INDEPENDENT VALIDATION BOOK

Facts • Studies • Proof ActivePure[®] (RCI) Certified Space Technology



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EXECUTIVE SUMMARY

Executive Summary

Research studies around the world have linked indoor air pollution to health effects such as headaches, respiratory problems, infectious diseases, chronic coughs, eye irritation and lethargy. Those suffering ailments such as asthma, allergies and lung diseases are also extremely vulnerable to indoor airborne pollution. The threat posed by indoor air contamination is a bigger problem than ever before and exposure in health care facilities is a major challenge.

This report presents the testing and research conducted to determine the effectiveness of ActivePure® Technology on contamination of air and surfaces. ActivePure Technology is the most powerful air and surface purification technology ever discovered, and it's also the only exclusive air and surface purification technology awarded the prestigious Certified Space Technology seal of approval by the Space Foundation, a NASA spin-off.

ActivePure Technology was previously named and referred to as Radiant Catalytic Ionization (RCI). In this report, various tests may refer to the technology as Radiant Catalytic Ionization (RCI), or as ActivePure Technology. The technologies are the same.

Testing and research of ActivePure Technology (Radiant Catalytic Ionization/RCI) shows that in nearly every instance, bacteria (including MRSA) was reduced to zero, as was fungi, and air quality and purity were massively improved. The same reductions are evident in surface contaminantion.

When used as suggested, ActivePure Technology maintains its effectiveness, resulting in several significant benefits, including:

- Protection from MRSA, E. coli and C. diff spores
- Protection from illness-causing bacteria and viruses
- Reduction in respiratory issues
- · Reduction in the number of common cold and flu outbreaks and sick days due to illness
- · Protection from allergy and asthma triggering contaminants
- · Improved productivity from healthier, cleaner air and surfaces

At Aerus, our mission is to be difference makers in people's lives by creating products and solutions that optimize health within homes, healthcare facilities and other environments. We believe that what you breathe, drink and eat should always be pure, safe, and contaminant free. And that everyone should have the opportunity to live a healthy life.





BIOLOGICAL REDUCTIONS ON SURFACES



ActivePure® Technology

ACTIVEPURE® TECHNOLOGY Previously called Radiant Catalytic Ionization (RCI) in prior years

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RADIANT CATALYTIC IONIZATION (RCI) Referred to in later years as ActivePure® Technology

ACTIVEPURE® TECHNOLOGY Previously called Radiant Catalytic Ionization (RCI) in prior years

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HOTEL INSTALLATION TESTING RESULTS

LABORATORY: Antimicrobial Testing Laboratory/Aerus 12 PRODUCT: Beyond Guardian Air + Beyond Treatment TECHNOLOGY: ActivePure[®] Technology (previously called RCI)/HEPA EXPOSURE TIME: 30 Days



MAJOR LEAGUE BASEBALL TEXAS RANGERS

LABORATORY: Antimicrobial Testing Laboratory/Aerus 13 PRODUCT: Beyond Guardian Air + Beyond Treatment TECHNOLOGY: ActivePure® Technology (previously called RCI)/HEPA EXPOSURE TIME: 30 Days



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MAJOR HOSPITAL OPERATING ROOM

LABORATORY: Antimicrobial Testing Laboratory/Aerus **PRODUCT: Beyond Guardian Air** TECHNOLOGY: ActivePure (previously called RCI)/HEPA **EXPOSURE TIME: 6 Days**







LABORATORY: Kansas State University **PRODUCT: Air Scrubber Plus** TECHNOLOGY: ActivePure[®] Technology (previously called RCI) **EXPOSURE TIME: 3 Months**



RADIANT CATALYTIC IONIZATION (RCI) Referred to in later years as ActivePure® Technology

ACTIVEPURE® TECHNOLOGY Previously called Radiant Catalytic Ionization (RCI) in prior years



ACTIVEPURE: NASA-Inspired, Certified Space Technology

ActivePure: NASA-Inspired Technology Certified by the Space Foundation

NASA-Inspired Technology

ActivePure Technology is the most powerful air and surface purification technology ever discovered. Originally developed by NASA researchers for use on the International Space Station, ActivePure Technology has been adapted for use in our air and surface purification units here on earth. The same powerful, exclusive technology trusted to protect NASA astronauts from bacteria, viruses, and other contaminants in outer space works continuously to purify the air and hard surfaces in your indoor environment.

