chemolithotrophic hydrogen-oxidizing bacteria can reduce oxygen with hydrogen, forming water, as a source of energy. Almost all of the hydrogen-oxidizers in the above genera are facultative chemolithotrophs, being able to grow on organic compounds. Many exhibit autotrophic growth on CO_2. Thus, the influx of CO_2 may have provided a selective environment for these bacteria, leading to their proliferation in the system.

Acidovorax delafieldii, a prominent isolate from both cooling loops, was one of 5 bacterial species isolated most commonly from domestic copper plumbing systems (Critchley et al, 2003). For this organism, cuprosolvency was related positively with chloride concentration and negatively with pH and Langeliers Index (a measure of aggressivity) of a variety of drinking water sources. Carbohydrate concentrations in biofilms of *A. delafieldii* were positively correlated ($p<0.001$) with cuprosolvency which was attributed to corrosive exopolysaccharides produced by the organism; it may also have been related to the rate of metabolism of the organism. This is the only report of a corrosion study using a hydrogen oxidizer that was found in an on-line literature search. The study demonstrates that these bacteria may be involved in metal corrosion, at least with copper.

Some of the hydrogen-oxidizing bacteria found in the IATCS coolant samples, including *R. eutropha*, may have nickel-dependent enzymes (Mulrooney and Hausinger, 2003). Micro-organisms with these enzymes are capable of sensing cellular nickel ion concentrations and taking up this nutrient. *R. eutropha* has a single gene responsible for Ni-specific transport. Among currently known nickel-dependent enzymes, those found in *R. eutropha* include a regulatory hydrogenase. A requirement for nickel in these organisms may be related to the coupling of nickel uptake with energy metabolism. This would lead to their selective growth in a system containing nickel.

Many of the species identified in IATCS samples, and probably some unidentified Gram-negative bacilli, fall into the former pseudomonas group of bacteria. Members of this group are well-known for their ability to form biofilms, in addition to having the potential to grow in a wide range of conditions with the ability to utilize a wide range of organic substrates. Thus, there is a high possibility of degradation of organic compounds, even antimicrobial agents or their breakdown products.

Culture-based methods used to grow planktonic bacteria do not necessarily (and probably do not) identify all of the microbes that live in the system as biofilms. However, the available data do give some indication of the predominant species that colonize the system.

Antimicrobial Efficacy

Most antimicrobial agents can be effective if they can come in contact with susceptible bacteria at sufficient concentration for a suitable length of time. Thus, for disinfection of water and other media, a relationship between concentration and time (Cxt) is developed under the conditions of use. In the case of a recirculating system, the stability of the active agent and the potential for repeated dosing determine the contact time. A number of factors may limit the concentration of an antimicrobial agent that can be applied, including material and chemical compatibility, and toxicological issues related to exposure risks to humans and other organisms.

It is well known that bacteria in biofilms are less susceptible to killing than their planktonic counterparts, for a number of reasons. The current theories include differences in physiological characteristics of biofilm cells, in addition to diffusion limitation within the biofilm matrix. The resistance of biofilms to antimicrobial agents is now attributed as much to physiological factors as to restricted antimicrobial penetration (Drenkard, 2003; Mah and O'Toole, 2001). Drenkard concluded:

> "During the early stages of biofilm development, changes in gene expression induced by surface attachment lead to the emergence of a biofilm-specific phenotype that potentially increases biofilm resistance. Later on, the production of the exopolysaccharide matrix contributes to increasing cell survival by delaying antimicrobial penetration. As biofilms mature, the increase in cell density creates gradients of nutrient and oxygen availability leading to a reduction in metabolic activity and growth rate. Furthermore, the increase in cell density also leads to the activation of quorum-sensing systems. On the other hand, nutrient starvation and oxygen limitation induce the general stress response and up-regulation of efflux pumps. Finally, environmental conditions present in the biofilm also induce or select for phenotypic/persister variants resistant to high concentrations of antimicrobials."

Many bacteria have intrinsic resistance mechanisms, such as the widespread occurrence of catalase which inactivates hydrogen peroxide. Thus, while peroxide may not penetrate a thick biofilm which produces catalase (Cochran et al, 2000), penetration is possible in a thick biofilm of *P. aeruginosa* that lacked a major catalase gene (Stewart et al, 2000). This exemplifies the potential effectiveness of combining antimicrobial agents to achieve a synergistic effect, for example if catalase can be inactivated to some extent by a treatment with a metal such as silver, the effects of oxidizing agents like peroxide or halogens may be significantly enhanced. Taking this approach, it may be possible to use antimicrobial agents at lower than the usual effective concentration which then brings them into the range of compatibility with system materials and toxicological requirements. Ultimately, biofilm eradication (or control) is dependent on the most resistant component of the population being treated (Gilbert et al, 2002).

Coolant pH

The IATCS was designed to have a coolant pH of 9.5±0.5. It appears that in the early stages of its operation, carbon dioxide penetrated the system through Teflon flexible hoses, causing the pH to fall and settle at 8.4. This was accompanied by growth of microbial populations which are now established at ca. 10^7 CFU/100ml (10^5 CFU/ml). While the coolant was chemically buffered, this was not sufficient to prevent development of the lower pH. In our opinion, it is of great importance that any agent added to the system should not cause any further lowering of the coolant pH. Thus, antimicrobial agents such as iodophors which contain strong acids are considered inappropriate for use in this system.

Recent reports (Critchley, 2003; Veazey, 2004) suggest that a high pH may be a key factor in controlling microbial influenced corrosion of copper. The original establishment of a high coolant pH was most likely based on similar considerations for nickel and other metals in the IATCS. Veazey quoted Marc Edwards (Virginia Tech), who said that limited bacterial activity was achieved in drinking water with a relatively high pH of 9.2, continuous chlorination, and the omission of any added organic matter, NH_3, or phosphate, although extreme pitting was reported. It was suggested that some non-microbial corrosion occurs at high biocide (in that case chlorine) levels, although MIC occurs in systems with inadequate water treatment. Carbon dioxide contributes to copper pitting in cold water. Therefore it is considered important that the coolant system pH should be brought back up towards the original target, in addition to prevention of CO_2 entering the system.

While phosphate was added to the coolant primarily as a corrosion inhibitor, it has shown little effectiveness (Holt, 2004). It has been progressively lost from the system since late 2002, since it combines with nickel and precipitates out of solution as nickel phosphate. In our opinion, it would be inadvisable to add more phosphate because it may not only tend to decrease pH but

also serve as a nutrient for bacterial growth. Phosphate is often a limiting factor for microbial growth in engineered systems, so phosphate levels may have contributed to microbial growth in the system.

Effects of Assimilable Organics (AOX)

Concentrations of Total Organic Carbon (TOC) in the system have consistently been above the specification limit of 5 ppm. Concentrations were stable at <25 ppm until early 2003. Even at the lower end of these concentrations, significant microbial growth is possible. A significant concern would be the addition of any antimicrobial agent that was not totally effective against all the bacteria in the system, including the most resistant biofilms. The hydrogen oxidizing bacteria, in addition to others likely to be present in the system, are well-known for their abilities to degrade complex organic compounds. Many antimicrobials break down naturally when mixed with water, forming less complex compounds that may have a lower antimicrobial activity in addition to being more prone to microbial degradation. These organisms would then not only derive energy from these compounds for growth, but also release assimilable organics which would serve as energy/growth sources for other surviving bacteria. We are not aware of any anti-microbial agent that can provide guaranteed killing of all bacteria in a system like this. Therefore, we submit that it would be extremely risky to add an organically-based antimicrobial agent without extensive ground testing at lower than pre-determined additive concentrations. Within a complex system such as the IATCS, it is inevitable that there will be dead-ends, pockets and crevices where the calculated antimicrobial concentration does not penetrate.

Glutaraldehyde and other organically-based antimicrobial agents may be subject to the phenomena described above. While glutaraldehyde may appear to be compatible and effective in similar systems, it may contribute to assimilable organic carbon, since the maximum use concentration dictated by toxicological concerns is 25 ppm (Perry, 2004), and this concentration is unlikely to be effective against bacteria in the system. Although the biocide minimum inhibitory concentration in IATCS coolant was determined to be 25 ppm (Wilson et al, 2003), evidence from the literature and experts suggest that it is highly unlikely that concentrations less than 100 ppm will achieve microbial control in this system.

RECOMMENDATIONS

The rationale for these recommendations is:

- It is critical that whatever is added to the system, assimilable organic carbon compounds (AOX) concentrations will not be increased either from chemical breakdown of the agent or by microbial degradation.

- Agents containing corrosive compounds like chlorine should be avoided, in addition to those that may contribute chloride ions.
- Agents containing phosphorous should be avoided because they may contribute to lowering the pH of the system in addition to stimulating microbial growth.
- If enhanced or synergistic activity of candidate agents can be achieved, it may be possible to use lower than usual concentrations and thus avoid serious effects on corrosion and pH.

Given the above factors, the majority of commercially-available antimicrobial agents were eliminated as candidate alternative biocides. On this basis, the following actions and antimicrobials are recommended:

System Restoration and Monitoring

Efforts should be made to return the system to within the original target pH of 9.5±0.5. This may require control of CO_2 input by reducing cabin CO_2 concentrations, wrapping gas-permeable Teflon flex hoses with a gas-impermeable material, or replacement of gas-permeable Teflon flex hoses with gas-impermeable hoses. Other possibilities for pH control include chemical additives, although care must be taken not to adversely influence other components such as chlorides which may stimulate corrosion. System monitoring over a period of time following any restoration event is critical, and it will assist in the interpretation of the results of remediation. For example, on-board monitoring of bacterial numbers following treatment during times when samples cannot be returned to the ground may provide extremely valuable results.

Monitoring should include microbial numbers and identification, pH, TOC, ammonia, nickel, phosphate, and filter and gas trap pressure, as has been done to date. In addition, thermal transfer efficiency and head-loss through the heat exchangers should be monitored. If possible, on-orbit monitoring of bacterial numbers should be conducted routinely, even if the results often exceed the upper detection level. Ultimately, comparison of results between ground-based and on-orbit measurements may prove significant, particularly in relation to monitoring the effects of new treatments.

While there is evidence that some bacteria, when growing as biofilms, may reduce or limit corrosion (Jayaraman et al, 1997a; Jayaraman et al, 1997b), this phenomenon should not be relied on because it is not known if bacteria such as *Pseudomonas mendocina* or *P. fragi* and others that may promote corrosion control are present in the system. In addition, the conditions for corrosion limitation by microorganisms may not be met in the IATCS.

System Remediation including Antimicrobial Treatment

This must be done with extreme care and caution, because it is possible that the effects of an antimicrobial agent on this closed system could themselves be catastrophic or at least enhance the risk of future hazards. For example, if a compound or its by-products build up in the system, this may ultimately stimulate further microbial growth or enhance corrosion or degradation of system components. Ongoing review of analytical results from the system, in addition to ground-based experiments in systems that simulate the IATCS as closely as possible are required.

Hydrogen Peroxide

This is an aggressive disinfectant that does not contribute any ions or nutrients to the system, and it degrades in time to H_2O and O_2. It can be used on membrane filters that do not tolerate chlorine. While relatively high concentrations have been recommended, it can be catalyzed by metals including iron, copper and silver (see below). Hydrogen peroxide is often used in combination with peracetic acid, but this may be inadvisable in this case because of potential effects on system pH, and also because peracetic acid is an organic compound which may contribute to AOX in the system.

Srinivasan and Eden (2004) showed that 1% (10,000 ppm) hydrogen peroxide caused substantial corrosion of Nedox (electro-less nickel) in an 8.4 pH Node 2 coolant fluid, and a further substantive increase in corrosion rate was observed with 3% (30,000 ppm) hydrogen peroxide. These concentrations are much greater than the 50 ppm minimum biocidal inhibitory concentration in IATCS coolant demonstrated earlier (Wilson et al, 2003). Corrosion experiments should be repeated at much lower concentrations of hydrogen peroxide to determine the effects of the most likely concentrations that would be used to treat the system.

Iodine

Iodine can be delivered from iodinated resins that release the halogen on demand. These resins were developed over 30 years ago (Taylor et al, 1970). The resins can be prepared to deliver I_2 concentrations up to around 30 ppm (Lambert et al, 1980). Iodine treatment later adopted for use on NASA spacecraft because the resins could be housed in an in-line delivery system called a Microbial Check Valve (MCV) (Atwater, 1996).

Dissolved iodine can be delivered from concentrated solutions containing appropriate concentrations of iodine and potassium iodide to ensure the presence of sufficient concentrations of the germicidally-active forms I_2, hypoidous acid (HOI) (Gottardi, 2001). Microbial control using iodine can be achieved in the pH range of 3-9 when using the appropriate formulation (Gottardi, 2001), although Richter and Cords (2001) suggest a pH range of 2-6 for iodine in

commercial sanitizers and disinfectants. The references cited in a footnote indicate that they are referring to iodophors which are typically used in the acidic range. Based on Gottardi's data, effective microbial control should be achieved at the higher pH levels of the IATCS.

While some microbes may be less-susceptible to iodine than others, studies have shown that bacteria similar to the majority colonizing the IATCS can be successfully treated with iodine, depending on the organism, growth conditions (Pyle and McFeters, 1989), and biofilm formation (Pyle and McFeters, 1990a). Although regrowth of survivors following iodination is possible (Pyle and McFeters, 1990b), it should be noted that low iodine concentrations and short treatment times were used in those studies.

Silver

This metal can be delivered either by ionization from an electrode (CAVion) or from ions bound to a ceramic carrier (Agion). Low concentrations, even in the ppb range, may be effective. Combination with oxidizing agents such as peroxide or iodine may result in strong synergistic activity, allowing for much lower than usual concentrations of these antimicrobials. Electrodes are available with either silver alone or a copper/silver alloy; the former may be preferable to avoid deposition of copper. While there may be some concerns regarding the possibility that silver will plate out on surfaces, there is little likelihood that this would have deleterious effects, apart from loss of silver from solution which could be replenished by continuous generation of silver ions. CRES 347 coupons with an alumina-based coating containing 10% (w/w) AgO as a hydrophilic antimicrobial demonstrated effective microbial control in accelerated tests with the equivalent of 10 years flowing water (Pickup and Zhou, 1998). Silver concentrations in condensate runoff were constantly around 50 ppb, which was greater than the 20 ppb found to provide effective microbial control. These metals are effective at higher pH values.

Silver nitrate and silver sulfadiazine in topical preparations have been used to treat conjunctivitis of the newborn and burn wounds (Weber and Rutala, 2001). An antimicrobial coating which combines an immobilized polymeric biocide with an insoluble silver salt releases antimicrobial silver on demand (Weber and Rutala, 2001). This preparation, which may be used for environmental surfaces and the skin, exemplifies the enhanced effects of combining biocides, as suggested by Pickup and Zhou (1998) for heat exchangers.

Hydrogen Peroxide – Silver

A formulation of this combination is commercially available, with recommended dosages of 0.5 ppm hydrogen peroxide and 10-20 ppb silver (Accepta 8101). This combination has been shown to be much more effective than H_2O_2 alone. It is possible that this combination is more effective

because microbial enzymes, possibly including catalases, are inhibited or inactivated by the metal, allowing a more effective dose of peroxide to reach targets on or in the cells.

Iodine – Silver

The synergistic effects of chlorine with low concentrations of metals such as copper and silver have been demonstrated (Yahya et al, 1990). Similar effects were observed with iodine, introduced as an iodine/potassium iodide solution at 200-500 ppb in the presence of 100-400 ppb copper and 11-44 ppm silver (Pyle et al, 1992). While the addition of copper to the IATCS is not recommended, it is very likely that similar synergistic effects would be found with a combination of iodine and silver at appropriate concentrations. It may be important that the electrolytic generation of silver ions, rather than chemical addition is used. Since the use of a MCV to deliver iodine is possible, this combination may prove to be both effective and relatively easy to install and operate onboard the ISS. We recommend a combination of iodine at around 1-10 ppm in combination with silver at 10-20 ppb. The issue of potential co-precipitation would have to be taken into account. We found no evidence of this in our experiments which were performed with relatively low concentrations of iodine and silver. As noted above, there may be a difference between the addition of a silver salt as opposed to generation of ionized silver from an electrode. In addition, an iodine solution similar to the one we used, rather than iodine released from resins in the MCV may be less likely to co-precipitate.

OVERALL RECOMMENDATIONS

In our opinion, the most prudent solution is to continue to gather relevant information from onboard IATCS data, analyze onboard samples returned to earth, and further ground-based experiments on antimicrobial efficacy and material compatibility of the alternatives we are proposing, prior to selecting any antimicrobial agent to treat the system.

The criteria for selection should be based, in order of priority on:

a. Toxicological assessment to determine upper limits for application of candidate antimicrobial agents.

b. Assessment of material and chemical compatibility within the toxicological limits.

c. Determination of antimicrobial efficacy within the toxicological limits.

If two agents can be used together at concentrations within the toxicological limits and levels for material and chemical compatibility for each, it may be possible to achieve antimicrobial efficacy even though either or both of the same two agents alone may not be effective at those concentrations. Tests for efficacy should be performed in ground-based model systems with

similar characteristics and degree of microbial colonization as those in the IATCS aboard the ISS.

There are several factors to be considered in terms of the urgency of a need to take remedial action in the form of biocide addition. One is the potential failure of a critical life support system due to biofouling, corrosion, reduced heat transfer efficiency, increased flow resistance across the cooling units, and blockage of filters and other components. While filters can be replaced, blockage of the narrow lumen of the heat exchangers by extensive biofilm sloughing could lead to major failure of the system. The other is the potential risk of exposing the crew to a potentially toxic concentration of an antimicrobial agent or its breakdown products. While breakthrough of refrigerant may be indicated by increased ammonia concentrations, microbial metabolism of ammonia may mask this. On the other hand, microbial ammonification may give a false indication of refrigerant breakthrough. It is recognized that balancing these risks is extremely difficult.

Criteria for system control may need to be revised. Although the present levels of IATCS biofouling may not be acceptable, it is quite likely that bringing the planktonic counts down to $10^2 – 10^3$ CFU/ml by the least hazardous means would reduce risks of system failure. The use of an antimicrobial agent or combination of agents at acceptable moderate, effective concentrations, may also achieve this goal. Whether or not it is advisable to change the pH requirement is another question. If it was possible to return the pH to closer to its original target value, significant remediation of the system may occur, permitting continued operation without the addition of any potentially harmful or controversial antimicrobial agent.

We are aware that this is a very complex situation, and that there are many engineering, chemical, physical, microbiological and other constraints to be taken into account in developing a treatment plan. Since the return of samples and components to earth is difficult and sporadic, on-board monitoring including microbiological analysis may need to be increased. Ground testing, including material compatibility and corrosion assessment should be performed at antimicrobial concentrations close to those likely to be used on board if the candidate antimicrobial is selected. There is no doubt that safety margins should be included in the plan, but we believe that the less invasive approaches may be safe and effective.

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Note: Copies of all articles cited and data appendices are attached to the printed report.

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Appendix C. MSFC Glutaraldehyde Toxicity Assessment

National Aeronautics and
Space Administration
George C. Marshall Space Flight Center
Marshall Space Flight Center, AL 35812

June 9, 2004

Reply to
Attn of: FD21(04-086)

TO: ED25/J. M. Holt

FROM: FD21/J. L. Perry

SUBJECT: Compatibility of a Candidate Internal Thermal Control System Biocide With the *International Space Station's* Environmental Control and Life Support System

At the request of the Internal Active Thermal Control System (IATCS) problem resolution team, an engineering assessment has been conducted to fully understand the Environmental Control and Life Support (ECLS) system-related impacts associated with changing the IATCS biocidal additive from silver to glutaraldehyde. A narrative report documenting this assessment is attached. The assessment was conducted according to standard practice for assessing the environmental impacts of payloads and within the bounds set by *International Space Station* (ISS) Program specifications for trace contaminant control.

Because the specification of the active trace contaminant control equipment for any spacecraft precedes those data necessary to fully validate its design, standard design practice dictates an approach whereby the active contamination control system performs its function unassisted by any other systems or processes in the cabin. This means that overboard atmospheric leakage and assists provided by other air processing systems such as CO_2 removal and humidity control equipment are not considered during the design and validation of the active trace contaminant control equipment. To maintain consistency, all new contamination loads are assessed in the same manner.

Within the context of ISS Program requirements, an additional loading of a chemical compound not contained in the design listing provided in SSP-41000Y, SSP-41162AN, or S683-29523P

constitutes a new, specific verification case. Therefore, this verification is constrained to consider only the active contamination control systems on board the *ISS*, unassisted by other serendipitous removal, for maintaining the added contamination load below individual compound spacecraft maximum allowable concentrations (SMACs). This maintains consistency with the active contamination control equipment's certification.

Specific findings from the detailed evaluation of glutaraldehyde as a candidate biocidal additive to the IATCS working fluid relating directly to contamination control equipment certification are the following:

1. Evaporation rates from concentrated aqueous solutions of glutaraldehyde are such that appropriate containment and personal protective equipment must be used when injecting the solution into the IATCS.

2. Basic, unassisted trace contaminant control capability as defined by *ISS* Program specification cannot accommodate the range of IATCS leakage rates for any glutaraldehyde concentration in the IATCS fluid. Therefore, the *ISS* active contamination control systems cannot be certified to control glutaraldehyde emissions into the cabin within the range of IATCS leakage specification.

Additional effort was undertaken by expanding the assessment's scope to address the fate of glutaraldehyde within the *ISS* cabin environment to address and understand the impact on all ECLS system processes—both atmospheric and water processing. This expansion considers an assist to the basic contamination control equipment provided via absorption by humidity condensate and the operation of contamination control equipment in the Russian On-orbit Segment (ROS). Contamination control system failure scenarios are also considered. Findings from the expanded evaluation are the following:

1. The combined ECLS trace contaminant control and water processing systems cannot be certified for IATCS fluid concentrations >25 mg/liter glutaraldehyde. If no other suitable additive can be found, however, glutaraldehyde concentrations <25 mg/liter may be used, based on the IATCS fluid leakage specification, to ensure long-term hazards to human health and ECLS system air quality control and water processing equipment are acceptable.

2. Any decision by the *ISS* Program to use glutaraldehyde as a biocidal additive to the IATCS fluid in the USOS must be reviewed by the International Partners within the Common Environments Team forum. This is necessary because fugitive emissions from the IATCS effect the common cabin environment and require removal by contamination control equipment on board the ROS to ensure acceptable cabin air quality is maintained.

Overall, measures must be taken to minimize the risk to human health and maintaining the ISS's cabin air quality as well as protecting the water processing systems. Although the active contamination control systems have proven themselves reliable, they are designed specifically to control the contamination loading from equipment off gassing and human metabolic processes alone. They are not designed to serve as a hazard control for chronic or acute chemical releases into the cabin. It should be noted that cabin air quality monitoring techniques employed by the *ISS* Program are not sensitive enough to monitor glutaraldehyde's concentration at or below the 180-day SMAC. Therefore, it is not possible to verify cabin air quality maintenance via existing monitoring techniques.

Based on the *ISS* ECLS engineering evaluation, it is found that the overall challenges and risks associated with using glutaraldehyde as a biocidal additive are significant and present long-term operational issues to the *ISS* Program if implemented. Therefore, it is recommended that other candidate biocidal additives be evaluated and a suitable alternative to glutaraldehyde selected. If no suitable alternative can be found, it is recommended that the existing silver additive or glutaraldehyde at concentrations <25 mg/liter be used on a periodic basis. Further, if glutaraldehyde is ultimately selected, its use must be reviewed and approved by the International Partners within the Common Environments forum.

Please contact me at 544-2730 concerning details of this assessment.

/original signed/
Jay L. Perry
Senior Engineer
ISS Air Quality Control Systems
Environmental Control and Life Support Group

Attachment
cc:
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NASA Engineering Analysis Report

Compatibility of a Candidate Internal Thermal Control System Biocide with the *International Space Station's* Environmental Control and Life Support System

J. L. Perry

George C. Marshall Space Flight Center
Flight Projects Directorate

June 2004

NASA ENGINEERING ANALYSIS

COMPATIBILITY OF A CANDIDATE INTERNAL THERMAL CONTROL SYSTEM BIOCIDE WITH THE *INTERNATIONAL SPACE STATION'S* ENVIRONMENTAL CONTROL AND LIFE SUPPORT SYSTEM

BACKGROUND

The *International Space Station's* (*ISS*) active thermal control system (ATCS) presently uses silver as a biocidal additive in the internal water working fluid. The silver concentration in the fluid declines within a few days as silver deposits upon metal surfaces, but microbial control is maintained by the specified 9.5 pH. Samples returned from flight have indicated that the internal ATCS fluid chemistry is affected by the on-orbit environment. Decreased pH and other changes have been traced to CO_2 permeation through the Teflon® flex hoses. Due to the combination of lower pH and lower biocidal additive concentration in the fluid concerns exist that microbially-induced corrosion (MIC) rates for internal ATCS wetted components may have increased, particularly for heat exchangers and cold plates.

The concern about MIC has led to a search for an alternative biocidal additive. Beyond periodically injecting more silver biocidal additive, hydrogen peroxide and glutaraldehyde are being considered as candidates.[1, 2] Material compatibility testing for glutaraldehyde has been completed while more work is pending for hydrogen peroxide. Since work to evaluate glutaraldehyde's suitability has reached a more advanced stage, a change request, SSCN 008447, was prepared that sought to implement glutaraldehyde on board the *ISS* U.S. On-orbit Segment (USOS).

One supporting basis for proceeding with the change request was an assessment of glutaraldehyde's toxicity hazard rating that stated that environmental control and life support (ECLS) system "charcoal filters should efficiently remove" glutaraldehyde vapors.[3] While a correct statement, it was not quantified and does not address the overall capability to control glutaraldehyde's concentration to below its 180-day spacecraft maximum allowable concentration (SMAC) of 0.002 mg/m^3. This SMAC is the lowest documented in JSC 20584. Chemical compounds with a very low SMAC are typically difficult for the ECLS system to control if persistent generation sources exist because the total effective flow rate through the contamination control equipment is limited. That is, active contamination control equipment on board the *ISS* is accomplished using fixed flow devices. The primary means for maintaining cabin concentration below the SMAC in such cases then becomes source control. With this in mind, an engineering assessment has been conducted to address the ECLS system's capability to accommodate routes by which glutaraldehyde can enter the cabin environment if it is employed as a biocidal additive to the internal ATCS working fluid.

Spacecraft Trace Contaminant Control Design Practice

Designing for spacecraft cabin trace contaminant control requires substantial design activity within the confines of the air quality standard. In the case of crewed spacecraft, that standard is the SMAC. Materials selection and control, hardware design, manufacturing processes, chemical process design, mission characteristics as well as crew size and activities are only a few of elements that must occur within the constraints of the air quality standards. A change to any of these, as is the case of a change in a thermal control system working fluid from a nonvolatile, inorganic silver ion biocidal additive to a semi-volatile, organic additive, can have an impact upon cabin atmospheric quality, to the ECLS system equipment, or both. A complete assessment by ECLS engineering is required when

such changes are proposed to ensure any potential impacts to the cabin environment, as well as the ECLS system equipment, are negligible.

Because the specification of the active trace contaminant control equipment for a spacecraft precedes those data necessary to fully validate its design, standard design practice dictates a conservative approach whereby the active contamination control system performs its function unassisted by any other systems or processes in the cabin.[4] This means that overboard atmospheric leakage and assists provided by other air processing systems such as CO_2 removal and humidity control equipment are not considered during the design and validation of the active trace contaminant control equipment. To maintain consistency, all new contamination loads are assessed in the same manner.

For the *ISS*, the key design requirements pertaining to trace contaminant control design and performance are found in the *ISS* System Specification (SSP-41000Y), the USOS Specification (SSP-41162AN), and the U.S. Laboratory Prime-Item Development Specification or PIDS (S683-29523P). In summary, these requirements state that trace contaminants shall be controlled to less than their respective SMAC for a normal equipment offgassing and crew metabolic load. More specifically, the U.S. Laboratory PIDS requires that the trace contaminant control subassembly (TCCS) maintain trace atmospheric component concentration from normal equipment offgassing and crew metabolic processes to less than 90% of individual contaminant SMACs.[5, 6, 7] These design specifications are for the active contamination control systems operating without assistance from other ECLS processes or overboard leakage. It is also important to note that they do not specify that the active contamination control systems on board the *ISS* must be designed to accommodate chronic, fugitive leaks from other systems or payloads nor do they specify that these systems' performance must be verified for such an additional contamination loading. Further, these requirements do not authorize using the active contamination control systems as hazard controls for other onboard systems or payloads.

Within the context of requirements, an additional loading of a chemical compound not contained in the design listing provided in SSP-41000Y, SSP-41162AN, or S683-29523P constitutes a new, specific verification case. As such, this verification must assume that only the active contamination control systems on board the *ISS* remove the added contamination load. This maintains consistency with the equipment's certification. It is informative to expand the assessment, however, to address the fate of the contamination to ensure that the impact upon all ECLS system processes—both atmospheric and water processing—are addressed.

APPROACH

Two basic assessments comprise the evaluation of glutaraldehyde's compatibility with the *ISS's* ECLS system. Concentrated aqueous solutions will be injected into the internal ATCS if glutaraldehyde's use as an alternative biocidal additive is implemented. Therefore, the first is an assessment of a bulk release of candidate stock solutions containing either 5% or 50% glutaraldehyde by mass. An additional subset of the first assessment is a case that considers a bulk release of 0.025% aqueous solution is considered as a gross leak from an internal ATCS failure. Second, is an assessment of the *ISS* ECLS system's capability to handle chronic, fugitive leaks from the internal ATCS for various concentrations of glutaraldehyde. This second assessment considers the ability of the ECLS atmospheric quality control equipment to accommodate chronic emissions from a range of internal ATCS leakage rates and glutaraldehyde concentrations. Appropriate equations and calculation techniques are developed to address these assessment cases.

Evaporation Rate

Estimating evaporation rate from a gross leak of stock solution or internal ATCS working fluid is accomplished using calculation techniques documented in the literature and employed by the U.S. Environmental Protection Agency (EPA) for assessing environmental impacts of chemical spills. Two equations are employed for calculating evaporation rate and the average result used for the purposes of this assessment. These equations require information on air velocity, vapor pressure, molecular weight, and leaked surface area. Equation 1 calculates the evaporation rate, q, in kg/s.[8]

$$q \cong (5.23\times10^{-9})U_S^{0.78}P_V M_W^{0.67}A_P^{0.94} \quad (1)$$

In Equation 1, U_S is air velocity in m/s, P_V is vapor pressure in N/m^2, M_W is molecular weight in g/mole, and A_P is leaked pool surface area in m^2. Similarly, Equation 2 estimates evaporation rate, QR, in lb/minute.[9] In Equation 2, M is molecular weight in g/mole, A is the leaked pool surface area in ft^2, T is absolute temperature in Kelvin, P_V is vapor pressure in mm Hg, and u is air velocity in m/s.

$$QR = \frac{0.284u^{0.78}M^{2/3}AP_V}{82.05T} \quad (2)$$

Both Equations 1 and 2 are used to estimate the evaporation rate from a leaked volume of a fluid with the average result from the two equations serving as the final estimate.

Cabin Mass Balance

Assessing the capability of the atmospheric quality control systems on board the *ISS* to effectively control glutaraldehyde concentration in the cabin as a result of fugitive emissions to below specified limits requires two stages. The first assumes the entire *ISS* cabin is a well-mixed volume and that the effective removal term, $\Sigma\eta v$, remains constant with time. This makes the solution of the basic mass balance equation, shown by Equation 3, fairly simple. The solved form of the equation is shown by Equation 4. Reference 10 documents the derivation of Equation 4. In Equations 3 and 4, m is the contaminant mass at time, t; m_o is the contaminant mass at time equal to zero; V is cabin volume; $\Sigma\eta v$ is the contaminant removal capacity; g is the contaminant generation rate; and t is time.

$$\frac{dm}{dt} = g - \left(\frac{\sum \eta v}{V}\right)m \quad (3)$$

$$m = m_o e^{-\left(\sum \eta v / V\right) t} + \left(gV / \sum \eta v\right)\left[1 - e^{-\left(\sum \eta v / V\right) t}\right] \quad (4)$$

The second stage assumes that in the case of a fugitive emission, conditions approach those of a steady state. At steady state conditions, Equation 4 reduces to a very simple form involving only the generation rate, cabin volume, and effective removal terms as shown by Equation 5.

$$m = gV / \sum \eta v \quad (5)$$

The second stage requires conducting a more rigorous mass balance on both the USOS and ROS to examine the effects of either the loss of ventilation flow between the USOS and ROS or the failure of active contamination control systems in either segment. As well, this assessment will provide a more detailed insight of the effects upon humidity condensate loading. This more rigorous mass balance requires the simultaneous solution of the mass balance equations for each individual segment. The mass balance equations for the USOS and ROS are provided by Equations 6 and 7, respectively. These equations define the change in contaminant mass as a function of time.

$$\frac{dm_U}{dt} = \frac{\dot{v}_R}{V_R} m_R - \frac{\dot{v}_U}{V_U} m_U - \frac{\sum \eta v}{V_U} m_U + g_U \quad (6)$$

$$\frac{dm_R}{dt} = \frac{\dot{v}_U}{V_U} m_U - \frac{\dot{v}_R}{V_R} m_R - \frac{\sum \eta v}{V_R} m_R + g_R \quad (7)$$

In Equations 6 and 7, m_U is the total mass of contaminant in the USOS, m_R is the total mass of the contaminant in the ROS, V_U is the USOS free volume, V_R is the ROS free volume, $\dot{v}_U$ is the intermodule ventilation flow from the USOS to ROS, $\dot{v}_R$ is the intermodule ventilation flow from the ROS to USOS, $\Sigma \eta v$ is the removal capacity in the respective segment, g_U is the generation rate in the USOS, and g_R is the generation rate in the ROS.

Simultaneous solution of Equations 6 and 7 provide an equation for each segment in the form of Equation 8. Details concerning the solution are provided in Appendix A. In Equation 8, m is the total mass of contaminant in the reference cabin volume; α, β, and γ are constants calculated from the segment cabin free volume, ventilation flow, removal capacity, and contaminant generation rate; and x_2 and x_3 are constants. The integration constants are calculated from the segment free volume, ventilation flow, and removal capacity parameters. Concentration is calculated by simply dividing the contaminant mass by the segment free volume.

$$m = \alpha + \beta e^{x_2 t} + \gamma e^{x_3 t} \quad (8)$$

If the entire cabin volume is assumed to be well mixed, or each segment is isolated, the total cabin mass balance equation can be defined more simply as Equation 4.

Cases Considered

Cases considered include several scenarios involving substantial leaks of stock solution as well as a range of fugitive emissions encompassing the range of leakage from the internal ATCS by specification. Effects upon the ability to maintain cabin air quality for the specified range of internal ATCS fluid leakage presented by normal operation of the trace contaminant control equipment on board the *ISS* and failure scenarios of this equipment are also considered.

Evaporation Rate

Evaporation rates were evaluated from a 1-liter spill of 5% aqueous glutaraldehyde, 100 ml of 50% aqueous glutaraldehyde, and 3.8 liters of 0.025% glutaraldehyde. All cases were evaluated at 20° C. The last case was also evaluated at 4.4° C because that case represents leakage from the internal ATCS while operating and the fluid would initially be at a lower temperature before warming to the cabin temperature. In all cases, it is assumed that the spill takes the form of a sphere as the minimum energy shape.

Control of Fugitive Emissions

Initial screening was conducted using Equation 5 to understand the effects of not only internal ATCS fluid leakage rate but also the glutaraldehyde concentration and available active contamination control capacity upon cabin atmospheric quality. The assessment bounds the capability dictated by specification documents and also assists in evaluating the potential impacts upon water processing systems. The leakage rates and concentrations listed in Table 1 were investigated. In addition, leakage rates of 0.2 mg/h and 2.7 mg/h were investigated because actual fluid leaks of these magnitudes have been experienced. Additional details on internal ATCS fluid leakage specifications defined by the internal ATCS System Problem Resolution Team (SPRT) are provided by Appendix B.

The initial concept involved using 250 mg glutaraldehyde/liter; however, subsequent review focused upon either 100 mg glutaraldehyde/liter or 50 mg glutaraldehyde/liter in the internal ATCS fluid. These latter concentrations are the focus for cases that consider a more rigorous cabin mass balance based upon Equations 6 and 7. Using the appropriate numerical values for the system variables in the solved form of Equation 8 for the USOS and ROS, the effects of various leakage rates of internal ATCS fluid containing either 100 mg/liter or 50 mg/liter glutaraldehyde on cabin atmospheric quality and humidity condensate loading were assessed.

Table 1. Internal ATCS Leakage Rates and Candidate Biocide Concentrations Investigated

PARAMETER	MAGNITUDE					
Leakage Rate (ml/h)	0.16	1.6	3.9	4.8	5.3	14.7
Biocide Concentration (mg/liter)	25	50	100	150	200	250

Vehicle Configuration

Two vehicle configurations are considered—the configuration as of Flight 4R and the *ISS* assembly complete 6-person crew capability. Estimated total cabin free volume for the 4R configuration is 371 m^3 comprised of the USOS free volume of 190.4 m^3 and the ROS free volume of 180.6 m^3. The U.S. assembly compete configuration expands the USOS volume to include the Japanese Experiment Module, Columbus Module, Centrifuge Accommodation Module, Node 2, and Node 3. It is assumed

that the ROS volume will not change appreciably to accommodate the 6-crew capability; therefore, the total *ISS* free volume will increase to approximately 928 m^3 as a result of the USOS free volume increasing to approximately 747.4 m^3. The Flight 4R configuration cases consider the present crew size of 2 people while the *ISS* assembly complete 6-crew capability cases consider only a crew of 3. Using only a crew of 3 for the assembly complete case is considered a greater challenge to overall trace contaminant control because the crew latent load is smaller than for the 6-person crew size. It is anticipated that a checkout period during assembly complete will have a 3-person crew.

In both the Flight 4R and assembly complete configurations, the TCCS and BMP provide the active contamination control on board the *ISS*. During both *ISS* assembly stages, the TCCS and BMP operate in parallel with each other to maintain the cabin atmospheric quality. The TCCS removes glutaraldehyde at 100% efficiency in its charcoal bed assembly. If the charcoal bed assembly becomes saturated, then the TCCS will remove the glutaraldehyde via its catalytic oxidizer assembly. The flows through the charcoal bed assembly and catalytic oxidizer assembly are 15.3 m^3/h and 4.6 m^3/h, respectively. The BMP removes glutaraldehyde at 100% efficiency at 27 m^3/h flow. This performance is estimated based upon activated charcoal's capacity for glutaraldehyde. Net intermodule ventilation (IMV) flow between the ROS to the USOS is typically 180 m^3/h. No attempt is made to account for the effects of IMV flow short circuiting. The challenges presented by failures of the TCCS and BMP, either individually or at the same time, are considered.

Absorption by Humidity Condensate as a Removal Device

In addition to removal by the active contamination control equipment, water soluble contaminants are also removed by absorption in humidity condensate. As noted earlier, the assist provided to the active contamination control equipment on board the *ISS* is considered only to address potential impacts to water processing systems. Absorption via humidity condensate is not considered when evaluating the capability for the active control systems to accommodate a new contaminant loading.

The primary condensate removal for the Flight 4R configuration is provided by the SKV in the ROS. Typical flow rate through the heat exchanger core is 144 m^3/h. The condensate loading normally ranges between a 3-person and 2-person latent load depending upon the crew size. Removal efficiency via absorption by humidity condensate is 86% for a 2-person latent load and 91% for a 3-person latent load. The calculation technique for estimating condensate absorption efficiency is documented by References 11 through 13. An average latent load is defined as 1.4 liters/day/person.

For the *ISS* assembly complete 6-person crew capability, the most challenging case exists during the time when the crew is limited to 3 people. The combination of added internal ATCS fluid loops and limited trace contaminant control scrubbing capacity are most severe during this time. It is assumed for these cases that a 2-person latent load is removed by the SKV and a 1-person latent load is removed by a CCAA in the USOS. At this rate of humidity condensate collection, the single pass removal efficiency is approximately 55% for the CCAA. Removal efficiency for the SKV is 86% as noted previously.

It must be noted that deviations from ideal Henry's Law behavior, as reported by References 12 and 13 are not accounted for in this assessment because specific data on glutaraldehyde are not available. For this reason, this aspect of the assessment is not conservative.

RESULTS AND DISCUSSION

The following discussion presents and discusses results for estimated evaporation rates from stock solutions, basic control of cabin atmospheric quality under varying internal ATCS fluid leakage conditions, and effects upon humidity condensate loading. Guidelines are presented for maintaining 2-failure tolerance with respect to ECLS atmospheric quality and water quality control functions.

Evaporation from Bulk Leakage

Evaporation rates from 1-liter of 5% aqueous solution, 100-ml of 50% aqueous solution, and 3.8-liters of 0.025% aqueous solution were calculated using Equations 1 and 2. The elapsed time to reach the 180-day SMAC is also calculated assuming no removal during the period of release. This is a standard, conservative approach to evaluating the time to reach the 180-day SMAC.

For the first case, the calculated evaporation rate is 3.5 mg/h. At this rate, the time to reach the 180-day SMAC in the USOS is 6.6 minutes. If allowed to disperse throughout the entire *ISS* cabin, the 180-day SMAC is reached in 13 minutes. As expected, the second case shows that the more concentrated solution gives the crew less time to react. The calculated evaporation rate from the 100-ml release of 50% aqueous solution is 9.9 mg/h. At this rate, the 180-day SMAC can be reached in the USOS within 2.3 minutes and for the entire *ISS* cabin within 4.5 minutes. Evaporation from the dilute solution containing 0.025% glutaraldehyde is 0.054 mg/h. At this rate, the 180-SMAC is reached within 7 hours in the USOS and 14 hours for the entire *ISS*.

Based upon the evaluation of evaporation rate, appropriate containment is required for any operation that involves handling aqueous glutaraldehyde solutions in the cabin. Also, depending upon the prevailing glutaraldehyde concentration in the internal ATCS fluid, evaporation from fugitive emissions is considered to be a concern making the rapid detection and remediation of any leak highly important to maintaining the *ISS's* cabin air quality. Evaporation from a 3.8-liter release of fluid (0.01 mg/h) is equivalent to the amount of glutaraldehyde introduced into the *ISS* cabin by a continuous 0.2 ml/h leak. Leaks of approximately 0.2 ml/h and 2.7 ml/h have been experienced on board the *ISS*.

Control of Fugitive Emissions

The ability to maintain cabin air quality in the presence of fugitive emissions must first consider the available equipment for actively removing the contamination. Figures 1 and 2 illustrate the overall scrubbing flow required to accommodate a range of internal ATCS fluid leakage containing 50 mg/liter and 100 mg/liter glutaraldehyde. These glutaraldehyde concentrations are considered to be the most likely implemented if approved by the *ISS* Program. Leakage rates of 3.9 ml/h and 5.3 ml/h most likely can be sustained for about 1 month while deliberating the need to shut down an internal ATCS fluid loop. For these leakage rates, Figures 1 and 2 show that effective removal flow rate ranges of 95 – 130 m^3/h and 195 – 265 m^3/h are necessary to maintain the concentration in the cabin below the 180-day SMAC for 50 mg/liter and 100 mg/liter glutaraldehyde in the fluid. This is far greater than the 15.3 m^3/h provide by the TCCS alone. The BMP provides an additional 27 m^3/h and removal via absorption by humidity condensate can vary.

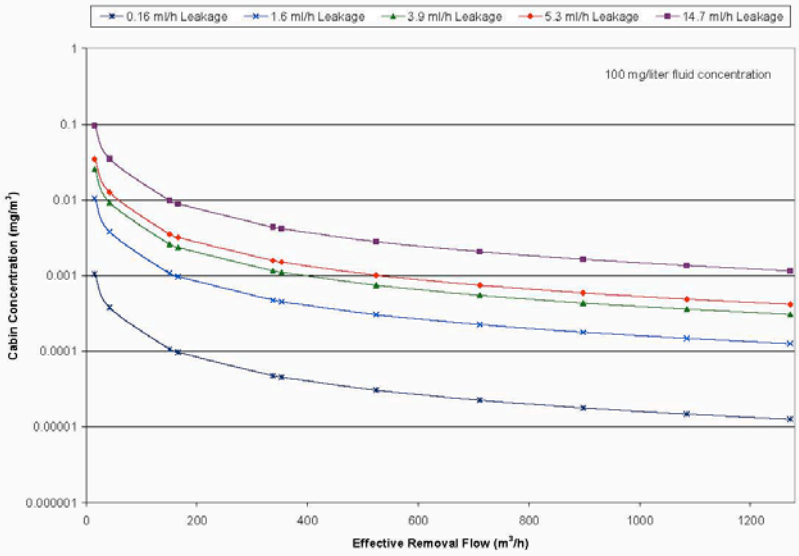

Figure 1. Effective Removal Flow to Maintain SMAC for 100 mg/liter Glutaraldehyde

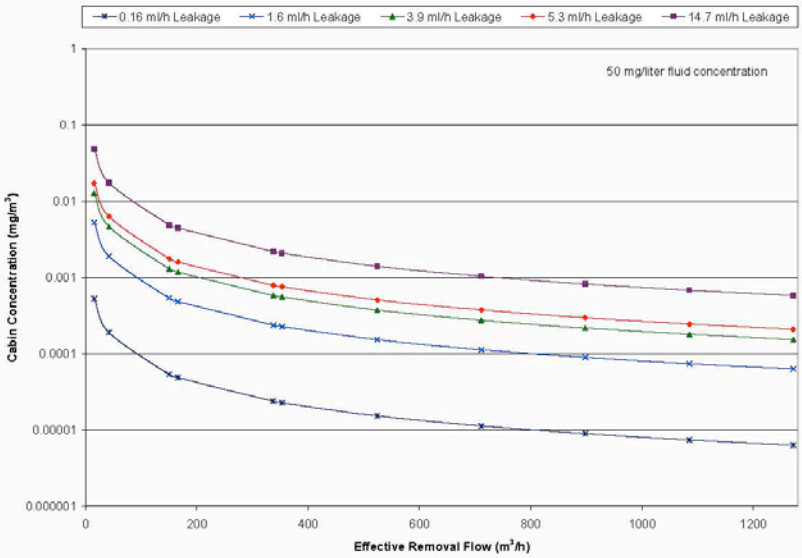

Figure 2. Effective Removal Flow to Maintain SMAC for 50 mg/liter Glutaraldehyde

Trace contaminant control for the *ISS* USOS was certified by engineering analysis using the constraint that the TCCS, with no assist from the Russian BMP or removal via absorption in humidity condensate, provides active control. Because any new contamination source represents an extension of the specified trace contaminant control design load, each new source is evaluated using the same criterion. This ensures that the same levels of safety apply for any known increase in the trace contaminant load. For information the assist provided to the TCCS by both the BMP and removal via absorption in humidity condensate are included. The additional cases allow the potential impact upon ECLS system water processing systems to be estimated; however, they do not serve as the primary basis for assessing trace contaminant control capacity for normal operations.

USOS TCCS Capability

A range of internal ATCS working fluid leakage rates and glutaraldehyde concentrations were evaluated. Figure 3 shows the steady state concentration that results when the TCCS provides the sole active removal. The TCCS, when operating alone, can provide effectively control for a glutaraldehyde source of no greater than 0.03 mg/h and still maintain the cabin concentration below the 180-day SMAC. This capability is equivalent to a sustained leakage from the internal ATCS up to 1.1 ml/h for 25mg/liter glutaraldehyde in the fluid. As the fluid's glutaraldehyde concentration increases, the magnitude of the sustained leak accommodated by the TCCS decreases to as low as 0.11 ml/h for 250 mg/liter glutaraldehyde in the fluid. These rates are much lower than those allowed for the internal ATCS by specification. Also, these rates are lower than the nearly 0.2 ml/h and 2.7 ml/h leakage rates that have been experienced on board the *ISS*.

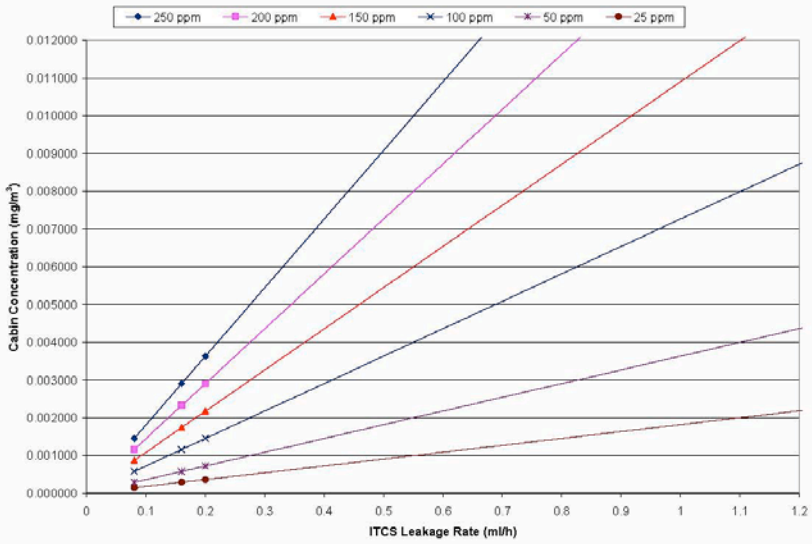

Figure 3. Leakage Accommodated by the USOS TCCS

TCCS and BMP Dual Capability

For the TCCS operating with an assist from the ROS's BMP, the range of leakage accommodated increases by nearly a factor of 3. Figure 4 shows that up to 3 ml/h and 0.3 ml/h fluid leakage can be accommodated for 25 mg/liter and 250 mg/liter glutaraldehyde in the fluid, respectively. This range of leakage rates is comparable to that observed on board the *ISS*.