Certified by the Space Foundation

ActivePure Technology has been recognized by the Space Foundation as being the only Certified Space Technology in its class. The Space Foundation is the space industry's leading advocate of the advancement of space exploration, technology, and awareness. Along with NASA's Technology Transfer Program, the Space Foundation's Certification Program honors life-changing space technologies that have been adapted for commercial use and positively impact life on earth. ActivePure Technology is the only space certified air and surface purification technology in the world.

Space Technology Hall of Fame Inductee

The Space Technology Hall of Fame® increases public awareness of the benefits of space exploration and encourages further innovation by recognizing individuals, organizations and companies that effectively adapt and market technologies originally developed for space to improve the quality of life for all humanity. Other prestigious inductees include, The Boeing Company, Canadian Space Agency, US Army Institute of Surgical Research, NASA Kennedy Space Center.

ActivePure cells are built in the U.S.A.









IN THE NEWS Hall of Fame Press Release

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AERUS' ACTIVEPURE TECHNOLOGY INDUCTED INTO PRESTIGIOUS SPACE TECHNOLOGY HALL OF FAME: ONE OF ONLY 75 TECHNOLOGIES TO RECEIVE SUCH AN HONOR IN 30 YEARS

ActivePure is based on technology originally developed by NASA. It is proven to reduce up to 99% of surface micro-organisms and dramatically reduce airborne contaminants and allergens. Today, the technology is available to consumers, promoting healthier lives through its Beyond by Aerus family of products featuring ActivePure Certified Space Technology.

Dallas, TX, April 6, 2017 -- Aerus LLC, the Healthy Home leader committed to providing cuttingedge technologies to create the healthiest homes around the world, is honored to announce the induction of its proprietary ActivePure Technology into the Space Technology Hall of Fame, a Space Foundation program aiming to increase public awareness of the benefits of space exploration and encouraging further innovation of NASA-adapted technologies to improve the quality of life for humanity. ActivePure Technology is one of only 75 technologies that have been inducted into the Space Technology Hall of Fame in the past 30 years. Past inductees have included energy-saving technologies, satellite and telecommunication technologies, practical commercial devices and health improvement technologies, including LASIK eye surgery, implantable pacemakers and hearing aids and many other devices that improve the quality of life for millions of people every day.

The induction ceremony takes place on April 6 in Colorado Springs, as the culminating event of the 33rd Space Symposium, a three-day conference attended by over 11,000 space leaders from around the world. The Space Technology Hall of Fame Dinner honoring the 2017 inductees will be co-sponsored by SpaceX, a space exploration company founded in 2002 by Elon Musk.

ActivePure Radiant Catalytic Ionization (RCI) Technology was initially developed by NASA scientists to eliminate ethylene gas onboard the International Space Station and has been adapted and enhanced by Aerus to benefit people all around the world.

Beyond by Aerus' ActivePure Technology has been tested in both university and laboratory environments, and also been used in numerous commercial and industrial settings that face particular high-risk issues. ActivePure Technology is engineered to eliminate contaminants in the air and on surfaces and is currently used in homes, offices, hospitals, daycares, hotels and professional sports facilities. It is proven to destroy airborne and surface viruses, mold, fungus, volatile organic compounds, and bacteria such as MRSA, E-coli and Staph. "We are very fortunate to live in a time when space-age technology and innovation have such remarkable implications for us here on Earth. Since ActivePure Technology was first deployed on the space shuttle Columbia and in subsequent successful applications aboard the International Space Station, we have been able to offer ActivePure Technology in a variety of products by Aerus, activTek, Vollara and Beyond by Aerus to improve people's lives," said Joe Urso, Aerus LLC CEO, who along with Andy Eide, Aerus LLC Vice President, Product Development and Manufacturing, will be individually inducted into the Space Technology Hall of Fame this year, "the induction into the Space Technology Hall of Fame is a great honor and is a culmination of years of research and testing to bring this product to the people who need it the most."

From state-of-the-art floor care systems to air and water purification systems and beyond, Aerus has created clean, safe and healthy indoor environments for over 50 million businesses and homes worldwide. Those who suffer from asthma or allergies, new parents or caregivers, pet lovers or anyone devoted to living a healthier life can benefit from the cleansing and purifying components of ActivePure Technology.