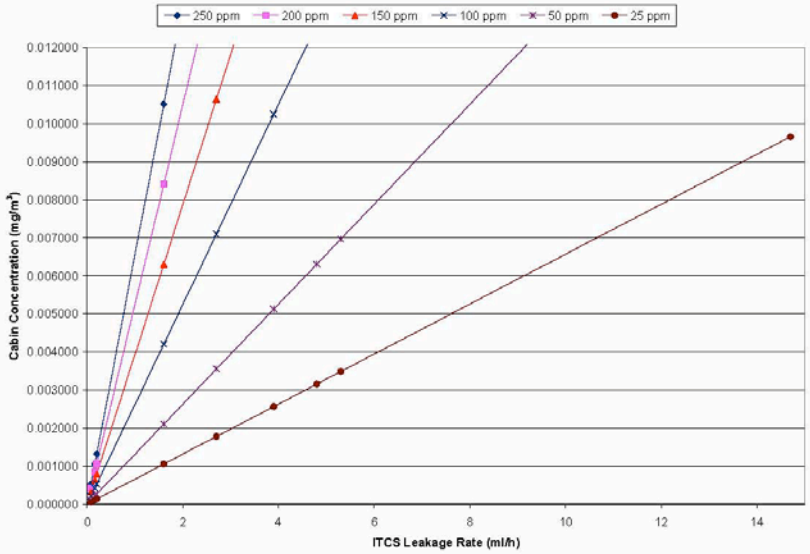

Figure 4. Leakage Accommodated by the USOS TCCS and ROS BMP

Absorption via Humidity Condensate and Impacts to Water Processing Equipment

Figure 5 shows the additional capability that absorption via humidity condensate provides. A single common cabin air assembly (CCAA) heat exchanger removing condensate at a 1-person equivalent latent load can remove glutaraldehyde via absorption at 55% efficiency. Similarly, the SKV heat exchanger on board the ROS can remove glutaraldehyde at 75% efficiency while removing condensate at a 1-person equivalent latent load. This increases to 86% for a 2-person latent load. Leakage ranging from 2.5 ml/h to nearly 13 ml/h leakage can be accommodated for 250 mg/liter and 50 mg/liter glutaraldehyde in the fluid, respectively. The 25 mg/liter glutaraldehyde concentration is accommodated across the full range of specified and observed leakage.

It is evident that removal via absorption by humidity condensate provides an effective assist to the active contamination control equipment. This is vividly illustrated by Figure 6 where the capabilities for the TCCS and BMP operating alone and when assisted by varying removal via absorption in humidity condensate are compared. The removal via absorption provided by a 2-person latent load can increase the capacity by more than a factor of 5 and a latent load equivalent to 3 people more than doubles that. While obviously effective, the impacts to water processing equipment must be accounted for. Water processing equipment engineers from both NASA and RSC Energia have indicated glutaraldehyde in humidity condensate must not exceed 5 mg/liter. Figures 4 and 5 show the

effect that varying cabin concentration and crew latent load can have upon humidity condensate loading for the CCAA and SKV units.

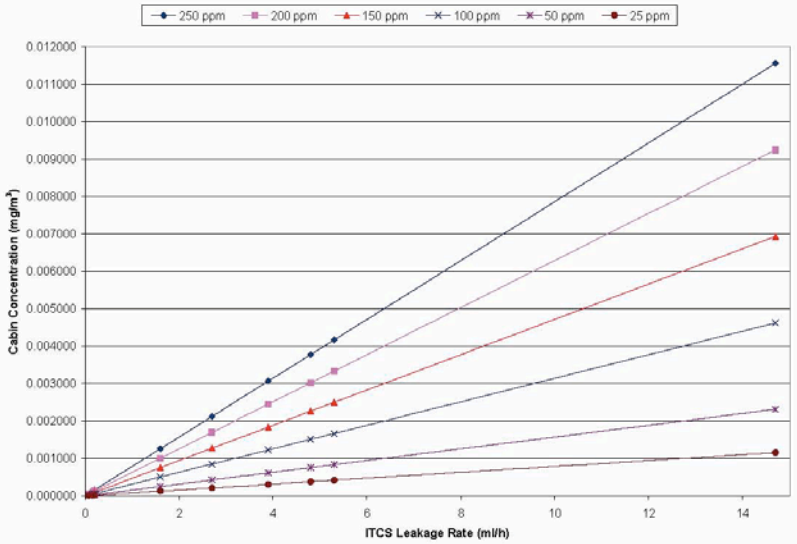

Figure 5. Leakage Accommodated by the USOS TCCS and ROS BMP Assisted by Humidity Condensate Absorption at Assembly Complete for a Crew of Three

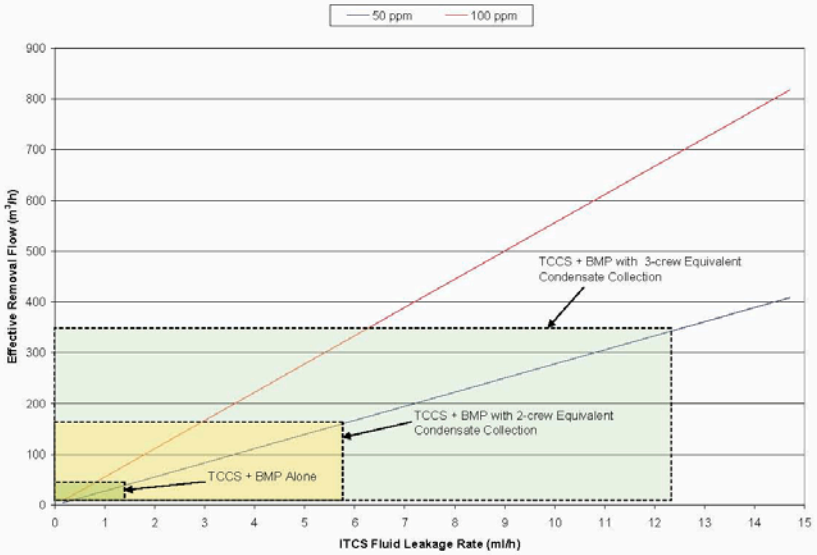

Figure 6. Comparison of Assisted and Unassisted Contamination Control Capacity

While removal via absorption by humidity condensate is a potentially effective removal route, the potential impact to the water processing systems can be significant and must be considered. Figures 7 and 8 show how the condensate loading varies when the latent load and the cabin concentration change. For the CCAA, Figure 7 shows the cabin concentration that can contribute to 5 mg/liter glutaraldehyde in the condensate ranges from 0.0015 mg/m^3 to 0.0032 mg/m^3 for latent loading up to 3 people. Similarly, Figure 8 shows that a cabin concentration ranging from 0.0027 mg/m^3 to 0.0066 mg/m^3 contribute to 5 mg/liter glutaraldehyde in the condensate collected by the SKV for latent loads up to 3 people.

To understand the potential impact upon humidity condensate loading for the Flight 4R and assembly complete configurations, the rigorous mass balance based upon the simultaneous solution of Equations 6 and 7 is used. Appendix C contains tabular results.

Figure 5 indicates that, with respect to maintaining cabin air quality, fluid containing up to 100 mg/liter glutaraldehyde can be used for nearly half the specified range of fluid leakage when all removal routes are considered. However, fluid containing <50 mg/liter glutaraldehyde has the least potential impact upon the cabin's atmosphere. Based upon the rigorous mass balance, the cabin concentration for the Flight 4R configuration can exceed the lower range for condensate loading acceptability for a CCAA when leakage is >1.8 ml/h for fluid containing 100 mg/liter glutaraldehyde. This increases to >3.6 ml/h for fluid containing 50 mg/liter glutaraldehyde. These leakage rates are within that allowed by specification for the Flight 4R configuration. Humidity condensate collected by the SKV will not be overloaded for the Flight 4R configuration unless total leakage exceeds 7.7 ml/h and 15.4 ml/h for fluid containing 100 mg/liter and 50 mg/liter glutaraldehyde, respectively.

For the assembly complete configuration, leakage >4.7 ml/h can overload the condensate collected by the CCAA for fluid containing 100 mg/liter glutaraldehyde. Similarly, leakage >9.4 ml/h containing 50 mg/liter glutaraldehyde can overload the condensate collected by the CCAA. Leakage much greater than allowed by specification is required to overload condensate collected by the SKV. For fluid containing 100 mg/liter glutaraldehyde, leakage >22 ml/h results in >5 mg/liter glutaraldehyde in the condensate. Sustained leakage >44 ml/h is necessary for fluid containing 50 mg/liter glutaraldehyde.

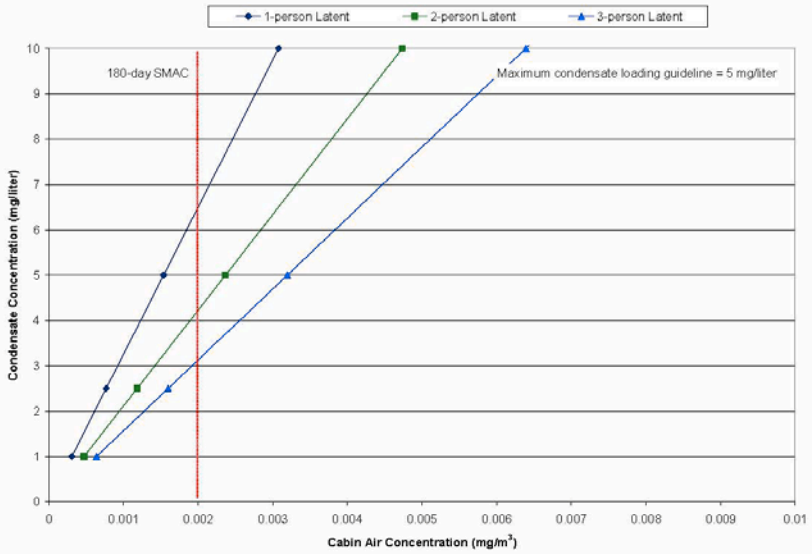

Figure 7. Effect of Cabin Glutaraldehyde Concentration upon Condensate Collected by the CCAA

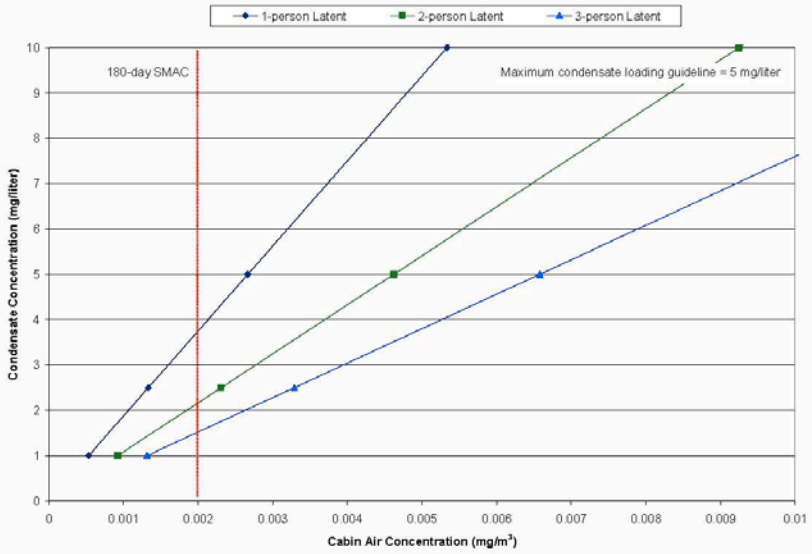

Figure 8. Effect of Cabin Glutaraldehyde Concentration upon Condensate Collected by the CCAA

Consideration for Air Quality Control System Failures

Given glutaraldehyde's very low 180-day SMAC and the fact that fluid leakage from the internal ATCS is expected, it is necessary to understand the potential effects that a failure of the TCCS and BMP either individually or simultaneously may have upon the *ISS's* overall trace contaminant control capability. The rigorous mass balance provided by simultaneous solution of Equations 6 and 7 was used to evaluate the effects. Internal ATCS fluid containing 100 mg/liter and 50 mg/liter glutaraldehyde was considered for both the Flight 4R and assembly complete configurations. Results are tabulated in Appendix C.

The worst case situation occurs when both the TCCS and BMP fail simultaneously. For such a situation, internal ATCS fluid leakage >1.9 ml/h for fluid containing 100 mg/liter glutaldehyde and >3.8 ml/h for fluid containing 50 mg/liter glutaraldehyde result in cabin concentration exceeding the 180-day SMAC. These leakage rates are within the range allowed by specification. For assembly complete, leakage >5.6 ml/h and >11.2 ml/h result in cabin concentration greater than the 180-day SMAC. Again, these leakage rates are within the range allowed by specification.

For individual failures of the TCCS and BMP for the *ISS* Flight 4R configuration, leakage rates >2.1 ml/h and >4.2 ml/h for fluid containing 100 mg/liter and 50 mg/liter glutaraldehyde, respectively, can result in cabin concentration greater than the 180-day SMAC. At assembly complete, the leakage rates increase to >5.9 ml/h for fluid containing 100 mg/liter glutaraldehyde and >11.8 ml/h for fluid containing 50 mg/liter glutaraldehyde.

If internal ATCS fluid leakage can be adequately controlled and monitored, leakage no greater than 1.8 ml/h for the Flight 4R configuration and 4.7 ml/h for the assembly complete configuration for internal ATCS fluid containing 100 mg/liter glutaraldehyde can achieve acceptable results. Likewise, for ATCS fluid containing 50 mg/liter glutaraldehyde, rates no greater than 3.6 ml/h for the Flight 4R configuration and 9.4 ml/h for the assembly complete configuration achieve acceptable results.

When considering the concentration threshold of 0.0015 mg/m^3 for avoiding adverse impacts upon humidity condensate loading in the USOS combined with a single trace contaminant control failure, leakage rates for the 4R configuration >1.6 ml/h and >3.2 ml/h for fluid containing 100 mg/liter and 50 mg/liter glutaraldehyde, respectively, exceed the threshold. Similarly, at assembly complete, leakage of fluid containing 100 mg/liter and 50 mg/liter glutaraldehyde exceeds the threshold at >4.4 ml/h and >8.8 ml/h, respectively. The range of leakage in both cases is within the range of internal ATCS leakage allowed by specification.

Summary

Overall, measures must be taken to minimize the risk to human health and maintaining the *ISS's* cabin air quality as well as protecting the water processing systems. Although the TCCS and BMP have proven themselves reliable, they are designed specifically to control the contamination loading from equipment offgassing and human metabolic processes alone. Further, cabin air quality monitoring techniques are not sensitive enough to monitor glutaraldehyde's concentration at or below the 180-day SMAC. Therefore, it is not possible to verify cabin air quality maintenance via existing monitoring techniques. Therefore, as shown by Figures 5 and 7 and presented earlier, to ensure that the risk to human health presented by potentially overwhelming the active air quality control systems and overloading humidity condensate, the internal ATCS fluid should contain <25 mg/liter glutaldehyde. For the entire range of specified internal ATCS fluid leakage, this concentration protects against all human health and ECLS equipment performance impacts as well as accommodates for the potential for air quality control equipment failures.

CONCLUSIONS

Based upon evaluation of glutaraldehyde as a candidate biocidal additive to the internal ATCS working fluid, conclusions are the following:

1. Evaporation rates from concentrated aqueous solutions of glutaraldehyde are such that appropriate containment and personal protective equipment must be used when injecting the solution into the internal ATCS.

2. Basic, unassisted trace contaminant control capability as defined by *ISS* Program specification cannot accommodate the range of internal ATCS leakage rates for any glutaraldehyde concentration in the fluid.

3. If no suitable alternative can be found, internal ATCS fluid must contain <25 mg/liter glutaraldehyde to ensure that long-term hazards to human health and operability of ECLS air quality control and water processing systems are acceptable.

RECOMMENDATIONS

Based upon *ISS* ECLS engineering evaluation, it is recommended that other candidate biocidal additives be evaluated. The overall challenges and risks associated with using glutaraldehyde as a biocidal additive are significant and present long-term operational issues to the *ISS* Program if implemented.

The USOS ECLS systems cannot be certified for glutaraldehyde concentration >25 mg/liter in the internal ATCS fluid. If no other suitable additive can be found, however, glutaraldehyde concentrations <25 mg/liter may be used within the range of internal ATCS fluid leakage specification to ensure long-term hazards to human health and ECLS system air quality control and water processing equipment are acceptable.

Further, any decision by the *ISS* Program to use glutaraldehyde as a biocidal additive to the internal ATCS fluid in the USOS must be reviewed by the International Partners within the Common Environments Team forum. This is necessary because fugitive emissions from the internal ATCS effect the common cabin environment.

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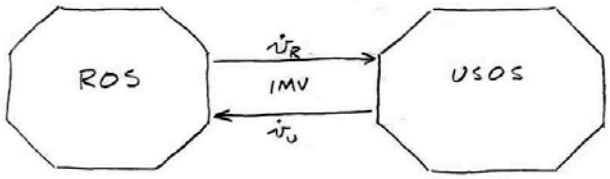
APPENDIX A—MASS BALANCE EQUATION DERIVATION
AND
EVAPORATIVE LOSS CALCULATIONS

CABIN MASS BALANCE - GENERALIZED

V.L. PERRY
MSFC/FD21
4-7-04



$V_R = 180.6\ m^3$	CABIN VOLUME	$V_U = 190.4 m^3$
$C_R = 0.02\ mg/m^3$	CONCENTRATION @ $t=\infty$	$C_U = 0.04\ mg/m^3$
$\dot{v}_R = 252.7\ m^3/h$	IMV FLOW	$\dot{v}_U = 118.6\ m^3/h$
$g_R = ?$	GENERATION RATE	$g_U = ?$
	REMOVAL DEVICES	
	TCCS →	$\eta\dot{v} = (1.0)(4.6) = 4.6 m^3/h$
$\eta\dot{v} = (0.9)(27)$	← BMP	
$\eta\dot{v} = (0.073)(144)$	← SKV	
$\Sigma\eta\dot{v}_R = 34.8 m^3/h$	TOTAL REMOVAL	$\Sigma\eta\dot{v}_U = 4.6 m^3/h$

USOS MASS BALANCE

$$\frac{dm_U}{dt} = \dot{v}_R C_R - \dot{v}_U C_U - (\Sigma\eta\dot{v}_U) C_U + g_U$$

$$= \left(\frac{\dot{v}_R}{V_R}\right) M_R - \left(\frac{\dot{v}_U}{V_U}\right) M_U - \left(\frac{\Sigma\eta\dot{v}_U}{V_U}\right) M_U + g_U \quad (1)$$

ROS MASS BALANCE

$$\frac{dM_R}{dt} = \dot{v}_U C_U - \dot{v}_R C_R - (\Sigma\eta\dot{v}_R) C_R + g_R$$

$$= \left(\frac{\dot{v}_U}{V_U}\right) M_U - \left(\frac{\dot{v}_R}{V_R}\right) M_R - \left(\frac{\Sigma\eta\dot{v}_R}{V_R}\right) M_R + g_R \quad (2)$$

(1)

DEFINE IN OPERATOR FORM

USOS

$$\frac{dM_U}{dt} = \frac{\dot{V}_R}{V_R} M_R - \frac{\dot{V}_U}{V_U} M_U - \left(\frac{\dot{r}_U}{V_U}\right) M_U + g_U$$

$$\frac{dM_U}{dt} = \frac{\dot{V}_R}{V_R} M_R - \left(\frac{\dot{V}_U}{V_U} + \frac{\dot{r}_U}{V_U}\right) M_U + g_U$$

SO $$\left[D + \left(\frac{\dot{V}_U}{V_U} + \frac{\dot{r}_U}{V_U}\right)\right] M_U - \left(\frac{\dot{V}_R}{V_R}\right) M_R = g_U \qquad (3)$$

RDS

$$\frac{dM_R}{dt} = \frac{\dot{V}_U}{V_U} M_U - \frac{\dot{V}_R}{V_R} M_R - \left(\frac{\dot{r}_R}{V_R}\right) M_R + g_R$$

$$\frac{dM_R}{dt} = \frac{\dot{V}_U}{V_U} M_U - \left(\frac{\dot{V}_R}{V_R} + \frac{\dot{r}_R}{V_R}\right) M_R + g_R$$

SO $$\left[D + \left(\frac{\dot{V}_R}{V_R} + \frac{\dot{r}_R}{V_R}\right)\right] M_R - \frac{\dot{V}_U}{V_U} M_U = g_R \qquad (4)$$

USING THE ANNIHILATOR METHOD

- TAKE DERIVATIVE OF BOTH SIDES OF (3) AND (4)

$$D\left[D + \left(\frac{\dot{V}_U}{V_U} + \frac{\dot{r}_U}{V_U}\right)\right] M_U - D\left(\frac{\dot{V}_R}{V_R}\right) M_R = 0 \qquad (3a)$$

$$D\left[D + \left(\frac{\dot{V}_R}{V_R} + \frac{\dot{r}_R}{V_R}\right)\right] M_R - D\left(\frac{\dot{V}_U}{V_U}\right) M_U = 0 \qquad (4a)$$

- ELIMINATE M_R

$$\left[D + \left(\frac{\dot{V}_R}{V_R} + \frac{\dot{r}_R}{V_R}\right)\right] D\left[D + \left(\frac{\dot{V}_U}{V_U} + \frac{\dot{r}_U}{V_U}\right)\right] M_U - \left[D + \left(\frac{\dot{V}_R}{V_R} + \frac{\dot{r}_R}{V_R}\right)\right] D\left(\frac{\dot{V}_R}{V_R}\right) M_R = 0$$

$$+ \left(\frac{\dot{V}_R}{V_R}\right) D\left[D + \left(\frac{\dot{V}_R}{V_R} + \frac{\dot{r}_R}{V_R}\right)\right] M_R - \left(\frac{\dot{r}_R}{V_R}\right) D\left(\frac{\dot{V}_U}{V_U}\right) M_U = 0$$

$$\left[D + \left(\frac{\dot{V}_R}{V_R} + \frac{\dot{r}_R}{V_R}\right)\right] D\left[D + \left(\frac{\dot{V}_U}{V_U} + \frac{\dot{r}_U}{V_U}\right)\right] M_U - \frac{\dot{V}_R \dot{V}_U}{V_R V_U} D M_U = 0$$

$$D\left[D + \left(\frac{\dot{r}_R}{V_R} + \frac{\dot{r}_R}{V_R}\right)\right]\left[D + \left(\frac{\dot{V}_U}{V_U} + \frac{\dot{r}_U}{V_U}\right)\right] M_U - D\frac{\dot{V}_R \dot{V}_U}{V_R V_U} M_U = 0$$

$$\left[D^2 + D\left(\frac{\dot{V}_U}{V_U} + \frac{\dot{r}_U}{V_U} + \frac{\dot{V}_R}{V_R} + \frac{\dot{r}_R}{V_R}\right) + \frac{\dot{V}_R \dot{V}_U}{V_R V_U} + \frac{\dot{V}_R \dot{r}_U}{V_R V_U} + \frac{\dot{r}_R \dot{V}_U}{V_R V_U} + \frac{\dot{r}_R \dot{r}_U}{V_R V_U} - \frac{\dot{V}_R \dot{V}_U}{V_R V_U}\right] M_U = 0$$

$$\left[D^2 + D\left(\frac{\dot{V}_U}{V_U} + \frac{\dot{r}_U}{V_U} + \frac{\dot{r}_R}{V_R} + \frac{\dot{r}_R}{V_R}\right) + \frac{\dot{V}_R \dot{r}_U}{V_R V_U} + \frac{\dot{r}_R \dot{V}_U}{V_R V_U} + \frac{\dot{r}_R \dot{r}_U}{V_R V_U}\right] M_U = 0$$

(7)

$D = 0$

$a = 1$

$b = \frac{\dot{v}_R}{V_R} + \frac{r_R}{V_R} + \frac{\dot{v}_U}{V_U} + \frac{r_U}{V_U}$

$c = \frac{\dot{v}_R r_U + r_R \dot{v}_U + r_R r_U}{V_R V_U}$

$\therefore \chi_1 = D \; ; \; \chi_2 = \frac{-b + \sqrt{b^2 - 4ac}}{2a} \; ; \; \chi_3 = \frac{-b - \sqrt{b^2 - 4ac}}{2a}$

and

$$\chi_2 = \frac{-\left(\frac{\dot{v}_R}{V_R} + \frac{r_R}{V_R} + \frac{\dot{v}_U}{V_U} + \frac{r_U}{V_U}\right) + \sqrt{\left(\frac{\dot{v}_R}{V_R} + \frac{r_R}{V_R} + \frac{\dot{v}_U}{V_U} + \frac{r_U}{V_U}\right)^2 - 4(1)(c)}}{2(1)}$$

$$\chi_3 = \frac{-\left(\frac{\dot{v}_R}{V_R} + \frac{r_R}{V_R} + \frac{\dot{v}_U}{V_U} + \frac{r_U}{V_U}\right) - \sqrt{\left(\frac{\dot{v}_R}{V_R} + \frac{r_R}{V_R} + \frac{\dot{v}_U}{V_U} + \frac{r_U}{V_U}\right)^2 - 4(1)(c)}}{2(1)}$$

ker

$$m_U = a + b e^{\chi_2 t} + c e^{\chi_3 t}$$

$$D_{m_U} = b\chi_2 e^{\chi_2 t} + c\chi_3 e^{\chi_3 t}$$

SUBSTITUTE D_{m_U} INTO (3)

$$b e^{\chi_2 t} + c e^{\chi_3 t} + \left(\frac{\dot{v}_U}{V_U} + \frac{r_U}{V_U}\right)\left(a + b e^{\chi_2 t} + c e^{\chi_3 t}\right) - \frac{\dot{v}_R}{V_R} m_R = g_U$$

$$\left(\frac{\dot{v}_R}{V_R}\right) m_R = \left(\frac{\dot{v}_U}{V_U} + \frac{r_U}{V_U}\right) a + \left(\chi_2 + \frac{\dot{v}_U}{V_U} + \frac{r_U}{V_U}\right) b e^{\chi_2 t} + \left(\chi_3 + \frac{\dot{v}_U}{V_U} + \frac{r_U}{V_U}\right) c e^{\chi_3 t} - g_U$$

$$* \quad m_R = \left(\frac{V_R}{\dot{v}_R}\right)\left[\left(\frac{\dot{v}_U}{V_U} + \frac{r_U}{V_U}\right) a + \left(\chi_2 + \frac{\dot{v}_U}{V_U} + \frac{r_U}{V_U}\right) b e^{\chi_2 t} + \left(\chi_3 + \frac{\dot{v}_U}{V_U} + \frac{r_U}{V_U}\right) c e^{\chi_3 t} - g_U\right]$$

(3)

DETERMINE CONSTANTS – a, b, c

FOR TOTAL MASS BALANCE, $M_T = M_U + M_R$

AT $t \to \infty$, $C_i = \frac{g_i}{\sum \eta \dot{v}}$ OR $\frac{M_T}{V_U + V_R} = \frac{(g_U + g_R)}{(r_U + r_R)}$

SO $M_T = \frac{(g_U + g_R)(V_U + V_R)}{r_U + r_R}$

SUBSTITUTE M_U AND M_R INTO EQUATION FOR M_T

$$\frac{(g_U + g_R)(V_U + V_R)}{r_U + r_R} = a + b e^{x_2 t} + c e^{x_3 t} + \left(\frac{V_R}{\dot{v}_R}\right)\left(\frac{\dot{v}_U + r_U}{V_U}\right) a + \left(\frac{V_R}{\dot{v}_R}\right)\left(x_2 + \frac{\dot{v}_U + r_U}{V_U}\right) b e^{x_2 t} + \left(\frac{V_R}{\dot{v}_R}\right)\left(x_3 + \frac{\dot{v}_U + r_U}{V_U}\right) c e^{x_3 t} - \frac{V_R g_U}{\dot{v}_R}$$

$$\frac{(g_U + g_R)(V_U + V_R)}{r_U + r_R} = \left[\left(\frac{V_R}{\dot{v}_R}\right)\left(\frac{\dot{v}_U + r_U}{V_U}\right) + 1\right] a + \left[\left(\frac{V_R}{\dot{v}_R}\right)\left(x_2 + \frac{\dot{v}_U + r_U}{V_U}\right) + 1\right] b e^{x_2 t} + \left[\left(\frac{V_R}{\dot{v}_R}\right)\left(x_3 + \frac{\dot{v}_U + r_U}{V_U}\right) + 1\right] c e^{x_3 t} - \frac{V_R g_U}{\dot{v}_R}$$

FOR x_2 AND $x_3 < 0$

$$\frac{(g_U + g_R)(V_U + V_R)}{r_U + r_R} = \left[\left(\frac{V_R}{\dot{v}_R}\right)\left(\frac{\dot{v}_U + r_U}{V_U}\right) + 1\right] a - \frac{V_R g_U}{\dot{v}_R}$$

$$\frac{(g_U + g_R)(V_U + V_R)}{r_U + r_R} = \left(\frac{V_R}{\dot{v}_R}\right)\left[\left(\frac{\dot{v}_U + r_U}{V_U} + \frac{\dot{v}_R}{V_R}\right) a - g_U\right]$$

$$\frac{(g_U + g_R)(V_U + V_R)\dot{v}_R}{(r_U + r_R) V_R} = \left(\frac{\dot{v}_U + r_U}{V_U} + \frac{\dot{v}_R}{V_R}\right) a - g_U$$

$$* \; a = \left[\frac{(g_U + g_R)(V_U + V_R)\dot{v}_R}{(r_U + r_R) V_R} + g_U\right] \Big/ \left(\frac{\dot{v}_U + r_U}{V_U} + \frac{\dot{v}_R}{V_R}\right)$$

4

DETERMINE CONSTANTS b AND c:

AT $t = 0$, $M_U = M_{U0}$, $M_R = M_{R0}$

SO, $M_{U0} = a + b + c$

$$M_{R0} = \left(\frac{V_R}{\dot{V}_R}\right)\left[\left(\frac{\dot{V}_U + r_U}{V_U}\right)a + \left(\chi_2 + \frac{\dot{V}_U + r_U}{V_U}\right)b + \left(\chi_3 + \frac{\dot{V}_U + r_U}{V_U}\right)c - g_U\right]$$

ELIMINATE b:

$$-\left[\left(\frac{V_R}{\dot{V}_R}\right)\left(\chi_2 + \frac{\dot{V}_U + r_U}{V_U}\right)\right]M_{U0} = -\left[\left(\frac{V_R}{\dot{V}_R}\right)\left(\chi_2 + \frac{\dot{V}_U + r_U}{V_U}\right)\right](a + b + c)$$

$$+ \quad M_{R0} = \left(\frac{V_R}{\dot{V}_R}\right)\left[\left(\frac{\dot{V}_U + r_U}{V_U}\right)a + \left(\chi_2 + \frac{\dot{V}_U + r_U}{V_U}\right)b + \left(\chi_3 + \frac{\dot{V}_U + r_U}{V_U}\right)c - g_U\right]$$

$$M_{R0} - \left[\left(\frac{V_R}{\dot{V}_R}\right)\left(\chi_2 + \frac{\dot{V}_U + r_U}{V_U}\right)\right]M_{U0} = -\left[\left(\frac{V_R}{\dot{V}_R}\right)\left(\chi_2 + \frac{\dot{V}_U + r_U}{V_U}\right)a + \left(\frac{V_R}{\dot{V}_R}\right)\left(\frac{\dot{V}_U + r_U}{V_U}\right)a - \left[\left(\frac{V_R}{\dot{V}_R}\right)\left(\chi_2 + \frac{\dot{V}_U + r_U}{V_U}\right)\right]c + \left[\left(\frac{V_R}{\dot{V}_R}\right)\left(\chi_3 + \frac{\dot{V}_U + r_U}{V_U}\right)\right]c - \frac{V_R g_U}{\dot{V}_R}$$

$$\frac{\dot{V}_R M_{R0}}{V_R} - \left(\chi_2 + \frac{\dot{V}_U + r_U}{V_U}\right)M_{U0} = -\left(\chi_2 + \frac{\dot{V}_U + r_U}{V_U}\right)a + \left(\frac{\dot{V}_U + r_U}{V_U}\right)a - \left(\chi_2 + \frac{\dot{V}_U + r_U}{V_U}\right)c + \left(\chi_3 + \frac{\dot{V}_U + r_U}{V_U}\right)c - g_U$$

$$\frac{\dot{V}_R M_{R0}}{V_R} - \left(\chi_2 + \frac{\dot{V}_U + r_U}{V_U}\right)M_{U0} = (\chi_3 - \chi_2)c - \chi_2 a - g_U$$

SOLVE FOR c:

$$* \quad c = \left(\frac{1}{\chi_3 - \chi_2}\right)\left[\frac{\dot{V}_R M_{R0}}{V_R} - \left(\chi_2 + \frac{\dot{V}_U + r_U}{V_U}\right)M_{U0} + \chi_2 a + g_U\right]$$

$$* \quad b = M_{U0} - a - c$$

(5)

GLUTARALDEHYDE EVALUATION - REVISED

J.L. PERRY
1-20-03
MSFC/FD24

PHYSICAL PROPERTIES

$M = 100.13$ g/mole
Sp. Gr. = 0.972 (PURE LIQUID @ 20°C) Sp. Gr. (aqueous) = 1.06-1.12
P_v (20°C) = 17 mm Hg

AQUEOUS GLUTARALDEHYDE VAPOR PRESSURE

AT 20°C AS A FUNCTION OF COMPOSITION*

$$PPM_v = 0.0122(\%\text{MASS})^2 + 1.9496(\%\text{MASS}) + 0.0172$$

AT VARYING TEMPERATURE*

0.1% MASS AQUEOUS SOLUTION

$$\text{Log}_{10} P_w = (-12.853)/\ln\left(\frac{1}{T}\right) - 73.739$$

0.01% MASS AQUEOUS SOLUTION

$$\text{Log}_{10} P_w = (-12.853)/\ln\left(\frac{1}{T}\right) - 74.739$$

* BASIS: J.D. OLSON: THE VAPOR PRESSURE OF PURE AND AQUEOUS GLUTARALDEHYDE. UNION CARBIDE, UNDATED.

HENEY'S GAS CONSTANT

$$\ln H = 29.1352 - 9187.99/T$$

REF: J.D. OLSON: THE VAPOR PRESSURE OF PURE AND AQUEOUS GLUTARALDEHYDE. UNION CARBIDE, UNDATED.

SPACECRAFT MAXIMUM ALLOWABLE CONCENTRATIONS

180-DAY	0.002 mg/m³	REF. JSC 20584 JUNE 1999.
30-DAY	0.012 mg/m³	
7-DAY	0.025 mg/m³	
24-HOUR	0.08 mg/m³	
1-HOUR	0.5 mg/m³	

REMOVAL MECHANISMS AND ISS CONFIGURATION

BMA — $\dot{V} = 27$ m³/h ; $\eta = 1.0$
TCCS — $\dot{V} = 15.3$ m³/h ; $\eta = 1.0$
EKU — $\dot{V} = 144$ m³/h ; $\eta = 0.88$ 76% BYPASS
CCAA — $\dot{V} = 68$ m³/h ; $\eta = 0$ 90% BYPASS - NO CONDENSATE
$V_{CABIN} = 371$ m³ $V_{USOS} = 190.6$ m³
CABIN AIR VELOCITY = 15 ft/minute (0.0762 m/s)
$T_{CABIN} = 20$ °C

(1)

EVALUATION CASES

- SPILLED 1 LITER 5% SOLUTION AT 20°C.
- SPILLED 100 ml 50% SOLUTION AT 20°C
- SPILLED 3.8 LITER (1 GALLON) 0.025% SOLUTION AT 4.4°C AND AT 20°C
- EVALUATE TIME TO EFFECT FROM 1/2 180-DAY SMAC TO 24-HOUR AND 1-HOUR SMACS
- EVALUATE DECAY TIME FROM 1-HOUR SMAC TO 180-DAY SMAC
  - TOTAL ISS VOLUME AND RESOURCES
  - USOS ISOLATED WITH TCCS ONLY
- EVALUATE ALLOWABLE MAGNITUDE OF FUGITIVE EMISSION
  - GLUTARALDEHYDE ALONE
  - GLUTARALDEHYDE IN CONTEXT WITH T-VALUE

ASSUMPTIONS

- SPILLED LIQUID FORMS MINIMUM ENERGY CONFIGURATION
  - SPHERICAL BLOB

$V = \frac{\pi}{6} D^3$ $\quad S = \pi D^2$

- FOR $V = 1000\ cm^3$, $D = 12.4\ cm$, $S = 483.05\ cm^2$ $0.0493\ m^2$ or $0.52\ ft^2$
  $V = 100\ cm^3$, $D = 5.76\ cm$, $S = 104.23\ cm^2$ $0.0104\ m^2$ or $0.11\ ft^2$
  $V = 3800\ cm^3$, $D = 19.4\ cm$, $S = 1182.37\ cm^2$ $0.118\ m^2$ or $1.27\ ft^2$
- CABIN AIR VELOCITY = 15 ft/minute or 0.0762 m/s

(2)

EVAPORATION RATE EQUATIONS

- $q \cong (5.23 \times 10^{-9}) U_s^{0.78} P_v M_w^{0.67} A_p^{0.94}$ REF. KUMAR, VATCHA, M SCHMELZLE. ENV. ENG. WORLD, NOV/DEC 1996

q[=] kg/s ; U_s[=] m/s ; P_v[=] N/m² ; M_w[=] g/mole ; A_p[=] m²

- $QR = \dfrac{0.284\, u^{0.78} M^{2/3} A P_{vp}}{82.05\, T}$ REF. J. PETRESS, CEP, APRIL 2003

QR [=] lb/min ; u[=] m/s ; P_{vp}[=] mm Hg ; M[=] g/mole
T [=] K ; A [=] ft²

CASE 1 - EVAPORATION RATE FROM 1 LITER, 5% AT 20°C

$P_v = 10.07\ ppm_v \times \dfrac{100.13}{24.044} = 41.94\ mg/m^3$

$\rho = \dfrac{MP}{RT}$

$P = \dfrac{\rho RT}{M} \times \dfrac{g}{1000\ mg} \times \dfrac{m^3}{10^6\ cm^3}$

$P = \dfrac{(41.94)(82.06)(293)}{(100.13)(1000)(10^6)} = 1.007 \times 10^{-5}$ atm
or 0.00765 mm Hg
or 1.02 Pa

$q \cong (5.23 \times 10^{-9})(0.0762)^{0.78}(1.02)(100.13)^{0.67}(0.0483)^{0.94}$

$q \cong 9.085 \times 10^{-10}$ kg/s or 3.27 mg/h

$QR = \dfrac{(0.284)(0.0762)^{0.78}(100.13)^{2/3}(0.52)(0.00765)}{(82.05)(293)}$

$QR = 1.36 \times 10^{-7}$ lb/min or 3.70 mg/h

AVERAGE = 3.485 mg/h

TIME TO 24-HOUR SMAC:
$t = (371)(0.02 - 0.00)/3.485$
$t = 8.5$ HOURS

TIME TO 180-DAY SMAC:
$t = (371)(0.002 - 0.00)/3.485 = 0.213$ HOURS (12.8 MINUTES)

(3)

CASE 2 - EVAPORATION RATE FROM 100 ml, 50% AT 20 °C

$P_w = 128\ ppm_v \times \frac{100.13}{24.044} = 533.04\ mg/m^3$

$P_w = \frac{(533.04)(82.06)(293)}{(100.13)(1000)(10^6)} = 1.22 \times 10^{-4}\ atm$

or

$0.0973\ mm\,Hg$

or

$12.97\ Pa$

$q = (5.23 \times 10^{-4})(0.0762)^{0.78}(12.97)(100.13)^{0.67}(0.0104)^{0.94}$

$q = 2.727 \times 10^{-9}\ kg/s$ or $9.82\ mg/h$

$QR = \frac{(0.284)(0.0762)^{0.78}(100.13)^{2/3}(0.11)(0.0973)}{(82.05)(293)}$

$QR = 3.66 \times 10^{-7}\ lb/min$ or $9.96\ mg/h$

AVERAGE = $9.89\ mg/h$

TIME TO 150-DAY SMAC

$t = (371)(0.002)/9.89 = 0.075\ h$ or 4.5 minutes

TIME TO 24-HOUR SMAC

$t = (371)(0.08)/9.89 = 3.0\ h$

CASE 3 - EVAPORATION RATE FROM 3.8 LITERS, 0.025% AT 4.4 °C AND 20 °C

$P_w(4.4°C) = \log_{10}(P_w) = -2.435$ FOR 0.01% SOLUTION

$P_w = 10^{-2.435} \times 2.5 = 0.00918\ ppm_v$

$P_w = 0.00918 \times \frac{100.13}{24.044} \times \frac{(82.06)(277.4)}{(100.13)(1000)(10^6)}$

$P_w = 8.694 \times 10^{-9}\ atm$

or

$6.607 \times 10^{-6}\ mm\,Hg$

or

$8.809 \times 10^{-4}\ Pa$

$q = (5.23 \times 10^{-4})(0.0762)^{0.78}(8.809 \times 10^{-4})(100.13)^{0.67}(0.118)^{0.94}$

$q = 1.82 \times 10^{-12}\ kg/s$ or $0.00654\ mg/h$

$QR = \frac{(0.284)(0.0762)^{0.78}(100.13)^{2/3}(1.27)(6.607 \times 10^{-6})}{(82.05)(277.4)}$

$QR = 3.03 \times 10^{-10}\ lb/minute$ or $0.00825\ mg/h$

AVERAGE = $0.0074\ mg/h$

(4)

TIME TO 180-DAY SMAC

$t = (371)(0.002)/0.0074 = 100.3\ h$

$P_w(20°C) = 0.0659$ ppmv

$P_w(20°C) = \frac{(0.0659)(82.06)(293)}{(24.044)(1000)(10^6)} = 6.595 \times 10^{-8}$ atm

or 5.012×10^{-5} mm Hg

or 6.682×10^{-3} Pa

$q = (5.23\times10^{-9})(0.0762)^{0.78}(6.682\times10^{-3})(100.13)^{0.67}(0.118)^{0.94}$

$q = 1.378 \times 10^{-11}$ kg/s or 0.0496 mg/h

$QR = \frac{(0.284)(0.0762)^{0.78}(100.13)^{2/3}(1.27)(5.012\times10^{-5})}{(82.05)(293)}$

$QR = 2.177 \times 10^{-5}$ lb/minute or 0.0592 mg/h

AVERAGE = 0.0544 mg/h

TIME TO 180-DAY SMAC

$t = (371)(0.002)/0.0544 = 13.6\ h$

DECAY RATE FROM 1-HOUR SMAC TO 180-DAY SMAC

$t = \frac{-V}{\sum \eta \dot{v}} \ln(C/C_0)$

$C_0 = 0.5\ mg/m^3$ $\quad \ln(H) = -3.9866$ $\quad H = 0.01856$ kPa/mole fr.

or $H = 1.832\times10^{-4}$ atm/mole fr.

$\sum \eta \dot{v} = 27 + 15.3 + 123.8 = 166.1$ (ISS)

$\sum \eta \dot{v} = 15.3$ (USOS)

C/C₀	ISS t	USOS t
0.95	0.11	0.64
0.75	0.64	3.6
0.5	1.5	8.6
0.25	3.1	17.3
0.1	5.1	28.7
0.05	6.7	37.3
0.004	12.3	68.8

(5)

MAXIMUM CABIN GENERATION

$$C = \frac{g}{\sum_i \eta_i \dot{v}_i}$$

USL PIDS = $0.9\,C_{SMAC}$ or $0.0018\ mg/m^3$

$$g = (\textstyle\sum_i \eta_i \dot{v}_i)\,C$$

$$g = (169)(0.0018) = 0.304\ mg/h$$

AT 250 mg/LITER, MAX LEAK = $\frac{0.304}{250} \times \frac{1000\ ml}{LITER}$

$= 1.22\ ml/h$

or

$29.2\ ml/day$

∴ COULD LEAK FOR 130 DAYS BEFORE REACH 1 GALLON LOSS

WITHOUT HUMIDITY CONDENSATE,

$g = (27 + 15.3)(0.0018) = 0.0761\ mg/h$ or $0.305\ ml/h$ or $7.3\ ml/day$

IN CONTEXT WITH T-VALUE - PRIMARY AIR QUALITY CONTRIBUTORS

	C (mg/m³)	SMAC
METHANOL	1.7	9
ETHANOL	5.9	2100
2-PROPANOL	0.34	150
n-BUTANOL	0.53	40
ACETALDEHYDE	0.25	4
ETHYL ACETATE	0.08	180
BUTYL ACETATE	0.025	190
DICHLOROMETHANE	0.4	10
TOLUENE	0.06	60
m-/p-XYLENES	0.05	220
o-XYLENE	0.1	220
ACETONE	0.38	52
2-BUTANONE	0.16	30
OMTS	3.75	12
DMPS	0.63	15
HMTS	4.4	9
METHANE	215	3800
HYDROGEN	22	340
CARBON MONOXIDE	1.6	11
FORMALDEHYDE	0.031	0.05
AMMONIA	0.3	7

OVERALL T-VALUE = 2.09

IRRITANTS T-VALUE = 0.73

∴ 0.27 FOR GLUTARALDEHYDE AND $0.00055\ mg/m^3$

$g = (169)(0.00055)$

$= 0.0929\ mg/h$

or $0.37\ ml/h$

or $8.9\ ml/d$

(6)

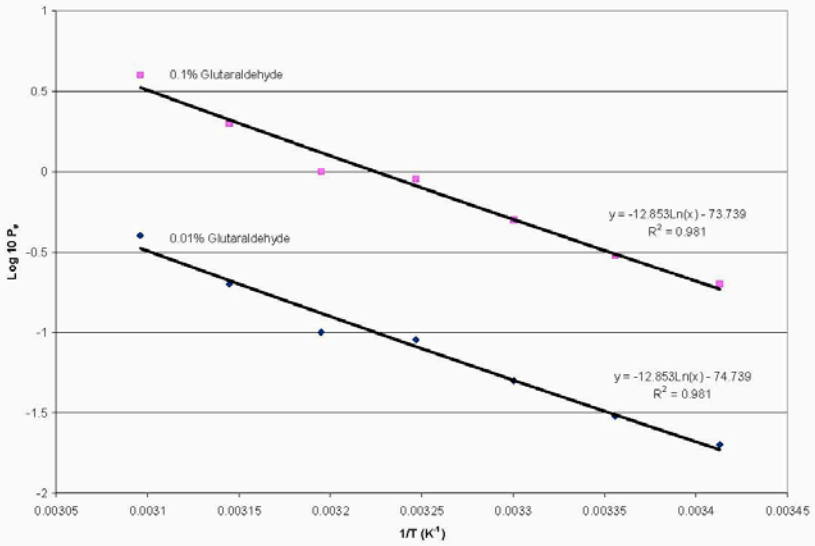


Figure A-1. Aqueous Glutaraldehyde Vapor Pressure

APPENDIX B—INTERNAL ATCS LEAKAGE SPECIFICATIONS
(Provided by Internal ATCS SPRT)

Spec. Leakage Rates (cc/hr)

	LTL	MTL
USL	0.80	0.80
Airlock	0.80	0.80
Node 1	0.80	0.80
Node 2	1.09	0.86
Node 3	1.50	2.00
CAM	0.48	0.48
MPLM	0.275	NA
Cupola	NA	0.026
APM	0.800	0.800
JEM	0.800	0.800
Combined spec. lkg =		14.71 cc/hr

Combined Spec. Leakage for current on-orbit IATCS in Single Loop Mode =	4.80 cc/hr
Threshold On-orbit leakage to initiate IFI (<1%/mo.) =	0.161624 cc/hr
Threshold On-orbit leakage at which a loop would be shut down (<1%/day) =	3.878967 cc/hr
Normal Leakage @ assembly complete (10 x's current IFI threshold) =	1.616236 cc/hr
Combined normal leakage & leakage at which a loop would be shut down =	5.333579 cc/hr

APPENDIX C—TABULAR RESULTS FROM USOS AND ROS MATERIAL BALANCE CALCULATIONS

100 ppm Loading/4R Configuration

Leak Rate (ml/h)	CONCENTRATION											
	All Operating			TCCS Off			BMP Off			TCCS & BMP Off		
	USOS (mg/m^3)	ROS (mg/m^3)	Total Cabin (mg/m^3)	USOS (mg/m^3)	ROS (mg/m^3)	Total Cabin (mg/m^3)	USOS (mg/m^3)	ROS (mg/m^3)	Total Cabin (mg/m^3)	USOS (mg/m^3)	ROS (mg/m^3)	Total Cabin (mg/m^3)
0.16	0.000134	5.66E-05	9.63E-05	0.000149	6.05E-05	0.000106	0.000152	7.6E-05	0.000115	0.000173	8.36E-05	0.000129
0.2	0.000168	7.07E-05	0.00012	0.000187	7.56E-05	0.000133	0.00019	9.5E-05	0.000144	0.000216	0.000105	0.000162
1.6	0.001341	0.000566	0.000963	0.001494	0.000605	0.001061	0.00152	0.00076	0.00115	0.001725	0.000836	0.001292
2.7	0.002262	0.000954	0.001626	0.002521	0.001021	0.00179	0.002565	0.001283	0.001941	0.002911	0.001411	0.002181
3.9	0.003267	0.001379	0.002348	0.003641	0.001474	0.002586	0.003705	0.001853	0.002804	0.004205	0.002038	0.00315
5.3	0.00444	0.001873	0.003191	0.004948	0.002003	0.003515	0.005035	0.002519	0.00381	0.005714	0.00277	0.004281

50 ppm Loading/4R Configuration

Leak Rate (ml/h)	CONCENTRATION											
	All Operating			TCCS Off			BMP Off			TCCS & BMP Off		
	USOS (mg/m^3)	ROS (mg/m^3)	Total Cabin (mg/m^3)	USOS (mg/m^3)	ROS (mg/m^3)	Total Cabin (mg/m^3)	USOS (mg/m^3)	ROS (mg/m^3)	Total Cabin (mg/m^3)	USOS (mg/m^3)	ROS (mg/m^3)	Total Cabin (mg/m^3)
0.16	6.7E-05	2.83E-05	4.82E-05	7.47E-05	3.02E-05	5.31E-05	7.6E-05	3.8E-05	5.75E-05	8.63E-05	4.18E-05	6.46E-05
0.2	8.38E-05	3.53E-05	6.02E-05	9.34E-05	3.78E-05	6.63E-05	9.5E-05	4.75E-05	7.19E-05	0.000108	5.23E-05	8.08E-05
1.6	0.00067	0.000283	0.000482	0.000747	0.000302	0.000531	0.00076	0.00038	0.000575	0.000863	0.000418	0.000646
2.7	0.001131	0.000477	0.000813	0.00126	0.00051	0.000895	0.001283	0.000642	0.000971	0.001456	0.000706	0.00109
3.9	0.001634	0.000689	0.001174	0.00182	0.000737	0.001293	0.001853	0.000927	0.001402	0.002102	0.001019	0.001575
5.3	0.00222	0.000937	0.001595	0.002474	0.001002	0.001757	0.002518	0.001259	0.001905	0.002857	0.001385	0.002141

100 ppm Loading/Assembly Complete												
Leak Rate (ml/h)	CONCENTRATION											
	All Operating			TCCS Off			BMP Off			TCCS & BMP Off		
	USOS (mg/m^3)	ROS (mg/m^3)	Total Cabin (mg/m^3)	USOS (mg/m^3)	ROS (mg/m^3)	Total Cabin (mg/m^3)	USOS (mg/m^3)	ROS (mg/m^3)	Total Cabin (mg/m^3)	USOS (mg/m^3)	ROS (mg/m^3)	Total Cabin (mg/m^3)
1.6	0.000513	0.000201	0.000453	0.000537	0.000206	0.000474	0.000544	0.000266	0.000491	0.000572	0.000276	0.000515
2.7	0.000866	0.000339	0.000765	0.000907	0.000348	0.000799	0.000918	0.00045	0.000828	0.000964	0.000466	0.000869
5.3	0.0017	0.000666	0.001501	0.001779	0.000684	0.001569	0.001802	0.000883	0.001625	0.001893	0.000915	0.001705
14.7	0.004714	0.001846	0.004163	0.004936	0.001896	0.004352	0.004998	0.002448	0.004508	0.005251	0.002539	0.00473

50 ppm Loading/Assembly Complete												
Leak Rate (ml/h)	CONCENTRATION											
	All Operating			TCCS Off			BMP Off			TCCS & BMP Off		
	USOS (mg/m^3)	ROS (mg/m^3)	Total Cabin (mg/m^3)	USOS (mg/m^3)	ROS (mg/m^3)	Total Cabin (mg/m^3)	USOS (mg/m^3)	ROS (mg/m^3)	Total Cabin (mg/m^3)	USOS (mg/m^3)	ROS (mg/m^3)	Total Cabin (mg/m^3)
1.6	0.000257	0.0001	0.000227	0.000269	0.000103	0.000237	0.000272	0.000133	0.000245	0.000286	0.000138	0.000257
2.7	0.000433	0.00017	0.000382	0.000453	0.000174	0.0004	0.000459	0.000225	0.000414	0.000482	0.000233	0.000434
5.3	0.00085	0.000333	0.00075	0.00089	0.000342	0.000784	0.000901	0.000441	0.000813	0.000947	0.000458	0.000853
14.7	0.002357	0.000923	0.002082	0.002468	0.000948	0.002176	0.002499	0.001224	0.002254	0.002625	0.001269	0.002365

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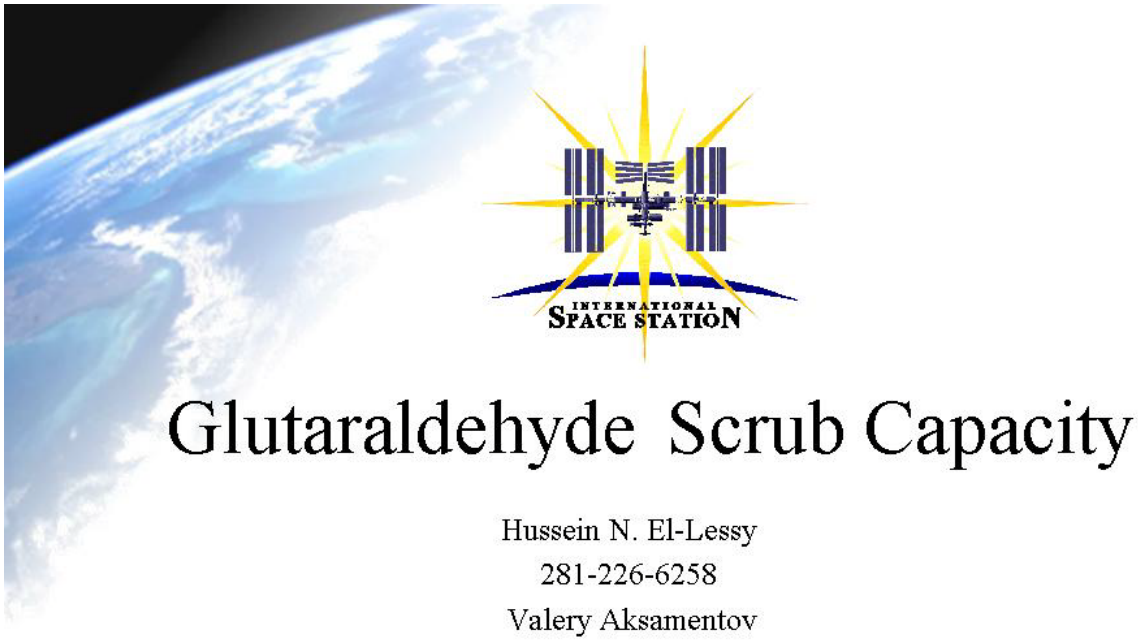
Appendix D. Boeing Glutaraldehyde Toxicity Assessment



Assumptions*

Assembly Complete	Current Configuration
• Cabin vol: 928 m^3	• Cabin vol: 371 m^3
• TCCS	• TCCS
– Flow 15.3m^3/hr	– Flow 15.3m^3/hr
– Efficiency 100%	– Efficiency 100%
• BMP	• BMP
– Flow 27m^3/hr	– Flow 27m^3/hr
– Efficiency 100%	– Efficiency 100%
• SKV	• SKV
– HX Flow 144 m^3/hr	– HX Flow 144 m^3/hr
– Efficiency 0.92	– Efficiency 0.92
• CCAA	• CCAA
– HX Flow 258 m^3/hr	– HX Flow 258 m^3/hr
– Efficiency 0.82	– Efficiency 0.82
• Spec leak rate 14.7 ml/hr @	• Spec leak rate 4.8 ml/hr @
– 50ppm ~ 0.735 mg/hr	– 50ppm ~ 0.240 mg/hr
• Nominal leak rate 1.6ml/hr @	• Nominal Leak rate 0.16ml/hr @
– 50ppm ~ 0.080 mg/hr	– 50ppm ~ 0.008 mg/hr

*All assumptions are constant for each given case unless otherwise indicated.