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Since 1924, Aerus LLC (formerly Electrolux USA) has been the Healthy Home leader committed to providing cutting-edge technologies and unparalleled service to create the healthiest homes and living environments in North America and in 70 countries around the world. The Aerus family of companies includes Aerus, Beyond by Aerus, Vollara, ActivTek, and AirScrubber Plus. They are the exclusive worldwide owners of ActivePure Technology, the most powerful air and surface purification technology ever discovered, and the only one in its class recognized by the Space Foundation as Certified Space Technology. ActivePure Technology has been engineered by Aerus based on technology originally developed by NASA.



IN THE NEWS *Mitsubishi Press Release*



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MITSUBISHI BRINGS NASA-INSPIRED TECHNOLOGY TO ITS VEHICLES IN BRAZIL

Auto giant partners with Aerus to protect drivers from deadly pollution, airborne viruses and bacteria

São Paolo, Brazil – Mitsubishi Motors of Brazil has announced plans to outfit its Pajero Full 2017 SUV with AutoPure, a technology developed and owned by Aerus, LLC, formerly Electrolux USA. An early stage version of the technology was developed by NASA scientists to manage contaminants on the International Space Station. Aerus has advanced and improved the original technology and it is now used in numerous consumer, commercial, and health care applications. ActivePure is the key science in the technology, and is used in many of Aerus' products, including its air purification systems; AutoPure is the next generation of ActivePure, engineered specifically for use in motor vehicles.

AutoPure technology is the result of three years of work, testing, and collaboration between Aerus and Mitsubishi to design a purification system for installation in Mitsubishi's cars that could address Brazilian driving conditions. The problem is acute, as testing demonstrates that the air in vehicles in Brazilian cities is contaminated with dangerous levels of poisonous gases. Extensive testing has shown AutoPure to be 99.99% effective at reducing air and surface contaminants, including E.Coli, MRSA, and H1N1, and AutoPure reduces exposure to carbon monoxide, other volatile organic compounds, and harmful air particulates in cars in Brazil to safe, non-toxic levels.

The technology, which is discreetly installed directly into the vehicle's air conditioning system, uses broad-spectrum UV light which, when exposed to a proprietary coating, creates super-charged hydroxyl radicals and super-oxides that destroy toxins, VOCs, and microorganisms in the air and on surfaces. AutoPure breaks down harmful pathogens into harmless elements like carbon, hydrogen, and oxygen, and is completely safe for people and animals.

In Brazil, air pollution kills nearly 49,000 every year. The World Health Organization (WHO) recently announced that air pollution represents the single biggest environmental risk to health, as only one in ten people around the globe breathes air that is considered "clean" by the organization's air quality guidelines.

Together, Mitsubishi Motors of Brazil and Aerus hope to address the issue of air pollution for Brazilian drivers who spend time in their cars every day by protecting them from air pollution and other threatening contaminants with AutoPure. "Aerus and Mitsubishi are pioneers in bringing the AutoPure system to the interior of vehicles, and our goal is to protect human health and make air quality safe for occupants in cars," said Joe Urso, CEO of Aerus.

AutoPure will be available to consumers starting with the 2017 Pajero Full SUV in Brazil, and Aerus hopes to install its AutoPure technology across Mitsubishi's vehicle lineup in future models. Aerus worked extensively through its colleagues in Brazil, Silux Ambientes Saudáveis, in collaboration with Mitsubishi Motors of Brazil.

Since 1924, Aerus has been the Healthy Home leader committed to providing cutting-edge technologies and unparalleled service to create the healthiest homes and living environments in North America and in 70 countries around the world. The Aerus family of companies includes Aerus, Beyond by Aerus, Vollara, ActivTek, and AirScrubber Plus. They are the exclusive worldwide owners of ActivePure and AutoPure Technology, the most powerful air and surface purification technology ever discovered, and the only one in its class recognized by the Space Foundation as Certified Space Technology. ActivePure and AutoPure have been engineered by Aerus based on technology originally developed by NASA.

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If you would like more information about this topic, please contact Laura Urso at 214-378-4003 or email at LUrso@aerusonline.com.



ACTIVEPURE: White Paper

BEYOND

BY AERUS

ACTIVEPURE® TECHNOLOGY

Safe, Effective, Proven, Certified

ActivePure^{*} is an exclusive environmental technology that can solve many everyday indoor air and surface contamination problems. Traditional passive technologies, such as HEPA, use filtration or electrostatic systems, which remove contaminants only if and when they travel through the purification unit. These traditional filtration systems can help reduce air pollution to a degree, but they do not reduce surface contamination at all and do not adequately reduce airborne contaminants. Our proprietary ActivePure^{*} Technology actively targets contaminants in the air and on surfaces, eliminating them on contact.

ActivePure^{*} Technology is derived from NASA Technology as used on the International Space Station. It is the only Certified Space Technology in the world in its class. ActivePure^{*} Technology utilizes a proprietary hydrophilic photo catalytic coating, consisting of non-nano titanium dioxide with a proprietary combination of additional transition elements to enhance efficiency. Activated by a specific wavelength of ultraviolet light, oxygen and humidity are extracted from the air to create a host of powerful oxidizers that target air and surface pollution. These oxidizers are extremely effective at destroying bacteria, volatile organic compounds (VOCs) and other environmental contaminants. ActivePure Technology does not create harmful chemicals but instead uses oxidizers found naturally occurring in the environment. These oxidizers are not harmful to humans, pets or plants and are complete safe for indoor use.

The key oxidizers created by ActivePure® Technology are the following:

- Hydrogen Peroxide (H₂O₂)
- Hydroxyls (OH-)
- Super Oxides (O₂-)

Hydrogen Peroxide

A major oxidizer created by ActivePure^{*} Technology is hydrogen peroxide (H_2O_2), which has proven to be effective against indoor pollutants and contaminants on surfaces and in the air. ActivePure^{*} Technology produces hydrogen peroxide molecules from the oxygen and humidity already present in the air. The hydrogen peroxide molecules are then carried throughout the indoor environment, neutralizing pollutants and contaminants in places that other technologies and filtration systems can't reach. Because hydrogen peroxide molecules have both positive and negative charges, they are drawn to pollutants and contaminants by the process of electrostatic attraction. Contaminants are then safely broken down into oxygen (O_2) and water (H_2O) vapor. Hydrogen peroxide is odorless, colorless and safe to use in occupied spaces. According to the Occupational Health and Safety Administration (OSHA), exposure to one part per million (1.0~ ppm) of hydrogen peroxide is considered safe throughout the day. ActivePure^{*} Technology produces only 0.02 ~ 0.04 ppm, well below the OSHA limit.

Hydroxyls

Another important oxidizer created by ActivePure[®] Technology is hydroxyls. Hydroxyls (OH-) are safe, naturally occurring, powerful oxidizers that quickly and safely neutralize many airborne and surface contaminants, odor-causing bacteria and chemical VOCs. As part of the ActivePure[®] process, hydroxyls are formed when an ultraviolet light of specific wavelengths is absorbed by the unit's proprietary coating. The coating strips the hydrogen (H) atoms from water molecules (H₂O) in the ambient air, forming negative hydroxyls (OH-). These hydroxyls break down carbon and hydrogen based VOCs and other organic contaminants, converting them into harmless carbon dioxide (CO₂) and water (H₂O) vapor.

While extremely effective at destroying odors, bacteria, VOCs and other contaminants, hydroxyls are completely safe for human, animal and plant exposure indoors. The hydroxyls produced by ActivePure[®] Technology are the same as those produced naturally in the earth's atmosphere by the reaction of UV rays and water vapor, and function to safely and naturally "scrub" and decontaminate indoor environments.

Super Oxides

Super oxides are oxygen molecules that arise when free hydrogen atoms (H) combine with ozone (O_3) are created in small amounts by nature in the air. When combined, they form the powerful oxidizers oxygen (O_2) and hydroxyls (OH-). ActivePure[®] Technology utilizes a UVC light source, naturally occurring ozone (O_3) , humidity and a photo catalyst to create powerful super oxides that eliminate bacteria, viruses, mold and other contaminants. This technology is not only safe for human exposure, but is significantly more effective at destroying contaminants than simple UV technology alone. In the process of creating super oxides, ActivePure[®] actually reduces the amount of ozone (O_3) that naturally exists in the air.

Super oxides have been utilized for decades in food processing plants, hospitals, and dental and doctor's offices to control environmental contamination and disinfect safely without chemicals.

Product Efficacy and Testing

ActivePure[®] Technology has consistently proven its ability to safely control and neutralize contaminants such as viruses, bacteria, mold, fungi and VOCs in numerous tests and studies, without harm to humans, animals and plant life. Extensive laboratory testing conducted at Kansas State University showed ActivePure[®] Technology to be effective against H1N1, H5N8, MRSA, Staph, Streptococcus, E-Coli, Listeria, Bacillus spp, Stachybotrys Chartarum and more. These university studies have shown that ActivePure[®] can reduce at least 96.4% and as much as 99.99% of surface and airborne contaminants within the first 24 hours.