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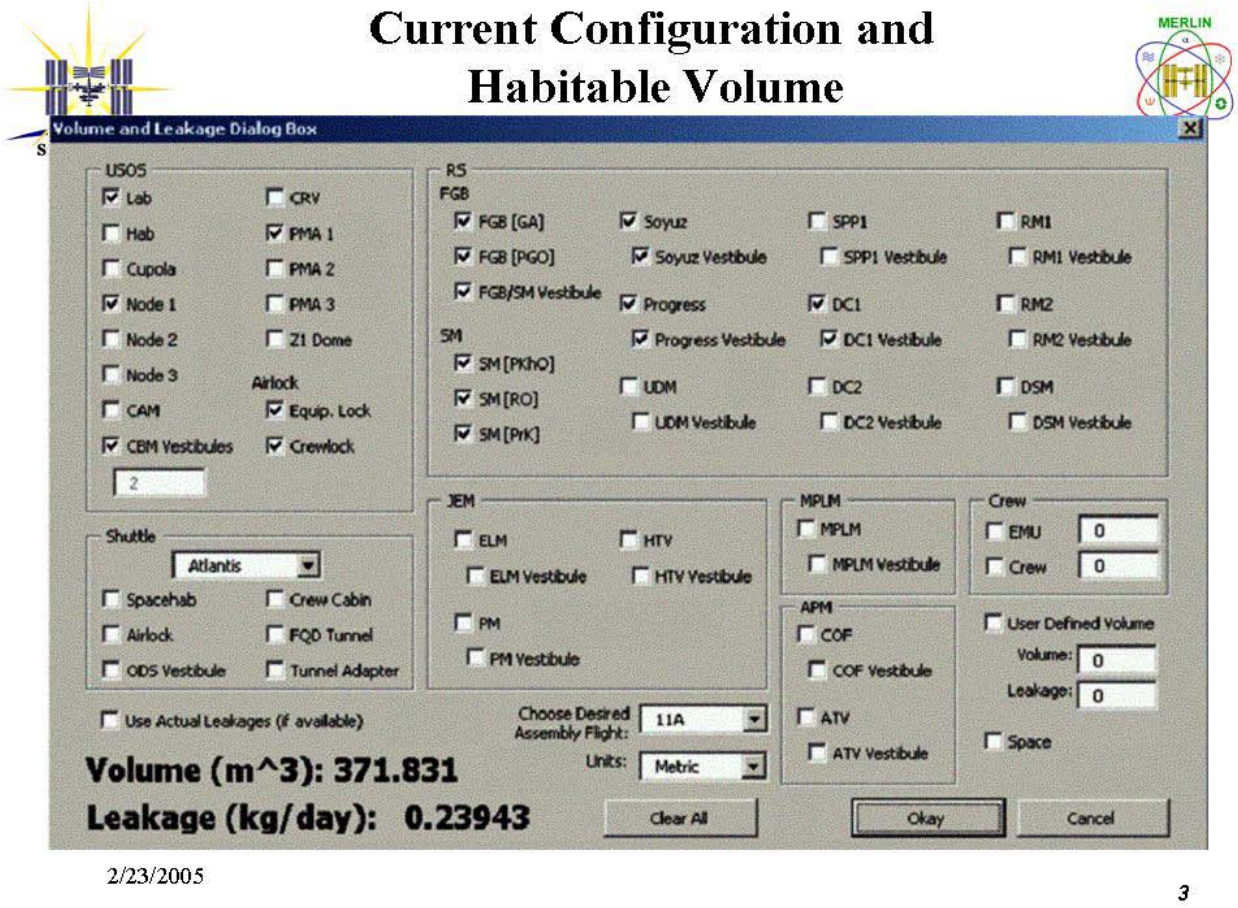
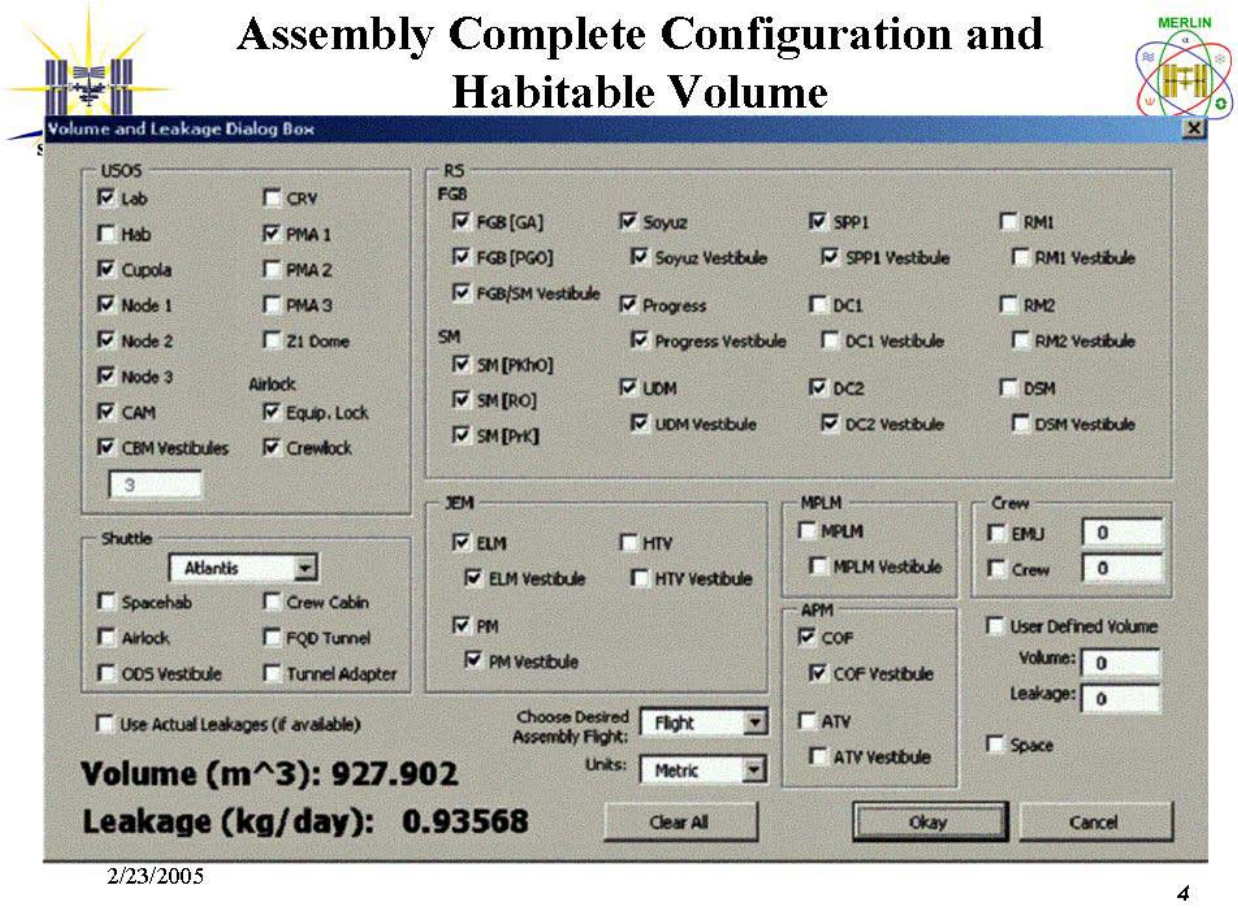
2


Current Configuration and Habitable Volume
MERLIN
Volume and Leakage Dialog Box
USOS
Lab
Hab
Cupola
Node 1
Node 2
Node 3
CAM
CBM Vestibules
2
CRV
PMA 1
PMA 2
PMA 3
Z1 Dome
Airlock
Equip. Lock
Crewlock
RS
FGB
FGB [GA]
FGB [PGO]
FGB/SM Vestibule
Soyuz
Soyuz Vestibule
Progress
Progress Vestibule
SM
SM [PkhO]
SM [RO]
SM [PrK]
UDM
UDM Vestibule
SPP1
SPP1 Vestibule
DC1
DC1 Vestibule
DC2
DC2 Vestibule
RM1
RM1 Vestibule
RM2
RM2 Vestibule
DSM
DSM Vestibule
JEM
ELM
ELM Vestibule
HTV
HTV Vestibule
PM
PM Vestibule
MPLM
MPLM
MPLM Vestibule
APM
COF
COF Vestibule
ATV
ATV Vestibule
Crew
EMU
0
Crew
0
User Defined Volume
Volume:
0
Leakage:
0
Space
Shuttle
Atlantis
Spacehab
Airlock
ODS Vestibule
Crew Cabin
FQD Tunnel
Tunnel Adapter
Use Actual Leakages (if available)
Choose Desired Assembly Flight:
11A
Units:
Metric
Volume (m^3): 371.831
Leakage (kg/day): 0.23943
Clear All
Okay
Cancel
2/23/2005
3


Assembly Complete Configuration and Habitable Volume
MERLIN
Volume and Leakage Dialog Box
USOS
Lab
Hab
Cupola
Node 1
Node 2
Node 3
CAM
CBM Vestibules
3
CRV
PMA 1
PMA 2
PMA 3
Z1 Dome
Airlock
Equip. Lock
Crewlock
RS
FGB
FGB [GA]
FGB [PGO]
FGB/SM Vestibule
Soyuz
Soyuz Vestibule
Progress
Progress Vestibule
SM
SM [PkhO]
SM [RO]
SM [PrK]
UDM
UDM Vestibule
SPP1
SPP1 Vestibule
DC1
DC1 Vestibule
DC2
DC2 Vestibule
RM1
RM1 Vestibule
RM2
RM2 Vestibule
DSM
DSM Vestibule
Shuttle
Atlantis
Spacehab
Airlock
ODS Vestibule
Crew Cabin
FQD Tunnel
Tunnel Adapter
JEM
ELM
ELM Vestibule
PM
PM Vestibule
HTV
HTV Vestibule
MPLM
MPLM
MPLM Vestibule
APM
COF
COF Vestibule
ATV
ATV Vestibule
Crew
EMU
Crew
0
User Defined Volume
Volume:
Leakage:
Space
Use Actual Leakages (if available)
Choose Desired Assembly Flight:
Flight
Units:
Metric
Volume (m^3): 927.902
Leakage (kg/day): 0.93568
Clear All
Okay
Cancel
2/23/2005
4

Air & Water Quality Limits

- SMAC values in various system and ISS configuration
  - 180 day SMAC= 0.002 mg/m^3
  - 30 day SMAC = 0.012 mg/m^3
  - 7 Day SMAC = 0.025 mg/m^3
  - 1 Day SMAC =0.08 mg/m^3
- Concentration of GA in collected humidity condensate and affects on ECLS hardware.
- Current RSOS SRV-K limit = 5mg/L [GA]
- Current USOS WPA limit = 5mg/L [GA]

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5

60 Possible System configuration Scenarios

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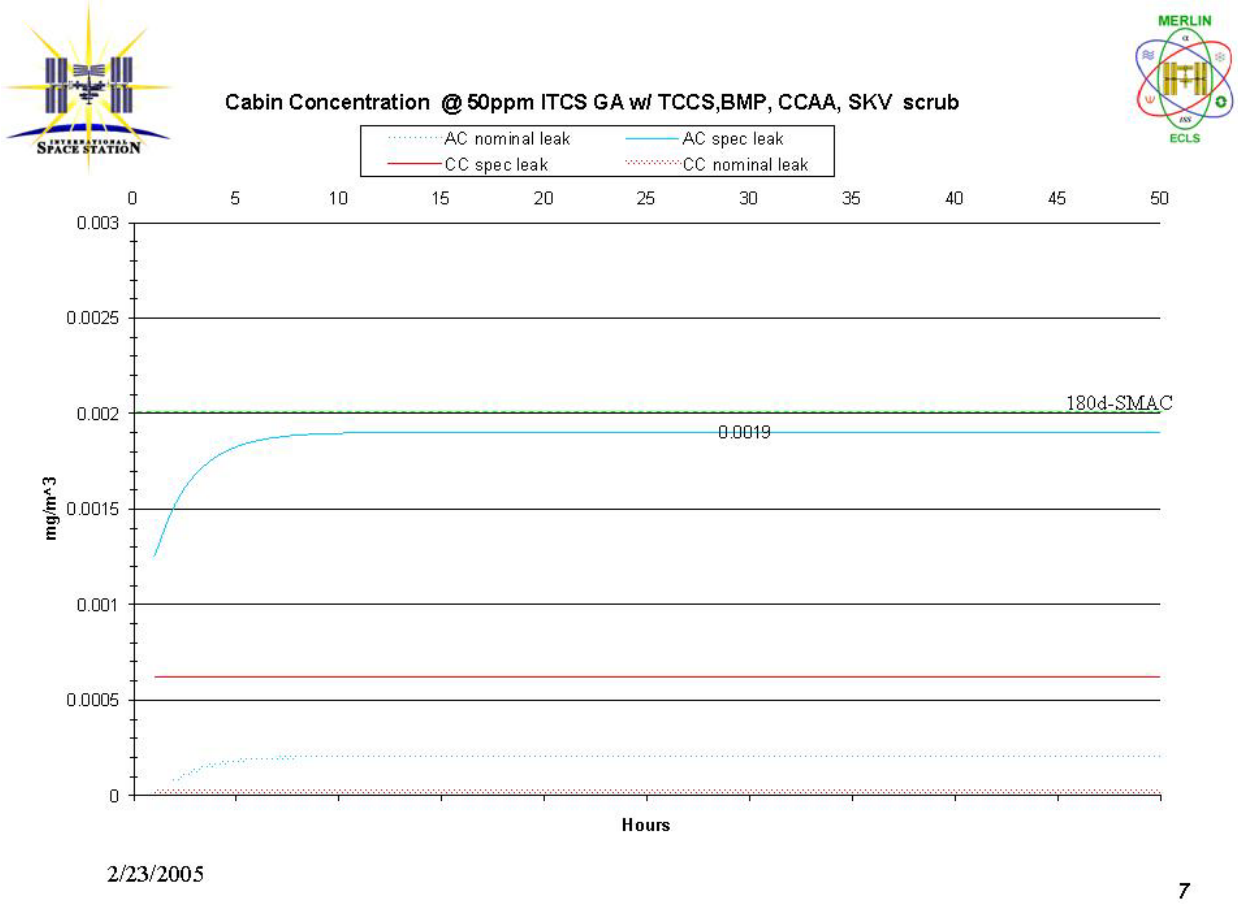
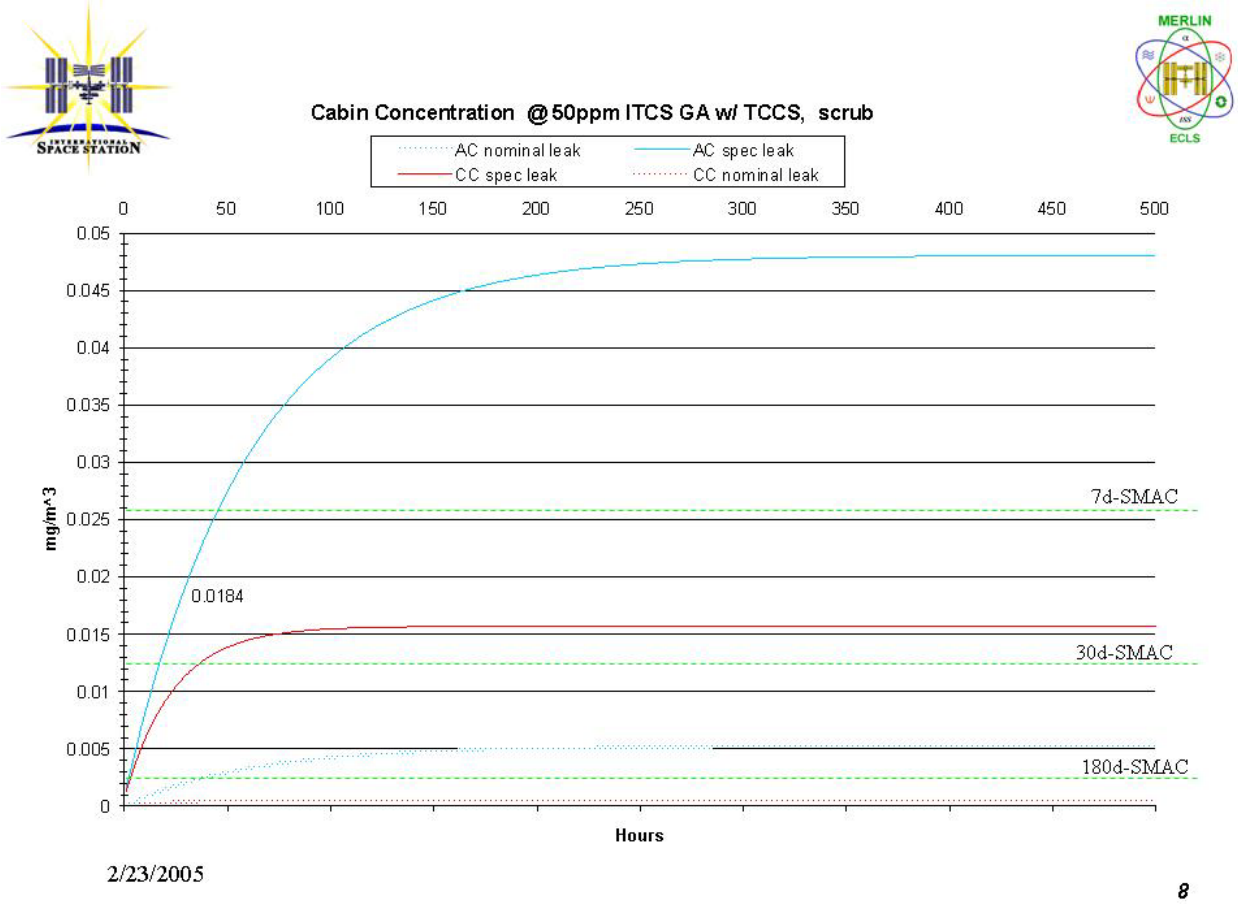
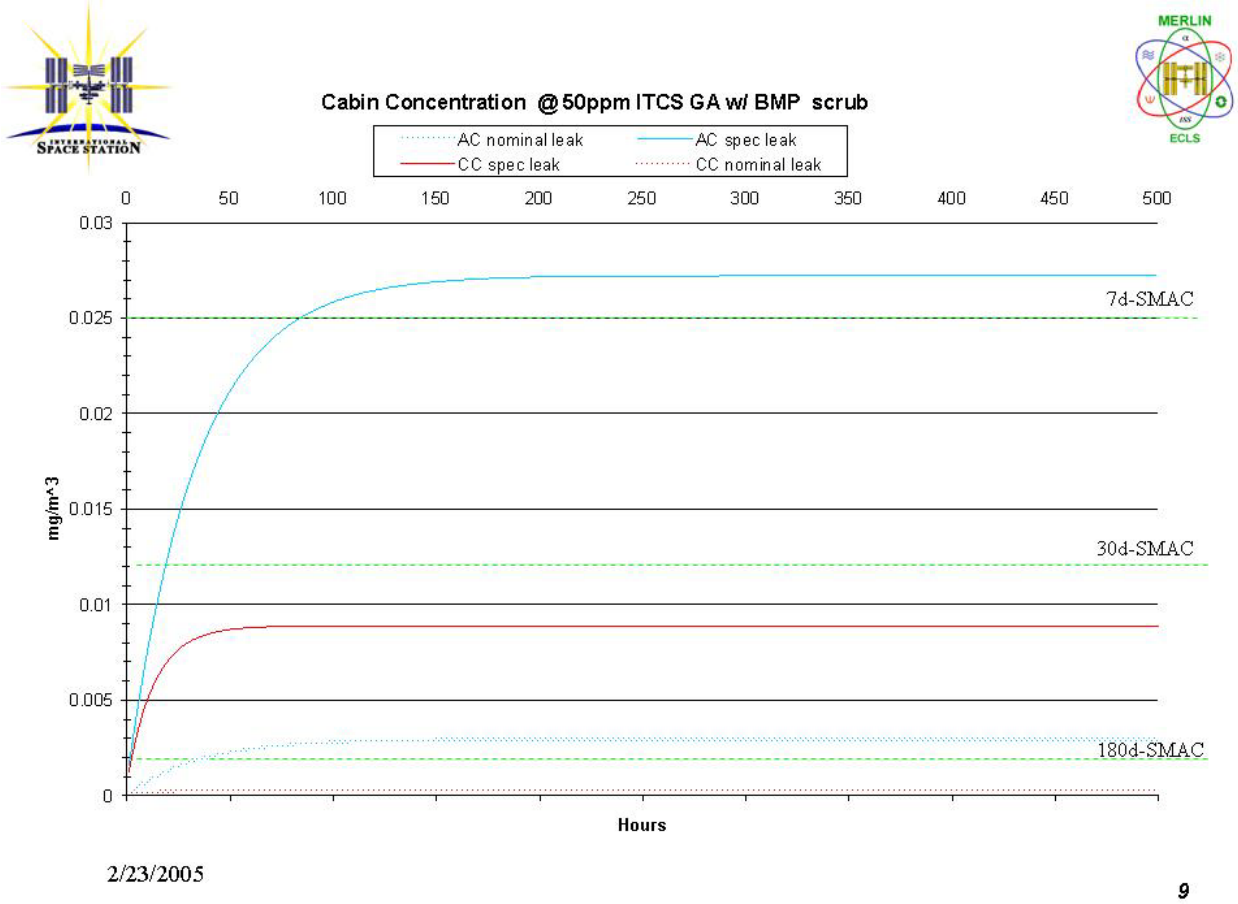
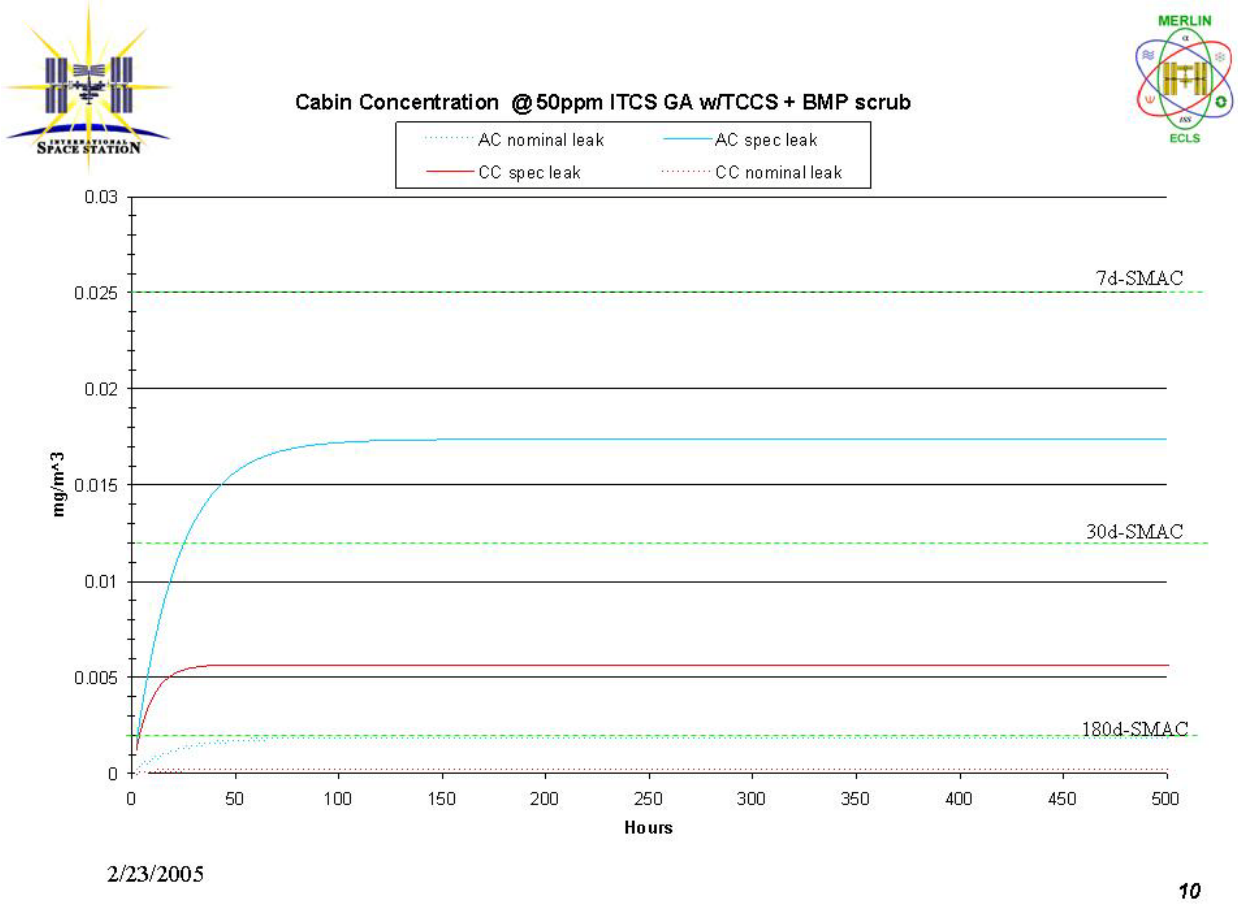
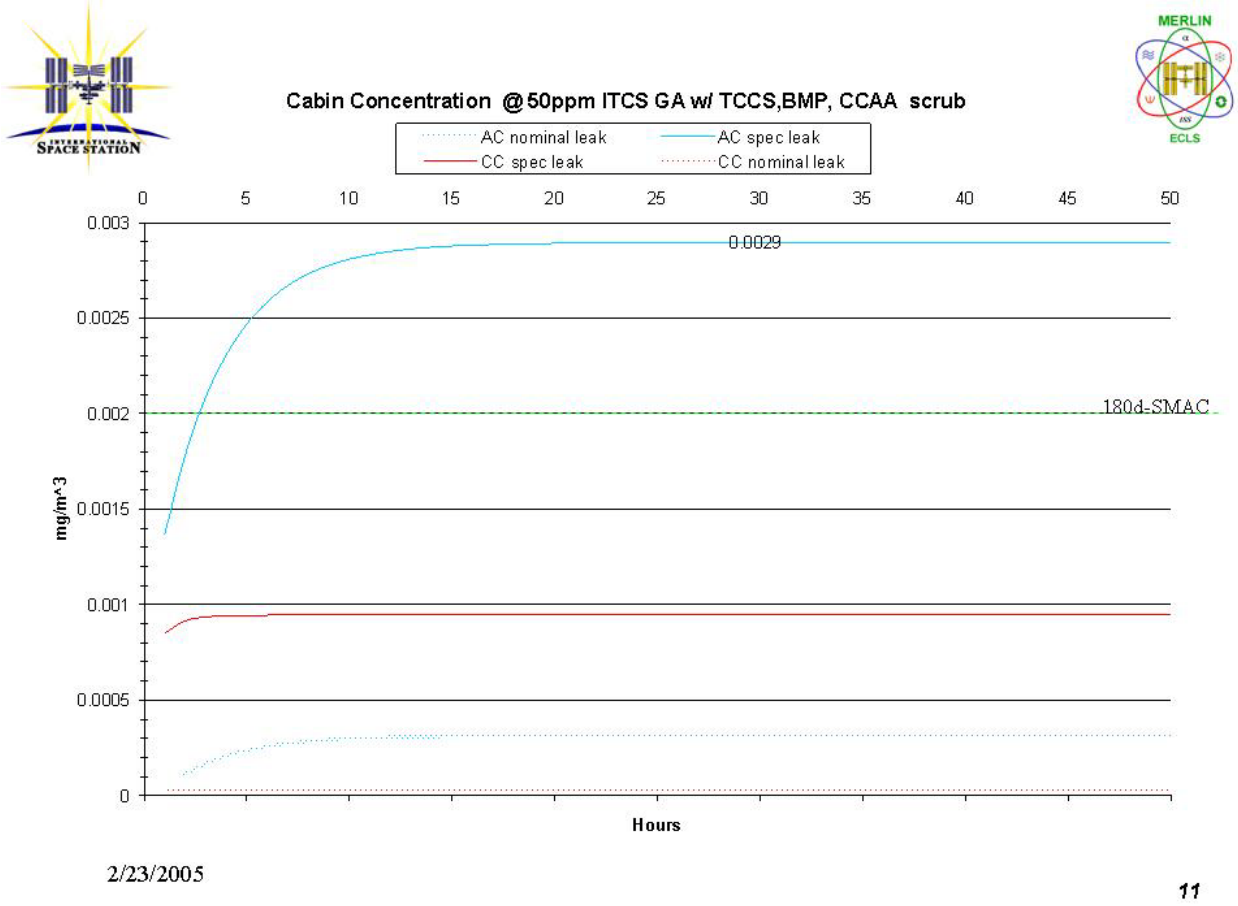
6

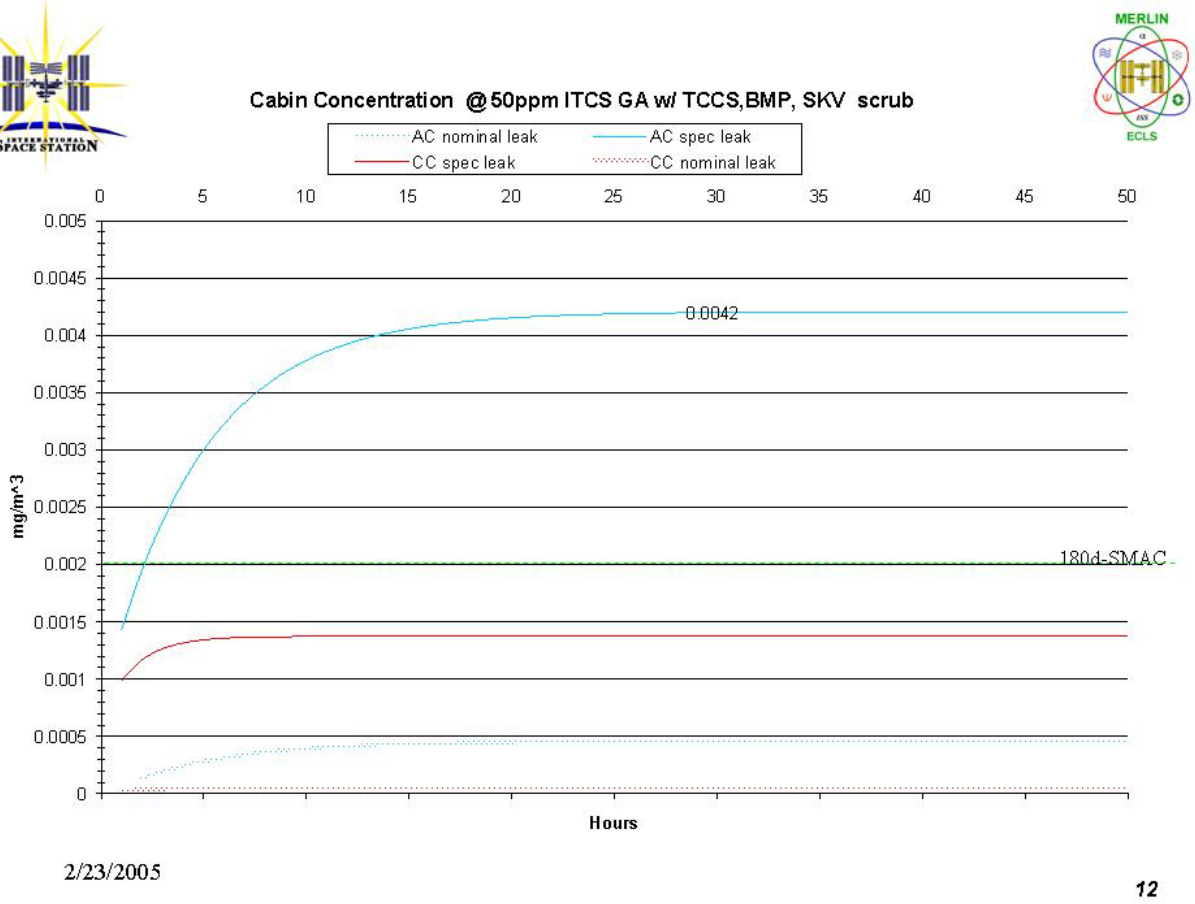

Cabin Concentration @ 50ppm ITCS GA w/ TCCS,BMP, CCAA, SKV scrub
AC nominal leak
AC spec leak
CC spec leak
CC nominal leak
0
5
10
15
20
25
30
35
40
45
50
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
mg/m^3
180d-SMAC
0.0019
Hours
2/23/2005
7

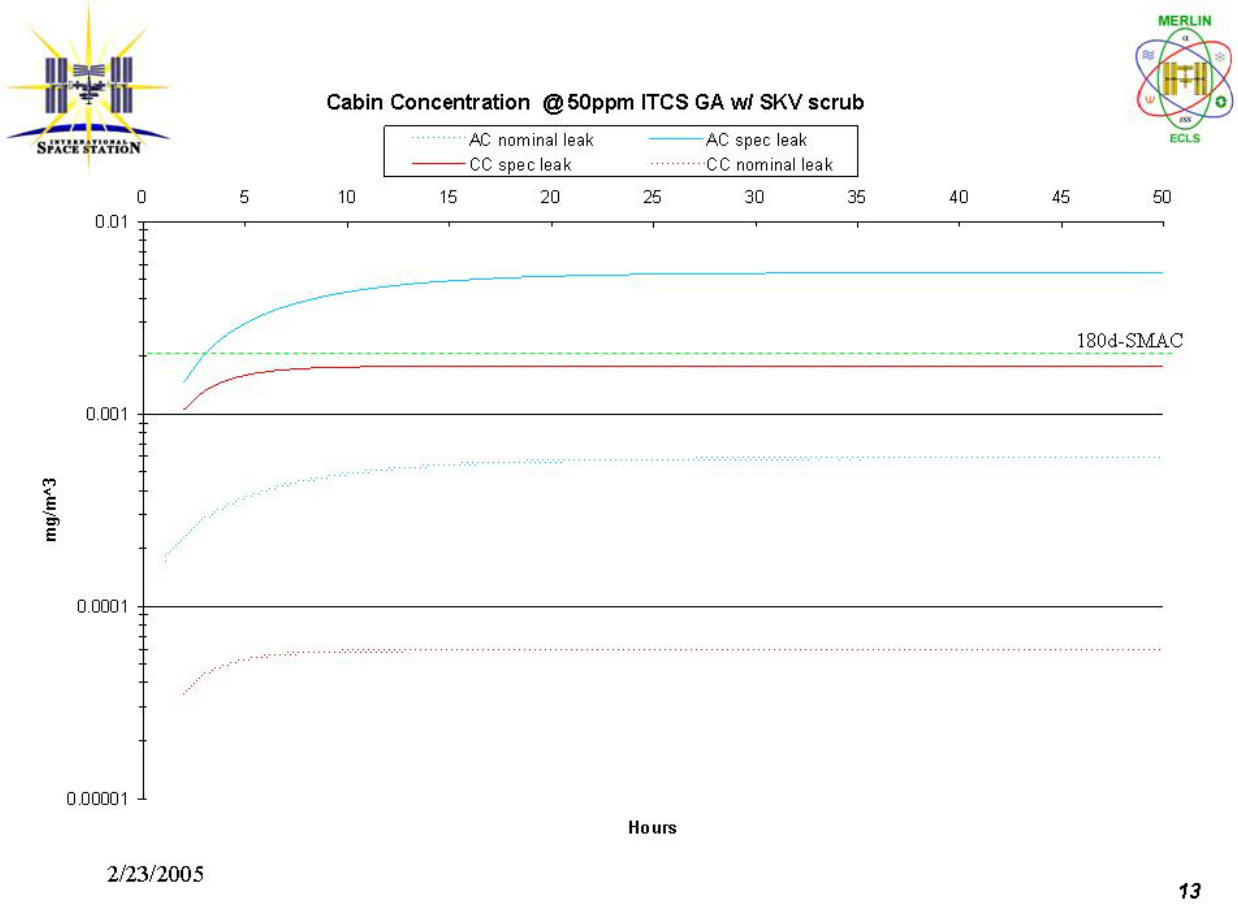

Cabin Concentration @50ppm ITCS GA w/ TCCS, scrub
AC nominal leak
AC spec leak
CC spec leak
CC nominal leak
0
50
100
150
200
250
300
350
400
450
500
0.05
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
mg/m^3
7d-SMAC
30d-SMAC
180d-SMAC
0.0184
Hours
2/23/2005
8

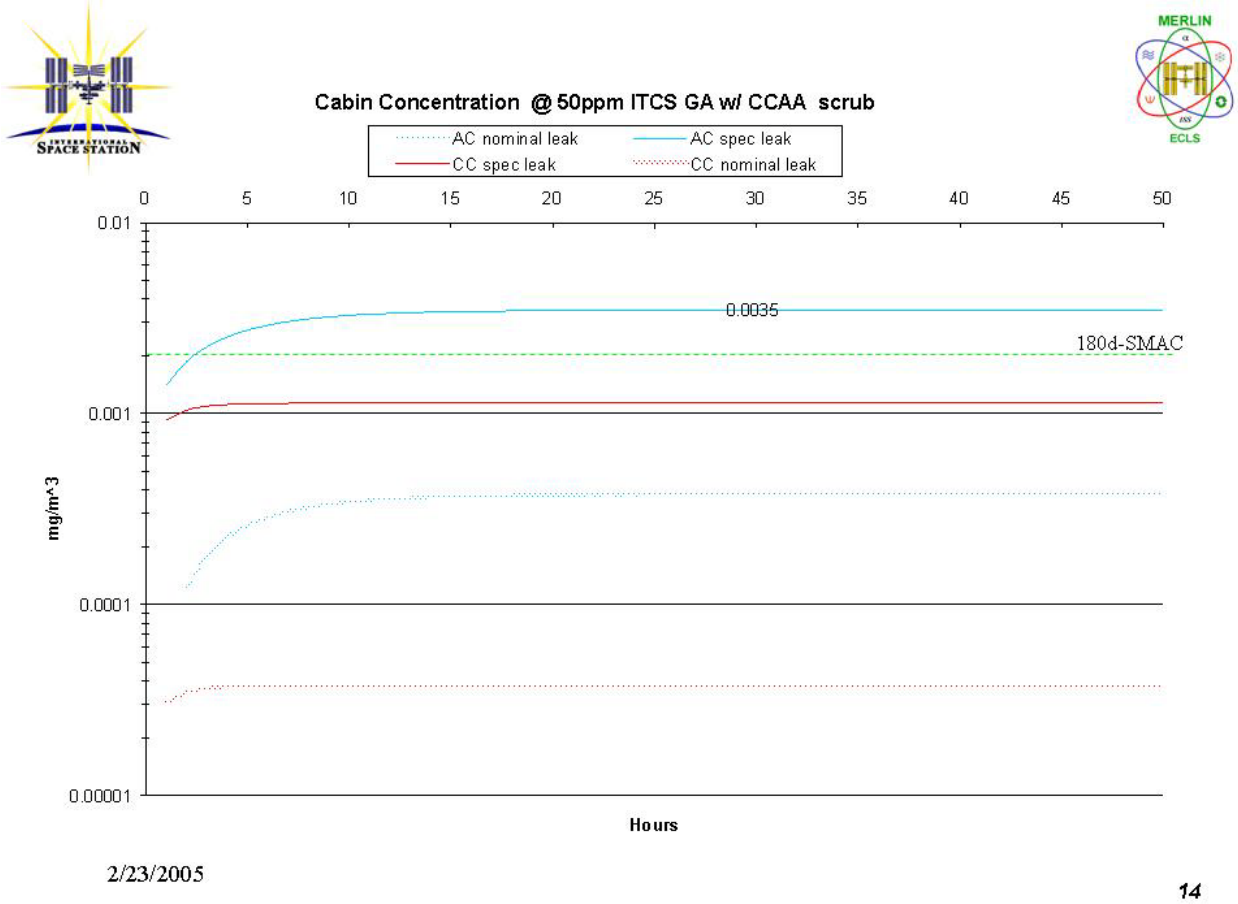

Cabin Concentration @ 50ppm ITCS GA w/ BMP scrub
AC nominal leak
AC spec leak
CC spec leak
CC nominal leak
0
50
100
150
200
250
300
350
400
450
500
0.03
0.025
0.02
0.015
0.01
0.005
0
mg/m^3
7d-SMAC
30d-SMAC
180d-SMAC
Hours
2/23/2005
9

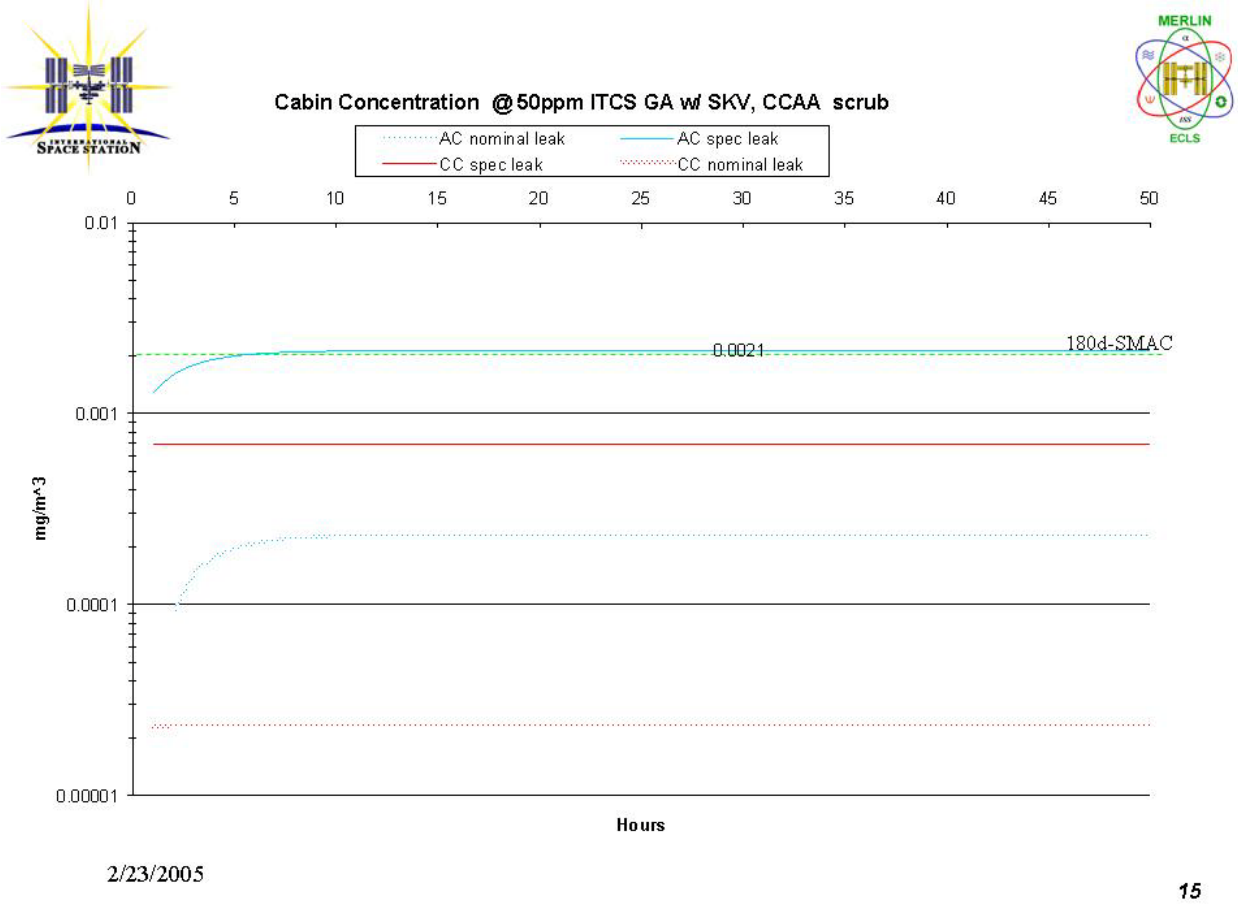

Cabin Concentration @50ppm ITCS GA w/TCCS + BMP scrub
AC nominal leak
AC spec leak
CC spec leak
CC nominal leak
0.03
0.025
0.02
0.015
0.01
0.005
0
mg/m^3
7d-SMAC
30d-SMAC
180d-SMAC
0
50
100
150
200
250
300
350
400
450
500
Hours
2/23/2005
10

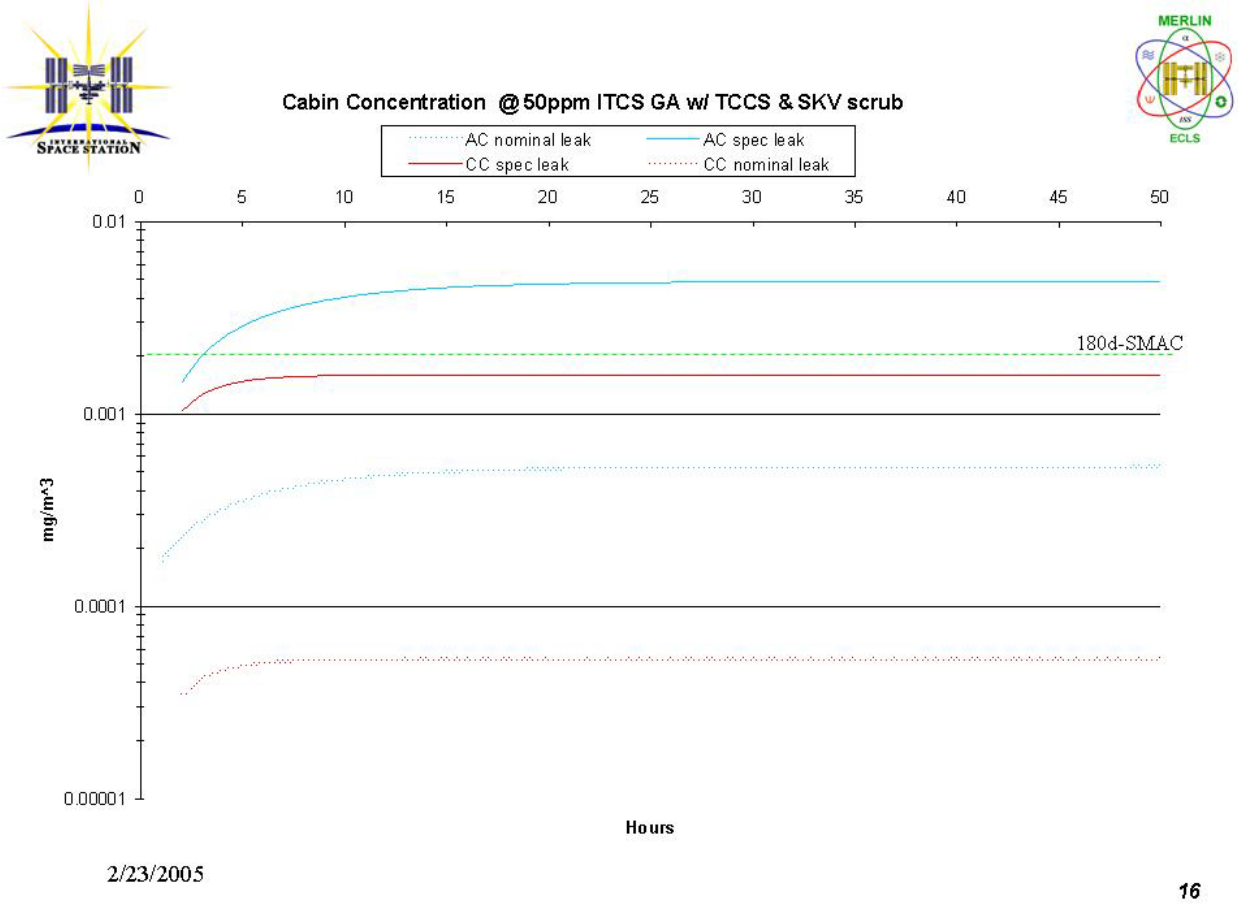

Cabin Concentration @50ppm ITCS GA w/ TCCS,BMP, CCAA scrub
AC nominal leak
AC spec leak
CC spec leak
CC nominal leak
0
5
10
15
20
25
30
35
40
45
50
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
0.0029
180d-SMAC
mg/m^3
Hours
2/23/2005
11

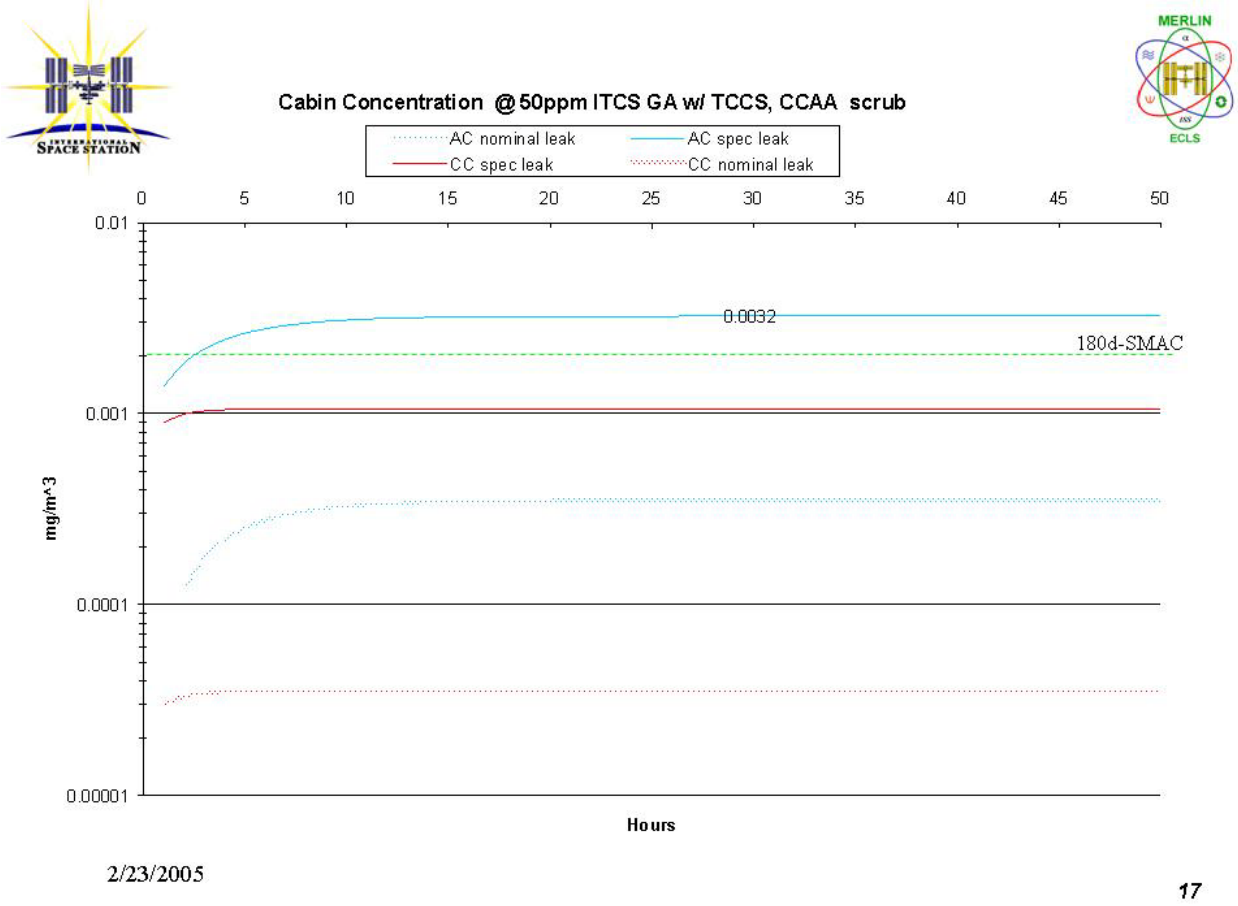

Cabin Concentration @50ppm ITCS GA w/ TCCS,BMP, SKV scrub
AC nominal leak
AC spec leak
CC spec leak
CC nominal leak
0
5
10
15
20
25
30
35
40
45
50
0.005
0.0045
0.004
0.0035
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
mg/m^3
0.0042
180d-SMAC
Hours
2/23/2005
12

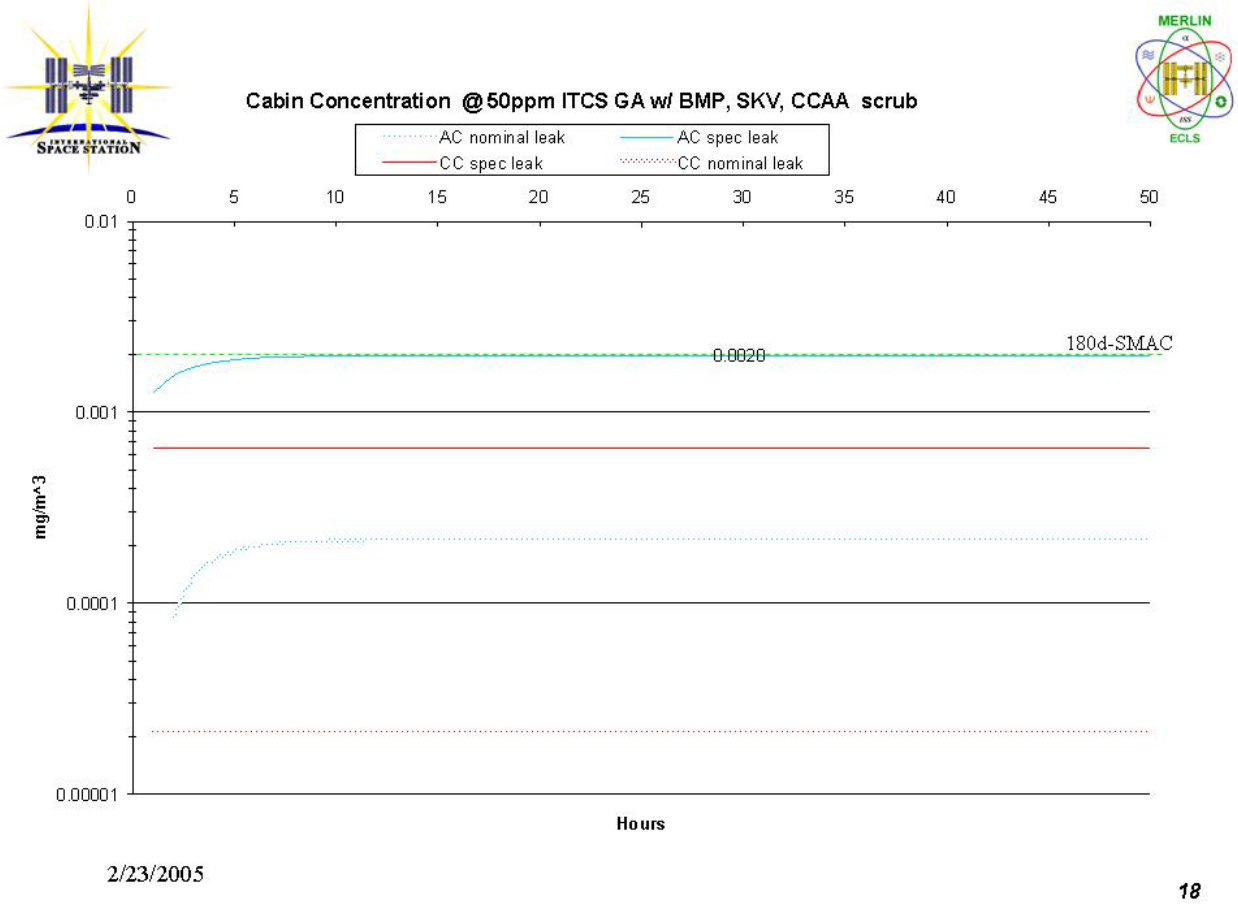

Cabin Concentration @ 50ppm ITCS GA w/ SKV scrub
AC nominal leak
AC spec leak
CC spec leak
CC nominal leak
0
5
10
15
20
25
30
35
40
45
50
0.01
0.001
0.0001
0.00001
180d-SMAC
mg/m^3
Hours
2/23/2005
13

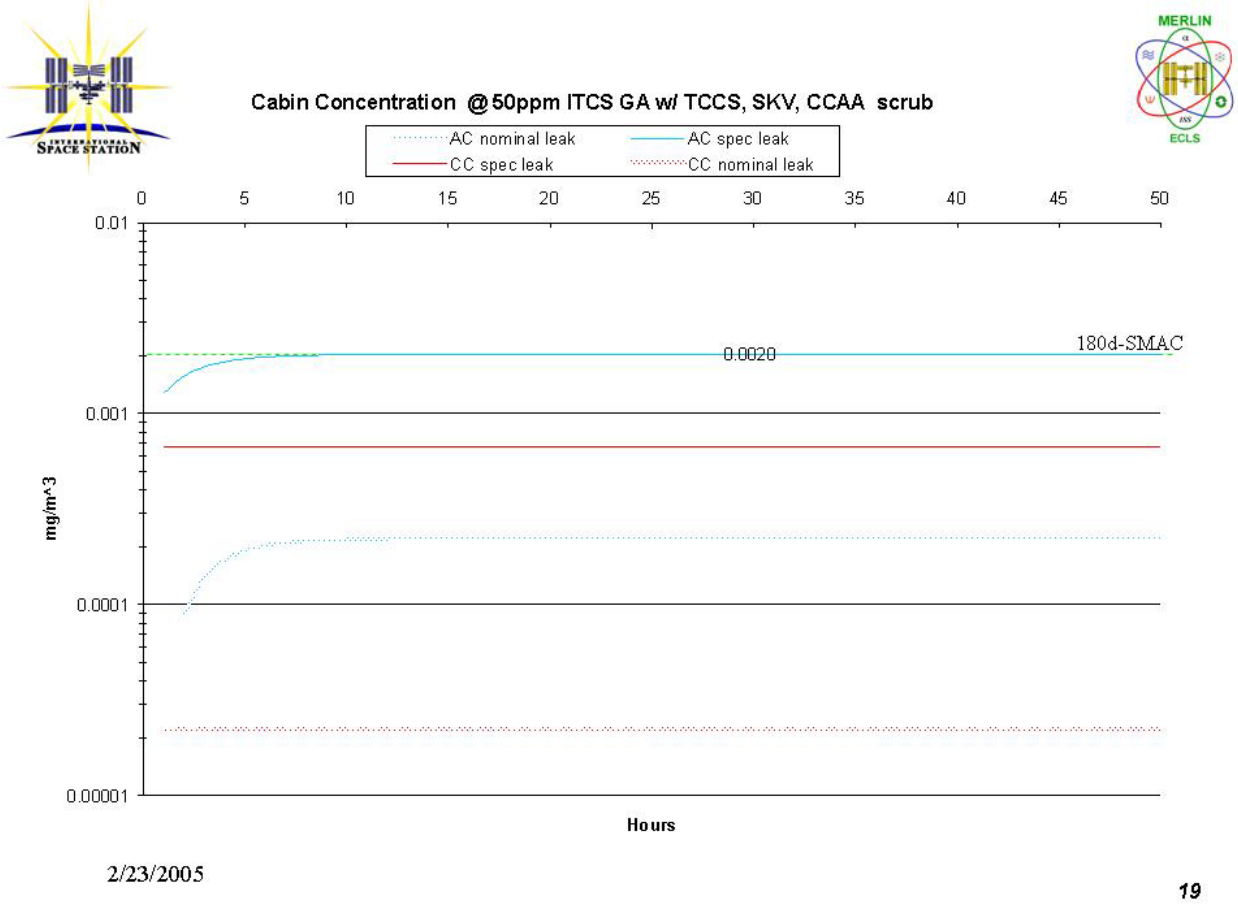

Cabin Concentration @ 50ppm ITCS GA w/ CCAA scrub
AC nominal leak
AC spec leak
CC spec leak
CC nominal leak
0
5
10
15
20
25
30
35
40
45
50
0.01
0.001
0.0001
0.00001
0.0035
180d-SMAC
mg/m^3
Hours
2/23/2005
14

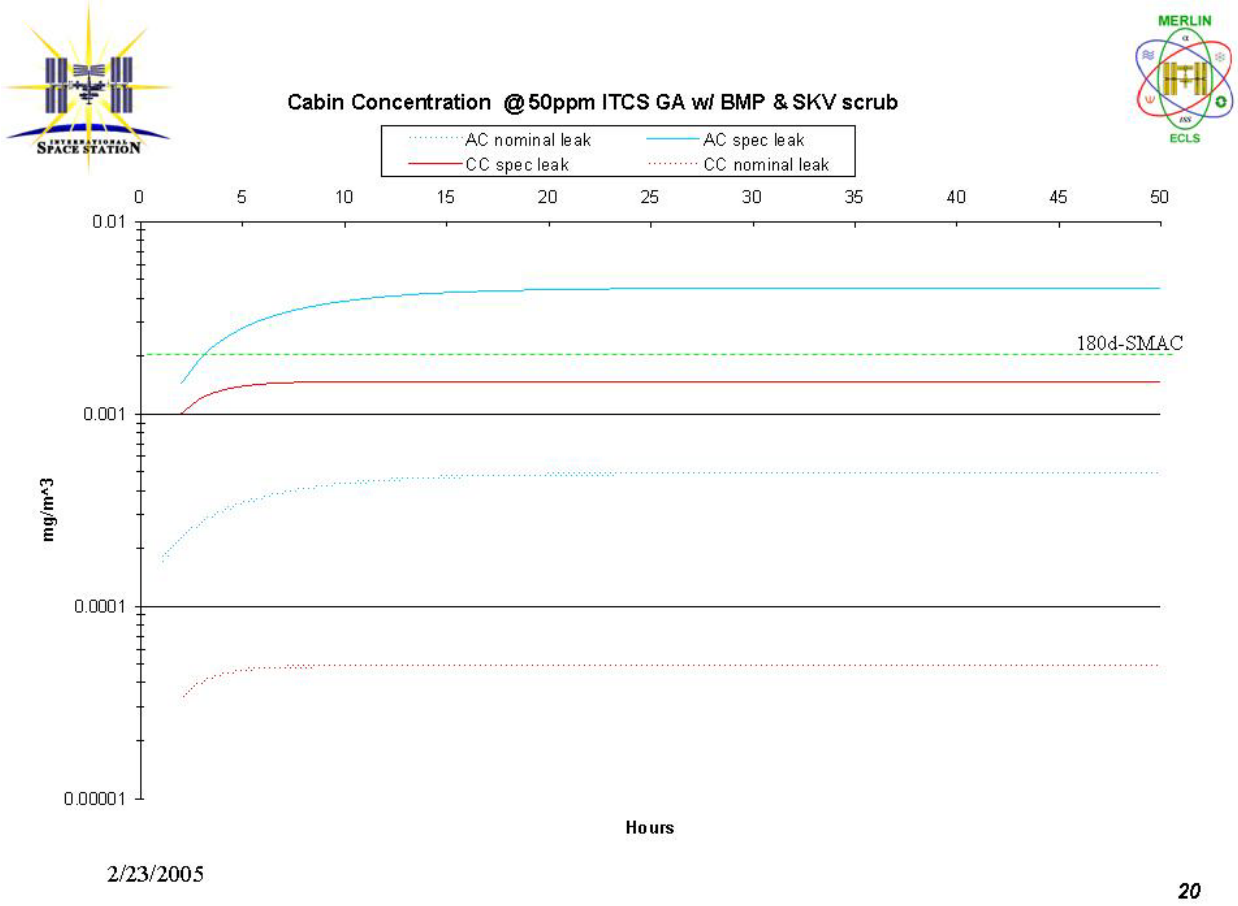

Cabin Concentration @50ppm ITCS GA w/ SKV, CCAA scrub
AC nominal leak
AC spec leak
CC spec leak
CC nominal leak
0
5
10
15
20
25
30
35
40
45
50
0.01
0.001
0.0001
0.00001
mg/m^3
0.0021
180d-SMAC
Hours
2/23/2005
15

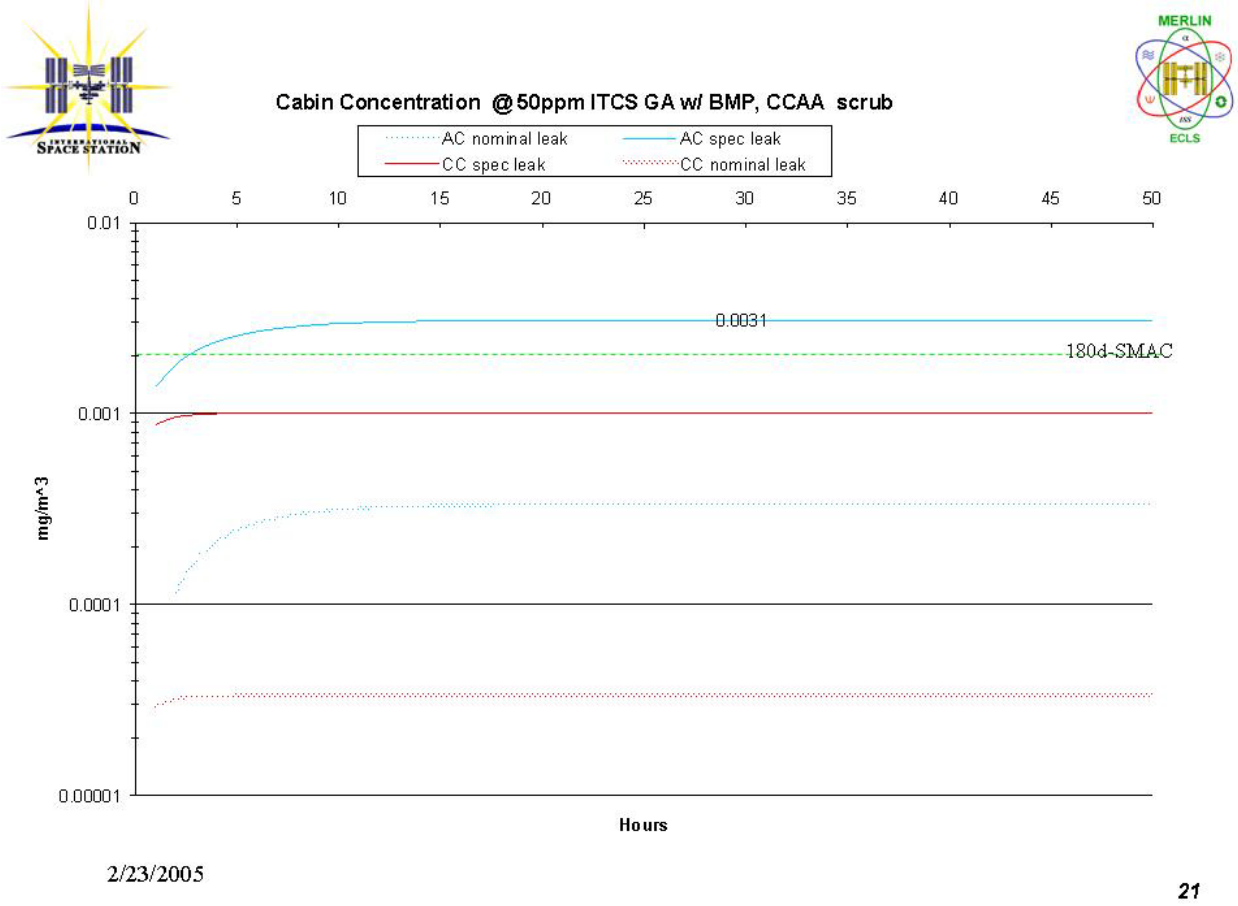

Cabin Concentration @50ppm ITCS GA w/ TCCS & SKV scrub
AC nominal leak
AC spec leak
CC spec leak
CC nominal leak
0
5
10
15
20
25
30
35
40
45
50
0.01
0.001
0.0001
0.00001
180d-SMAC
mg/m^3
Hours
2/23/2005
16


Cabin Concentration @ 50ppm ITCS GA w/ TCCS, CCAA scrub
AC nominal leak
AC spec leak
CC spec leak
CC nominal leak
0
5
10
15
20
25
30
35
40
45
50
0.01
0.001
0.0001
0.00001
0.0032
180d-SMAC
mg/m^3
Hours
2/23/2005
17


Cabin Concentration @50ppm ITCS GA w/ BMP, SKV, CCAA scrub
AC nominal leak
AC spec leak
CC spec leak
CC nominal leak
0
5
10
15
20
25
30
35
40
45
50
0.01
0.001
0.0001
0.00001
mg/m^3
0.0020
180d-SMAC
Hours
2/23/2005
18


Cabin Concentration @50ppm ITCS GA w/ TCCS, SKV, CCAA scrub
AC nominal leak
AC spec leak
CC spec leak
CC nominal leak
0
5
10
15
20
25
30
35
40
45
50
0.01
0.001
0.0001
0.00001
mg/m^3
0.0020
180d-SMAC
Hours
2/23/2005
19


Cabin Concentration @50ppm ITCS GA w/ BMP & SKV scrub
AC nominal leak
AC spec leak
CC spec leak
CC nominal leak
0
5
10
15
20
25
30
35
40
45
50
0.01
0.001
0.0001
0.00001
mg/m^3
180d-SMAC
Hours
2/23/2005
20


Cabin Concentration @50ppm ITCS GA w/ BMP, CCAA scrub
AC nominal leak
AC spec leak
CC spec leak
CC nominal leak
0
5
10
15
20
25
30
35
40
45
50
0.01
0.001
0.0001
0.00001
0.0031
180d-SMAC
mg/m^3
Hours
2/23/2005
21

Mass Balance and Crew driven Maximum Collected Condensate Concentration of GA (Humidity removal device only @ 50ppm leak)

[GA](mg/L)		Bio-driven Humidity condensate collection rate						1.4	L/person/day
		# crew	0	1	2	3	4	5	6
	Leak ml/hr	mg/hr	Assume all GA by mass from leak dissolves into collected condensate including liquid portion of leaked fluid.						
AC (928m^3)	14.7	0.735	0.00	10.06	6.26	4.20	3.02	2.47	2.08
	1.6	0.08	0.00	1.10	0.68	0.46	0.33	0.27	0.23
CC (371m^3)	4.8	0.08	0.00	3.29	2.04	1.37	0.99	0.81	0.68
	0.16	0.008	0.00	0.11	0.07	0.05	0.03	0.03	0.02

Table values in mg/L of collected condensate

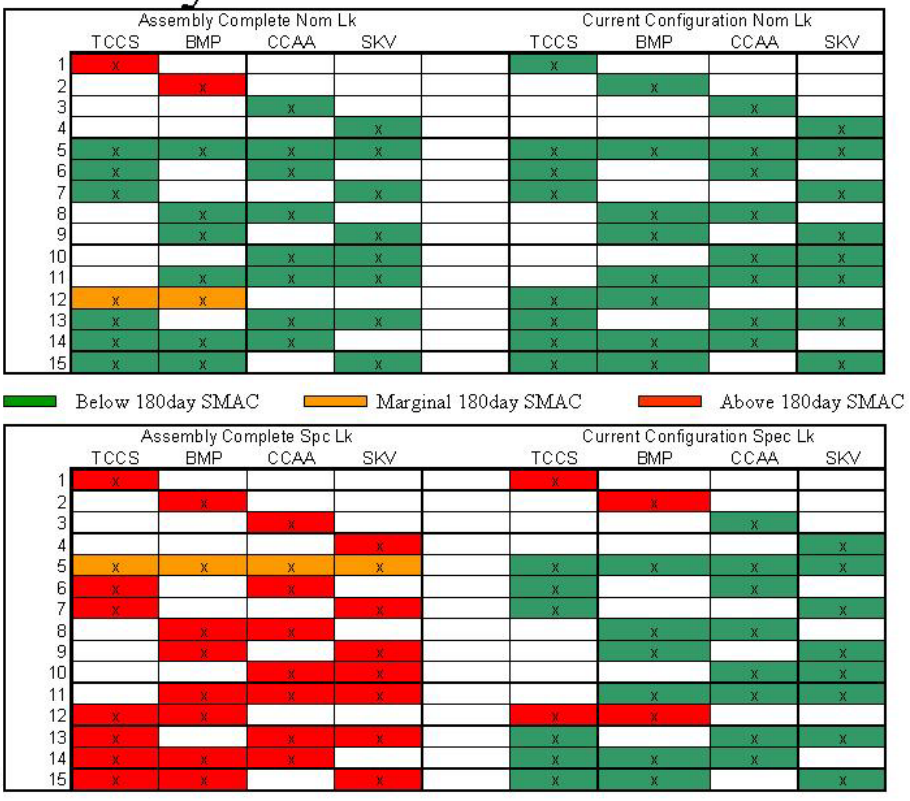
[GA](mg/day)									
		# crew	0	1	2	3	4	5	6
	Leak ml/hr	mg/hr	Assume all GA by mass from leak dissolves into collected condensate.						
AC (928m^3)	14.7	0.735	0.00	17.64	17.64	17.64	17.64	17.64	17.64
	1.6	0.08	0.00	1.92	1.92	1.92	1.92	1.92	1.92
CC (371m^3)	4.8	0.08	0.00	1.92	1.92	1.92	1.92	1.92	1.92
	0.16	0.008	0.00	0.19	0.19	0.19	0.19	0.19	0.19

Table values in mg/day of GA in total collected condensate

2/23/2005

22

Analysis Results Matrices



2/23/2005

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Agreements/Open Discussion

- NASA/Boeing agree with air concentration of GA given certain HW operation.
- NASA/Boeing agree with max GA in condensate values depicted in chart 22.
- NASA/Boeing in open discussions regarding interpretation of SSP41000 and USL PIDS requirements. When and where to apply such requirements.

2/23/2005

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Analysis Interpretation

- Assembly complete spec leak is found to be least desirable scenario.
- 2 conditions which include any combination of TCCS and/or BMP are also found to be non-desirable scenarios. **Note** that this is not a nominal condition under nominal operations. It is, however, expected in an un-manned platform since humidity control is not in effect .
- An assembly complete w/ spec leak and all scrubbing hardware on seems to allow for a marginal scenario. Not a viable recommendation since on-orbit operations have seen less than this optimum condition. (case in point SKV shutdown during 11/2001 time frame)
- All else is found to be within acceptable air and water quality conditions.

2/23/2005

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Current Positions and Ongoing Discussions

- Boeing internal GA scrub model is found to be in coherence with Customer model driven values.
- Customer has concurred with Boeing's cabin air and condensate max conc. of GA.

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Back Up Charts

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Condensate Concentration Sample Calculations

1) Given historical data of condensate removal from crew and utilizing the principles of mass balance:

Assume 1.4 L/person/day of humidity condensate as CCAA heat exchanger removal rate
Assembly complete volume (as we saw on the shared Gas.tools spreadsheet) @ 928 m^3
Nominal leak rate (as agreed to by Mike holt and Rusty Morrison) of 1.6ml/hr (0.08mg GA/hr).
GA concentration in ITCS loops @ 50ppm
Crew = 6 (not including rats ~ 1 persons)

Predicted concentration of [GA] in removed humidity condensate assuming no scrubbing by AR systems and 100% saturation by GA
= [(0.08mg/hr)(24hrs)]/[(6)(1.4L/person/day)] = 0.23 mg GA / L of condensate

Current Configuration volume = 371 m^3
Nominal leak rate 0.16ml/hr (0.008 mg/hr)
GA concentration in ITCS loops @ 50 ppm
Crew = 2

Predicted concentration of [GA] in removed humidity condensate assuming no scrubbing by AR systems and 100% saturation by GA
=[(0.008mg/hr)(24hrs)]/[(2)(1.4L/person/day)] = 0.069 mg GA / L of condensate.

At any given humidity condensate loading total removal is independent of SKV and CCAA operational split.

2)An alternate method of calculating condensate loading is using the CCAA HX moisture removal model.
Assuming Cabin temp of 77 F, LTL 45 F, LTL water flow rate of 559 kg/hr and IMV flow of 730 m^3/hr the calculated cabin moisture content is found to be 3.0 L H2O.

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Sample of Model Spreadsheet

Cab vol m^3(AC)	928					
LKG rt(mg/hr)	0.08	nom				
Scrub Tool	*TCCS*	*BMP*	*SKV*	*CCAA*	**Total (m^3/hr)**	
	15.3	27	132.5	211.56	**386.34**	
hr	50ppm Total GA(mg)	[Cabin]mg/m^3	scrubbed (mg)	Residual (mg)	Net [Cab]mg/m^3	ISS Cabin turnover
1	0.08	0.000215633				
2	0.16	0.000431267	0.083307817	0.076692183	8.26424E-05	
3	0.24	0.0006469	0.03192808	0.124764103	0.000134444	1
4	0.32	0.000862534	0.051941125	0.152822979	0.00016468	2
5	0.4	0.001078167	0.063622446	0.169200533	0.000182328	2
6	0.48	0.001293801	0.070440662	0.178759871	0.000192629	2
7	0.56	0.001509434	0.074420354	0.184339517	0.000198642	3
8	0.64	0.001725067	0.076743243	0.187596275	0.000202151	3
9	0.72	0.001940701	0.078099078	0.189497196	0.0002042	4
10	0.8	0.002156334	0.07889046	0.190606736	0.000205395	4
11	0.88	0.002371968	0.079352378	0.191254359	0.000206093	5
12	0.96	0.002587601	0.079621992	0.191632366	0.0002065	5
13	1.04	0.002803235	0.079779362	0.191853004	0.000206738	5
14	1.12	0.003018868	0.079871217	0.191981787	0.000206877	6
15	1.2	0.003234501	0.079924831	0.192056955	0.000206958	6

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Appendix E. Altran Report

NOTE: **Following Altran's 87-page report is Altran's response to Boeing's comments on the report (dated December 2, 2003).**

Microbial Influenced Corrosion of International Space Station Heat Exchanger Materials: Simulation Test of Worst Case Conditions

Technical Report 02660-TR-001

Revision 0

Volume 1 of 1

Prepared for:

The Boeing Company

December, 2003

altran

Report Record

altran

Report No. : 02660-TR-001 Rev. No.: 0 Sheet No. 2

QA Status: 10CFR50 ☐, 21CFR820 ☐, ISO 9000 ☐, CG ☒, Other ☐ Total Pages: 87

Title: Microbial Influenced Corrosion of International Space Station Heat Exchanger Materials: Simulation Test of Worst Case Conditions

Client: The Boeing Company Facility:

Revision Description: Original Copy

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Originator(s)	Date	Verifier(s)	Date
Signed 12-5-03 Patrick Macuch		Signed 12-4-03 Ockert Van Der Schijff, Pr. Eng., Ph.D.	
Signed 12-4-03 Thomas McKrell, Ph.D.		Signed 12-11-03 Marc Mittelman, Ph.D.	
Signed 12-4-03 Thomas H. Service, Ph.D., P.E.		Signed 12-11-03 Ralph Mitchell, Ph.D.	
		Signed 12-11-03 Ron Latanision, Ph.D.	
		Signed 12-4-03 Thomas McKrell, Ph.D.	

Verification: Verification is performed in accordance with EOP 3.4 as indicated below

☐ Design review as documented on the following sheet or N/A

☐ Alternate calculation as documented in attachment or N/A

☐ Qualification testing as documented in attachment or N/A

Approved for Release:

Signed 12-11-03
Patrick Macuch, Project Manager Date

altran

Laboratory M&TE Index

Report No. 02660-TR-001 Rev.: 0 Sheet: 4

By.: Patrick Macuch Date: Chk.: Deb Wiebe Date:

The following laboratory test equipment control was used in preparation of this report

M&TE Equipment	ID No.	Calibration Date	Calibration Due Date	Operator Name	Test Procedure
Gilson Pipette (1000-P)	R60173L	7-11-2003	6-24-2004	P. Macuch	Manufacturer
Gilson Pipette (200-P)	R53498L	7-11-2003	6-24-2004	P. Macuch	Manufacturer
Gilson Pipette (100-P)	R58856E	7-11-2003	6-24-2004	P. Macuch	Manufacturer
Gilson Pipette (20-P)	R58430D	7-11-2003	6-24-2004	P. Macuch	Manufacturer
Corning pH Meter	7475	6-24-2003	6-24-2004	P. Macuch	Manufacturer
Orion pH Meter	008417	6-24-2003	6-24-2004	P. Macuch	Manufacturer
Monarch Tachometer	1335094	6-24-2003	6-24-2004	P. Macuch	Manufacturer
Fisher Pipette (2-10mL)	N33977	6-24-2003	6-24-2004	P. Macuch	Manufacturer
Perkin Elmer VMP Mulichannel Potentiostat	0055	Project Specific	Project Specific	T. McKrell	TP 11.16
EG&G 273 Potentiostat	07102	Project Specific	Project Specific	T. McKrell	TP 11.16
ZView's DMP 1000 Image Software	14705	10/17/03	4/17/04	V. Roy	TP 11.10
Scanning Electron Microscope	8009-11-08	Project Specific	Project Specific	V. Christie	TP 11.05
Energy Dispersive Spectrometer	3168	3-19-03	3-19-04	V. Christie	TP 11.06
Nikon SMZ-U Stereoscope	111251	6/24/03	6/24/04	T. McKrell	TP 11.10
Nikon Optiphot Microscope	243912	6/24/03	6/24/04	V. Roy	TP 11.10
0.01 mm/Division Stage Micrometer	02B00421	1/5/99	1/5/04	T. McKrell	As Described in Report

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Executive Summary

Experimental results previously reported by Hamilton Sundstrand Corporation (HSC) suggested that components of the International space station (ISS) Internal Thermal Control System (ITCS) heat exchanger could be susceptible to localized corrosion that could potentially result in through-wall penetration. NASA expressed concerns that the ISS water/ammonia based heat exchanger (HX) could be at risk for failure and consequently expose the crew to hazardous chemical agents. This microbial influenced corrosion accelerated test (MAT) was designed to assess and quantify the potential damage from microbial accumulation on the surface of the ISS ITCS heat exchanger materials. The MAT system was designed to simulate the expected "worst case" operating parameters of the ISS ITCS to enable an assessment of material corrosion rates in a 6 month exposure test.

During the MAT the materials were exposed to a laminar flow of 80°F ITCS fluid continuously controlled at pH values of 8.3 or 9.4 and in the presence or absence (controls) of bacteria isolated from on-orbit ITCS fluid samples. Eight independent bioreactors (four test conditions times two sample types) were used in the MAT. Additionally, each bioreactor supplied a triplicate series (30, 90 and 180 day samples) of flow cells containing ISS HX BNi-2 or BNi-3 coupon materials. At the end of each exposure period, flow cells were removed from the MAT system and the coupons assessed for either microbial colonization (one coupon replicate) via culture and biofilm evaluation by SEM or corrosion damage (3 replicates). In this study, a total of approximately 1,000 measurements/evaluations were made on 96 test specimens.

A number of techniques were used to evaluate the extent of corrosion damage of the samples. These techniques included pre- and post-exposure macro-photographs of the samples, SEM of the sample surfaces and cross sections and measurement of braze thickness on cross-sectioned samples using optical microscopy. A total of 72 end point samples were analyzed with the SEM in three regions (nickel, fillet and braze regions) at various magnifications, with no discernable damage or corrosion product deposits observed. The same three regions of the 24 cross-sectioned and metallurgically prepared end point samples were examined with SEM at various magnifications, with no discernable damage observed. The minimum and maximum braze thickness values of the cross-sectioned samples were then determined at five locations, and the fillet and nickel regions photo documented. No corrosion damage of either the fillet or nickel braze regions was observed.

Statistical and graphical analyses were performed on the braze thickness values. The results of the statistical analyses indicated that with a 95% confidence level, but not 99%, the minimum thickness values of two outlying samples were significantly different than the unexposed sample minimum. However, these differences were attributed to scatter because the same analysis showed, with 99% confidence, that a number of samples had increased in thickness by a significant amount as compared to the unexposed samples. Additionally, these two outliers were attributed to scatter because the other analyses did not show any indications of corrosion damage, either material wastage or corrosion product accumulation. The graphical analysis did not show any significant differences between the end point samples and the unexposed samples. The graphical analysis criterion was that no significant difference had occurred provided that when plotted the range defined by an endpoint sample's average and associated standard deviation overlaid the corresponding range defined by the unexposed sample average and standard deviation. Using this criterion to determine if a significant change had occurred a typical detection limit was determined to be 0.10 mil. This limit was not set by the measuring equipment, but on the observed variations in the as-received condition of the test materials.

Additional corrosion analysis was performed under a microbial colony. The ISS microorganisms colonized and produced localized biofilms on the surface of the BNi-2 and BNi-3 test materials under the simulated ISS heat exchanger conditions. Examination of the surface underneath an approximately 1 mm^2 microbial colony on a BNi-2 coupon sample showed no evidence of corrosion damage both in terms of metal loss or corrosion product deposits on the materials.
In the absence of measurable changes in thickness detected over several time points, it is not possible to extrapolate these data to longer exposure times. The time line of the 6 month MAT exposure period was originally determined by the space shuttle's schedule and NASA's requirement to obtain data on the ISS HX material performance prior to authorizing the retrieval of an on-orbit ISS HX for investigation. It is recommended that the MAT challenge study be repeated increasing the exposure time to a minimum of 12 months in order to better assess the corrosion performance of the ISS HX materials and the potential safety risk to the ISS crew.

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1.0 INTRODUCTION

Test results from Hamilton Sundstrand indicate that there is a possibility of localized pitting corrosion of the nickel-based braze filler (BNi – 2)/CRES 347 combination and BNi-3 braze materials used to construct the heat exchangers (HX) of the International Space Station (ISS) (Reference 1). It is reported that the pitting occurs at the location of silver deposits on the braze surface. The water/ammonia HX in the Internal Thermal Control System (ITCS) loop is considered the weakest point in the loop because of the material thickness (~7 mils).

The fluid in the ITCS of the ISS is water-based, containing phosphate (corrosion control), borate (pH buffer) and silver sulfate (microbial control). The ITCS is composed of two loops, the Moderate Temperature Loop (MTL) and the Low Temperature Loop (LTL). The loops operate independently and are maintained at different temperatures, however, the loops can be cross-connected and have been mixed for short periods of time in the ISS. The ISS specifications require the pH of the solution to be maintained at 9.5 +/- 0.5. The pH in the flight fluid has dropped from 9.5 to 8.3 due to diffusion of CO_2 through Teflon hoses. It was reported that the concentration of antimicrobial silver in the solution drops quickly after the solution is in contact with the hardware, due to plating on the metals. This drop in the silver concentration, to negligible levels, has provided an environment conducive to microbial growth. Microbial levels in the fluid range from 10^2 cfu/100 mL to 10^6 cfu/100 mL. The drop in the solution pH coupled with the plating of the silver on the surface of the materials and microbial growth in the fluid are a cause for concern.

The objective of these tests were to assess and quantify the damage from microbial accumulation on the surface of the ISS ITCS heat exchanger materials at pH 8.3 and 9.5. This microbial influenced corrosion accelerated test (**MAT**) was designed to simulate the operating parameters of the ISS ITCS and provide an assessment of in situ "worst case" material corrosion rates over a 6 month time frame.

2.0 INPUT

All BNi-2 and BNi-3 samples were received from Hamilton Sundstrand in a preconditioned state. The composition of the BNi-2 and BNi-3 brazing materials are given by SAE Aerospace Material Specifications AMS 4777 and AMS 4778, respectively (Reference 2). It was reported that the samples were preconditioned by processing them such that silver deposits formed on their surfaces. The thermal treatment process is proprietary and details were not disclosed.

One hundred liters of ITSC fluid was received from Boeing for the MAT exposure testing. The solution was unique in that it was silver free. The pH of the "as received" ITCS fluid was measured to be 9.4 and was stored at ambient temperature until used for exposure tests.

Eight bacterial strains were received from Boeing for use in the MAT exposure tests. It was reported that the strains provided were archived isolates from the ITCS fluid of the ISS HX. Also provided

were 3 recent ISS ITCS fluid samples for microbial characterization and isolation of new stains for the MAT exposure test by Altran Corporation.

3.0 BACKGROUND (DEVELOPMENT OF THE MAT SYSTEM)

3.1. pH probe selection

The pH of the ITCS test solutions were to be maintained at 8.3 or 9.4, within a tolerance of ±0.2, throughout the test period (Reference 3). If required, the pH of the solutions were to be lowered using a CO_2/O_2 gas sparging technique adopted from Hamilton Sundstrand (Reference 4). In order to meet the proposed tolerance, Altran developed a Labview based automated pH control system that would read and record the pH with an in-line pH electrode, and automatically sparge the solution with the CO_2/O_2 gas mixture if required.

The criteria for the selection of a suitable pH electrode were: 1) low leakage rate of electrode electrolyte, 2) low drift and 3) high accuracy. Commercially available pH electrodes are based on liquid, or gel or solid state ion sensitive field effect transistor (ISFET) technologies. Liquid electrodes were immediately ruled out due to their high leakage rate of electrolyte. ISFET probes were initially ruled out due to published data that indicated high drift.

Published leakage rates for gel pH electrodes are given as "negligible", and testing by Altran for both accuracy and drift proved them to be within acceptable limits. Based on these findings, Altran selected an inflow type gel electrode from Cole Parmer (Model 05993-90). This probe showed high accuracy and no indication of drift during 10 days of exposure to ITCS.

Initially, the acceptable chloride concentration in the ITCS test solutions was not specified. However, after the technical readiness review meeting, (1/14/2002) Boeing raised concerns that the selected electrode may introduce unacceptable levels of chlorides, and specified that the end point chloride concentration be no greater than 1 ppm. At this time Altran was also requested to reinvestigate the use of an ISFET probe. An ISFET pH electrode from Topac (Hingham, Ma) was obtained for testing. The probe required the use of an electrolyte to produce a reference voltage.

Accordingly, Altran investigated the chloride leaching characteristics of the selected gel pH electrode and the ISFET probe. Leach testing of the probes immersed in 250 mL of sterile filtered ITCS fluid for 10 days at ambient temperature indicated that the electrodes would leach chloride levels greater than the MAT test tolerance of 1 ppm (Table 1). Additionally, the ISFET probe showed the highest drift of any electrode type tested in ITCS and its accuracy was unacceptable for maintaining the desired tolerance (Figure 1).

These findings suggested that technology for continuous automated pH monitoring and control of the MAT ITCS solutions, that would not add potentially corrosive levels of

contaminants, did not exist. After discussions with Boeing it was decided to perform the pH monitoring manually by periodically measuring grab samples of the ITCS fluids from the MAT system. The system developed for continuous and automated pH control and monitoring was modified for use in the grab sample system. The system was modified such that it read a grab sample's pH and if required automatically sparged the associated bioreactor. To insured that precise control of the pH could be achieved the effects of sparge time and ITCS volume on pH were determined (Figure 2).

3.2. Pre-MAT prototype test loop

Before beginning the MAT, a prototype single flow cell loop was run for 44 days at a pH of 8.3. This served to qualify the data acquisition/pH control system (Labview based), and the system's design and components. The methods and materials employed for the prototype system were similar to those developed for conducting the MAT exposure test (Section 4). The prototype system included a single flow cell loop that contained the following:

- inflow type gel electrode (Cole Parmer, model 05993-90)
- 1L Erlenmeyer flask with 500 mL of pH 8.3 ITCS fluid (bioreactor)
- peristaltic pump with size 17 Norprene™ tubing
- CO_2/O_2 gas mixture (50:50) that automatically sparged into the ITCS for pH control
- BNi-2 and BNi-3 samples
- Labview based data acquisition system, for continuous and automated monitoring and control of pH

The prototype system was operated at ambient temperature and a flow rate of 0.33 ft/s. The test loop was run for 2 weeks without inoculation of microbes isolated from the ISS heat exchangers. Once the pH of the test loop ITCS fluid was shown to be stable for 2 weeks, without CO_2 gas adjustment, the bioreactor was inoculated with the ISS isolates. The test loop ITCS fluid pH was then automatically monitored and controlled by the MAT data acquisition system. The pH was also verified by measurement of grab samples using a Corning model 220 pH meter for comparative purposes.

The pH of the ITCS was stable for approximately 40 days after initial adjustment with the CO_2 gas mixture (Table 2). The pH of the prototype system fluid was also unaffected by the inoculation of the challenge microorganisms. The pH did not require CO_2 adjustment for 12 days following inoculation.

3.3. Flow cell fluid dynamics

Flow cells were designed to hold 4 replicate test coupons in a stream of ITCS solution such that a laminar flow environment was achieved and turbulent currents across the coupon surfaces were minimized (Figures 3-7 and Reference 5). Two critical aspects of the flow cell design were the ITCS solution flow channel dimension and the positioning of the ISS sample coupon materials within the channel. The distance between samples and the cross sectional area above them in the flow channel, allowed for laminar flow characteristics of the ITCS through the flow cells.

During the MAT, BNi-2 and BNi-3 coupon materials were tested separately at pH 8.3 or 9.4 and with and without the presence of the ISS microbial isolates. Drilled holes and cropped corners on the coupons were arranged downstream of the direction of the ITCS fluid flow to minimize eddy current formation. Flow characteristics within the cell was confirmed by injecting crystal violet dye through the cell and observing the flow dynamics recorded on video tape (videotape recording enclosed). The video shows that the device provided a laminar flow environment for the test materials when operated at a flow rate of 0.33 ft/s. The crystal violet dye front showed no sign of turbulence (mixing) as it passed between the in-flow positioned test coupon materials.

3.4. Investigation of electrochemical sample conditioning

The objective of this testing was to investigate the possibility of reproducing the pitting morphology observed in the vicinity of silver deposits during an immersion test of a BNi-2 sample. This immersion test was performed by Hamilton Sundstrand, and is described in Reference 1. BNi-2 and BNi-3 samples were cyclically polarized in sterile filtered ITCS fluid, at room temperature. The pH of the fluid was adjusted to 7.3 and 8.3 by sparging a 50/50 mix of CO_2 and O_2 through the solution. The testing was performed with a potentiostat and a standard flat cell following methods provided by Hamilton Sundstrand (Reference 6) and in general accordance with published standard methods (Reference 7).

The benchmarks used to determine if the electrochemical sample conditioning was successful were:

- Is the removal of material occurring at the Ag deposits?
- Is the pitting uniform over the sample's surface?
- Is it certain that exposure of the base material (347 stainless steel) is not occurring?

The sample conditioning would be considered to be successful only if all these questions could be answered affirmatively.

A number of analytical techniques were used to validate these benchmarks. Specifically, cross sections and surfaces of electrochemically polarized and unpolarized samples were examined using optical microscopy, scanning electron microscopy (SEM), and atomic force microscopy (AFM). The cross-sectioned samples were metallurgically mounted and polished prior to examination.

Each of these analytical techniques has its own strengths and weaknesses. Optical microscopy provides the lowest magnification images of a surface, and SEM provides higher magnification images with a greater depth of field. AFM allows for high magnification, and the added benefit of three-dimensional imaging of surfaces. In using AFM to quantify the damage, depth measurements are referenced upon the material heights adjacent to the affected area. The disadvantage of this technique is that if material loss adjacent to the area of attack has occurred, the depth measurement will be artificially low. The same issue exists with using optical microscopy or SEM to characterize depths of material loss on cross-sectioned samples. However, the interface

between the brazing and base material can be used as reference point. The disadvantage of this technique is that inherent surface roughness and variations in brazing thickness will determine the detection limits of the material loss measurements.

Cyclic polarization curves for BNi-2 and BNi-3 samples immersed in pH 7.3 and 8.3 ITCS fluid are shown in Figures 8-11. Similar behavior is shown in all four of these polarization plots, i.e. a positive hysteresis that is indicative of pitting. Post testing macrophotographs of some of the samples used in this testing are shown in Figures 12-15. As suggested by the polarization plots, the macrophotographs show the corrosion attack to be localized, and in most cases to be associated with the crevice introduced by the seal of the flat cell. Figures 16 and 17 show that in some instances the pitting has penetrated to the base metal. SEM examination of the surfaces before and after testing did not show any of the desired localized pitting in the vicinity of the silver deposits, but rather often showed coarsening of the silver deposits (Figures 18 and 19). AFM images of the before and after conditioning surfaces showed a similar trend (Figures 20-23).

The results of the cyclic polarization testing did not meet the established benchmarks. After discussions with Boeing, it was decided not to electrochemically condition the samples prior to commencing the MAT.

3.5. Microbial strains provided from the ISS

Previously archived bacterial strains isolated from ISS ITCS fluid samples were received from Boeing for inoculation of the MAT exposure test. Table 3 lists the strains received. The isolates were cultured aerobically on R2A medium (Northeast Laboratories, Waterville, ME) at 30°C. The isolates were used to prepare a consortium to inoculate the MAT system.

3.6. ITCS fluid samples provided from the ISS

On-orbit heat exchanger ITCS fluid samples were also provided by Boeing and were cultured for their microbial content using selective and non-selective media. The samples were serially diluted 1:10 into sterile 0.1% (v/v) peptone solution and 0.1 mL of each dilution plated onto R2A, Tripticase soy (TSA), Nutrient (NA), TSA with 5% sheep blood, Sucrose peptone (SPA), Potato dextrose (PDA) and Sabouraud (SAB) agar. Culture plates were incubated aerobically at 30°C for up to 7 days then enumerated. Colonies showing different morphologies were isolated by streak plating single colonies onto the same medium from which they were initially grown. The isolates were incubated aerobically at 30°C for up to 5 days. Table 4 summarizes the culture results of the ISS ITCS fluid samples.

Thirteen bacterial isolates were obtained from the ITCS fluid samples based on their different colony morphologies and were subsequently identified genetically. Isolates were identified using phylogenetic analysis of their 16S rRNA. Briefly, the approximately 1500 base pairs of the microbial 16S rRNA were sequenced using polymerase chain reaction (PCR) methods. The isolate 16S rRNA sequence obtained was then compared to a library of microbial sequences, and a relatedness index was

obtained. Table 5 summarizes the identification of the isolates obtained from the on-orbit ITCS samples. Isolates 02036-2, 02080-1 and 02080-4 were used as inocula for the MAT test in addition to the isolates provided by Boeing.

3.7. Microbial strain maintenance

Single colonies of pure isolates were transferred to fresh R2A media approximately every ten days and incubated aerobically at 30°C for maintenance. Maintenance cultures were used to re-inoculate the MAT tests as needed. Stock seed cultures were prepared from all strains provided by Boeing or isolated from on-orbit ITCS fluid by suspending pure culture cells in 1 mL of sterile 50% (v/v) glycerol solution in cryogenic vials and freezing at -20°C.

4.0 METHODS (MAT EXPOSURE SYSTEM)

The MAT system (Figures 24 and 25) was designed to expose the test coupons to a laminar flow of ITCS solution in the presence and absence (control) of a microbial consortium composed of isolates from the on-orbit heat exchangers. Sterilized Pyrex kettles (bioreactors) containing 2.0 L of sterile filtered (0.2µ cellulose acetate membrane) ITCS solution (Figure 26) were housed in microbiological incubator cabinets set at 80°F (+/- 2.0). ITCS solutions were maintained at pH 8.3 or 9.4 by the addition of a CO_2 gas mixture. The ITCS solution was pumped through each test loop at a linear velocity of 0.33 ft/s using peristaltic pumps (Figure 27). The laminar flow and velocity simulated the ISS HX operating conditions on-orbit. The fluid re-circulated on a continuous basis using size 17 Norprene™ food grade tubing (Cole Parmer), and was continuously mixed using a magnetic stirrer. A triplicate series of flow cells (for 30, 90, and 180 day sample exposure testing) received ITCS solution from a single bioreactor and pump head (Figure 28). The MAT bioreactors were vented through sterile air filters, with silicone tubing connected to a water lock system to prevent back flow addition of air, and alleviate any pressure from CO_2 addition and/or microbial activity (Figure 29).

4.1. Coupon loading into flow cells

Preconditioned BNi-2 and BNi-3 coupons were fixed onto high density polyethylene (HDPE) plastic backing plates (flow cell middle plate) with food-safe silicone adhesive (Dow Corning, RTV 732). The sample coupons were sufficiently separated (distance between samples = 50 x flow channel height) to minimized turbulence. The coupon backing plate allowed for removal of the coupons from the flow cell without damaging or contaminating them with debris from the silicone adhesive after MAT exposure testing. When the silicone adhesive holding the coupons had cured, the flow cells were assembled, filled with 70% isopropyl alcohol (IPA) and allowed to stand for 5 min for disinfection. After disinfection, the flow cells were flushed once with 200 mL of sterile filtered ITCS (approximately ten times the volume of the flow cell), at the appropriate pH, to remove the IPA. The flow cells were then loaded into the MAT system and the flow of ITCS solution started.

4.2. MAT system inoculation

The MAT system bioreactors A1 through A4 were inoculated with the strains isolated from the ISS and with the sulfate reducing bacterium (SRB) *Desulfovibrio desulfuricans*. Pure cultures of bacterial strains, provided by Boeing and isolated from on-orbit ITCS solution, were grown aerobically on R2A medium for 5 days at 30°C for MAT test inoculum preparation. Cells of the pure bacterial strains were harvested from R2A medium and suspended to a concentration of approximately 1 x 10^9 cells/mL (McFarland Standard, Biomerieux) in 5 mL of filter sterilized ITCS solution. The SRB was grown at 30C for 7 days anaerobically in a Forma Model 1025 anaerobic chamber on Baar's medium (Reference 8), and also prepared for inoculation of the MAT system. The cell suspensions were first washed 2 times in ITCS solution by centrifuging the cells at 3500 X G and re-suspending in 5 mL of sterile ITCS solution. The single strain cell suspensions were then combined to prepare an inoculum of all strains for the MAT tests. Fifteen mL of the suspension was used to inoculate each bioreactor for a final concentration of approximately 10^8 cfu/100mL of each stain in the MAT exposure tests. Control bioreactors were not inoculated. Table 6 indicates the inoculum levels of the MAT test strains at the start of the exposure testing (t = 0). The data also shows that all of the strains were inoculated to within 1.3 logs (range 0.0-1.3) of the desired starting concentration of 1 x 10^6 cells/mL.

The on-orbit ITCS fluid samples, provided for inoculation of the MAT test, were not used as direct inocula as originally proposed. The ITCS samples were submitted before initiation of the exposure study and were not suitable for use after long term storage. Bacterial isolates cultured from the on-orbit samples, however, were used in conjunction with the isolates provided by Boeing for inoculation of the MAT tests.

4.3. MAT Exposure Test Monitoring and Control

4.3.1. pH monitoring and control

The ITCS solution was buffered with borate to a pH of 9.5. The pH of the ITCS solutions in the MAT bioreactors was maintained at either 8.3 or 9.4 (+/- 0.2). The pH of ITCS solution in the bioreactors was maintained at 8.3 by sparging a mixture of sterile filtered (in-line vent filter, 0.2µ) compressed CO_2 and O_2 gas (50:50) into the ITCS solutions. A control program (Labview) was used to monitor and adjust the pH of the ITCS solutions via measurement of time-course grab samples (once per week). When the pH increased above 8.3 by the preset tolerance of ≥0.05 units, solenoid valves opened to allow CO_2/O_2 gas flow into the bioreactor ITCS solutions via glass dispersion tubes (sparging stones). The pH values of the ITCS solutions were recorded weekly over the duration of the test (180 days).

4.3.2. Temperature monitoring and control

The MAT system bioreactors and flow cells were housed in microbiological incubator cabinets set at 80°F +/-2.0 (27°C +/-1.1). The MAT system

automatically measured the temperature of the ITCS solutions and the room temperature in which the test was conducted every 10 minutes throughout the 180 day exposure period.

4.3.3. Microbial growth monitoring and control

The number of inoculated bacteria in the bioreactor ITCS solutions was monitored once a week using viable culture methods (Reference 9). Aerobic heterotrophic medium (R2A) was used to determine the viable count of the bioreactors. Re-inoculation of the MAT test bioreactors was performed at Boeing's direction, based upon maintaining a titer of $\geq 1 \times 10^5$ cfu/mL.

4.3.4. Total organic and inorganic carbon (TOC and TIC) monitoring

Forty mL of ITCS solution were collected biweekly from each bioreactor into EPA approved, organic free bottles (Level 1, amber environmental sample vials, Eagle Picher, Miami, OK) and analyzed for TOC and TIC levels (EPA Method 415.1).

4.3.5. Dissolved oxygen (DO) monitoring

Five mL of ITCS solution were collected biweekly from each bioreactor into 25 mL beakers and analyzed for dissolved oxygen (DO) levels using a DO probe (Extech, Model 407510, Waltham, MA).

4.4. MAT Exposure Test End Point Analysis of Coupon Samples at 30, 90, and 180 Days

Coupons were evaluated for corrosion damage and biofilm formation at 30, 90 and 180 days exposure to the ITCS solutions. Flow cells for each time point were removed from the MAT system and the sample backing plates removed. Coupons were removed using a sterile scalpel to cut the silicone adhesive along the edge of each coupon. The second coupon downstream of the flow cell inlet was evaluated for biofilm using viable culture and SEM techniques. The other three replicate coupons were analyzed for corrosion damage.

4.4.1. Biofilm coupon swab viable culture

A sterile cotton tipped swab was used to sample a defined surface area of the biofilm test coupons for viable count determination. A sterile stainless steel plate, with a 1 cm diameter hole, was used as a template for collecting a surface sample using the swab. The area sampled on the coupon, determined by the template hole, was swabbed and the swab placed into 5 mL of sterile ITCS solution of the appropriate pH and 3mm glass beads to aid in dispersion. The biofilm swab sample was sonicating in an ice cold water bath 3 times for 10s each. The sample tube was then vortexed at high speed for 60s. The dispersed sample was serially diluted into ITCS solution and 0.1 mL of each dilution spread-plated in duplicate onto R2A agar. Additionally, 3 mL of the swab dispersion buffer was membrane filtered (Nalgene filter funnel, 0.45µ gridded

cellulose nitrate) and the membranes plated onto R2A. R2A plates were incubated aerobically at 30°C for 7 days then enumerated for colony forming units (cfu).

One mL of the swab dispersion buffer was inoculated into Butlin's medium vials for detection of sulfate reducing bacteria (SRB) (Reference 10). The Butlin's medium vial contained a seal with a butyl rubber septum, for inoculation of ITCS fluid samples via syringes, and a mild steel nail for detection of hydrogen sulfide generation. The inoculated media vials were then incubated at 30°C for 14 days. Formation of a black precipitate on the nail and in the bottom of the vial indicated growth of SRB.

4.4.2. Biofilm coupon SEM

Samples for biofilm analysis using SEM were removed from test coupons by cutting an approximately 0.25" X 0.25" sample section from the overall coupon using isopropyl alcohol cleaned sheet metal snips. The sample section was removed from the upper right corner of the coupon for all SEM biofilm evaluations at each test time point. Coupon sample sections were placed in 2 mL of sterile filtered (0.45µm cellulose acetate membrane) fixing solution containing 3% (v/v) glutaraldehyde in 0.1M sodium cacodylate solution for at least 5 min. Coupons were then fixed in 2 mL of sterile filtered 1% (v/v) osmium tetroxide in 0.1 M sodium cacodylate solution for 2 min. After fixing, the coupons were washed in sterile filtered 0.1M sodium cacodylate solution 3 times for 2 min then rinsed with dilute 1:1 (v/v) 0.1M sodium cacodylate solution. Coupons were then dehydrated by immersing them for 2 min in a series of increasing concentration of ethanol washes beginning with 40% and increasing to 80% in 10% (v/v) increments. The ethanol washing series was then increased in concentration by 5% increments to 100% and coupons stored in the 100% ethanol at 5°C prior to critical point drying (Reference 11).

Dehydrated coupons were critical point dried (CPD) in liquid CO_2 using a critical point dryer (Smdri PVT-3B, Tousimis Research Co., Rockville, MD). After CPD coupons were sputter coated with gold using an SPI-Module Controller and Sputter Coater (Structure Probe Inc., Westchester, PA). The unit is equipped with a pure gold anode for sputtering. Coupons were pumped down to approximately $5x10^{-1}$ mbar and purged with a small amount of inert argon gas during coating. Coupons were then sputtered up to 4 times for 40 seconds each run depending on the coating thickness desired (determined by SEM). Gold-coated coupons were analyzed for presence of biofilm and photographed using a Cambridge Stereoscan Model S-240 scanning electron microscope (Leo Electron Optics, NY).

4.4.3. Characterization of unexposed samples (baseline analyses)

After discussion with Boeing it was decided that the baseline/unexposed samples would not be characterized prior to testing, but rather that unexposed samples would be characterized using the same techniques as employed to

quantify the damage on the end point samples. The basis for this decision was that it would not be possible to characterize the same location prior to and post exposure and that it was believed that the sample processing procedure produced samples that were similar to one another. This allowed for efficient and identical characterization of the unexposed and exposed samples.

The methodology used to analyze the unexposed and exposed samples is given in section 4.4.4. Slight modifications to the optical microscopy of the cross sectioned samples (section 4.4.4.5) methodology were made. These differences were: 1) the unexposed samples were sectioned three times, and all three sections were mounted and the braze thickness quantified as described in section 4.4.4.5, and 2) two BNi-2 and two BNI-3 samples were examined at a total of 12 randomly spaced locations and 5 observed minimum values were documented per sample type. For each sample type one sample was sectioned normal to the Ni strip and the other parallel to the strip. This allowed for the investigation of any anisotropic behavior of the braze thickness, and the determination of any braze thickness variation in the vicinity of the fillet.

4.4.4. End point analysis at 30, 90, 180 days (corrosion damage analyses)

At each end point (30, 90 180 day) eight flow cells were removed from the MAT system, and the samples examined for damage. Tables 7 and 8 show the flow cell numbers, sample numbers, sample type and test conditions for the 30, 90 and 180 day end points. In these tables the flow cell sample position numbers indicate their position in flow cell, i.e. #1 is closest to the inlet and #4 is farthest from the inlet. Each flow cell contained 4 samples, one for microbial analysis and three for corrosion analysis. The microbial sample was taken from the second position from the inlet of each flow cell, position #2.

After removal of the corrosion samples they underwent the following processing and analysis to quantify the corrosion damage:

- cleaning with isopropanol to remove the biofilm
- digital imaging (macrophotographs)
- scanning electron microscope (SEM) examination of the sample surfaces
- sectioning, mounting and metallographically polishing the resulting cross sections
- sputter coating the mounts with gold and examination by SEM
- repolishing the mounts then etching and examination by optical microscopy.

This listing is chronological. For comparative purposes new unexposed samples underwent the same processing and analysis, except as detailed in section 4.4.3.

4.4.4.1. Prior and Post Exposure Macrophotographs

Prior to initiation of the test, digital images of the samples mounted to the flow cells were taken to compare to the samples after testing. Post testing images were taken after the samples were removed from

the flow cell and were cleaned with isopropanol. After removal from the flow cell samples were stored in a desiccator when not being analyzed.

4.4.4.2. SEM of Sample Surfaces

As shown in Figure 30, SEM images of the samples' surfaces were acquired in three regions on the sample. In the braze and nickel strip regions samples were scanned at low magnification until the area showing the observed worst case damage was located. At this location images were acquired at magnifications of 50X, 500X, 1,000X, 5,000X and 10,000X. All SEM magnifications are approximate. Since the fillet area showed a great deal of morphological variation only a 50X image was obtained. Figure 31 shows the data entry sheet used for this analysis.

4.4.4.3. Sample Sectioning and Mounting

From each flow cell, the second sample from the outlet, position #3, of the flow cell was sectioned. The sectioning line passed through the cut corner and nickel strip of the sample to allow for subsequent analysis of these regions. The larger remnant and all other uncut samples were stored in a desiccator, for possible future analysis. The smaller piece resulting from the cut was cold mounted in epoxy and metallographically polished.

4.4.4.4. SEM of Cross Sectioned Samples

As the mounting material was nonconductive the cross sectional mounts were sputter coated with gold to allow for examination by the SEM. Imaging of the three regions shown in Figure 30 was carried out at magnifications of 5,000X and 10,000X. One location on the fillet and nickel strip and three locations on the brazing were examined. Additionally, one location at the cut corner edge was documented. For all regions the perceived worst case locations were documented. The magnifications used did not allow for thickness measurements to be made from these images, therefore the focus of this examination was to locate surface areas exhibiting possible damage. Figure 32 shows the data entry sheet used for this analysis.

4.4.4.5. Optical Microscopy of Cross Sectioned Samples

AFM Figures 20-23 show that the brazing is not flat, therefore for the braze region of the sample the average minimum and maximum thickness and associated standard deviation were determined. These values were chosen to quantify any attack rather than depth measurements because of the subjective nature of depth measurements. Specifically, the topography of the samples makes it difficult to locate small areas of attack and to establish a baseline

from which to measure depths. The circled areas of Figures 157, 158, 162, 183, 188 and 193 of Attachment D show the difficulty associated with setting a reference point. Additionally, these figures show that localized areas of the unexposed samples exhibit a similar morphology as may be expect if localized attack was to have already occurred. Accordingly, braze thickness measurements were chosen because the braze to base material interface provided a reference point that was unaffected by exposure. See section 3.4 for an additional discussion of the difficulties associated with determining a suitable reference point for depth measurements.

After the mounted cross section samples were examined with the SEM the samples were repolished to remove the gold plating and etched to reveal the braze to base material interface. A magnification was chosen such that both the brazing outer surface and brazing/substrate interface could be imaged, allowing for brazing thickness measurements. One location at the cut corner edge, fillet and nickel regions and four locations approximately equally spaced in the braze region were photo documented. Additionally, the brazing was scanned to photo document the observed worst case/thinnest location. Figure 33 shows the data entry sheet used for this analysis.

Braze thickness was measured with an imaging and measurement software program. Measurement of a fixed 0.01 mm distance on a stage micrometer indicated that measurements were reproducible to ± 0.0039 mil. Additionally, the average of ten measurements equaled the calibrated distance of 0.01 mm. All measurements were made in mm, to the ten thousandths digit, and were subsequently converted to thousandths of an inch (mil).

4.4.4.6. Under Biofilm Corrosion Evaluation of BNi-2 Sample 215 (180 days exposure)

BNi-2 sample 215 was chosen for evaluation of corrosion damage associated with biofilm features. This sample was chosen because one of the microcolonies found on its surface was distributed over an area of approximately 1 mm^2. This allowed the mapping of its location on the coupon at lower SEM magnification before and after removal of the biofilm. Most microcolonies that were found on the other test coupon samples were smaller, making them difficult to locate after cleaning. After the microcolony's location had been mapped the biofilm on the coupon was removed by immersing the coupon in an ultrasonic acetone bath for 30 sec.

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5.0 RESULTS

5.1. MAT Exposure System

Tables 7 and 8 indicate the overall MAT exposure test parameters for the ISS sample coupon materials. All samples were maintained at 27°C. The flow rate of 0.33 ft/s was maintained for the duration of the test.

5.1.1. Coupon loading into flow cells

The MAT test coupon samples were glued onto the flow cell backing plates using silicone adhesive. After the adhesive had cured, a subset of the coupons were measured for their height (thickness) above the flow cell backing plates. This was done to determine the average sample height and determine the peristaltic pump rate that would be required to achieve an average flow rate of 0.33 ft/sec of ITCS fluid across the surfaces of the coupon samples when in the MAT system flow cells. Briefly, the cross sectional area above the coupons, relative to the flow cell channel height dimension, required that the ITCS fluid pump be set at a the calculated rate of 49 rpm using size 17 Norprene™ tubing (rated at 2.8 mL/rev). The pump rate was verified by testing the flow rate of water through a peristaltic pump set at 49 rpm. The result was that the pump rate needed to be set at 52 rpm to achieve the required 0.33 ft/sec flow rate of ITCS fluid across the coupon samples.

It was recognized that the Norprene™ tubing would deform over time in the pump housing during the MAT exposure test. The flow rates of the 30 and 90 day pumps were verified after removal from the MAT system. Verification of the 30 day pumps resulted in the 90 and 180 day pump rates to be adjusted to 57 rpm. Verification of the 90 day pumps required the 180 day pump rates to be adjusted to 59 rpm. Verification of the 180 day pumps showed that the flow rate of ITCS did not change from the 90 day verification and was maintained at 0.33 ft/sec to the end of the test.

5.2. MAT Exposure Test Monitoring and Control

5.2.1. pH monitoring and control

Figure 34 illustrates the MAT bioreactors ITCS fluid pH over the course of the exposure period. The data indicate that the ITCS solutions were maintained at pH 8.3 or 9.4 ±0.2 throughout the 180 day exposure test period. The pH of the 9.4 ITCS bioreactors equilibrate to the reported 9.2 pKa of the borate buffering system in the solution after 60 days. Maintenance of the 8.3 pH ITCS bioreactors at the desired tolerance required that they be sparged with the CO_2/O_2 gas mixture at a flow rate of 85 scc/m for one minute approximately twice per week. Table 9 presents the raw pH data of all bioreactors over the course of the exposure test.

There was no significant difference in the bioreactor pH (prior to entering the flow cells) and the flow cell pH (returning to the bioreactors)(Table 10).

5.2.2. Temperature monitoring and control

Figure 35 illustrates the average daily room and MAT test bioreactor/incubator temperatures over the course of the exposure test period. The data show that the bioreactors were maintained at a temperature of 27°C ±1.0 throughout the 180 day exposure test period.

5.2.3. Microbial growth monitoring and control

Figure 36 illustrates the MAT bioreactor viable culture counts over the course of the exposure test period. The test bioreactors were inoculated with the bacterial consortia at the required concentration of 1 x 10^6 cfu/mL. After two weeks of operation, the MAT bioreactors A3 and A4 dropped below the required viable count level of 1 x 10^6 cfu/mL and were re-inoculated with the test strains. All of the inoculated bioreactors dropped approximately one log in growth level after 60 days of operation and maintained levels of approximately 1 x 10^5 cfu/mL to the end of the exposure test period. The bioreactors were maintained at the 1 x 10^5 cfu/mL level and were not re-inoculated with the test species. Table 11 presents the raw viable culture data of all bioreactors over the course of the exposure test.

Control bioreactor B6 showed growth of bacteria after 40 days of operation. Control bioreactors B5, B7 and B8 showed no signs of growth throughout the 180 day test period.

5.2.3.1. Sulfate reducing bacteria recovery from MAT bulk ITCS fluid and biofilm coupon surface swabs

The sulfate reducing bacterium (SRB) *Desulfovibrio desulfuricans* (ATCC 7757) was inoculated into the test bioreactors A1-A4 at the start of the exposure testing. The organism was only recovered from the MAT ITCS fluid of bioreactor A2, one week after it was inoculated. The organism was not recovered from any of the inoculated bioreactors ITCS fluid or from surface swabs of the biofilm coupons at the 30 day exposure time point.

Sterile filtered sodium sulfate (Sigma Chemical, product S6547) solution was added to all of the MAT bioreactors to a final concentration of approximately 1.8 ppm at day 40 of the exposure test.

The SRB was re-inoculated into bioreactors A1-A4 at day 50 of the exposure test. The organism was not recovered from any of the

inoculated bioreactors ITCS fluid or from surface swabs of the biofilm coupons at either the 90 or 180 day exposure time points.

5.2.4. Total organic and inorganic carbon (TOC and TIC) monitoring

Figure 37 illustrates the MAT bioreactor total inorganic carbon (TIC) levels over the course of the exposure test. The pH 8.3 bioreactors (A1, A2, B5 and B6) had significantly higher TIC levels than the 9.4 pH bioreactors (A3, A4, B7 and B8). This is attributed to the controlling of the pH 8.3 bioreactors using the carbon dioxide gas mixture. Bioreactor B5 showed consistently higher TIC levels that the other pH 8.3 bioreactors. The pH 9.4 bioreactors showed a trend of increasing TIC levels throughout the exposure test period and achieved levels of approximately 25 mg/L by the test end. The anomalous 111 day data for Bioreactor A2 is likely due to an error in sampling. Table 12 presents the raw TIC data of all bioreactors over the course of the exposure test.

Figure 38 illustrates the MAT bioreactor total organic carbon (TOC) levels over the course of the exposure period. At the start of the test, the bioreactors ranged from 35.0 to 98.0 mg/L TOC. After 2 weeks of operation, the bioreactor TOC levels dropped an average 26 mg/L. Table 13 presents the raw TOC data of all bioreactors over the course of the exposure test.

5.2.5. Dissolved oxygen (DO) monitoring

Figure 39 illustrates the MAT bioreactor total dissolved oxygen (DO) levels over the course of the exposure test. Table 14 shows that all of bioreactors showed increased DO over the course of the test.

5.3. MAT Exposure Test End Point Analysis of Coupon Samples at 30, 90, and 180 Days

5.3.1. Biofilm coupon swab viable culture

Figure 40 and Table 15 illustrate the MAT biofilm coupon viable counts at 30, 90 and 180 days exposure testing. The data indicate that the coupon materials were colonized at increasing levels over time. The lack of growth obtained from the 180 day coupon sample from control bioreactor B6 was unexpected. The bulk ITCS fluid of bioreactor B6 indicated that the contaminating bacteria were still at the expected levels at the end of the test, Figure 36.

Except for the 30 day biofilm viable count from the coupon of bioreactor A1, there was no significant difference in colonization levels between the different ISS heat exchanger materials under the different test conditions at each time point. The significantly higher viable count from the 30 day bioreactor A1 coupon sample could be attributed to sampling error associated with presence of biofilm slime, evident in the flow cell, and sample clumping. Most of the inoculated flow cells had visual evidence of yellow slime formation on interior surfaces at all time points. The 180 day inoculated flow cell interiors were

documented photographically (Figure 41 and Attachment J). Viable surface swab samples were collected from the interior surfaces of the inoculated 8.3 pH, 180 day flow cells and their associated Norprene ITCS fluid distribution tubing to compare colonization levels with the coupon test materials. Figure 42 illustrates that there was no significant difference between the colonization level of the materials from which the flow cells were manufactured, the tubing used to distribute the ITCS test fluids, and the ISS heat exchanger materials tested.

5.3.2. Biofilm coupon SEM

Attachments A, B and C exhibit SEM photomicrographs of the 30, 90 and 180 day biofilm coupon colonization results for the ISS heat exchanger materials. The images show that the ISS test material surfaces were colonized by the test isolates in the form of biofilms composed of single cells and heterogeneous, localized micro-colonies that ranged from 5 μm^2 (Attachment A, Figure 3) to 1 mm^2 (Attachment C, Figure 2). There was no apparent difference between the braze material and nickel strip of the ISS materials for colonization characteristics. There was also no apparent difference between the test conditions for colonization characteristics of the inoculated test materials.

Except for the 90 day exposure coupon from bioreactor B6, control uninoculated coupons showed no evidence of colonization over the course of the exposure test. The 90 day exposure coupon from bioreactor B6 showed evidence of colonization (Attachment B, Figure 6), however, the 180 day exposure coupon from B6 showed no evidence of single cells or micro-colonies (Attachment C, Figure 6). This was consistent with the lack of recovery of viable bacteria from the swab sample taken from the coupon. The bulk ITCS fluid from B6 at the 180 day time point indicated that the contaminating bacteria were at the expected viable count level.

5.3.3. Characterization of pretest samples (baseline analyses)

Unexposed/baseline samples were characterized using the same techniques as the end point samples, except as noted in section 4.4.3. Results for the unexposed BNi-2 and BNi-3 samples are given in Attachment D. The maximum and minimum brazing thickness as determined by optical microscopy of cross sectioned unexposed samples are given as the zero time point data in Tables 16-19. Additionally, no anisotropic behavior of the braze thickness was observed, but thickening of the braze in the vicinity of the fillet was observed. For this reason locations close to the fillet region of the end point and unexposed samples were not analyzed.

5.3.4. End point analysis at 30, 90, 180 days (corrosion damage analyses)

5.3.4.1. Prior to and Post Exposure Macrophotographs

Prior to and post exposure images of the 30, 90 and 180 day BNi-2 and BNi-3 samples are shown in Attachment E.

5.3.4.2. SEM of Sample Surfaces

Surface SEM images of BNi-2 and BNi-3 samples post 30, 90 and 180 days of exposure are shown in Attachments F, G and H. All surfaces of the corrosion samples were analyzed with the SEM. However, for brevity, only the samples that were subsequently cross sectioned are included in the end point attachments. These samples were chosen because their cross sections were subsequently examined by SEM and optical microscopy. Therefore, their inclusion insures complete documentation, by this report, of one sample per flow cell/test condition.

5.3.4.3. SEM of Cross Sectioned Samples

SEM images of the cross sectioned BNi-3 and BNi-2 samples post 30, 90 and 180 days of exposure are shown in Attachments F, G and H.

5.3.4.4. Optical Microscopy of Cross Sectioned Samples

Attachments F, G and H show optical images of BNi-2 and BNi-3 cross-sectioned samples post 30, 90 and 180 days of exposure. Similar images of unexposed samples are given in Attachment D. It was from these images that minimum and maximum braze thickness measurements were made.

6.0 DISCUSSION

This discussion focuses on the results of the corrosion damage analysis.

6.1. Prior to and Post Exposure Macrophotographs

The 30 day BNi-2 and BNi-3 samples did not show any discernable differences relative to the unexposed samples after exposure. Some of the 90 day samples (324, 326, 327, 338, 356 and 358) did show some darkening post exposure. Orange staining and/or significant darkening of some of the 180 day samples (328, 330, 331, 340, 350, 351, 360, 372) was noted. It is interesting to note that all of these samples are of the BNi-3 type. It was observed that the samples would darken with exposure to light.

6.2. SEM of Sample Surfaces

Attachment D shows the presence of needle like features on BNi-2 samples. EDS of these features showed them to be Cr rich. Similar features were not observed on the

BNi-3 samples. Unexposed and exposed samples also showed pores at grain boundary triple points and a dark phase, especially in the vicinity of the fillet, between grains. At higher magnifications 180 day samples 250, 231 and 262 may show some indications of attack at the braze grain boundaries. However, as shown in the following sections these features were too minor to be quantified by the other analytical techniques employed in this study. In addition, baseline samples that were not exposed showed similar features. As such we are reluctant to conclude that the observed features are indeed corrosion damage. The only way to resolve this is to expose the material for a longer period. If these features are indeed the result of localized corrosion, longer exposure times will result in better resolution and perhaps measurable corrosion damage. If a feature of interest was observed EDS was performed to determine if a corrosion product was present. These EDS spectra did not indicate the presence of corrosion products, which is another telltale indication of active corrosion.

6.3. SEM of Cross Sectioned Samples

Both the BNi-2 and BNi-3 samples did not show any significant differences relative to the unexposed samples, Attachments D, F, G and H. If a feature of interest was observed EDS was performed to determine if a corrosion product was present. These EDS spectra did not indicated the presence of corrosion products.

6.4. Optical Microscopy of Cross Sectioned Samples

6.4.1. Introduction

Tables 16-19 show the average minimum and maximum braze thickness values and associated standard deviation for the approximately evenly spaced locations of the unexposed and end point samples. Figures 43-50 show this data graphically. To facilitate comparison, each sample type is plotted on identical Y axes, and the thickness is plotted over the same range (1.10 mil) independent of sample type. The averages for the end point samples are represented by either a hollow square (minimum) or a filled square (maximum) and the standard deviation by the error bars. The average minimum and maximum thickness of the unexposed samples are represented by horizontal dashed lines. Additionally, a shaded region, with a height defined to be ± the standard deviation of the associated averages, is centered on the baseline average values. The error bars and shaded regions then provide a measure of the scatter associated with the minimum and maximum values, and the distance between the maximum and minimum was a measure of the samples' roughness. Graphing both the unexposed and end point data in this manner allows for comparison of the unexposed and end point samples and of inoculated versus uninoculated effects.

6.4.2. Unexposed Samples

A number of interesting trends concerning the unexposed samples are shown in Figures 43-50. The BNi-2's unexposed average minimum thickness (1.13 mil)

is greater than BNi-3's average unexposed maximum thickness (1.04 mil), i.e. the BNi-2 braze is initially thicker. Additionally, the increased distance between the maximum and minimum average thickness values of the BNi-3 samples indicates that these samples are initially rougher than the BNi-2 samples.

6.4.3. End point samples

The end point braze thickness data was analyzed graphically and statistically.

6.4.3.1. Graphical analysis

The first analysis of the braze thickness data was to define a significant difference as when an "end point sample's" maximum or minimum value that did not graphically fall within the corresponding maximum or minimum shaded region of the unexposed samples. The "end point sample" was defined as the entire range represented by the error bars. As none of the samples showed the formation of corrosion products on their surfaces, end point samples whose thickness values were above the corresponding shaded region were considered not to have experienced corrosion related damage. However, samples whose end point values fell below their corresponding unexposed shaded region were considered to be candidates that may have experienced corrosion related damage. Reviewing Figures 43-50 with this last criterion in mind none of the samples showed corrosion related damage. There are two 180 day samples whose minimum values do almost meet this criterion. Namely, samples 362 (exposed to pH 9.4 and inoculated conditions) and 374 (exposed to pH 9.4 and uninoculated conditions). However, the other analyses performed on these samples did not show any indications of corrosion damage.

As shown above, the criteria used in the graphical analysis sets the detection limit for corrosion related damage. Consider sample 374 for example, for the BNi-3 samples the standard deviation of the minimum thickness of the unexposed samples was 0.06 mil and the standard deviation of sample 374's minimum thickness was 0.04 mil. Therefore, for this sample the detection limit was 0.10 mil.

6.4.3.2. Statistical analysis

As the baseline samples are not smooth and the braze thickness is not uniform from sample to sample, statistics have been used as a second technique to help determine if a significant difference between the baseline and exposed samples existed.

The student's t-test was used to determine if there was a statistically significant difference between the average minimum thickness

measured at time zero (unexposed) and after the different exposure times. This technique initially hypothesizes that there is no significant difference between the two mean values. Calculations can then be made whether to accept or reject this hypothesis based on a desired confidence level. In practice confidence levels of 95% or 99% are typically used. That is, there are about 95 and 99 chances out of 100 that the populations are the same, i.e. no statistical difference in the mean values. Additionally, the student's t distribution is a useful distribution function to compare differences between mean values that were calculated from small sample sizes such as those in this study.

The description, derivation and distribution percentiles for the t-distribution are readily available in standard statistical References 12 and 13. Briefly, consider two sample sizes N1 and N2 that have means and standard deviations given by X1, X2 and s1, s2 respectively. To test the hypothesis that the samples come from the same population the t-statistic, t, is calculated from:

$$t = \frac{X_1 - X_2}{\sigma\sqrt{(1/N_1 + 1/N_2)}} \qquad (1)$$

where:

$$\sigma = \sqrt{\frac{(N_1 s_1^2 + N_2 s_2^2}{\nu}}$$

where ν is the degree of freedom given by ν = N1+N2-2. This t-statistic can then be compared to the students t-distribution for the appropriate confidence level and specific degree of freedom.

Table 20 summarizes the results of the significance testing. Significance tests were performed to determine if the average minimum brazing thickness for each exposure condition was different than the initial baseline average minimum thickness. The tests were carried out at the 95% and 99% confidence limits. The results show in most cases that there is no significant difference at the 95% and 99% confidence levels in the minimum brazing thickness after the exposure conditions. In some cases there is a significant difference at the 95% but not at 99%. This indicates that there may be a significant difference. In the cases where there is a difference at both 95% and 99% it can be concluded that there is a significant difference between the values. In all cases where a 95% and 99% confidence level of a significant difference exists the post

exposure minimum thickness values had actually increased after exposure.

It is interesting to note that the two samples flagged in the graphical analysis, samples 362 and 374, showed a post exposure reduction in the braze thickness that were shown to have a 95% confidence level of being different but not a 99% confidence level. According to this analysis this indicates a possible significant difference. However, as the other analyses performed on these samples did not show any signs of corrosion damage it was concluded that these samples showed no corrosion related damage. The idea that such outliers inherently exist in the population of unexposed samples is further supported by the fact that a number of samples, 254, 338 and 370, show minimum thickness values that are shown to be significantly greater than the baseline samples with a 99% confidence level.

6.4.4. Fillet Region

The fillet regions of the endpoint samples did not show any discernable differences relative to the unexposed samples. The etchant used for the optical braze thickness measurements appeared to preferentially attacked the fillet regions making analysis difficult. Additionally, these areas where not uniform.

6.5. Under biofilm corrosion evaluation of BNi-2 sample 215 (180 days exposure)

Attachment I illustrates the results of biofilm removal from the surface of BNi-2 sample 215 for examination of the condition of the underlying brazing. The position of the targeted microcolony on the coupon was mapped using SEM at low magnification (Figures 1, A and B). The vein-like features extending out from the fillet of the sample served as landmarks for locating the microcolony after cleaning of the coupon. The result of the cleaning was that the microcolony was removed, along with its gold coating, leaving a footprint of the area it had once covered (Figures 1, C and D). The footprint resulted from the contrast between the areas underneath the microcolony that were exposed (dark color) from cleaning and the areas outside of the microcolony that remained coated with gold after cleaning (light color).

Figure 2-E shows the characteristics of the microcolony in the area indicated by the square in Figure 1-B. The microcolony was heterogeneous in its distribution and thickness of microbial cells. Likewise, Figure 2-F shows the area depicted in Figure 2-E with approximately 4X less magnification after cleaning. The contrast between the gold coated areas with the areas cleaned of biofilm are apparent. The brazing of the coupon, underneath where the microcolony was removed showed no damage. Figures 2, G and H show the areas depicted by the arrow and square (respectively) in Figure 2-F. These figures do not show evidence of corrosion attack of the brazing grains or grain boundaries underneath the microcolony. Additionally, no attack in the immediate vicinity of the microcolony was observed.

7.0 CONCLUSIONS

- The MAT System maintained the exposure test parameter constants of pH, temperature, flow rate, and microbial growth levels within the tolerances set for simulating the ISS heat exchanger environment.
- The microbial challenge isolates were able to colonize and produce localized biofilms on the surface of the BNi-2 and BNi-3 test materials under the simulated ISS heat exchanger conditions.
- Examination of the surface underneath and in the vicinity of an approximately 1 mm^2 microbial colony, on a BNi-2 coupon sample, showed no evidence of corrosion associated with this microbial colony.
- The optical braze thickness measurements and the other analyses performed did not show any significant/detectable differences between the unexposed, 30, 90 and 180 day end point samples. Therefore, no significant corrosion damage and specifically no MIC was identified in this study.

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Table 1: Results of Leach Testing of pH electrodes in ITCS

Boeing Lab Sample No:	Sample Description	ITCS Chloride (ppm)	ITCS Silver (ppm)
2003-02-05-00297	Control unexposed ITCS	0.02	0.003
2003-02-05-00298	Topac solid state electrode	28.3	0.003
2003-02-05-00299	Cole Parmer gel electrode	23.5	0.022

(Data provided by The Boeing Company)

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Table 2: Pre-MAT Prototype Test Loop pH

Test Day	MAT System pH	Corning Meter pH	Delta pH (Corning vs. MAT)
1	8.25	8.25	0.00
3	[A]8.37	8.34	0.03
4	8.33	8.24	0.09
5	8.34	8.22	0.12
8	8.26	8.25	0.01
9	8.23	8.24	0.01
10	8.22	8.26	0.04
12	8.15	8.26	0.11
12	[B]8.25	8.26	0.01
17	8.12	8.31	0.19
19	8.07	8.30	0.23
22	7.97	8.29	0.32
23	7.90	8.30	0.40
24	7.88	8.30	0.42
25	7.83	8.30	0.47
26	7.85	8.33	0.48
29	7.89	8.28	0.39
30	7.93	8.33	0.40
32	7.87	8.37	0.50
33	7.84	8.31	0.47
33	[C]8.26	8.31	0.05
36	8.02	8.32	0.30
37	7.91	8.34	0.43
38	7.85	8.29	0.44
39	7.82	8.35	0.53
40	7.83	8.35	0.52
43	7.71	8.34	0.63
44	7.66	8.31	0.65

A = CO_2 Sparge Occurred
B = MAT System Calibration
C = MAT System Calibration and Inoculation

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Table 3: Boeing Isolates for MAT Inoculation

Boeing Stain Number	Altran Identifier	Species	Colony Description
02-0420-0816-3	02660-B1	*Sphingomonas paucimobilis*	yellow, entire, convex
02-0420-0807-2	02660-B2	*Variovorax paradoxus*	large, white, entire, convex
02-0420-0813-1	02660-B3	*Acidovorax delafieldii*	white, entire convex
02-0420-0816-2	02660-B4	*Stenotrophomonas maltophilia*	white entire convex
02-0718-1522-1	02660-B5	*Hydrogenophaga pseudoflava*	small, translucent, entire
02-0718-1509-2	02660-B6	*Pseudomonas stutzeri*	translucent, flat, entire
02-0622-1173-2-1	02660-B7	*Comamonas acidovorans*	small, translucent, entire, convex
01-1219-2119-3	02660-B8	Unidentified Gram negative rod (*Rhizobium*)	white, entire, convex

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Table 4: Results of Culture of ISS Heat Exchanger ITCS Fluid Samples

ISS ITCS Sample ID	Culture Medium	Viable Count (cfu/mL)	Isolate(s)	Colony Description
01510	NA	210	01510-5	orange, irregular margin
	TSA	230	01510-1	white, entire
			01510-2	cream, irregular margin
	R2A	<10	NA	NA
	SPA	30	*No isolate	NA
	SAB	<10	NA	NA
	PDA	<10	NA	NA
	TBA	300	01510-3	small, translucent, entire
			01510-4	white, wrinkled, haemolytic
02036	NA	<10	NA	NA
	TSA	120	02036-3	cream, wrinkled
	R2A	<10	NA	NA
	SPA	10	*No isolate	NA
	SAB	<10	NA	NA
	PDA	<10	NA	NA
	TBA	20	02036-1	small, translucent
			02036-2	white, entire, convex
02080	NA	800	02080-1	cream, entire
			02080-2	orange, radial striae, irregular margin
			02080-3	white, wrinkled
			02080-4	small, translucent, convex
	TSA	20	** No isolate	like 02080-1
	R2A	570	** No isolate	like 02080-1 and 2
	SPA	380	*No isolate	NA
	SAB	<10	NA	NA
	PDA	<10	NA	NA
	TBA	50	02080-5	cream, entire

NA = nutrient agar, TSA = tripticase soy agar, R2A = heterotrophic medium, SPA = sucrose peptone agar, SAB = Sabouraud dextrose agar, PDA = potato dextrose agar, TBA = TSA with 5% sheep blood
* = no apparent slime forming organisms
** = colonies present were like those chosen as isolates from the sample plated on nutrient agar (NA)

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Table 5: Identification of Strains Isolated from ISS Heat Exchanger ITCS Samples

Strain Number	Closest Matches	Similarity Index (0.0 – 1.0)	Phylogenetic Analysis	GenBank Accession No.
01510-1	*Staphylococcus capitus*	1.000	Staphylococcaceae	L37599
	Staphylococcus epidermis	1.000		D83362
	Staphylococcus caprae	1.000		AB009935
01510-2	*Variovorax paradoxus*	0.912	Neisseriaceae	AY127900
	Beta proteobacterium	0.887		AF423075
	Variovorax paradoxus	0.885		AF250030
01510-3	*Variovorax paradoxus*	0.979	Neisseriaceae	AY127900
	Beta proteobacterium	0.959		AF423075
	Variovorax sp.	0.959		AB003627
01510-4	*Bacillus sp.*	1.000	Bacillaceae	AJ315067
	Bacillus pumilus	1.000		AY030327
	Bacillus pumilus	0.989		AB020208
01510-5	*Bacillus vallismortis*	0.974	Bacillaceae	AB021198
	Bacillus sp.	0.974		AB017587
	Bacillus subtilis	0.961		AF287011
02036-1	*Micrococcus luteus*	0.996	Micrococcaceae	AJ409096
	Micrococcus luteus	0.963		AF057289
	Micrococcus luteus	0.955		M38242
02036-2	*Ralstonia sp.*	0.949	Burkholderiaceae	Y10824
	Uncultured ralstonia sp.	0.932		AF526928
	Ralstonia eutropha	0.917		D87999
02036-3	*Bacillus pumilus*	0.972	Bacillaceae	AY030327
	Bacillus sp.	0.962		AJ315067
	Bacillus pumilus	0.952		AB020208
02080-1	*Acidovorax temperans*	0.933	Comamonadaceae	AF078766
	Acidovorax sp.	0.926		AJ012071
	Acidovorax sp.	0.926		AJ012070
02080-2	*Bacillus vallismortis*	0.988	Bacillaceae	AB021198
	Bacillus sp.	0.978		AB017587
	Bacillus subtilis	0.978		AF287011
02080-3	*Bacillus sp.*	0.978	Bacillaceaea	AB017591
	Bacillus sp.	0.976		AF411118
	Bacillus sp.	0.970		AB017589
02080-4	*Acidovorax sp.*	0.932	Comamonadaceae	AJ012070
	Acidovorax sp.	0.932		AJ012071
	Acidovorax temperans	0.930		AF078766
02080-5	*Acidovorax temperans*	0.928	Comamonadaceae	AF078766
	Acidovorax sp.	0.922		AJ012071
	Acidovorax sp.	0.922		AJ012070

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Table 6: Inoculation of MAT System Bioreactors (A1-A4) with ISS Isolates

Strain Number	Species	Final MAT Cell Concentration at t = 0 (cells/mL)	Log Difference Between Final and Target Cell Concentration 1×10^6 cells/mL
*02-0420-0816-3	*Sphingomonas paucimobilis*	7×10^6	0.8
*02-0420-0807-2	*Variovorax paradoxus*	5×10^6	0.7
*02-0420-0813-1	*Acidovorax delafieldii*	2×10^6	0.3
*02-0420-0816-2	*Stenotrophomonas maltophilia*	2×10^7	1.3
*02-0718-1522-1	*Hydrogenophaga pseudoflava*	2×10^6	0.3
*02-0718-1509-2	*Pseudomonas stutzeri*	2×10^7	1.3
*02-0622-1173-2-1	*Comamonas acidovorans*	1×10^6	0.0
*01-1219-2119-3	Unidentified gram negative rod	5×10^6	0.7
**02036-2	*Ralstonia* species	7×10^5	0.2
**02080-1	*Acidovorax temperans*	5×10^4	1.3
**02080-4	*Acidovorax* species	7×10^4	1.2
***7757	*Desulfovibrio desulfuricans*	9×10^5	0.1

*Boeing Company isolate
**Altran Corporation isolate
***American Type Culture Collection (ATCC)

Table 7: MAT Test Parameters of Inoculated Bioreactors

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Bioreactor Identification	Bioreactor ITCS pH	Sample Coupon Material	Sample Test Day	Flow cell Number	Coupon Sample Number	Flow cell Sample Position
A1	8.3	BNi-3	30	1	320	1
					321	2
					322	3
					323	4
			90	2	324	1
					325	2
					326	3
					327	4
			180	3	328	1
					329	2
					330	3
					331	4
A2	8.3	BNi-2	30	13	200	1
					201	2
					202	3
					205	4
			90	14	206	1
					207	2
					210	3
					211	4
			180	15	212	1
					215	2
					216	3
					217	4
A3	9.4	BNi-3	30	7	352	1
					353	2
					354	3
					355	4
			90	8	356	1
					357	2
					358	3
					377	4
			180	9	360	1
					361	2
					362	3
					363	4
A4	9.4	BNi-2	30	19	243	1
					222	2
					240	3
					241	4
			90	20	266	1
					245	2
					246	3
					267	4
			180	21	265	1
					249	2
					250	3
					264	4

Table 8: MAT Test Parameters of Un-inoculated (Control) Bioreactors

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Bioreactor Identification	Bioreactor ITCS pH	Sample Coupon Material	Sample Test Day	Flow cell Number	Coupon Sample Number	Flow cell Sample Position
B5	8.3	BNi-3	30	4	332	1
					333	2
					334	3
					335	4
			90	5	336	1
					337	2
					338	3
					376	4
			180	6	340	1
					345	2
					350	3
					351	4
B6	8.3	BNi-2	30	16	220	1
					221	2
					223	3
					224	4
			90	17	225	1
					226	2
					227	3
					228	4
			180	18	242	1
					230	2
					231	3
					233	4
B7	9.4	BNi-3	30	10	364	1
					365	2
					366	3
					367	4
			90	11	368	1
					369	2
					370	3
					371	4
			180	12	372	1
					373	2
					374	3
					375	4
B8	9.4	BNi-2	30	22	252	1
					253	2
					254	3
					255	4
			90	23	256	1
					257	2
					258	3
					259	4
			180	24	260	1
					261	2
					262	3
					263	4

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Table 9: MAT Bioreactor pH Raw Data

	Bioreactor							
Test Day	**A1**	**A2**	**A3**	**A4**	**B5**	**B6**	**B7**	**B8**
0	8.55	8.56	9.52	9.51	8.53	8.53	9.51	9.51
1	8.64	8.43	9.42	9.42	8.47	8.53	9.42	9.42
4	8.44	8.29	9.39	9.40	8.36	8.46	9.44	9.45
6	8.45	8.40	9.47	9.47	8.42	8.47	9.49	9.46
7	8.25	8.27	9.39	9.34	8.25	8.36	9.38	9.36
12	8.38	8.36	9.42	9.40	8.37	8.46	9.42	9.43
15	8.32	8.29	9.38	9.39	8.37	8.45	9.42	9.42
19	8.36	8.33	9.38	9.37	8.38	8.46	9.40	9.39
25	8.39	8.37	9.39	9.38	8.40	8.48	9.40	9.40
32	8.40	8.40	9.39	9.38	8.48	8.44	9.43	9.42
39	8.39	8.39	9.38	9.39	8.45	8.45	9.44	9.45
46	8.36	8.31	9.38	9.37	8.41	8.44	9.44	9.40
53	8.42	8.38	9.39	9.39	8.45	8.48	9.45	9.45
61	8.33	8.30	9.26	9.28	8.42	8.46	9.36	9.34
73	8.37	8.36	9.23	9.24	8.43	8.44	9.29	9.27
82	8.35	8.33	9.21	9.21	8.43	8.41	9.30	9.28
89	8.42	8.45	9.30	9.30	8.54	8.51	9.36	9.34
92	8.38	8.38	9.22	9.22	8.42	8.40	9.29	9.26
96	8.39	8.35	9.22	9.21	8.44	8.41	9.27	9.21
103	8.51	8.46	9.26	9.24	8.54	8.48	9.25	9.23
104	8.43	8.40	9.26	9.27	8.52	8.50	9.33	9.30
110	8.36	8.34	9.23	9.22	8.41	8.42	9.32	9.29
119	8.39	8.37	9.20	9.20	8.39	8.41	9.28	9.24
120	8.52	8.50	9.29	9.26	8.50	8.50	9.32	9.31
121	8.46	8.47	9.29	9.26	8.50	8.49	9.33	9.30
125	8.33	8.32	9.23	9.21	8.40	8.42	9.28	9.26
127	8.36	8.34	9.24	9.22	8.42	8.44	9.30	9.27
132	8.39	8.35	9.22	9.19	8.46	8.48	9.24	9.22
131	8.35	8.35	9.21	9.20	8.41	8.44	9.26	9.23
136	8.40	8.42	9.25	9.23	8.45	8.48	9.31	9.28
137	8.33	8.34	9.24	9.21	8.38	8.44	9.27	9.23
143	8.34	8.35	9.20	9.21	8.39	8.44	9.27	9.23
150	8.37	8.34	9.19	9.16	8.39	8.43	9.22	9.18
151	8.38	8.39	9.20	9.18	8.41	8.48	9.24	9.20
158	8.32	8.32	9.15	9.14	8.34	8.43	9.22	9.19
159	8.35	8.42	9.22	9.19	8.40	8.48	9.28	9.24
164	8.33	8.30	9.20	9.18	8.38	8.46	9.26	9.23
171	8.40	8.38	9.21	9.17	8.39	8.46	9.25	9.22
178	8.35	8.31	9.21	9.19	8.43	8.48	9.24	9.19
180	8.34	8.32	9.15	9.12	8.42	8.46	9.19	9.13

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Table 10: Comparison of Bioreactor ITCS pH with Flow Cell ITCS pH

Sample	Test Day	Bioreactor pH	Flow cell pH	Delta pH
A1	30	8.42	8.38	0.04
A1	90	8.38	8.42	0.04
A1	180	8.34	8.38	0.04
A2	30	8.37	8.33	0.04
A2	90	8.38	8.41	0.03
A2	180	8.32	8.36	0.04
A3	30	9.41	9.39	0.02
A3	90	9.22	9.27	0.05
A3	180	9.15	9.13	0.02
A4	30	9.41	9.38	0.03
A4	90	9.22	9.26	0.04
A4	180	9.12	9.11	0.01
B5	30	8.41	8.41	0.00
B5	90	8.42	8.43	0.01
B5	180	8.42	8.44	0.02
B6	30	8.39	8.40	0.01
B6	90	8.40	8.43	0.03
B6	180	8.46	8.49	0.03
B7	30	9.41	9.43	0.02
B7	90	9.29	9.30	0.01
B7	180	9.19	9.18	0.01
B8	30	9.40	9.42	0.02
B8	90	9.26	9.28	0.02
B8	180	9.13	9.14	0.01

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Table 11: MAT Bioreactor ITCS Viable Culture (cfu/mL) Raw Data

	Bioreactor							
Test Day	**A1**	**A2**	**A3**	**A4**	**B5**	**B6**	**B7**	**B8**
0-Baseline (pre-inoculation)	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
0	6.0E+06	6.0E+06	6.0E+06	5.0E+06	0.0E+00	0.0E+00	0.0E+00	0.0E+00
6	5.0E+06	5.0E+06	1.0E+06	1.0E+06	0.0E+00	0.0E+00	0.0E+00	0.0E+00
14	2.0E+06	4.0E+06	7.0E+04	9.0E+04	2.0E+01	0.0E+00	0.0E+00	0.0E+00
18 (post re-inoculation)	NA	NA	1.0E+06	6.0E+05	NA	NA	NA	NA
20	9.0E+05	7.0E+06	5.0E+05	1.0E+06	0.0E+00	0.0E+00	0.0E+00	0.0E+00
26	4.0E+05	2.0E+06	3.0E+05	6.0E+05	0.0E+00	0.0E+00	1.0E+01	0.0E+00
31	3.0E+05	2.0E+06	4.0E+05	5.0E+05	0.0E+00	0.0E+00	0.0E+00	0.0E+00
39	6.0E+05	1.0E+06	6.0E+05	4.0E+05	0.0E+00	0.0E+00	0.0E+00	0.0E+00
46	5.0E+05	2.0E+06	7.0E+05	7.0E+05	0.0E+00	1.0E+03	0.0E+00	0.0E+00
53	4.0E+05	1.0E+06	5.0E+05	5.0E+05	0.0E+00	6.0E+05	0.0E+00	0.0E+00
60	2.0E+05	1.0E+06	3.0E+05	3.0E+05	0.0E+00	7.0E+06	0.0E+00	0.0E+00
67	2.0E+05	1.0E+06	3.0E+05	5.0E+05	0.0E+00	6.0E+04	0.0E+00	0.0E+00
74	1.0E+05	4.0E+05	1.0E+05	3.0E+05	0.0E+00	2.0E+04	0.0E+00	0.0E+00
81	6.0E+04	6.0E+05	2.0E+05	2.0E+05	0.0E+00	3.0E+04	0.0E+00	0.0E+00
88	8.0E+04	5.0E+05	2.0E+05	3.0E+05	0.0E+00	3.0E+04	0.0E+00	0.0E+00
95	1.0E+05	5.0E+05	2.0E+05	2.0E+05	0.0E+00	3.0E+04	0.0E+00	0.0E+00
102	1.0E+05	5.0E+05	2.0E+05	3.0E+05	0.0E+00	3.0E+04	0.0E+00	0.0E+00
109	1.0E+05	3.0E+05	2.0E+05	3.0E+05	0.0E+00	2.0E+04	0.0E+00	0.0E+00
116	1.0E+05	3.0E+05	3.0E+05	3.0E+05	0.0E+00	2.0E+04	0.0E+00	0.0E+00
123	2.0E+05	3.0E+05	2.0E+05	4.0E+05	0.0E+00	3.0E+04	0.0E+00	0.0E+00
130	2.0E+05	4.0E+05	4.0E+05	5.0E+05	0.0E+00	3.0E+04	0.0E+00	0.0E+00
137	1.0E+05	3.0E+05	3.0E+05	3.0E+05	0.0E+00	2.0E+04	0.0E+00	0.0E+00
144	1.0E+05	1.0E+05	3.0E+05	3.0E+05	0.0E+00	2.0E+04	0.0E+00	0.0E+00
151	1.0E+05	2.0E+05	4.0E+05	3.0E+05	0.0E+00	1.0E+04	0.0E+00	0.0E+00
158	1.0E+05	2.0E+05	5.0E+05	3.0E+05	0.0E+00	1.0E+04	0.0E+00	0.0E+00
165	1.0E+05	2.0E+05	4.0E+05	3.0E+05	0.0E+00	2.0E+04	0.0E+00	0.0E+00
172	1.0E+05	2.0E+05	5.0E+05	3.0E+05	0.0E+00	6.0E+04	0.0E+00	0.0E+00
180	2.0E+05	3.0E+05	3.0E+05	2.0E+05	0.0E+00	4.0E+04	0.0E+00	0.0E+00

NA = Not Applicable

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Table 12: MAT Bioreactor Total Inorganic Carbon (TIC) Levels (mg/L) Raw Data

	Bioreactor							
Test Day	**A1**	**A2**	**A3**	**A4**	**B5**	**B6**	**B7**	**B8**
0	150.00	150.00	0.00	0.00	150.00	150.00	0.00	0.00
12	191.00	196.00	7.10	7.10	204.00	194.00	4.90	5.30
27	186.00	194.00	10.80	10.00	328.00	194.00	7.00	7.40
40	165.00	171.00	12.10	11.10	362.00	154.00	8.20	8.20
55	162.00	163.00	14.60	13.90	268.00	198.00	9.80	10.10
69	183.00	181.00	16.30	16.10	300.00	169.00	11.40	12.20
83	166.00	171.00	18.10	18.30	296.00	168.00	11.80	14.30
97	307.00	331.00	20.80	21.30	426.00	319.00	13.70	17.10
111	172.00	*22.50*	22.00	22.70	310.00	179.00	15.30	17.70
125	170.00	181.00	24.20	25.20	286.00	176.00	16.40	20.70
139	189.00	180.00	26.60	29.00	298.00	164.00	20.60	24.40
180	147.00	153.00	29.80	31.30	242.00	139.00	22.50	26.70

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Table 13: MAT Bioreactor Total Organic Carbon (TOC) Levels (mg/L) Raw Data

	Bioreactor							
Test Day	**A1**	**A2**	**A3**	**A4**	**B5**	**B6**	**B7**	**B8**
0	88.00	98.00	52.00	35.00	80.00	60.00	73.00	53.00
12	36.50	58.70	27.80	27.80	49.50	37.80	41.10	48.20
27	47.00	66.00	38.70	27.50	71.00	44.00	53.00	42.00
40	42.00	66.00	46.10	34.90	62.00	40.00	56.00	46.00
55	43.00	58.00	37.80	28.30	57.00	39.00	50.70	47.50
69	46.40	58.00	40.70	32.30	61.00	48.50	57.70	47.70
97	43.00	70.00	40.80	31.90	70.00	49.00	71.00	50.80
111	44.00	30.80	41.30	31.10	62.00	39.00	56.60	48.10
125	42.00	66.00	38.50	31.50	68.00	47.00	55.00	49.00
139	46.00	67.00	43.60	31.10	67.00	51.00	65.00	53.00
180	40.00	56.00	30.40	28.20	68.00	41.00	54.70	48.90

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Table 14: MAT Bioreactor Dissolved Oxygen (DO) Levels (mg/L) Raw Data

	Bioreactor							
Test Day	**A1**	**A2**	**A3**	**A4**	**B5**	**B6**	**B7**	**B8**
0	7.2	7.4	7.4	6.7	7.5	7.8	6.3	7.3
12	7.0	7.9	7.7	7.8	7.3	7.4	7.2	7.4
27	7.9	7.8	7.9	8.0	7.9	8.0	7.8	7.9
40	8.5	8.4	8.1	8.1	8.3	8.3	8.0	8.0
55	8.2	8.5	8.5	8.6	8.8	8.9	8.8	8.8
69	8.4	8.2	8.3	8.0	8.2	7.9	7.9	8.0
83	8.5	8.3	8.3	8.3	8.2	8.3	8.3	8.3
97	8.8	8.5	8.5	8.2	8.5	8.6	8.5	8.6
111	8.3	8.2	8.3	8.2	8.5	8.5	8.4	8.5
139	8.6	8.7	8.6	8.6	9.1	9.0	8.6	8.7
180	ND	ND	ND	ND	ND	ND	ND	ND

ND = Not Determined

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Table 15: Colonization of ISS Heat Exchanger Materials: Biofilm Coupon Viable Culture (cfu/cm^2) Raw Data

	ISS Material-Bioreactor							
Test Day	**BNi3-A1**	**BNi2-A2**	**BNi3-A3**	**BNi2-A4**	**BNi3-B5**	**BNi2-B6**	**BNi3-B7**	**BNi2-B8**
30	3.0E+05	8.0E+01	4.0E+02	7.0E+01	0.0E+00	0.0E+00	0.0E+00	0.0E+00
90	5.0E+03	3.0E+04	2.0E+04	2.0E+04	0.0E+00	4.0E+03	0.0E+00	0.0E+00
180	1.0E+06	5.0E+05	2.0E+06	2.0E+05	0.0E+00	0.0E+00	0.0E+00	0.0E+00

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Table 16: Brazing Thickness Values for pH 8.3 and Inoculated Conditions, Values Given as Average, Standard Deviation – Unexposed Samples Given at Time 0

	BNi-2			BNi-3		
Time (Days)	Sample Number(s)	Minimum Thickness (mil)	Maximum Thickness (mil)	Sample Number(s)	Minimum Thickness (mil)	Maximum Thickness (mil)
0	204 & 213	1.13, 0.08	1.30, 0.06	303 & 308	0.75, 0.06	1.04, 0.07
30	202	1.07, 0.08	1.26, 0.11	322	0.68, 0.07	1.04, 0.03
90	210	1.17, 0.07	1.37, 0.04	326	0.84, 0.12	1.09, 0.12
180	216	1.20, 0.07	1.36, 0.06	330	0.76, 0.08	0.97, 0.09

Table 17: Brazing Thickness Values for pH 8.3 and Uninoculated Conditions, Values Given as Average, Standard Deviation – Unexposed Samples Given at Time 0

	BNi-2			BNi-3		
Time (Days)	Sample Number(s)	Minimum Thickness (mil)	Maximum Thickness (mil)	Sample Number(s)	Minimum Thickness (mil)	Maximum Thickness (mil)
0	204 & 213	1.13, 0.08	1.30, 0.06	303 & 308	0.75, 0.06	1.04, 0.07
30	223	1.11, 0.05	1.29, 0.08	334	0.78, 0.09	1.09, 0.07
90	227	1.20, 0.13	1.35, 0.14	338	0.95, 0.04	1.14, 0.10
180	231	1.19, 0.06	1.38, 0.04	350	0.78, 0.06	1.10, 0.04

Table 18. Brazing Thickness Values for pH 9.4 and Inoculated Conditions, Values Given as Average, Standard Deviation – Unexposed Samples Given at Time 0

	BNi-2			BNi-3		
Time (Days)	Sample Number(s)	Minimum Thickness (mil)	Maximum Thickness (mil)	Sample Number(s)	Minimum Thickness (mil)	Maximum Thickness (mil)
0	204 & 213	1.13, 0.08	1.30, 0.06	303 & 308	0.75, 0.06	1.04, 0.07
30	240	1.20, 0.11	1.48, 0.05	354	0.76, 0.06	1.00, 0.09
90	246	1.15, 0.19	1.43, 0.21	358	0.88, 0.15	1.19, 0.03
180	250	1.12, 0.04	1.28, 0.04	362	0.61, 0.16	1.02, 0.07

Table 19. Brazing Thickness Values for pH 9.4 and Uninoculated Conditions, Values Given as Average, Standard Deviation – Unexposed Samples Given at Time 0

	BNi-2			BNi-3		
Time (Days)	Sample Number(s)	Minimum Thickness (mil)	Maximum Thickness (mil)	Sample Number(s)	Minimum Thickness (mil)	Maximum Thickness (mil)
0	204 & 213	1.13, 0.08	1.30, 0.06	303 & 308	0.75, 0.06	1.04, 0.07
30	254	1.32, 0.11	1.58, 0.15	366	0.76, 0.12	1.06, 0.09
90	258	1.09, 0.05	1.25, 0.03	370	0.87, 0.03	1.20, 0.10
180	262	1.11, 0.07	1.30, 0.07	374	0.67, 0.04	0.99, 0.14

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Table 20. Summary of Statistical Significance Analysis of Minimum Braze Thickness

	BNi-2 Inoculated pH 8.3			BNi-3 Inoculated pH 8.3		
Time (Days)	Sample Number(s)	95% Confident	99% Confident	Sample Number(s)	95% Confident	99% Confident
30	202	No Difference	No Difference	322	No Difference	No Difference
90	210	No Difference	No Difference	326	No Difference	No Difference
180	216	No Difference	No Difference	330	No Difference	No Difference

	BNi-2 Uninoculated pH 8.3			BNi-3 Uninoculated pH 8.3		
Time (Days)	Sample Number(s)	95% Confident	99% Confident	Sample Number(s)	95% Confident	99% Confident
30	223	No Difference	No Difference	334	No Difference	No Difference
90	227	No Difference	No Difference	338	Different	Different
180	231	No Difference	No Difference	350	No Difference	No Difference

	BNi-2 Inoculated pH 9.4			BNi-3 Inoculated pH 9.4		
Time (Days)	Sample Number(s)	95% Confident	99% Confident	Sample Number(s)	95% Confident	99% Confident
30	240	No Difference	No Difference	354	No Difference	No Difference
90	246	No Difference	No Difference	358	Different	No Difference
180	250	No Difference	No Difference	362	Different	No Difference

	BNi-2 Uninoculated pH 9.4			BNi-3 Uninoculated pH 9.4		
Time (Days)	Sample Number(s)	95% Confident	99% Confident	Sample Number(s)	95% Confident	99% Confident
30	254	Different	Different	366	No Difference	No Difference
90	258	No Difference	No Difference	370	Different	Different
180	262	No Difference	No Difference	374	Different	No Difference

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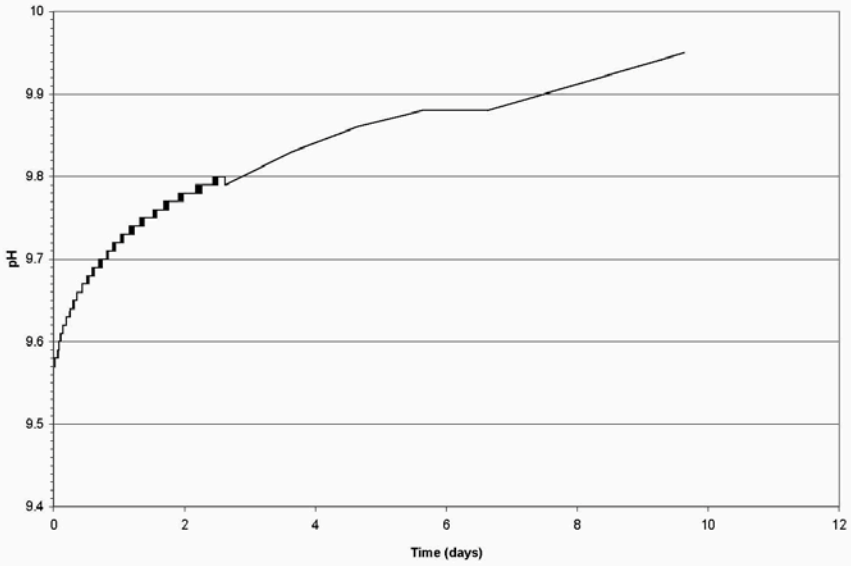

Figure 1: ISFET (Solid State) pH Electrode Drift

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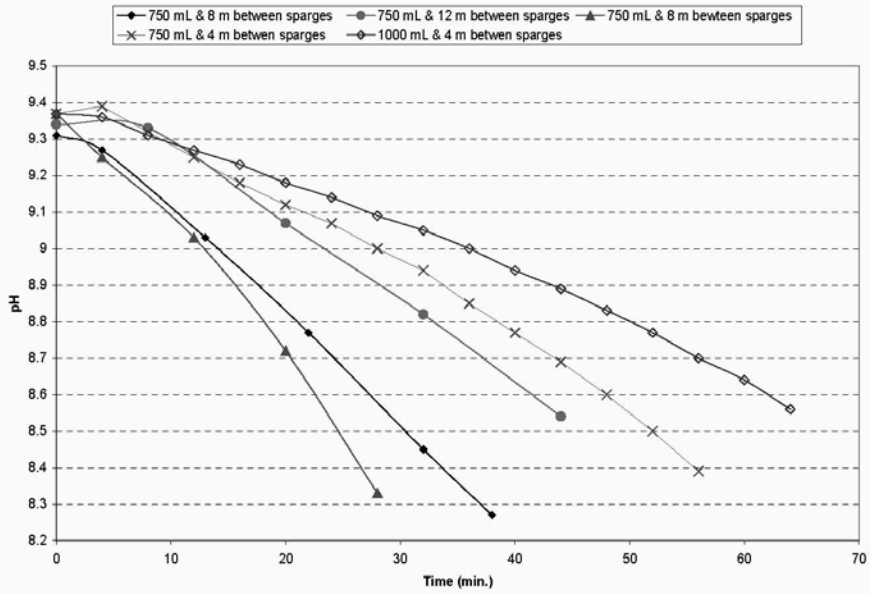

Figure 2: Effect of CO_2 Sparge Time and ITCS Volume on pH

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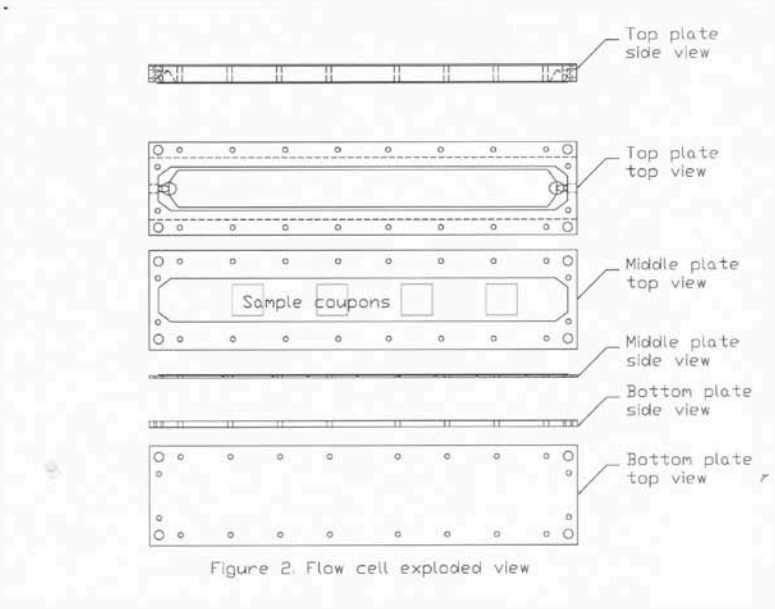

Figure 2. Flow cell exploded view

Figure 3: Overall Schematic of MAT Flow Cell

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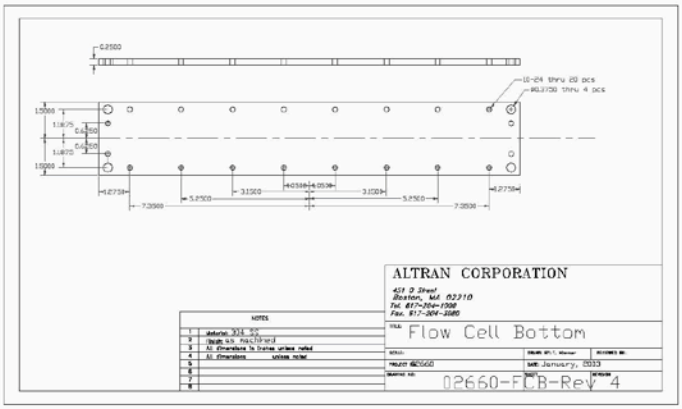

Figure 4: MAT Flow Cell Bottom Plate Specifications

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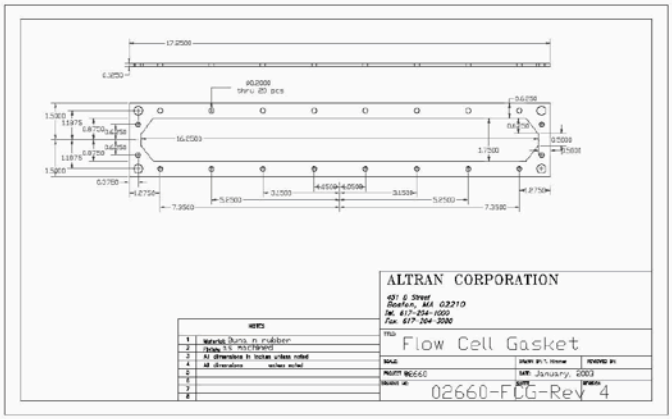

Figure 5: MAT Flow Cell Gasket Specifications

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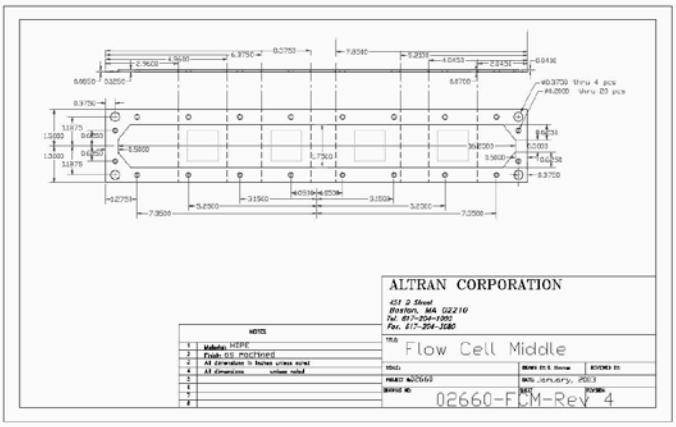

Figure 6: MAT Flow Cell Middle Plate Specifications

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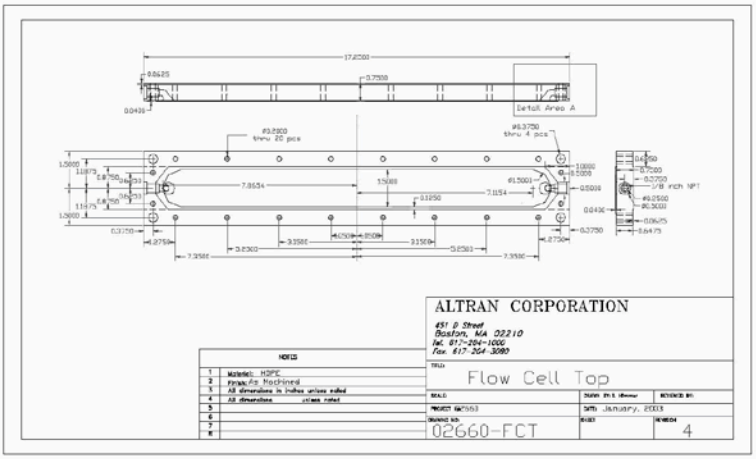

Figure 7: MAT Flow Cell Top Plate Specifications

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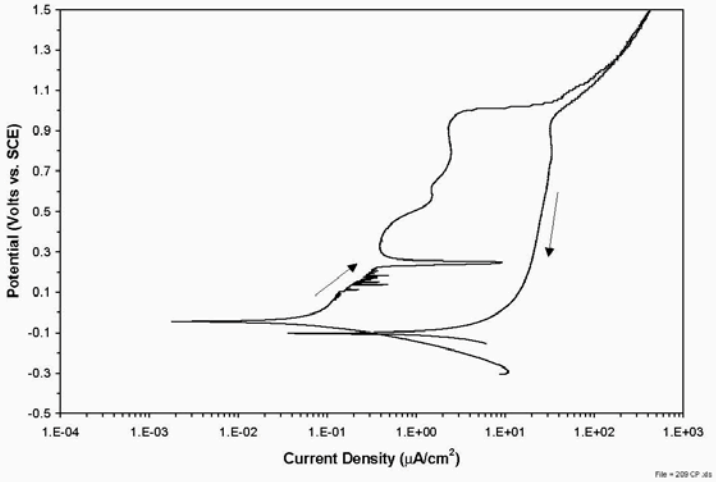

Figure 8: Cyclic Polarization Curve for a BNi-2 Sample in ITCS Fluid at pH 7.3

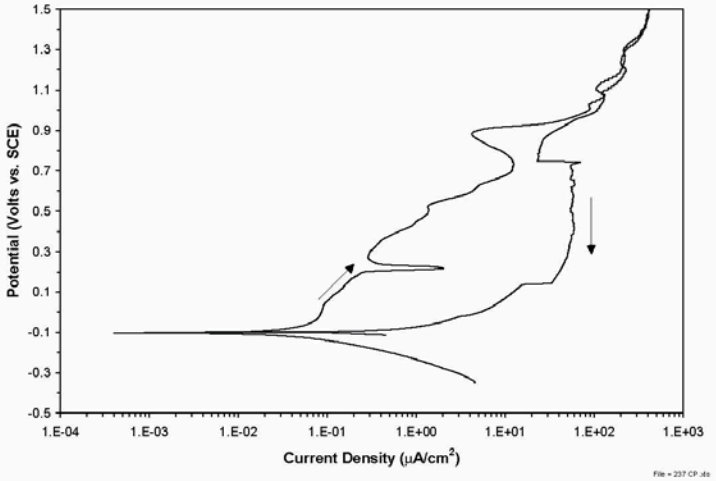

Figure 9: Cyclic Polarization Curve for a BNi-2 Sample in ITCS Fluid at pH 8.3

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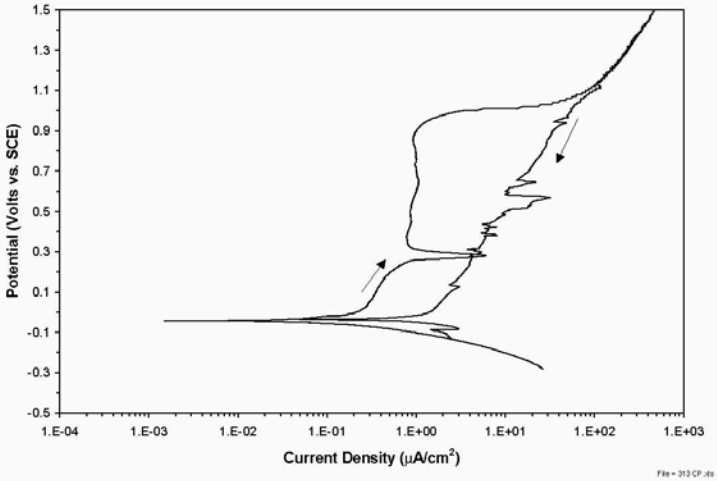

Figure 10: Cyclic Polarization Curve for a BNi-3 Sample in ITCS Fluid at pH 7.3

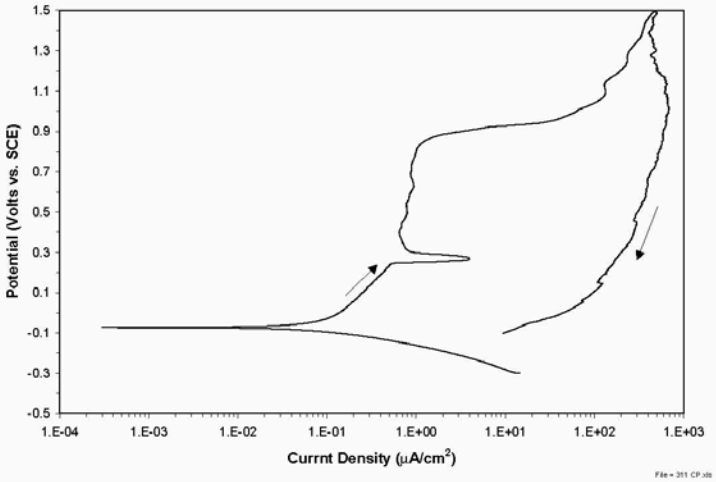

Figure 11: Cyclic Polarization Curve for a BNi-3 Sample in ITCS Fluid at pH 8.3

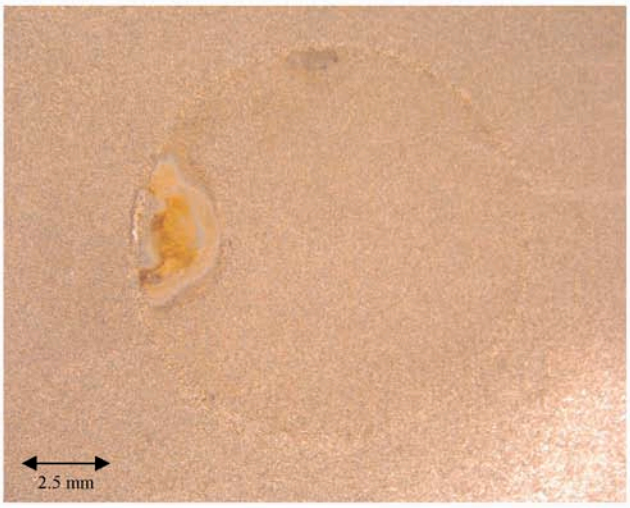

Figure 12: Macrophotograph of a BNi-2 Sample Post Cyclic Polarization Testing in ITCS Fluid at pH 7.3

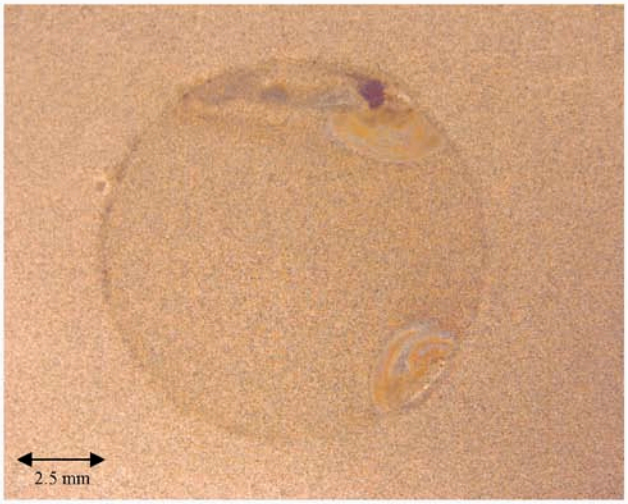

Figure 13: Macrophotograph of a BNi-2 Sample Post Cyclic Polarization Testing in ITCS Fluid at pH 8.3

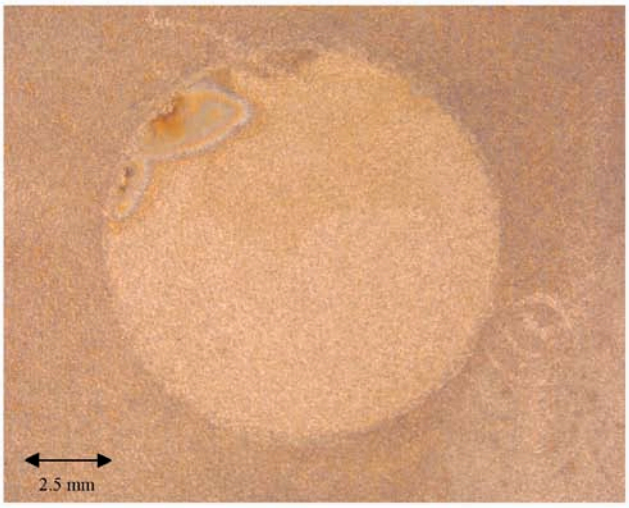

Figure 14: Macrophotograph of a BNi-3 Sample Post Cyclic Polarization Testing in ITCS Fluid at pH 7.3

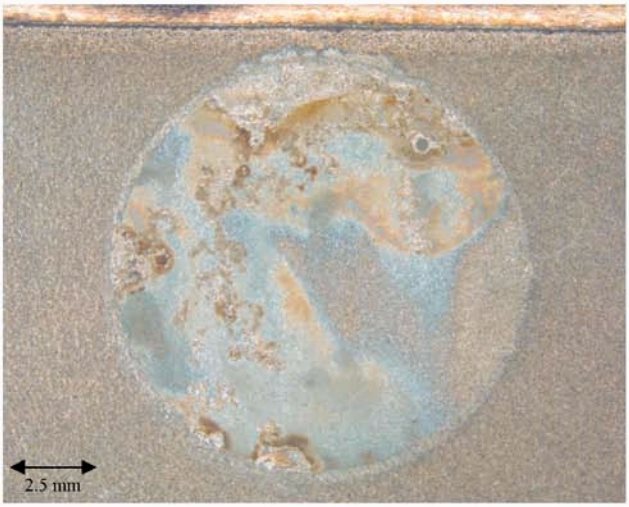

Figure 15: Macrophotograph of a BNi-3 Sample Post Cyclic Polarization Testing in ITCS Fluid at pH 8.3

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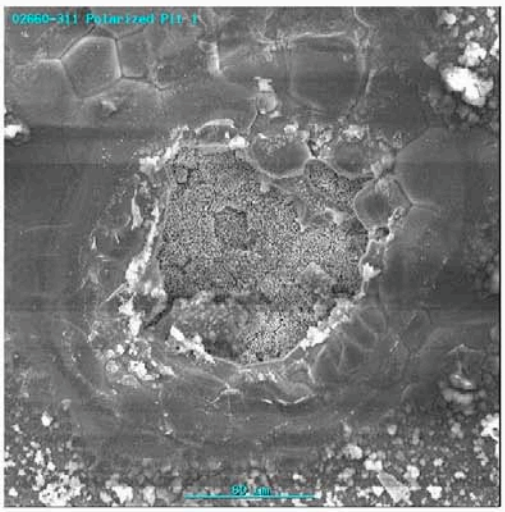

Figure 16: SEM Image of Pitting to the Base Metal of a BNi-3 Sample

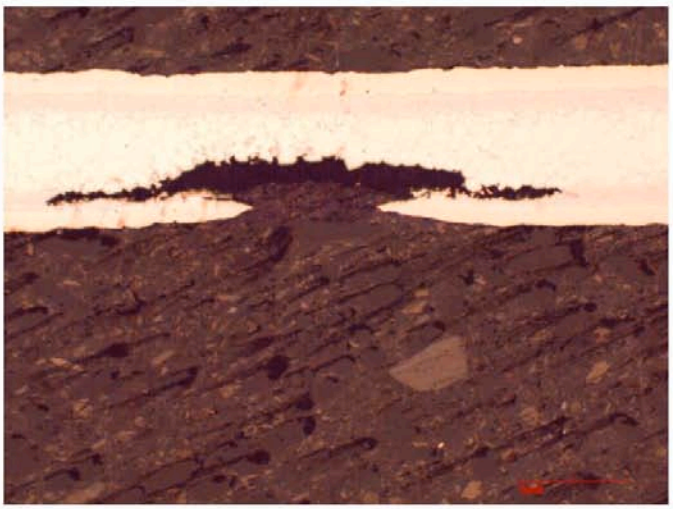

Figure 17: Photomicrograph of a Pit in Cross Section, the BNi-3 Sample has been Mounted and Polished

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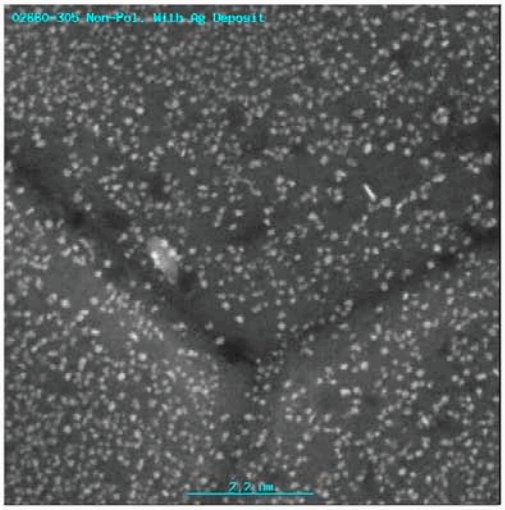

Figure 18: Typical SEM Surface Image of a BNi-3 Sample Prior to Testing

Figure 19: Typical SEM Surface Image of a BNi-3 Sample Post Testing

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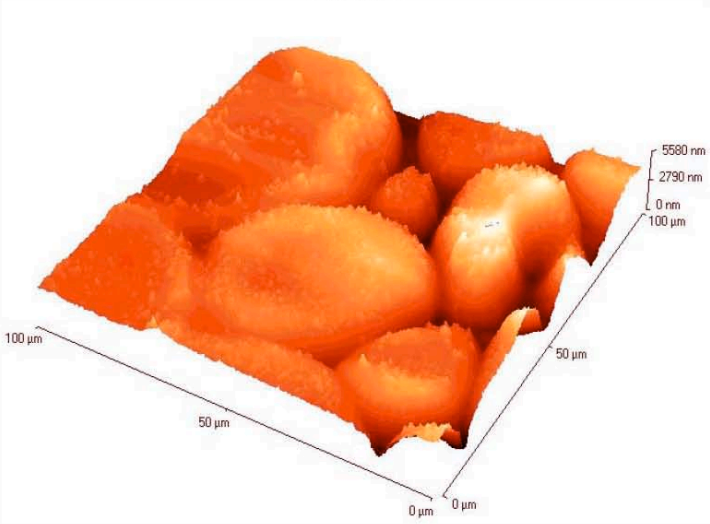

Figure 20: AFM Image of a BNi-2 Sample Prior to Conditioning

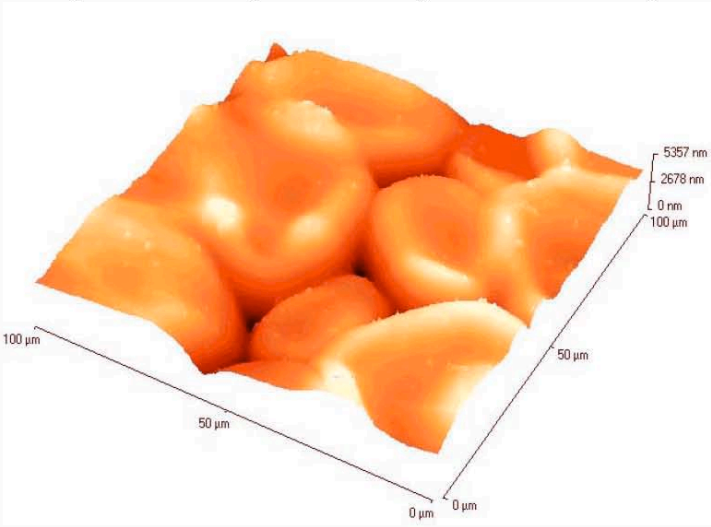

Figure 21: AFM Image of a BNi-2 Sample Post Conditioning

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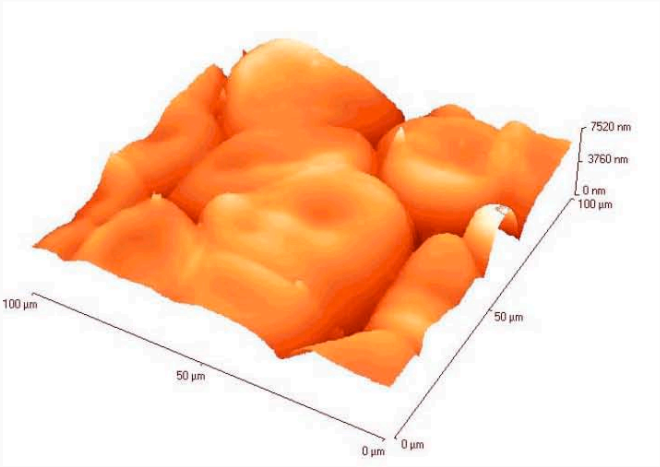

Figure 22: AFM Image of a BNi-3 Sample Prior to Conditioning

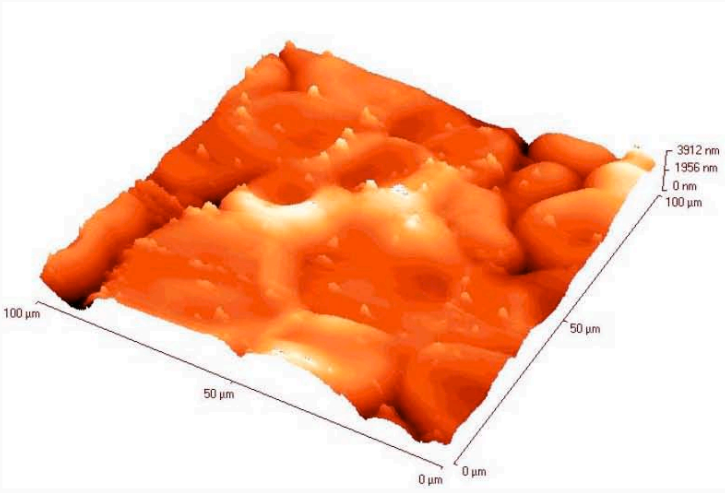

Figure 23: AFM Image of a BNi-3 Sample Post Conditioning

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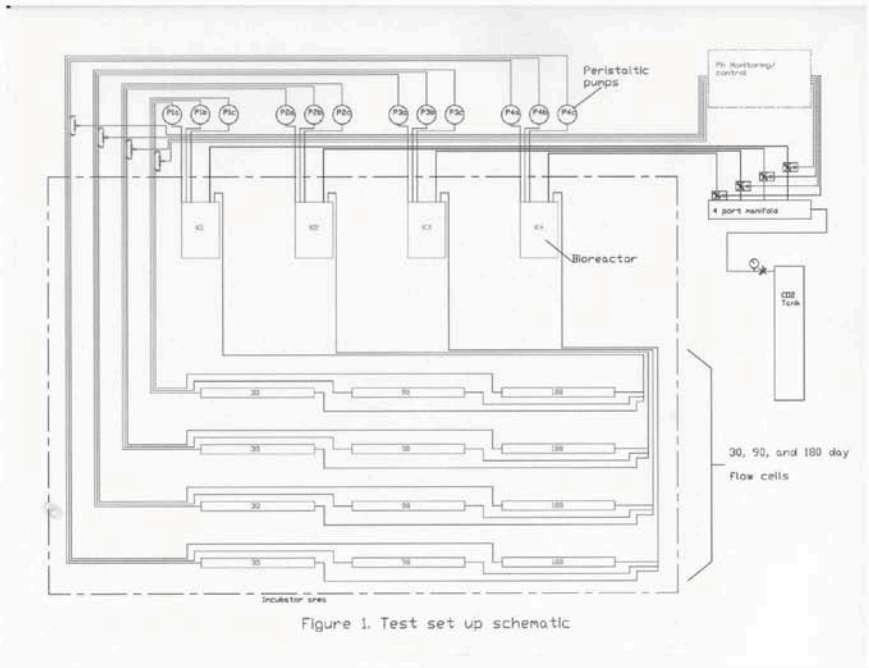

Figure 1. Test set up schematic

Figure 24: Schematic of Overall MAT System Setup

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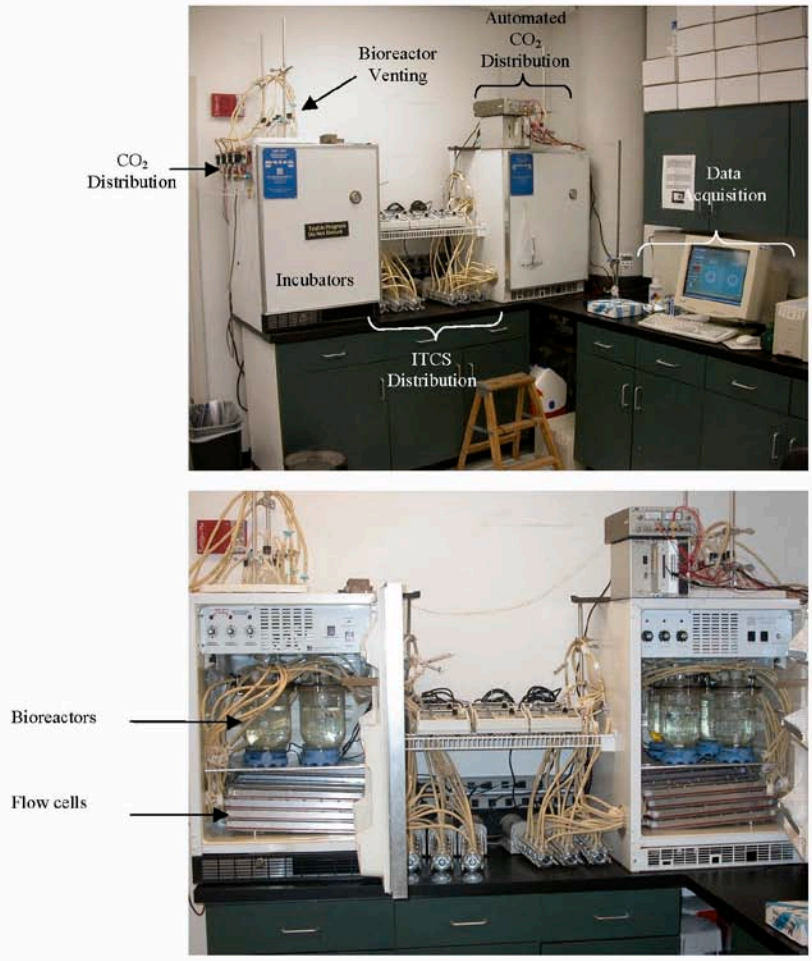

Figure 25: Overall MAT System Setup

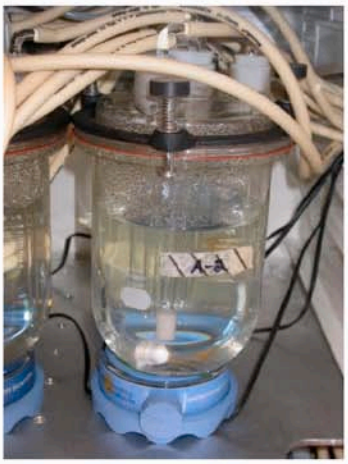

Figure 26: MAT Bioreactor

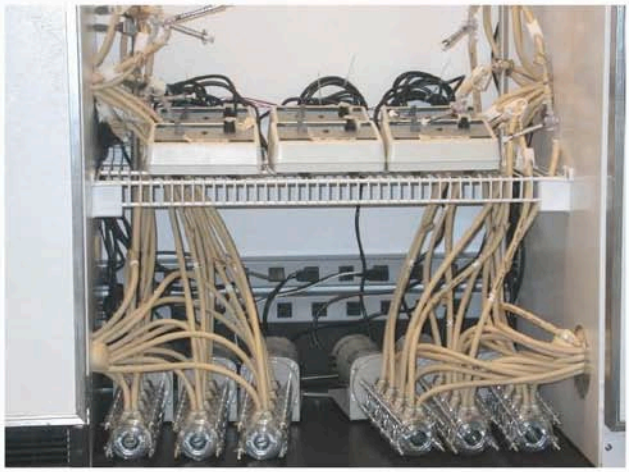

Figure 27: MAT ITCS Distribution by Peristaltic Pumps

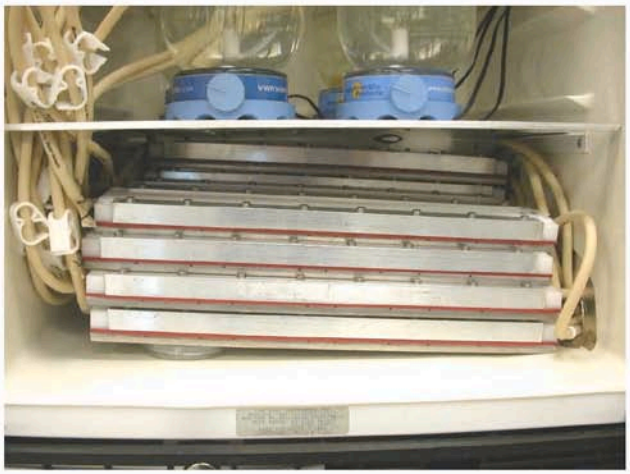

Figure 28: MAT Flow Cells

Figure 29: MAT Bioreactor Venting

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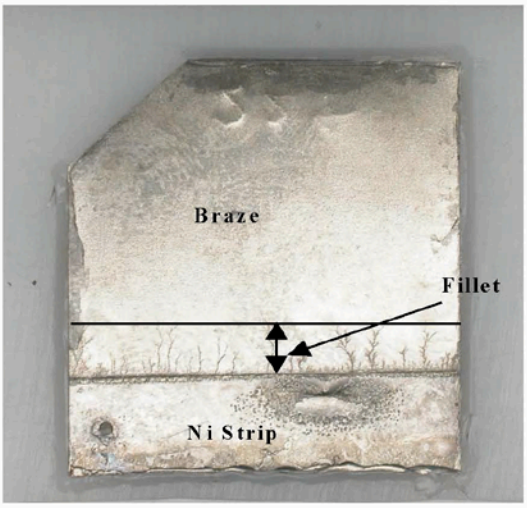

Figure 30: Three Regions of the Samples (a BNi-2 sample prior to exposure)

File = xxx day surface SEM data entry sheet.xls Altran Project # 02660

NOTE: WORST CASE LOCATIONS ARE TO BE EXAMINED, FOR BRAZE MUST BE SUFFICIENTLY AWAY FROM FILLET

Date:

Sample	Region	Magnification (X)	Filename	Comment
___ ___ ___	Braze	50	"sample #"-braze-lo_1.tif	
		500	"sample #"-braze-med_1.tif	
		1,000	"sample #"-braze-hi_1.tif	
		5,000	"sample #"-braze-xhi_1.tif	
		10,000	"sample #"-braze-xxhi_1.tif	
	Fillet	50	"sample #"-fillet-lo_1.tif	
	Ni Strip	50	"sample #"-Ni-lo_1.tif	
		500	"sample #"-Ni-med_1.tif	
		1,000	"sample #"-Ni-hi_1.tif	
		5,000	"sample #"-Ni-xhi_1.tif	
		10,000	"sample #"-Ni-xxhi_1.tif	
	Additional-			
	Additional-			
	Additional-			
	Additional-			
	Additional-			

Originator: Verifier:

Figure 31: Data Entry Sheet for Surface SEM Analysis

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File = xxx day cross section SEM data entry sheet.xls Altran Project # 02660

NOTE: WORST CASE LOCATIONS ARE TO BE EXAMINED

Date:

Sample	Region	Magnification (X)	Filename	Comment
———	Braze	5,000	"sample #"A-braze-lo.tif	
		10,000	"sample #"A-braze-hi.tif	
		5,000	"sample #"B-braze-lo.tif	
		10,000	"sample #"B-braze-hi.tif	
		5,000	"sample #"C-braze-lo.tif	
		10,000	"sample #"C-braze-hi.tif	
	Fillet	5,000	"sample #"-fillet-lo.tif	
		10,000	"sample #"-fillet-hi.tif	
	Ni Strip	5,000	"sample #"-Ni-lo.tif	
		10,000	"sample #"-Ni-hi.tif	
	Cut Corner Braze	5,000	"sample #"-Corner-lo.tif	
		10,000	"sample #"-Corner-hi.tif	
	Additional-			
	Additional-			
	Additional-			
	Additional-			
	Additional-			

Originator: Verifier:

Figure 32: Data Entry Sheet for SEM Analysis of Cross Sectioned Samples

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File = xxx day data sheet for braze thickness.xls

File - Sample ID	Max Thickness as Measured (mm)	Min Thickness as Measured (mm)	Actual Max Thickness (in)	Actual Min Thickness (in)
Micro, 2xx-A.jpg				
Micro, 2xx-B.jpg				
Micro, 2xx-C.jpg				
Micro, 2xx-D.jpg				
Micro, 2xx-E.jpg				
Micro, 2xx-F.jpg				
Micro, 2xx-A.jpg				
Micro, 2xx-B.jpg				
Micro, 2xx-C.jpg				
Micro, 2xx-D.jpg				
Micro, 2xx-E.jpg				
Micro, 2xx-F.jpg				
Micro, 2xx-A.jpg				
Micro, 2xx-B.jpg				
Micro, 2xx-C.jpg				
Micro, 2xx-D.jpg				
Micro, 2xx-E.jpg				
Micro, 2xx-F.jpg				
Micro, 2xx-A.jpg				
Micro, 2xx-B.jpg				
Micro, 2xx-C.jpg				
Micro, 2xx-D.jpg				
Micro, 2xx-E.jpg				
Micro, 2xx-F.jpg				
Micro, 3xx-A.jpg				
Micro, 3xx-B.jpg				
Micro, 3xx-C.jpg				
Micro, 3xx-D.jpg				
Micro, 3xx-E.jpg				
Micro, 3xx-F.jpg				
Micro, 3xx-A.jpg				
Micro, 3xx-B.jpg				
Micro, 3xx-C.jpg				
Micro, 3xx-D.jpg				
Micro, 3xx-E.jpg				
Micro, 3xx-F.jpg				
Micro, 3xx-A.jpg				
Micro, 3xx-B.jpg				
Micro, 3xx-C.jpg				
Micro, 3xx-D.jpg				
Micro, 3xx-E.jpg				
Micro, 3xx-F.jpg				

Note:
A - At cut corner edge
B - Minimum observed thickness over entire section length.
C-F - From edge to fillet evenly spaced
Images of the Ni and fillet were also taken.

Figure 33: Data Entry Sheet for Optical Brazing Thickness Measurements

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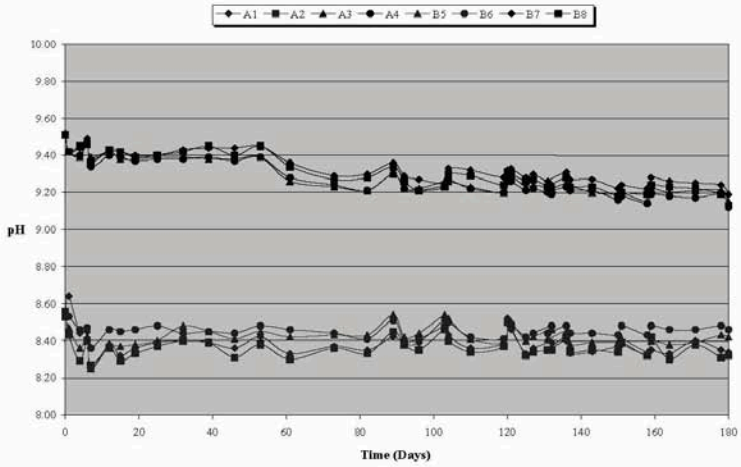

Figure 34: MAT Bioreactor pH

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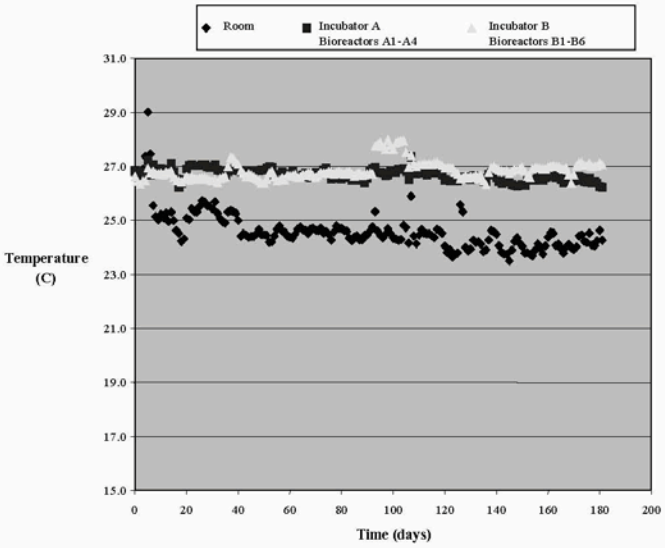

Figure 35: Average Daily Room and MAT Incubator/Bioreactor Temperatures

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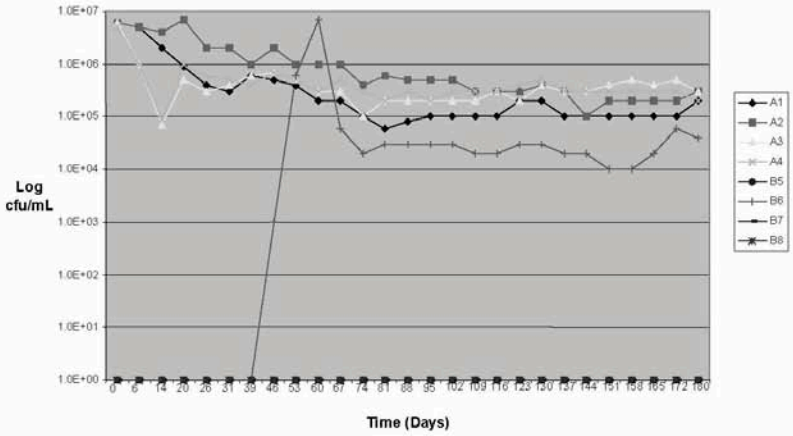

Figure 36: MAT Bioreactor Viable Counts

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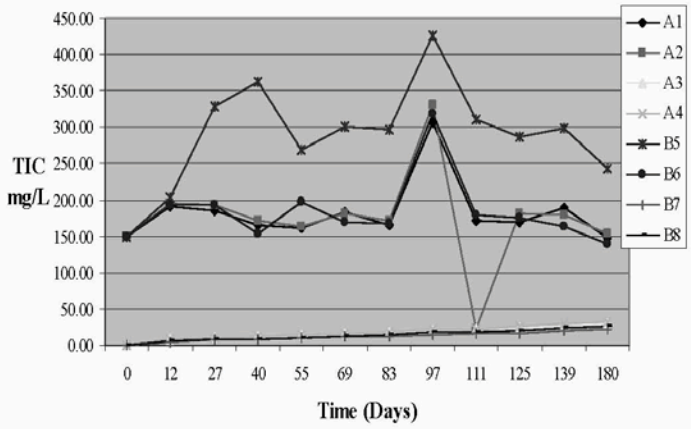

Figure 37: MAT Bioreactor Total Inorganic Carbon (TIC)

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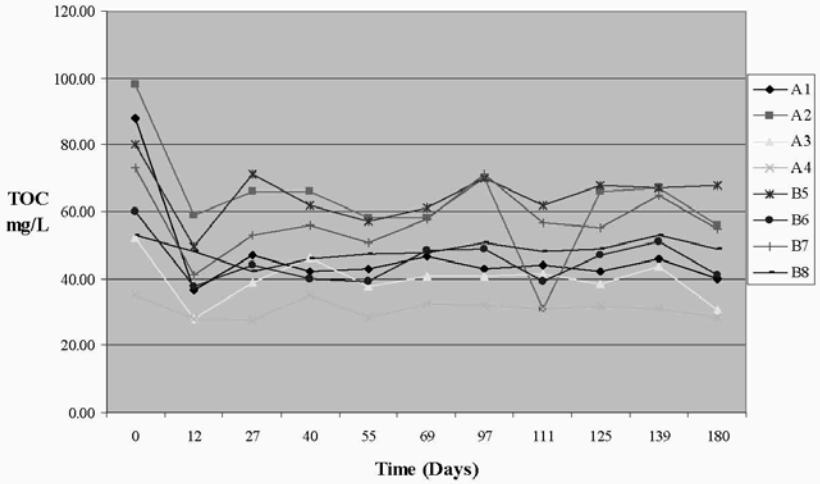

Figure 38: MAT Bioreactor Total Organic Carbon (TOC)

75

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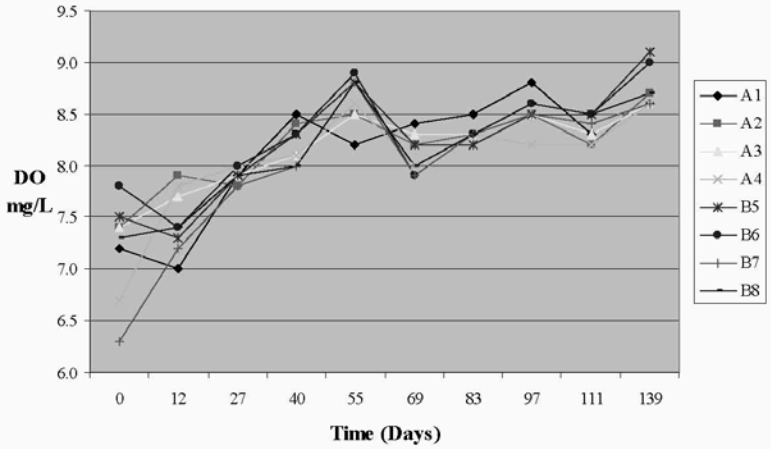

Figure 39: MAT Bioreactor Dissolved Oxygen (DO)

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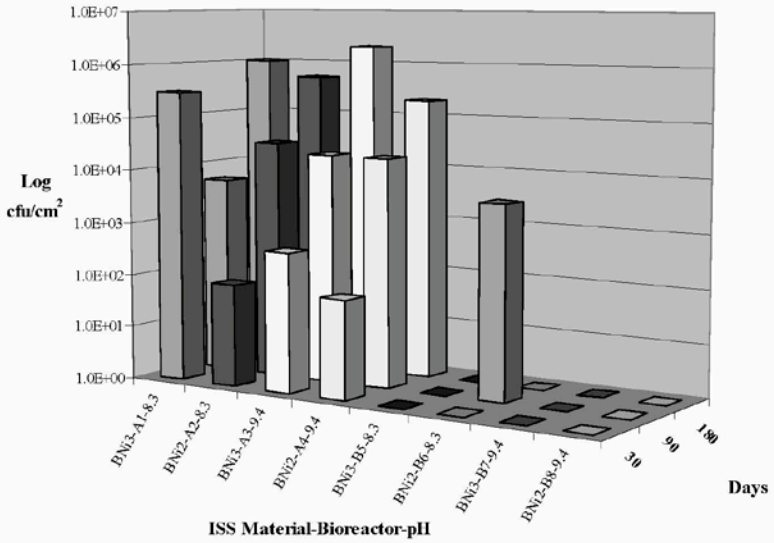

Figure 40: Colonization of ISS Heat Exchanger Materials

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Figure 41: 180 Day Flow cell #3 as Removed From the MAT System and Opened to Expose the Test Coupons

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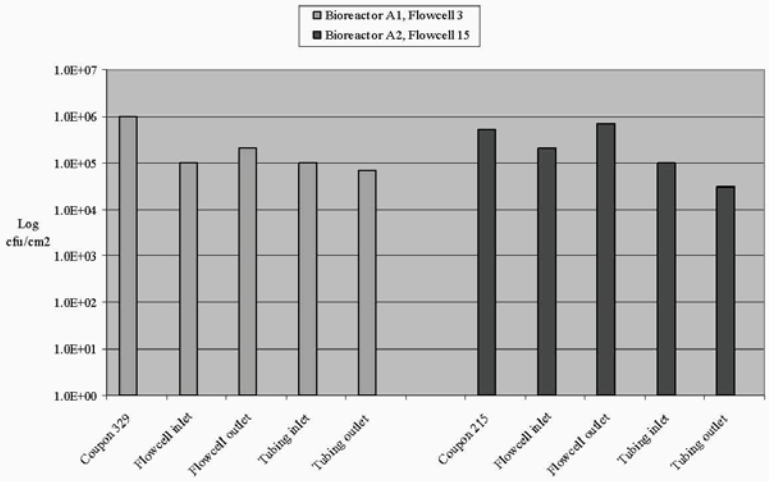

Figure 42: Comparison of the Colonization Levels of Flow Cell Materials of Construction, ITCS Distribution Tubing, and ISS Heat Exchanger Sample Materials at 180 Day Test Time Point

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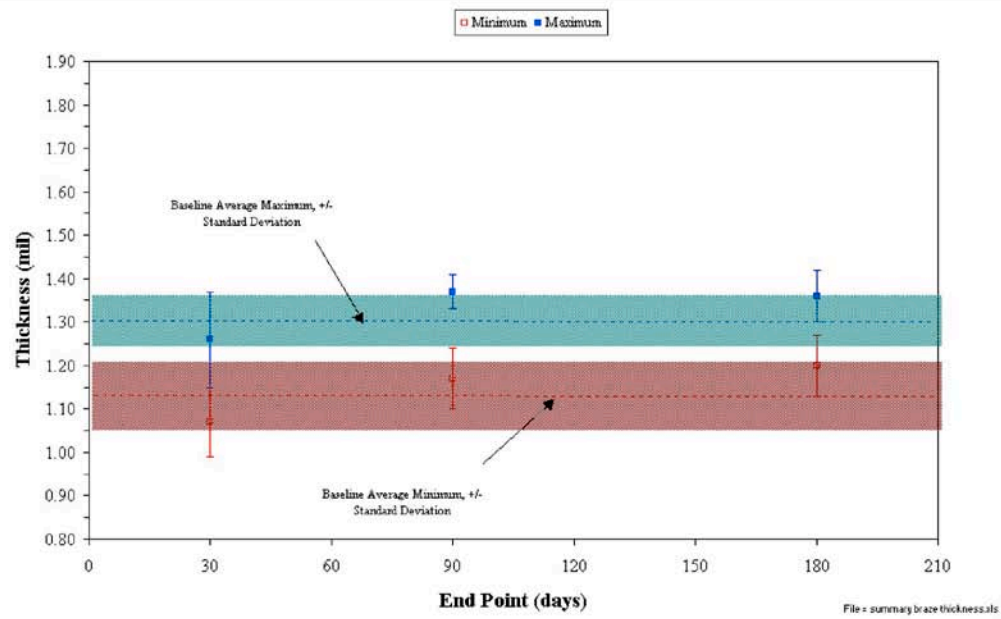

Figure 43: Brazing Thickness Values for BNi-2 Samples Exposed to pH 8.3 and Inoculated Conditions

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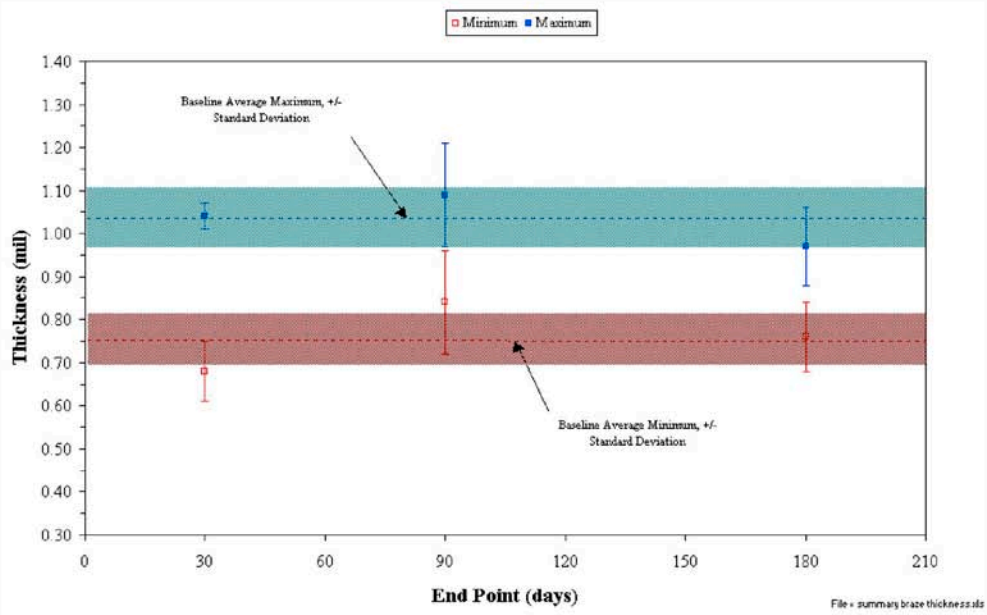

Figure 44: Brazing Thickness Values for BNi-3 Samples Exposed to pH 8.3 and Inoculated Conditions

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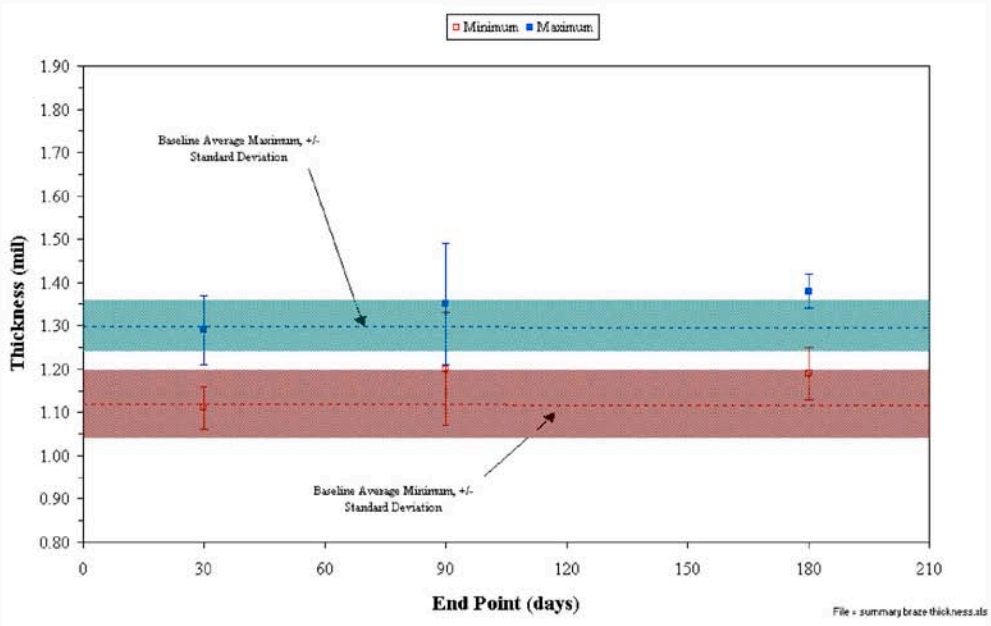

Figure 45: Brazing Thickness Values for BNi-2 Samples Exposed to pH 8.3 and Uninoculated Conditions

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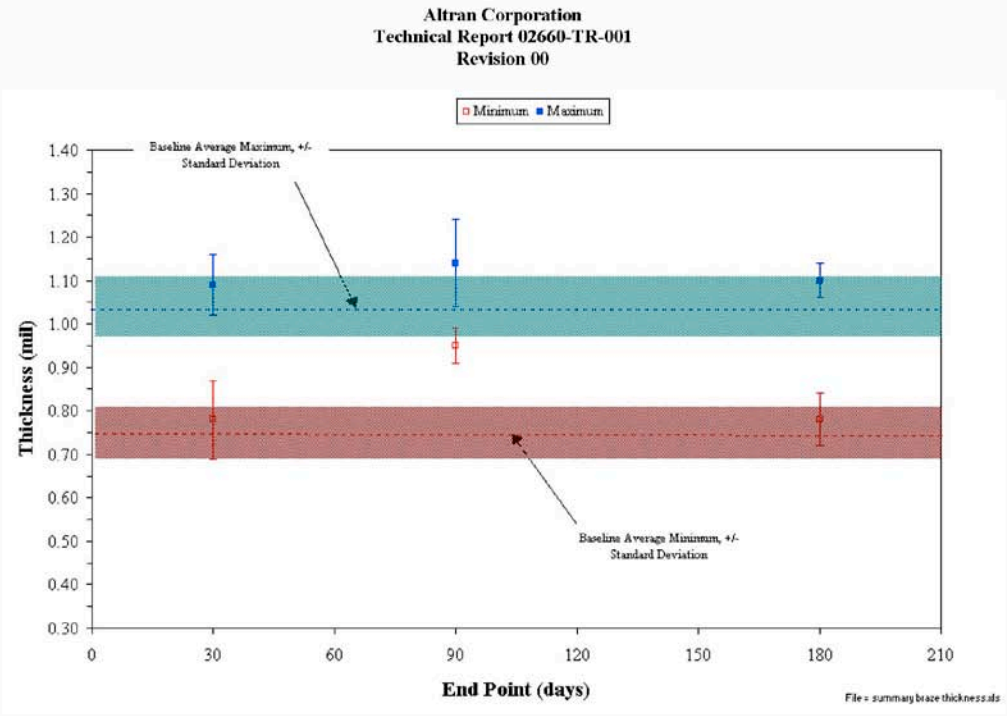

Figure 46: Brazing Thickness Values for BNi-3 Samples Exposed to pH 8.3 and Uninoculated Conditions

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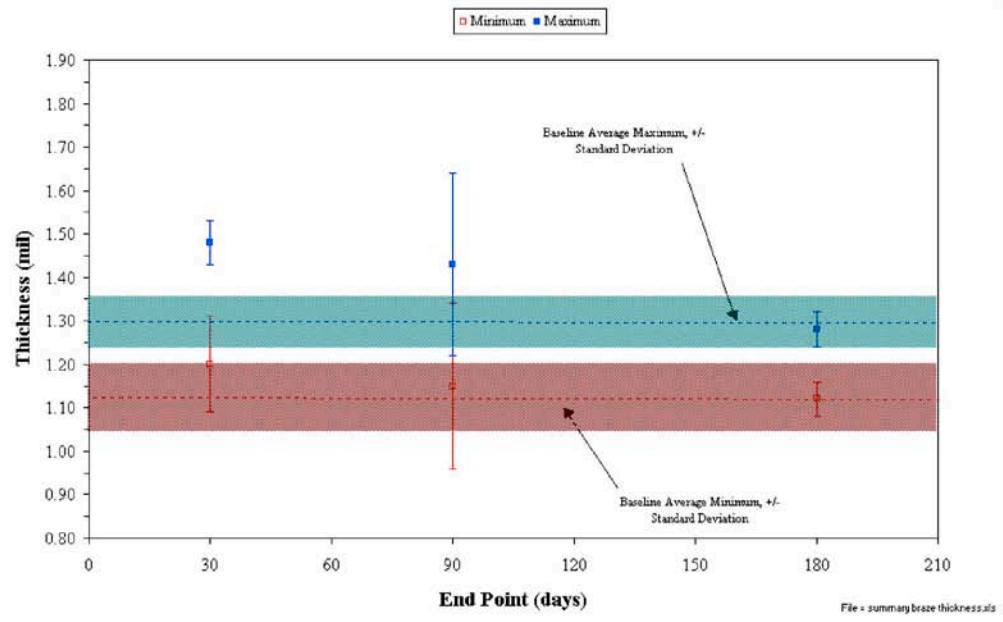

Figure 47: Brazing Thickness Values for BNi-2 Samples Exposed to pH 9.4 and Inoculated Conditions

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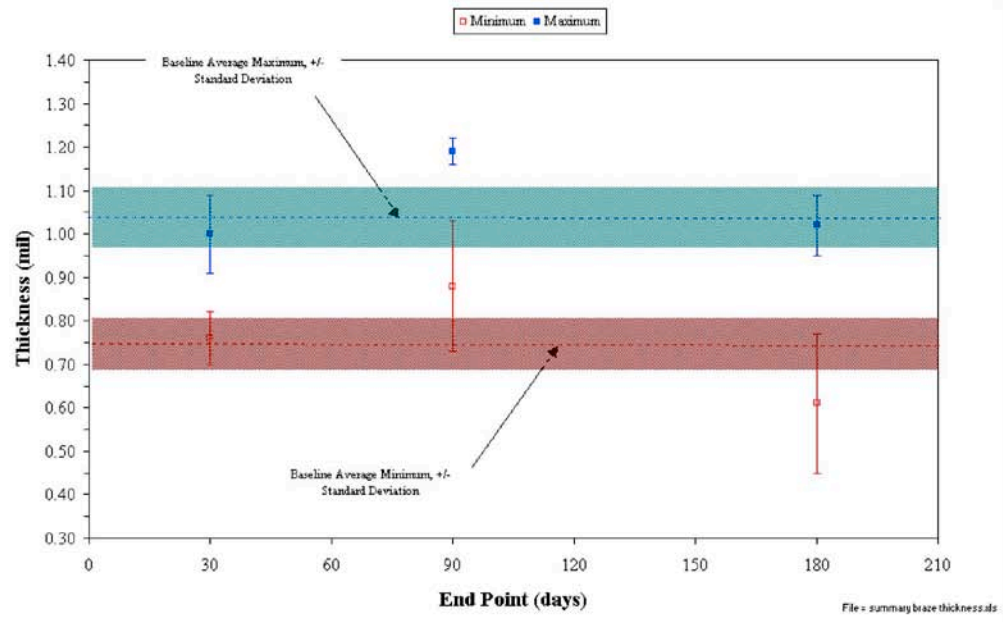

Figure 48: Brazing Thickness Values for BNi-3 Samples Exposed to pH 9.4 and Inoculated Conditions

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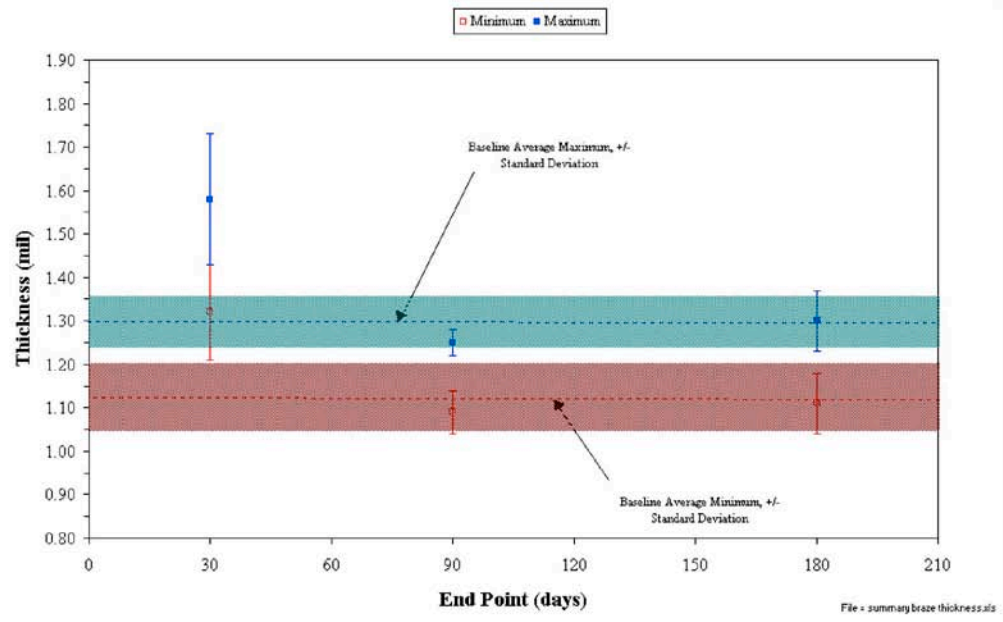

Figure 49: Brazing Thickness Values for BNi-2 Samples Exposed to pH 9.4 and Uninoculated Conditions

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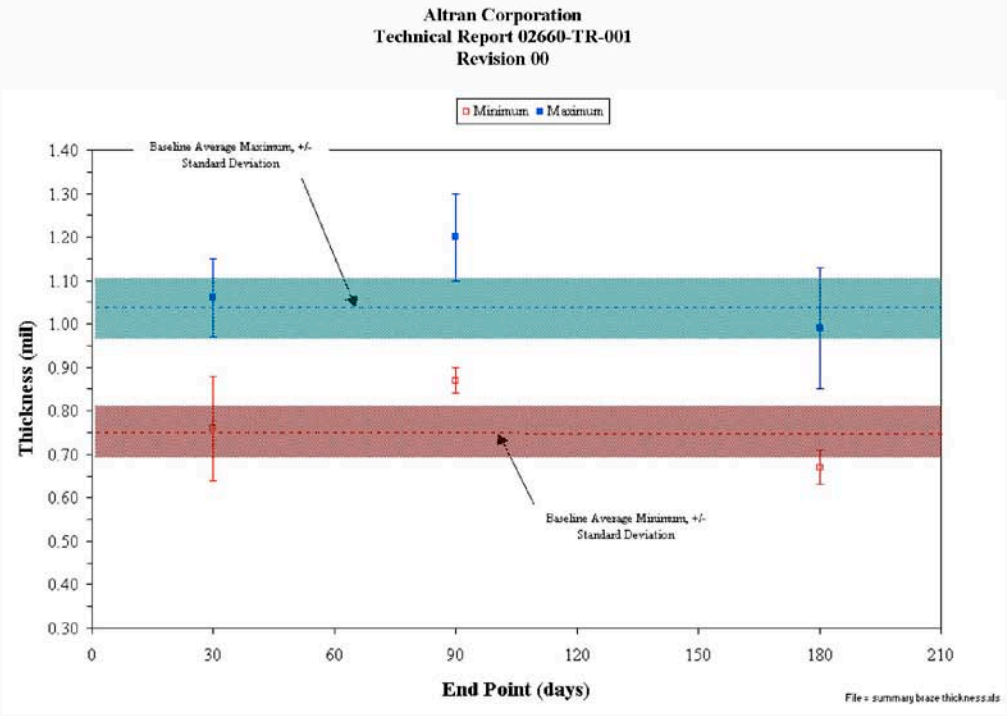

Figure 50: Brazing Thickness Values for BNi-3 Samples Exposed to pH 9.4 and Uninoculated Conditions

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altran

ALTRAN CORPORATION
451 D Street • Boston, MA 02210 • 617/204-1000 • Fax: 617/204-1010

December 2, 2003
02660-L-049

R. Steven Daugherty
The Boeing Company
Integrated Defense Systems - NASA Systems
ISS Thermal & Environmental Control-Houston
M/S HB2-20

SUBJECT: Altran Corporation's Responses to the Boeing Company's Comments on Altran's MAT Draft Technical Report 02660-TR-001 Rev. 0

Dear Mr. Daugherty:

This letter is written in response to comments that the Boeing Company had after review of Altran Corporation's draft technical report 02660-TR-001 Rev. 0, "Microbial Influenced Corrosion of International Space Station Heat Exchanger Materials: Simulation Test of Worst Case Conditions." All comments were e-mailed to Altran Corporation from The Boeing Company. Three groups of comments were received by Altran and are discussed in the order received. This document is structured such that each comment (given in *italics*) is immediately followed by Altran's response.

1.0 MIC TEAM COMMENTS – RECEIVED NOVEMBER 19, 2003

Before I provide the comments from the MIC Team to the report, I would like to mention that all the team members had a positive comment about the report and the test. Mostly very positive feedback was provided at the meeting. Altran did a very good job summarizing the data that was compiled from the test. There is an incredible amount of information from the analyses performed. There are several details from some of the analyses that we will like to have more details about. Below is a list of those comments.

1. *Did you notice a microbial colonization pattern/coverage difference between the BNi 2 and BNi3 coupons? Any difference between the materials at all?- even small differences might be good to mention.*

   A review of the swab-test results did not reveal a significant difference between the coupon types. As you know, the surface structure of the coupons was not ideal for sampling. Therefore, the only way small differences between coupon type could be detected would be to employ flat coupon surfaces for swab/SEM analysis.

02660-L-049
December 2, 2003

However, we can also state that our SEM observations did not reveal any apparent differences.

2. *Did you notice a microbial colonization pattern/coverage difference between the 2 pH? Any difference between the materials at all?- even small differences might be good to mention.*

Similarly, we did not see any differences based on either swab recoveries or SEM observations.

3. *Do you have an idea of the type of colonization thickness that the coupons had that cannot be appreciated by looking at the SEM pictures?*

We did not observe multi-layered biofilms on coupon surfaces. It is likely that the microcolony thicknesses were less than 10 µm. As you know, the desiccation that is associated with ethanol dehydration and critical-point drying significantly reduces the volume of biomass.

4. *Please provide information that might explain why the microbial population declined initially, but then stayed stable for the rest of the test- there was TOC in the fluid, but it was not declining (at least in any measurable levels)*

TOC measurements reflect both assimilible and non-assimilable carbon. It is possible that there was a constant source of assimilible organic carbon (AOC) from material leaching and/or from microbial metabolites. In a steady-state system, growth (cell numbers and cell biomass) is determined by AOC concentration and availability. The initial changes in bacterial numbers likely reflected changes in the relative make-up of populations best adapted to the carbon sources and prevailing environmental conditions.

5. *If available, we would like to see pictures of the 'slime' on the coupons/tubing or other surfaces. Did you document with pictures any of this slime?*

Altran will incorporate pictures of the 90 and 180 day inoculated flow cell interiors that were taken immediately after removal from the MAT system and opened for processing the coupons. No pictures were taken of the ITCS distribution tubing.

6. *Please provide a table that lists the SEM pictures in the report and contains: type of coupon, pH of the fluid, control/test. It will be a great reference when you are looking at the pictures in the attachments.*

If this table was to be constructed it would have approximately 1,000 entries, as there are this many Figures. An alternative would be to use the sample numbers, given in each Figure caption, in conjunction with Tables 7 and 8 to determine all of

the information requested. Tables 7 and 8 list all the MAT study samples relative to their exposure conditions.

7. *Please provide if possible, a few examples of pictures in the same frame or page that shows the effect of time and pH and type of coupon over time (in one page to have unexposed sample, 1 month, 3 months, 6 months). It will be good to be able to see the effect(s) over time. We would like to have a disk with the pictures in a file that can be manipulated (so we can move around the pictures) not in acrobat reader.*

Altran Corporation will provide a CD with all of the report's image files for manipulation by The Boeing Company.

8. *It is the opinion of one of the team members that there is some visible corrosion on the surface where the microbial colony was "lifted", Could you clarify or provide additional information about why you report it as insignificant?*

These images, taken from Attachment I, have been inserted below to facilitate this discussion. We will have to discuss the specific location of interest. However, in comparing the area in the vicinity of the colony to the baseline samples and other 180 day samples we did not see any indications of accelerated attack in this area.

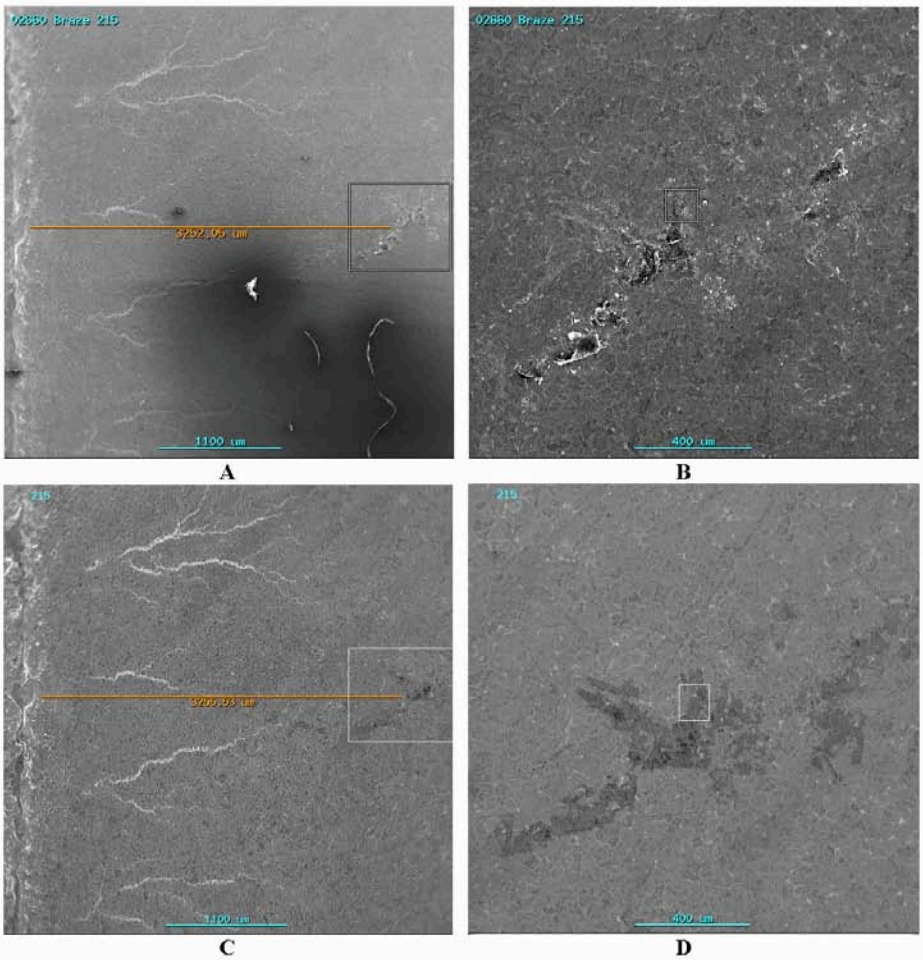
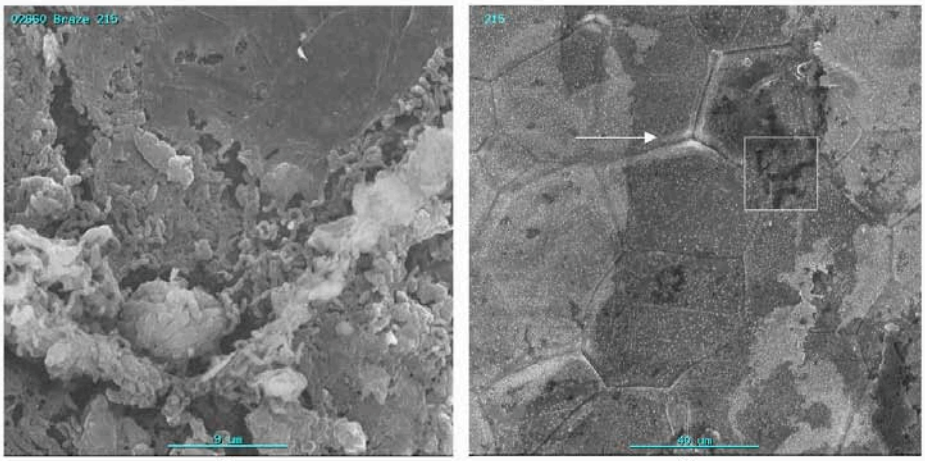


Figure 1. Under Biofilm Corrosion Evaluation of BNi-2 Sample 215 from Bioreactor A2, Flow Cell 15 (180 days exposure)

A = Location of biofilm on test coupon
B = Magnification of area depicted by square in photomicrograph A
C = Location of biofilm area after cleaning of coupon for under deposit analysis
D = Magnification of area depicted by square in photomicrograph C



E F G H

Figure 2. Under Biofilm Corrosion Evaluation of BNi-2 Sample 215 from Bioreactor A2, Flow Cell 15 (180 days exposure)

E = Magnification of area depicted by square in Figure 1, photomicrograph B
F = Magnification of area depicted by square in Figure 1, photomicrograph D
G = Magnification of area depicted by arrow in photomicrograph F
H = Magnification of area depicted by square in photomicrograph F

9. *From your experience and the literature that you are familiar with in testing materials for MIC, How long is the necessary time to expose a material to a fluid with microorganisms to establish that there is a cause for MIC concern?*

The literature contains reports of MIC in various metals occurring within days to months relative to abiotic environments. The time of initial appearance and rate of microbially-mediated corrosion damage is dependent upon three factors: 1) metallurgy, 2) environmental physicochemistry (AOC, pH, temperature, etc.), And 3) microbial population. There are no standard tables describing the relationship between these variables and corrosion rates. With the possible exception of titanium, all metals used in industry have been found to be susceptible to MIC. Therefore, it is always necessary to conduct empirical studies to assess the susceptibility of a given metal/alloy to microbial populations in a defined exposure environment. In our studies, we did not find any difference between the inoculated and control environments—nor was there any difference between these groups and unexposed coupons. Therefore, using the analytical techniques employed in this study one would have to test for greater than 180 days to determine a corrosion rate for these materials and conditions, provided that these materials will corrode under the test conditions.

2.0 COMMENTS FROM J. L. GOLDEN – RECEIVED NOVEMBER 20, 2003

1. *Section 5.2.3. Observation: Microbial level in the test fluid equilibrated at 1x10E5 cfu/ml, good agreement with on-orbit maximum levels observed.*

No response is required.

2. *Section 5.2.3.1. Observation: No SRB survival; biofilm sampled.*

No response is required.

3. *Section 5.3.1. Observation: Yellow slime was on everything, not just the plastic components of the test set-up. Comment: Photos would be helpful.*

Altran will provide photos as describe in section 1.0, question 5 above.

4. *Section 6.1. Observation: Darkening of coupons when exposed to light. This is a reaction of the silver deposition produced during coupon "conditioning", and is consistent with what has been observed by the Corrosion Team.*

No response is required.

5. *Section 6.5. Observation: Great microbe pictures in attachement I, photo 2-E.*

No response is required.

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6. *Attachment H Observations consistent with Corrosion Team test results.*
   p. 10, 12, and 21: good photos of intermetallic phase attack on BNi-2 coupons.

The referenced photos (pages 10, 12 and 21) from Attachment H (180 Day Metallurgy) are inserted below. For comparative purposes pages 10, 15 and 16 of Attachment D (Baseline Metallurgy) have been inserted immediately following these images. Relative to the baseline images some of the BNi-2 180 day samples appear to possibly have some attack at the grain boundaries. Section 6.2 of 02660-TR-001 has been revised (highlighted text has been added) to read:

"6.2 SEM of Sample Surfaces

Attachment D shows the presence of needle like features on BNi-2 samples. EDS of these features showed them to be Cr rich. Similar features were not observed on the BNi-3 samples. Unexposed and exposed samples also showed pores at grain boundary triple points and a dark phase, especially in the vicinity of the fillet, between grains. At higher magnifications 180 day samples 250, 231 and 262 may show some indications of attack at the braze grain boundaries. As shown in the following sections these features were too minor to be quantified by the other analytical techniques employed in this study. If the test duration was extended beyond 180 days perhaps measurable damage would have resulted. If a feature of interest was observed EDS was performed to determine if a corrosion product was present. These EDS spectra did not indicate the presence of corrosion products."

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PAGE 10 OF ATTACHMENT H

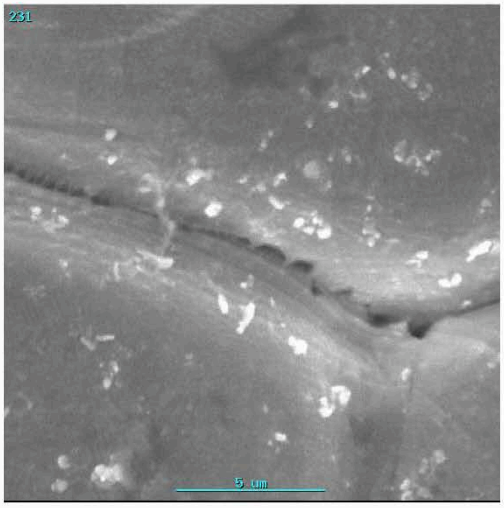

Figure 15. "5,000X" SEM Image of the Braze Region of Sample 231 (180 day pH 8.3 Un-inoculated)

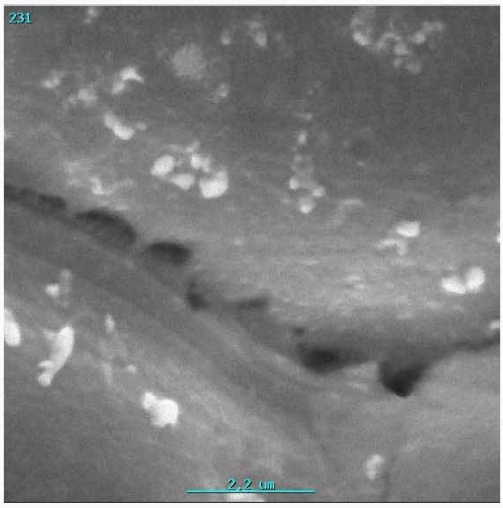

Figure 16. "10,000X" SEM Image of the Braze Region of Sample 231 (180 day pH 8.3 Un-inoculated)

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PAGE 12 OF ATTACHMENT H

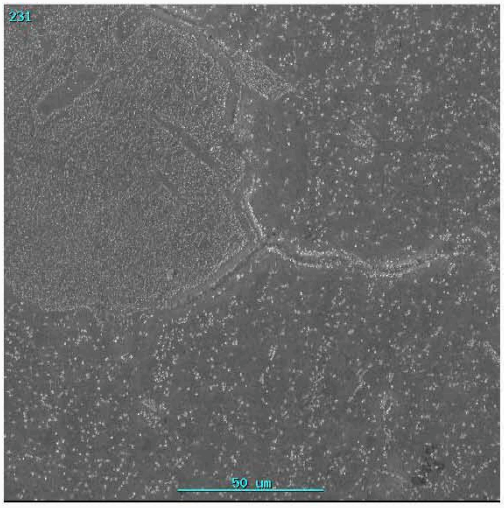

Figure 19. "500X" SEM Image of the Ni Strip Region of Sample 231

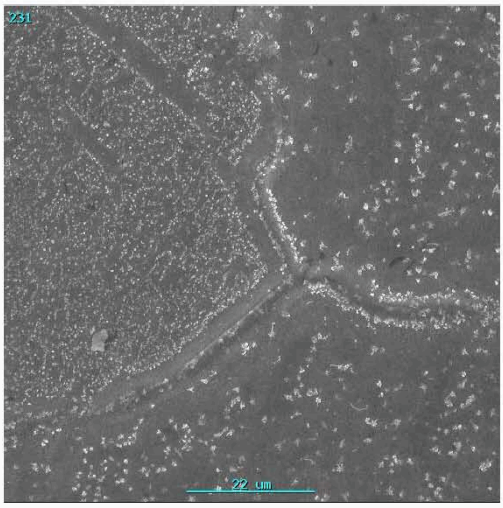

Figure 20. "1,000X" SEM Image of the Ni Strip Region of Sample 231

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PAGE 21 OF ATTACHMENT H

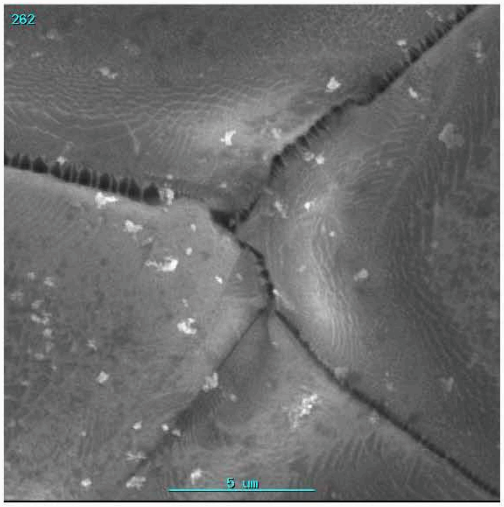

Figure 37. "5,000X" SEM Image of the Braze Region of Sample 262 (180 day pH 9.4 Un-inoculated)

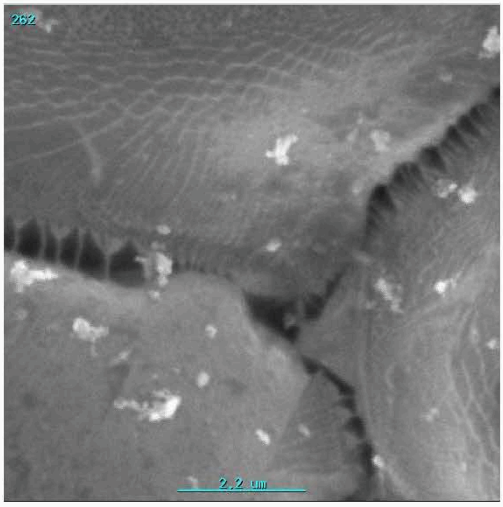

Figure 38. "10,000X" SEM Image of the Braze Region of Sample 262 (180 day pH 9.4 Un-inoculated)

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PAGE 10 OF ATTACHMENT D (Baseline Metallurgy)

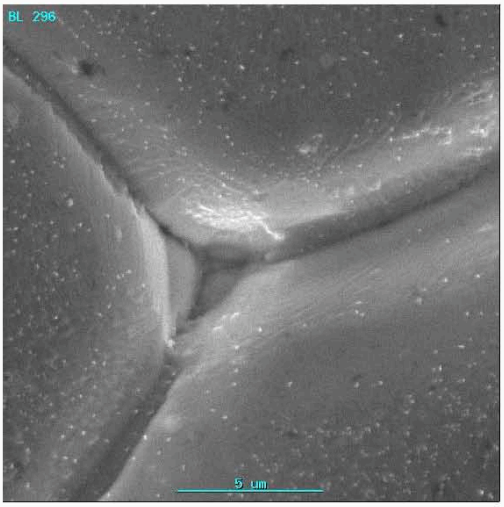

Figure 15. "5,000X" SEM Image of the Braze Region of Sample 296 (baseline)

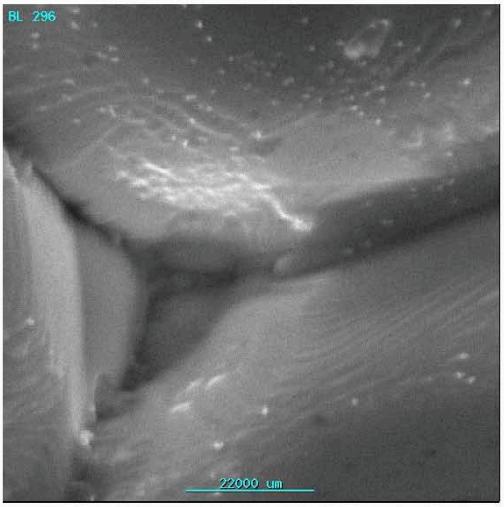

Figure 16. "10,000X" SEM Image of the Braze Region of Sample 296 (baseline)

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PAGE 15 OF ATTACHMENT D (Baseline Metallurgy)

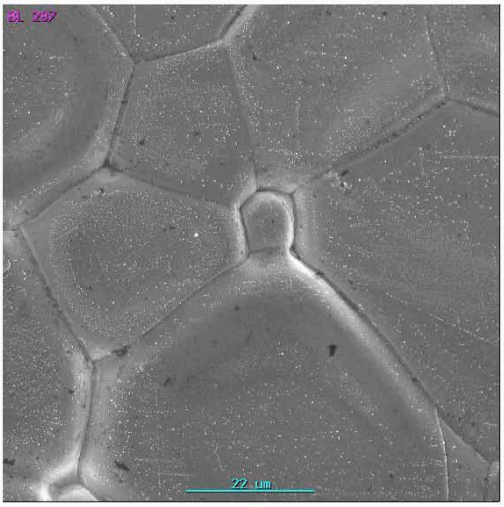

Figure 25. "1,000X" SEM Image of the Braze Region of Sample 297 (baseline)

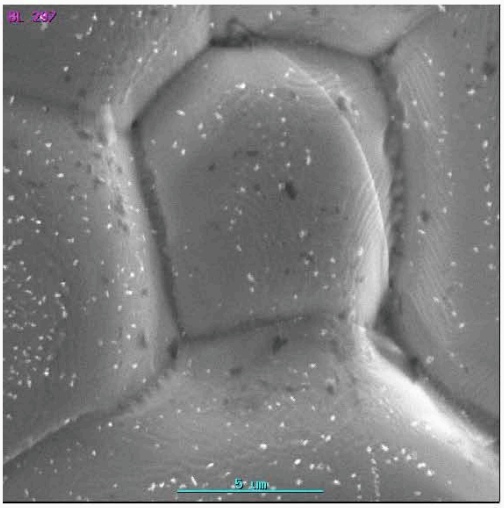

Figure 26. "5,000X" SEM Image of the Braze Region of Sample 297 (baseline)

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PAGE 16 OF ATTACHMENT D (Baseline Metallurgy)

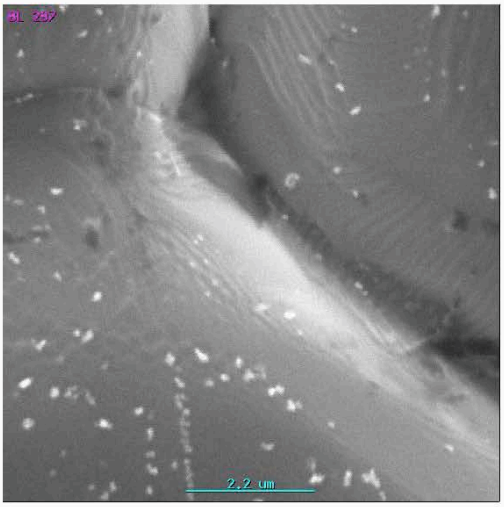

Figure 27. "10,000X" SEM Image of the Braze Region of Sample 297 (baseline)

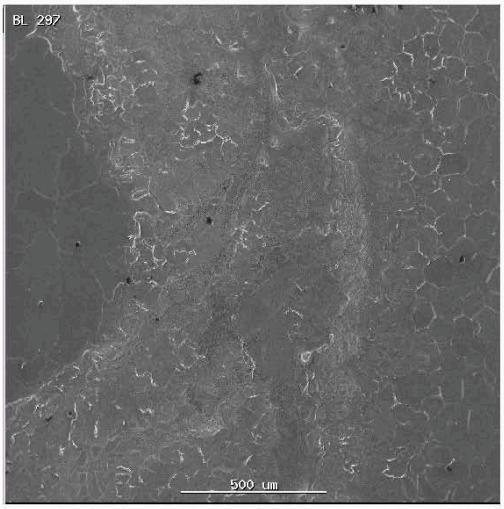

Figure 28. "50X" SEM Image of the Fillet Region of Sample 297 (baseline)

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p. 29, 30: apparent Ag relocation onto surface etched ridgelines of BNi-3.

The referenced photos (pages 29 and 30) from Attachment H (180 Day Metallurgy) are inserted below. These images show Ag decorating terrace like features on the Ni regions of BNi-3 samples. For comparative purposes page 45 of Attachment D (Baseline Metallurgy) has been inserted immediately following these images. Relative to the baseline image these images appear to be very similar.

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PAGE 29 OF ATTACHMENT H (180 Day Metallurgy)

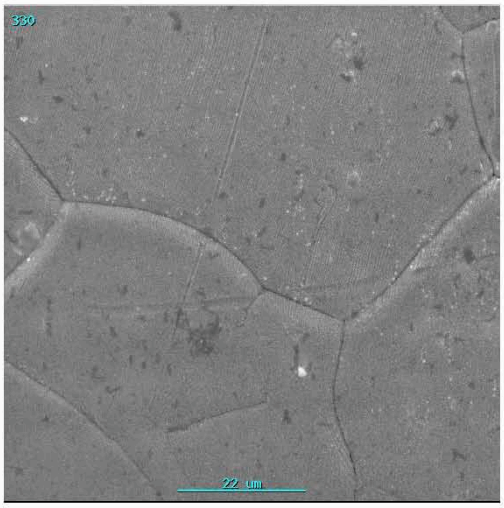

Figure 53. "1,000X" SEM Image of the Ni Strip Region of Sample 330

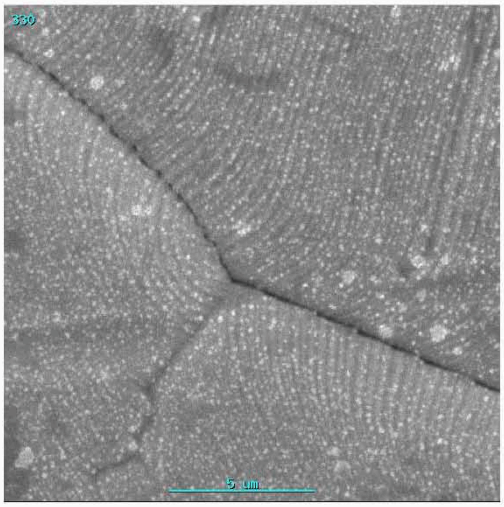

Figure 54. "5,000X" SEM Image of the Ni Strip Region of Sample 330

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PAGE 30 OF ATTACHMENT H (180 Day Metallurgy)

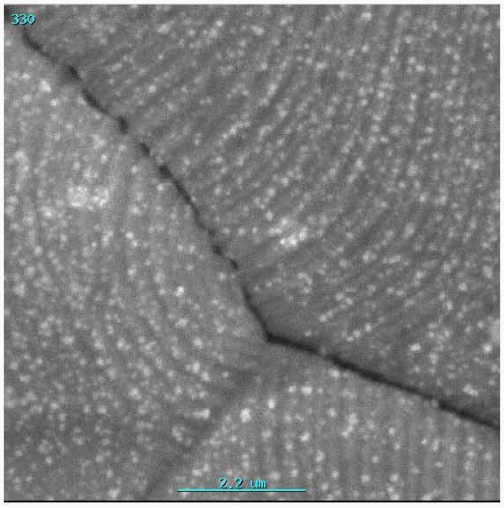

Figure 55. "10,000X" SEM Image of the Ni Strip Region of Sample 330

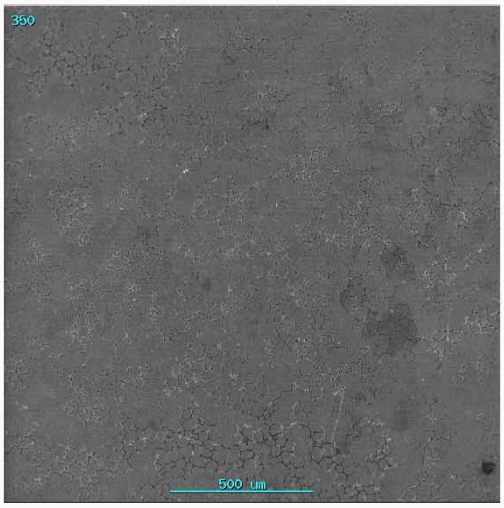

Figure 56. "50X" SEM Image of the Braze Region of Sample 350

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PAGE 45 OF ATTACHMENT D (Baseline Metallurgy)

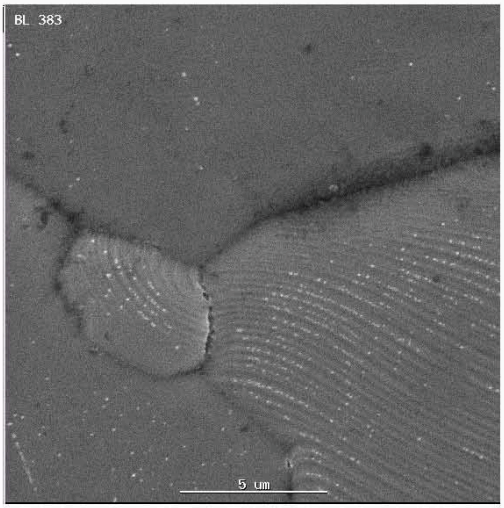

Figure 85. "5,000X" SEM Image of the Ni Strip Region of Sample 383 (baseline)

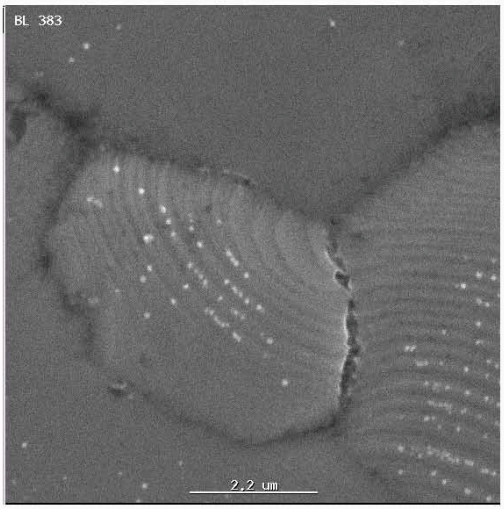

Figure 86. "10,000X" SEM Image of the Ni Strip Region of Sample 383 (baseline)

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p. 31: apparent relocation of Ag onto matrix phase during etch of intermetallic phase on BNi-3.

The referenced photos (page 31) from Attachment H (180 Day Metallurgy) are inserted below. These images shows the braze region of a BNi-3 sample. For comparative purposes Figure 103 of page 54 of Attachment D (Baseline Metallurgy) has been inserted immediately following this image. It was found to be common to see such intergranular features in the fillet regions of the baseline samples.

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PAGE 31 OF ATTACHMENT H (180 Day Metallurgy)

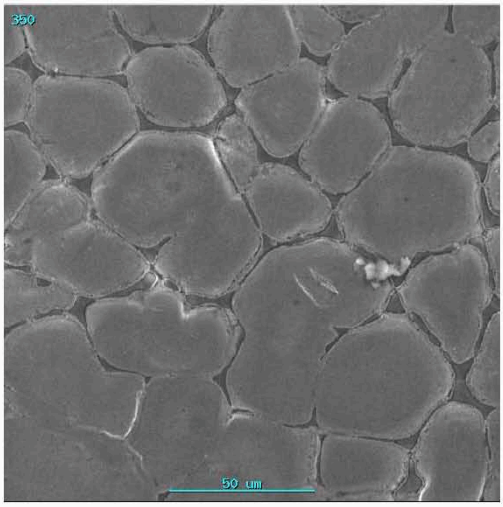

Figure 57. "500X" SEM Image of the Braze Region of Sample 350

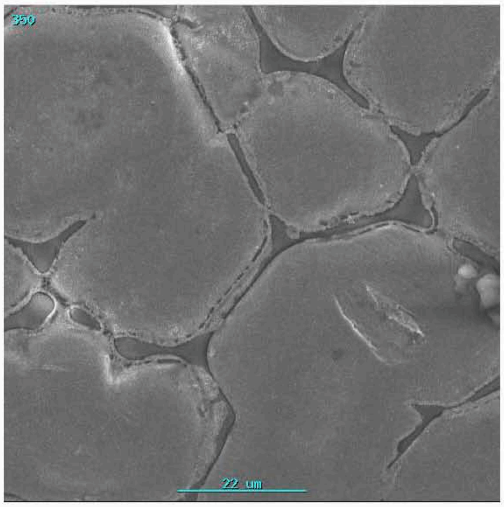

Figure 58. "1,000X" SEM Image of the Braze Region of Sample 350

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PAGE 54 OF ATTACHMENT D (Baseline Metallurgy)

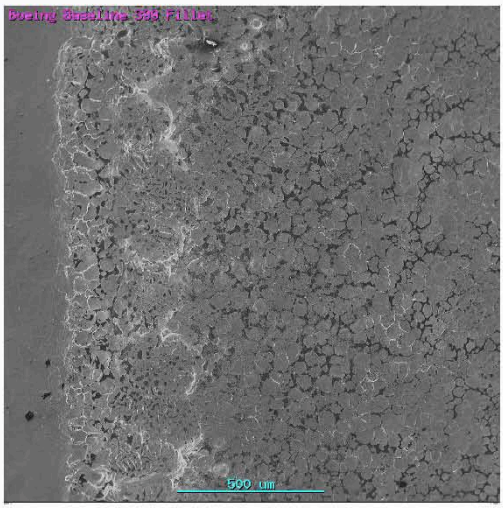

Figure 103. "50X" SEM Image of the Fillet Region of Sample 399

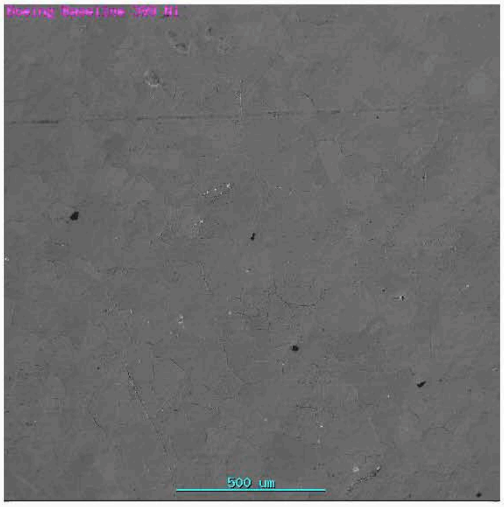

Figure 104. "50X" SEM Image of the Ni Strip Region of Sample 399

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p. 81: availability and etching of intermetallics in BNi-3 fillet region.

The referenced photos (page 81) from Attachment H (180 Day Metallurgy) are inserted below. For comparative purposes Figure 135 of page 71 of Attachment D (Baseline Metallurgy) has been inserted immediately following this image. Based on comparison with the baseline photographs, we do not conclude that there is a significant difference.

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PAGE 81 OF ATTACHMENT H (180 Day Metallurgy)

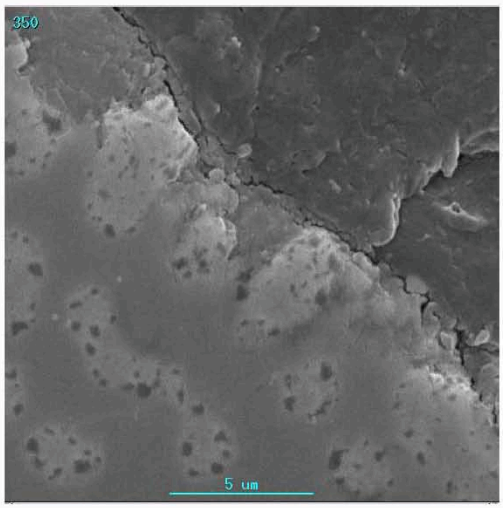

Figure 155. "5,000X" SEM Image of the Fillet Region of Sample 350

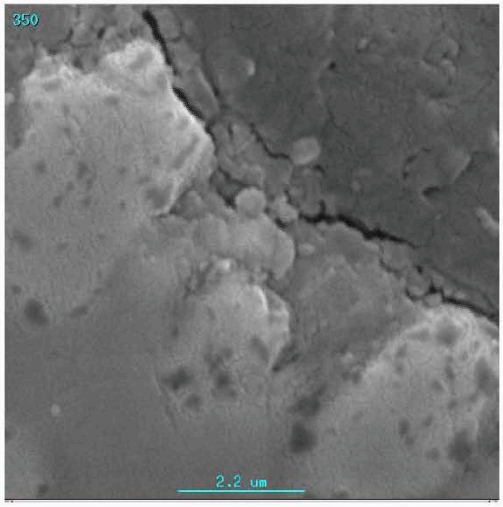

Figure 156. "10,000X" SEM Image of the Fillet Region of Sample 350

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PAGE 71 OF ATTACHMENT D (Baseline Metallurgy)

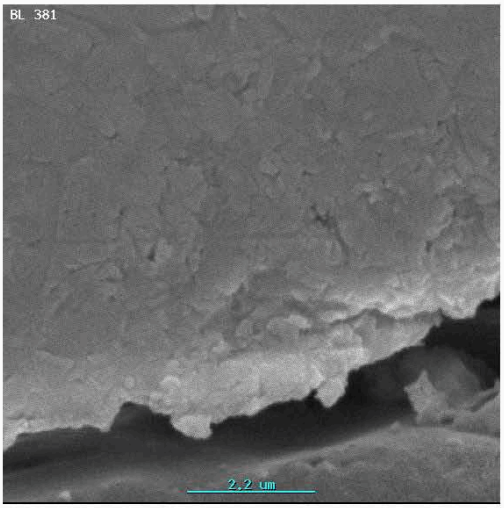

Figure 135. "10,000X" SEM Image of the Fillet Region of Sample 381

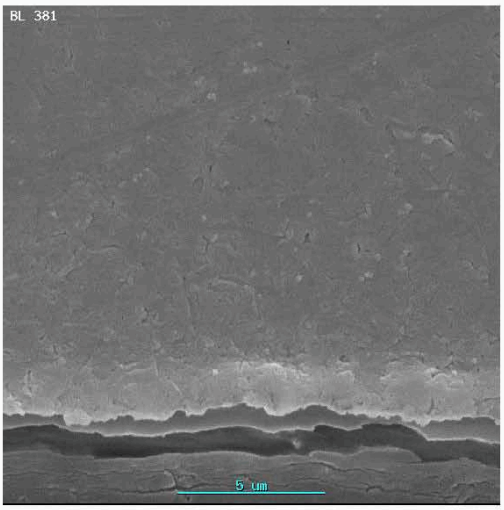

Figure136. "5,000X" SEM Image of the Ni Strip Region of Sample 381

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p. 112: good example of intermetallic corrosion in BNi-2 fillet region.

The referenced photo (page 112) from Attachment H (180 Day Metallurgy) is inserted below. For comparative purposes pages 82 and 85 of Attachment D (Baseline Metallurgy) have been inserted immediately following this image. Based on comparison with the baseline photographs, we do not conclude that there is a significant difference.

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PAGE 112 OF ATTACHMENT H (180 Day Metallurgy)

Figure 214. Optical Microscopic Image of the Fillet Region of Sample 262

Figure 215. Optical Microscopic Image of the Ni Strip Region of Sample 262

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PAGE 82 OF ATTACHMENT D (Baseline Metallurgy)

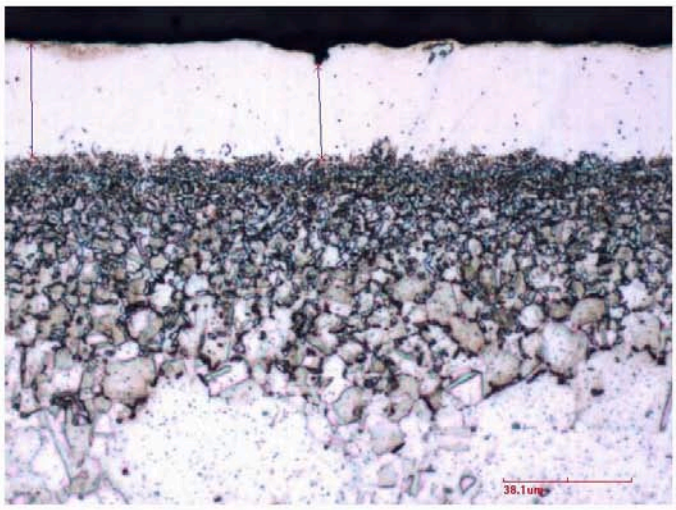

Figure 154. Optical Microscopic Image of the Braze of Sample 204 – Section 1, Location D

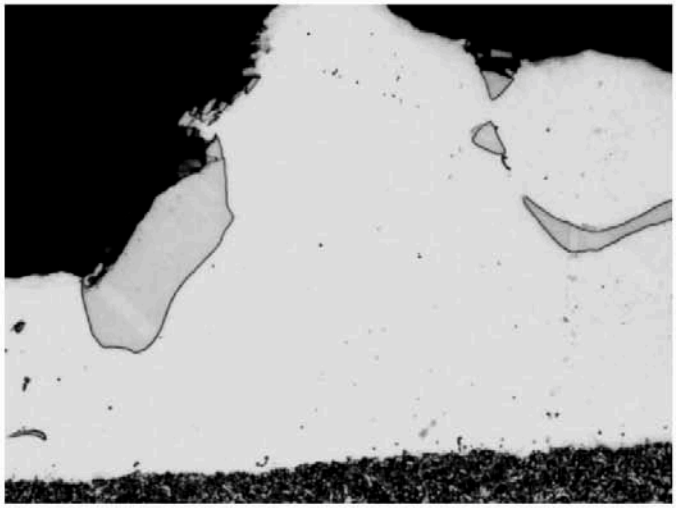

Figure 155. Optical Microscopic Image of the Fillet Region of Sample 204 – Section 1

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PAGE 85 OF ATTACHMENT D (Baseline Metallurgy)

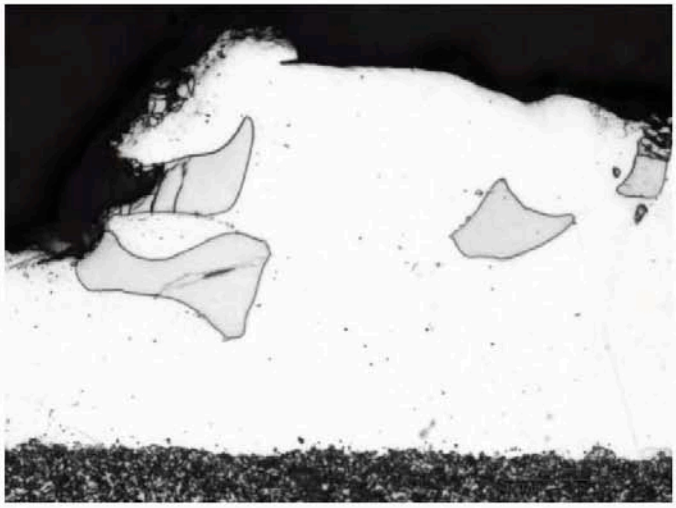

Figure 160. Optical Microscopic Image of the Fillet Region of Sample 204 – Section 2

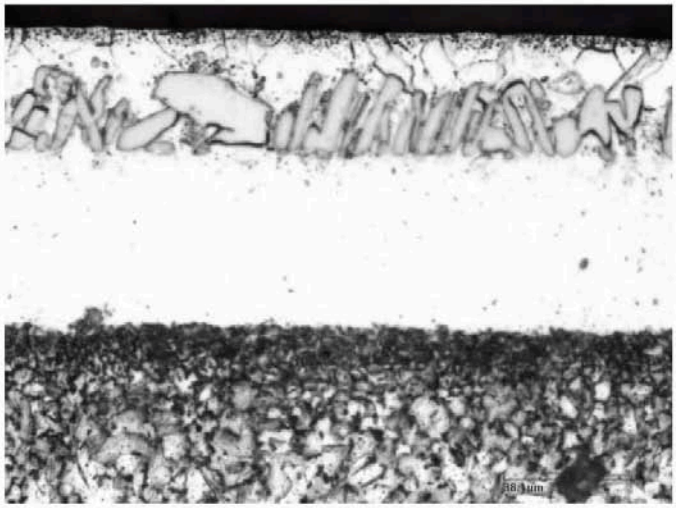

Figure 161: Optical Microscopic Image of the Ni Strip Region of Sample 204 – Section 2

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p. 120: good example of intermetallic corrosion in BNi-3 fillet region, with what appears to be precipitate formation.

The referenced photo (page 120) from Attachment H (180 Day Metallurgy) is inserted below. For comparative purposes pages 98 and 102 of Attachment D (Baseline Metallurgy) have been inserted immediately following this image. Based on comparison with the baseline photographs, we do not conclude that there is a significant difference.

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PAGE 120 OF ATTACHMENT H (180 Day Metallurgy)

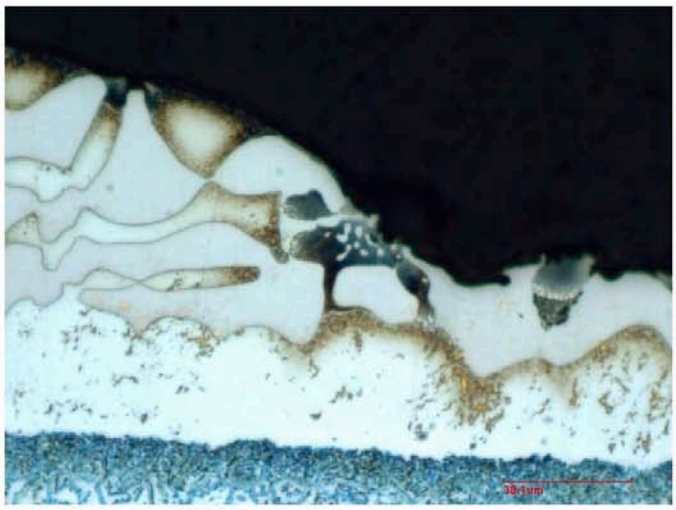

Figure 230. Optical Microscopic Image of the Fillet Region of Sample 350

Figure 231. Optical Microscopic Image of the Ni Strip Region of Sample 350

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Figure 186. Optical Microscopic Image of the Fillet Region of Sample 308 – Section 1

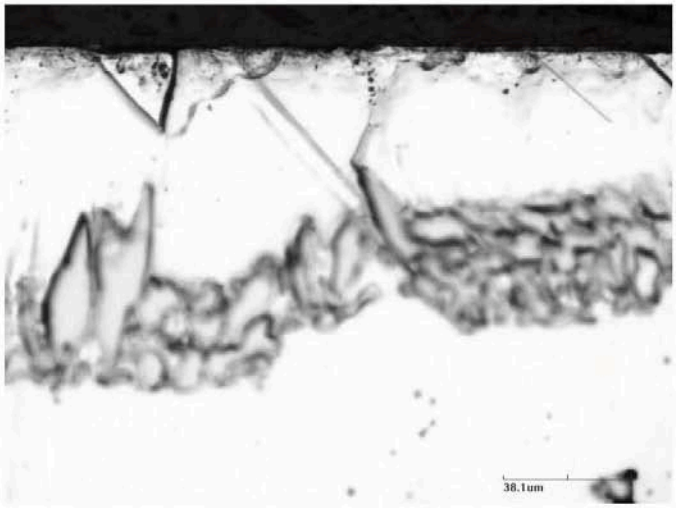

Figure 187: Optical Microscopic Image of the Ni Strip Region of Sample 308 – Section 1

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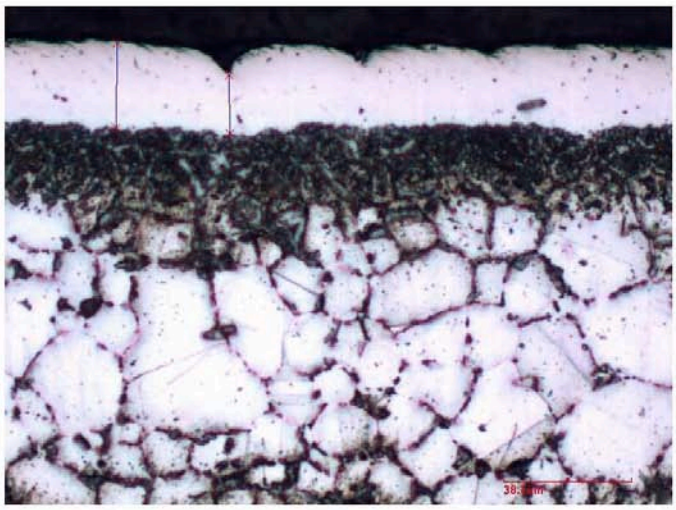

Figure 194. Optical Microscopic Image of the Braze of Sample 308 – Section 3, Location C

Figure 195. Optical Microscopic Image of the Fillet Region of Sample 308 – Section 3

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7. *Attachment H, p.82 (Fig. 157). Comment: Figure is identified as Nickel strip region on a BNi-3 coupon. This may be near the nickel strip, but the nickel strip is not in view. This is a fillet near the nickel strip, as identified by the extensive amount of intermetallic. Extensive corrosion into the intermetallic phase is observed.*

This image was mislabeled and unfortunately the Ni strip region was not available for this sample. Accordingly, this page was changed as shown on the next page.

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Photo Not Available

Figure 157. "5,000X" SEM Image of the Ni Strip Region of Sample 350

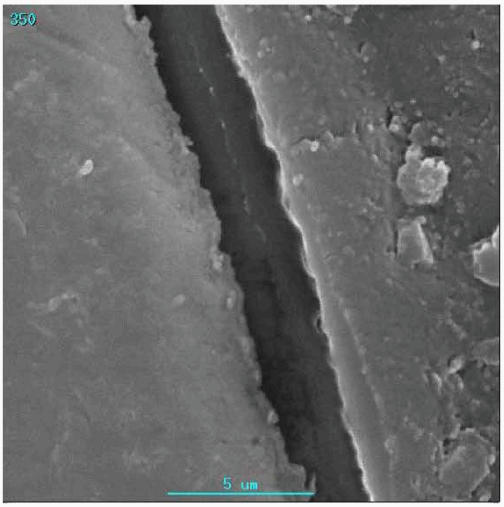

Figure 158. "5,000X" SEM Image of the Braze Region at the Cut Corner of Sample 350

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8. *General Comment: I thought the report was quite good; excellent documentation of test conditions. I agreed with the interpretation of the results and the conclusions.*

   No response is required.

3.0 COMMENT FROM MONSI ROMAN – RECEIVED NOVEMBER 24, 2003

1. *"If it is OK, I will like to add one more question to the questions I sent to you earlier for the Altran report next week -- Could Altran provide us with information about any difference observed between the controls (not inoculated) and the test coupons? It happens that Altran ran the best corrosion test that could have been performed, because the corrosion on the coupons from the vessels that were NOT inoculated will provide information about the chemical corrosion that can not be provided by the data that HS has gathered-I think that will be a great addition to the data we have."*

   As detailed in the discussion section of the report, the analysis performed did not indicate any significant difference between the controls (not inoculated) and the test coupons.

We look forward to discussing these comments and responses on Wednesday December 3rd at 9 AM CST.

Very truly yours,

ALTRAN CORPORATION

Patrick Macuch
Project Manager

Thomas J. McKrell, Ph.D.
Project Engineer

cc: R. Mitchell
M. Mittelman
O. Van Der Schijff
R. Latanision
Boeing employees to be designated by Mr. Daugherty

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Appendix F. Toxicologist Assessment of IATCS Coolant Biocides

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Cell Phone: 205-427-1043
mecoleman33@sbcglobal.net

To: Monsi Roman/MSFC/EV51
Chief Microbiologist

FROM: Martin E. Coleman, Ph.D.

DATE: March 4, 2005

SUBJECT: Toxicological Assessment of IATCS Coolant Biocides

MESSAGE

You requested a toxicological hazard assessment of two biocidal agents that are candidates for usage in the International Space Station (ISS) Internal Active Thermal Control System (IATCS) Coolant Loops. A silver salt is currently used for this purpose. However, since the silver deposits out on the IATCS metallic component, it will be replaced by another antimicrobial agent*. At present, the two leading candidates are ortho-phthaldehyde (OPA) and glutaraldehyde (GA). You requested that I make a toxicological comparison between the two chemicals agents in order to facilitate making this decision. The present report, which addresses these biocides, will be confined to their relative toxicity/irritancy hazards. The report will not address the bactericidal efficacy, materials compatibility, or effects of these agents on the Environmental Control and Life Support Systems (ECLSS); these issues will be addressed by other specialists.

I was told by Mr. Jay Perry, MSFC Senior Engineer, ISS ECLSS Air Quality Control, that the IATCS coolant loop is normally expected to leak somewhat. He also told me that the various racks in the ISS are frequently taken down or installed, so there is always a chance of a bad connection and subsequent leak in any of them. Since some of the racks are in the avionics system where they are not readily visible, a leakage in these coolant systems might go undetected for several weeks or longer, giving the coolant fluids this long a time to evaporate into the ISS habitable areas. It is also possible that the crew may not enter certain modules, such as the ISS airlock for some time, possibly allowing a leak to go undetected for a long time. Mr. Perry noted that the IATCS could leak as much as 1 gallon (4 liters) of IATCS fluid before the system shuts down. In the present memo, the total amounts of fluid in the IATCS loops, the maximum potential leak rates from the various racks and modules, and the toxic hazard potentials in the event that leaks should occur will be reviewed.

Table 1 in the enclosure gives the total amount of IATCS coolant fluids and their temperature ranges in both the low and high temperature loops, which serve all of the ISS modules and racks that require cooling. Table 2 gives the maximum permissible leak rates for many of the major ISS modules and racks, the maximum permissible leak rates for the combined racks and modules, the total combined leakage rates for all racks and modules that would require an inflight maintenance initiative (IMI) to stop the leaks, and the amount of IATCS fluid loss that would cause the system to shut down. A toxicological hazard assessment of both OPA and GA, in the liquid and vapor forms, is given in Table 3. Table 3 also gives some physical characteristics of these chemicals, such as vapor pressures and pH, that might be related to their toxic hazards or escape potentials.

Note in Table 3 that the vapor pressure of highly concentrated GA is rather high, while that of a 2% aqueous solution is much lower. This is apparently due to the very high affinity of GA for water. Thus if a dilute GA solution were to escape into a modular atmosphere, it would vaporize rather slowly. If it were soon detected, it could probably be removed by a crewmember(s) with the vacuum vent system, wet wipes, or by another method (after a crewmember donned suitable protective gear) before a significant amount vaporized. However, if the GA solution were to escape into a module, such as the airlock, where the crewmembers seldom entered, or if it were to escape into certain lockers or racks, it might not be detected for several days to several weeks or longer. Thus, as the water evaporated, the GA would become more and more concentrated and its rate of vaporization would progressively increase. As the concentration increased, the vapor would become more and more toxic and irritating. Note in Table 3 that a GA vapor concentration of only 0.3 ppm causes notable eye and respiratory irritation, and 0.38 ppm causes severe irritation and headache.

GA vapors can cause allergic reactions at lower vapor concentrations. For example, one report noted that repeated exposure to vapor concentrations as low as 0.12 ppm caused allergic symptoms, including respiratory difficulty (see enclosure). Due to the high toxicity, irritancy and allergenicity of GA vapors, the JSC Toxicology Group has set some very low spacecraft maximum allowable concentration (SMAC) limits, especially for long term exposures. Thus the 30-day SMAC for GA vapors is only 0.003 ppm (Table 3). This concentration could be exceeded by a wide margin over a long period of time after an undetected escape of a GA solution.

On the other hand, it is much less likely that the vapor from OPA would cause any significant irritation or allergic response. Note in Table 3 that the vapor pressure of pure OPA at room temperature is only 5.2 X 10^{-3} torr (mm Hg), which is much lower than that of pure GA. The vapor pressure of Cidex OPA Solution (0.55% OPA) is only 7.6 X 10^{-7} torr. This concentration of OPA in the Cidex OPA Solution is still more than 10 times higher than the maximum OPA concentration that is under consideration for use as a biocidal agent in the IATCS (0.039%). Therefore, the OPA vapor pressure in the IATCS fluid would be expected to be even lower. Even at elevated temperatures (55 – 60° C), the vapor pressure of OPA is still very low (see Table 3). Due to the low vapor pressure, the odor from Cidex OPA is "barely perceptible" or "virtually odorless," while GA has a "strong pungent odor." (see Table 3). The primary risk of OPA-induced allergies (in rare instances) is apparently from repeated skin contact with residual liquids or solids on OPA disinfected instruments (see Table 3).

The planned maximum GA concentration in the IATCS liquid solution is 100 ppm (0.01%) (see Table 3). This concentration is 25 times lower than the minimum critical eye hazard level (toxicity/irritancy hazard level 1), established by the JSC Toxicology Group (0.25%, 2500 ppm) (l). Therefore, it would initially not be a significant irritancy or toxicity hazard. But after the GA solution escaped and became more and more concentrated due to water evaporation, it might eventually become a catastrophic eye hazard (at 1% or 10,000 ppm concentration), probably capable of causing permanent eye damage upon direct contact. The critical and catastrophic hazard levels of eye irritants are further defined in Table 3.

On the basis of several long-term dosing studies in animals, GA does not appear to be a carcinogenesis threat. There was a significantly increased incidence of one type of leukemia in female rats that ingested GA in the drinking water for two years. But since this type of leukemia occurs spontaneously at about 24 – 25% in the strain of rats tested, the GA was believed to be a facilitator, rather than a direct cause of this leukemia (Table 3).

The OPA solution would probably be much less irritating than a GA solution in the event of eye or skin contact. Note in Table 3 that one report indicated that the Cidex OPA solution is not a known irritant. Another report indicated that it was only slightly irritating to the rabbit's eyes. The Johnson & Johnson

materials safety data sheet (MSDS) indicated that it may cause "stinging, tears and redness" upon direct eye contact. However, no eye irritancy data was cited. As is the case of many materials safety data sheets, it is likely that this statement may have been prompted by worst-case conservativism.

Note in Table 3 that in animal studies, the systemic toxicity of GA is rather high, as is indicated by rather low mouse and rat oral and i.v. LD50 values (lethal dose in 50% of animals). The much higher rabbit dermal LD50 for GA (indicating a much lower level of toxicity when administered by this route) is probably because the GA-exposed skin forms a barrier to further adsorption. The rat oral and dermal LD50 values for OPA are much higher, indicating a much lower level of systemic toxicity. This is a further indication that OPA is a much lesser toxicity concern than is GA.

Many hospitals in the United States and other countries are changing over from GA to OPA as a primary low temperature disinfectant. The lower risk of toxicity, irritancy and allergenicity is a major factor for this change. For the same reasons, it seems that for the enclosed environments of the ISS modules, OPA would be much preferable to GA as the antimicrobial agent in the IATCS water loop.

.....

*Wilson, Mark, et. al. Selection of an Alternate Biocide for the International Space Station Active Thermal Control System
Coolant Loops. NASA Document 2003- 01-2568. SAE International, 2003.

Original signed by: Martin E. Coleman, Ph.D.
Wyle Consulting Toxicologist
Enclosure
IATCS-Tox Memo

c.c.
John T. James/SF23
Axel Larsen/MA2
Mike Pedley/EM2
Gary Rankin/EC3
Tom Limero/Wyle/SF23
Hector Garcia/Wyle/SF23
Monsi Roman/MSFC/EV5
Jay Perry/MSFC/EV51
Mike Holt/MSFC/EV34

ENCLOSURE

TABLE 1

Total ITCS Fluid Volumes and Temperatures In the ISS[a]

Loops	Fluid Volume	Fluid Temperature
Low Temperature Loop (LTL)	63 liters	3.3 – 6.1°C
Medium Temperature Loop (MTL)	200 liters	16.1 – 18.3°C
Total Volume	263 liters	

TABLE 2

Permissible Leak Rates of IATCS Coolant Loops Within Some Modules of the ISS[b]

ISS Modules or Racks	Permissible Leak Rate (cc/hr)	
	LTL	MTL
USL	0.80	0.80
Airlock	0.80	0.80
Node 1	0.80	0.80
Node 2	1.09	0.86
Node 3	1.50	2.00
CAM	0.48	0.48
MPLM	0.275	NA
Cupola	NA	0.026
APM	0.800	0.800
JEM	0.800	0.800
Design Specifications for combined leakage rate (all modules)	14.7 cc/hour (352.8 cc/24 hr.)	
Specifications for combined leakage in a single loop mode	4.80 cc/hour	
Threshold on-orbit leakage at which a loop would be shut down	3.88 cc/hour (<1%/day) Note: One would actually have to lose about a gallon (4 liters) of fluid before the IATCS system would shut down. This would be expected to take about one month.	
Threshold on-orbit leakage rate to initiate an inflight maintenance initiative (IFI) (for entire IATCS configuration).	0.161624 cc/hour	

Legend: ISS: International Space Station
IATCS: Internal Active Thermal Control System
USL: United States Laboratory (100 m^3 volume)
MPLM: Mini-Pressurized Logistics Module
APM: Attached Pressurized Module (as European Laboratory Module) (62 m^3 Volume)
JEM: Japanese Experiment Module (125 m^3 volume)
CAM: Centrifuge Accommodation Module

TABLE 3
Toxicological Characteristics of Ortho Phthaldehyde and Glutaraldehyde

Characteristics	Ortho Phthladehyde (OPA)	Glutaraldehyde (GA)
Water Solubility	Very soluble	Very soluble
Planed Ranges of Concentrations in IATCS Fluid	130 – 390 ppm (in solution)[c] (0.013 – 0.039%)	30 - 100 ppm (in solution)[c] (0.003 – 0.01%)
Vapor Pressure (1 torr = 1 mm Hg)	5.2 X 10-3 torr (pure OPA, 21°C)[d] 7.6 X 10^{-7} torr (0.55% solution, 25°C[d] 1.75 X 10^{-5} torr (concentrate, 35°C)[e] 9.12 X 10^{-6} torr (1% solution, 55°C)[e] 1.3 X 10^{-4} torr (1.7% solution, 60°C)[e]	17 torr (pure GA, 20°C)[f,g] 0.0152 (1.52 X 10^{-2}) torr (50% solution)[h] 0.0012 (1.2 X 10^{-3}) torr (2% solution, 20°C)[h] 0.0012 (1.2 X 10^{-3}) torr (2.4% activated solution, pH 8.2-8.9)[i]
Need for Additives	Normally, OPA solution requires no activation or mixing with additives (see exception below).	Needs activation with an alkaline buffer for disinfectant efficacy (see pH below)
pH	Normal usage: pH about 6.5 Increasing pH to 8 increases sporicidal activity[j].	3.0 – 4.6 normal, unactivated - Not very effective as an antimicrobial agent[i] 8.2 – 8.9 – activated with alkali. Very effective as an antimicrobial agent[i].
Irritancy (liquid solution)	"OPA….is not a known irritant to the eyes and nasal passages…"[j] Cidex OPA solution: "eye contact may cause stinging, excess tearing and redness."[k] Cidex OPA solution: Eye irritancy (rabbit): "slightly irritating, but reversible in 7 days"*	Liquid Solutions: A strong eye and skin irritant[l] A catastrophic (Tox. level 2) eye hazard: ≥ 1%[m] (potential for causing permanent damage) A critical (Tox. level 1) eye hazard: 0.25% to <1%[m] (may cause irritation for longer than 30 minutes, but no significant risk of permanent damage) No adverse effect level (NOAEL): < 0.25%[m]
Human Toxicity, and Irritancy (vapors)	In most reports, vaporization was so low (see above) that the vapors would generally not be a problem.	GA Vapor: A strong eye and respiratory irritant Human studies: (a) Notable irritating response level: 0.3 ppm[h] (b) 12-minute exposure to 0.38 ppm during cold sterilization procedures (2% solution): severe eye, nose and throat irritation, sudden headache[h]
Animal Toxicity Data	Rat oral LD50: >5000 mg/kg[n] Rabbit dermal LD50: >2000 mg/kg[n]	Animal Toxicity Data[o] Rat inhalation LC50: 5000 ppm/4hrs Mouse i.v. LD50: 15.4 mg/kg Rat i.v. LD50: 15.3 mg/kg Mouse oral LD50: 100 mg/kg Rat oral LD50: 134 mg/kg Rabbit dermal LD50: 2560 mg/kg

TABLE 3 – continued
Toxicological Characteristics of Ortho Phthaldehyde and Glutaraldehyde

Characteristics	Ortho Phthladehyde (OPA)	Glutaraldehyde (GA)
Allergic potential	In rare cases it may cause allergic sensitization in patients or health care workers upon repeated contact with OPA disinfected instruments[p].	Repeated exposure to the vapor (at 0.12 ppm in the breathing zone) has caused allergic sensitization, such as watering of eyes, rhinitis (runny nose), respiratory difficulty, nausea and headache[q]. GA exposure in hospitals is a recognized cause of occupational asthma in many industrialized nations[r].
Human Exposure Limits to Vapors	No vapor exposure limit has been set due to the very low vaporization.	NASA Official SMACs[s]: 1-hr: 0.12 ppm (0.5 mg/m^3) 24-hr: 0.04 ppm (0.08 mg/m^3) 7-day: 0.006 ppm (0.025 mg/m^3) 30-day: 0.003 ppm (0.012 mg/m^3) 180-day: 0.0006 ppm (0.002 mg/m^3) ACGIH 8-hr/day TLV: 0.05 ppm (0.2 mg/m^3)[g]
Odor	Barely perceptible[l] Virtually odorless due to low vapor pressure[d]	Strong pungent odor[s]
Potential Carcinogenicity of Vapors	Has not been tested (as far as we know).	Male and female rats and mice exposed to GA vapor for 6 hr/day, 5 days/week and for 104 weeks (0, 250,500, and 750 ppb for rats and 0, 62.5, 125, or 250 ppb for mice) showed no evidence of carcinogenicity[t]. GA in drinking water for 2 years at 0, 50, 250 and 1000 ppm showed a significantly increased incidence of large-granular-cell leukemia in females only, in all treated groups. However, this condition occurs spontaneously at a lower incidence (23-24%) in females of the strain used[h]. Results indicated that this was a modifying influence on a spontaneous occurrence, not a direct carcinogenic effect[i].
Toxic Hazard Potential of Stock Solutions Used to Replenish IATCs Water Loop Biocides	Not known	GA: 5 – 25%[a] Both concentrations: Toxicity level 2 (catastrophic) eye hazard[a]. Also a vapor hazard if it should escape and vaporize.

Legend:
ACGIH: American Conference of Governmental Industrial Hygienists
TLV: Threshold Limit Value. An ACGIH recommended industrial workplace concentration limit for airborne contaminants, based on 8-hour-per day, 5-day-per-week human exposures.
SMAC: Spacecraft maximum allowable concentration (established by the JSC Toxicology Group). The SMACs vary, depending on the expected duration of crew exposure.

References:

a. Wilson, Mark, et.al. Selection of an Alternate Biocide for the ISS Internal Thermal Control System Coolant – Phase II. NASA Document 2004-01-2472. SAE International, 2004.

b. Information supplied by Mr. Jay Perry/MSFC/EV52, Senior Engineer, ISS ECLSS Air Quality Control.

c. Information supplied by Monsi Roman/MSFC/EV51, MSFC Chief Microbiologist, a member of the IATCS Biocide Working Group.

d. Feldman, L.A. Attachment to Customer Letter: CL-136, Rev. B, "Vapor Pressure of OPA in Air from Cidex OPA Solution." Advanced Sterilization Products, 12/06/01.

e. Unpublished data supplied by Advanced Sterilization Products, March 6, 2003.

f. SPI Supplies manufacturer's brochure, "SPI-ChemTM Glutaraldehyde for Electron and Light Microscopy."

g. Ladd Research Industries , Materials Safety Data Sheet (MSDS) for Glutaraldehyde, August 12, 2002.

h. American Conference of Governmental Industrial Hygienists (ACGIH), Documentation of the Threshold Values and Biological Exposure Indexes, Glutaraldehyde. 6th Edition, Volume 1, 1991.

i. Advanced Sterilization Products, MSDS 006, Rev. B. Cidex™ Activated Dialdehyde Solution (Dec. 6, 1999)

j. Rutala, William A. and David J Weber. "New Disinfection and Sterilization Methods, New Edition," Emerging Infectious Diseases, 348 – 352, 2001.

k. Advanced Sterilization Products (Johnson and Johnson Co.). "Cidex OPA Solution." MSDS #MS-09588-0-001, Rev. B, 6/26/01

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m.. JSC Toxicology Group: Procedural Guidelines for Assessing the Toxic Hazard Levels of Chemicals and Biological Agents, in Guidelines for Assessing the Toxic Hazard of Spacecraft Chemicals and Materials, JSC 26895, p. 11, 1997.

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r. Werley, MS, et.al. Respiratory peripheral sensory irritation and hypersensitivity studies with glutaraldehyde vapor. Toxicology and Industrial Health 11:489-501 (1995). Cited in Sustainable Hospitals, Lowell Center for Sustainable Productions, 2001.

s. Committee on Toxicology: National Research Council. Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants: Glutaraldehyde. Volume 3, pp 271-291. National Academy Press, Washington DC, 1996
Note: The spacecraft maximum allowable concentrations (SMACs) listed in this publication were derived and documented by members of the JSC Toxicology Group. Then these SMACs and their support documentation were reviewed by the National Research Council Committee on Toxicology and were published by the National Academy Press.

t. Toxicology and Carcinogenesis Studies of Glutaraldehyde in F344/N Rats and B6C3F1 Mice p. 5, Technical Report Series No. 490 (1999). NIH publication No. 99-3980. National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.