Further testing was commissioned at the University of Cincinnati Center for Health-Related Aerosol Studies to investigate ActivePure^{*} Technology's kill rate for airborne bio-contaminants. These tests established the extraordinary effectiveness of ActivePure^{*} Technology in safely destroying contaminants. In the tests, 90% of the airborne pathogens measured were reduced in only 30 minutes, a rate 50 times more effective than normal filtration.

No Ozone

Ozone (O_3) is created naturally by nature and is present in our air, and can also be created by man-made technologies. The EPA has determined that ozone at levels in excess of 0.5 ppm may be damaging to health. ActivePure^{*} Technology has been proven to create only minuscule amounts of ozone, at levels over one thousand times lower than the EPA safeguard levels. Moreover, ActivePure^{*} Technology actually converts and lowers naturally occurring ozone as described above, as it creates safe super oxides (O_3) which in turn eliminate harmful pathogens.

Space Foundation - Technology Certified

The Space Foundation has recognized ActivePure^{*} and our company for utilizing technologies originally invented for use in space programs to eliminate VOCs and other contaminants, and adapting these technologies for everyday use. ActivePure^{*} Technology is based on a variation of technology originally developed for use by NASA on the International Space Station, and is recognized globally as the only Certified Space Technology in its class. You can only get this technology from us.

Applications

Our ActivePure[®] Technology has been successfully and safely used in hospitals, homes, doctor's offices, professional sports facilities and other applications across the world.

Should you have any further questions on our technology please feel free to contact us.

Dr. Troy Sanford, CN, ND

RESEARCH REPORT: *Biological Reductions on Surfaces*

SCIENTIFIC VALIDATION







Reduction of Surface Contaminants

ActivePure® (RCI) Technology 24 hour testings conducted by Kansas State University

Results based on laboratory testing

Testing by Kansas State University. Results may vary based on environmental conditions.

*Scientific testing has demonstrated the use of ActivePure Technology to substantially reduce microbial populations on surfaces – including but not limited to Escherichia coli, Listeria monocytogenes, Streptococcus spp., Pseudomonas aeruginosa, Bacillus spp., Staphylococcus aureus, Candida albicans, methicillin-resistant staphylococcus aureus (MRSA) and S. chartarum. Field results may vary based on environmental conditions. No claim with respect to airborne microbials is made based on these results. These results have not been evaluated by the FDA. This product is not a medical device intended to diagnose, treat, cure, or prevent any disease.

Efficacy of EcoQuest Radiant Catalytic Ionization Cell and Breeze AT Ozone Generators at Reducing Microbial Populations on Stainless Steel Surfaces

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Summary and Implications

This study was conducted to determine the potential use of EcoQuest Radiant Catalytic Ionization Cell for the inactivation of *Escherichia coli, Listeria monocytogenes, Streptococcus* spp., *Pseudomonas aeruginosa, Bacillus* spp., *Staphylococcus aureus, Candida albicans*, and *S. chartarum*, on stainless-steel surfaces at diverse contact times in a controlled airflow cabinet. In addition, the EcoQuest Breeze AT Ozone generator was evaluated under the same conditions for the inactivation of *Candida albicans* and *S. chartarum*. Better disinfection technologies for food contact surfaces are needed to control food borne pathogens in processing environments. Ozone technologies have only recently been approved for use on food contact surfaces. This study evaluated the application of gaseous ozone and other oxidative gases on stainless-steel surfaces against the microorganisms listed above. Both technologies reduced populations of all microorganisms tested on stainless-steel surfaces by at least 90% after 24 h exposure. The Radiant Catalytic Ionization Cell was more effective at reducing microbial counts for shorter exposure times than was the Breeze AT Ozone Generator.

INTRODUCTION

The food and beverage industries face a number of issues when it comes to producing a safe, wholesome product. Foodborne pathogens such as *E. coli* 0157:H7, *Listeria moncytogenes*, and *Salmonella* spp. have been a growing concern throughout the years. Processors are also concerned about spoilage microorganisms that shorten shelf life and cost companies millions every year in spoiled product. Industries impacted include the meat, seafood, poultry, produce, baking, canned foods, dairy, and almost all other segments of the market.

The U.S. Department of Agriculture estimates the costs associated with food borne illness to be about \$5.5 to \$22 billion a year. This doesn't include the billions lost every year due to spoiled product, which must be disposed of or sold as a lesser valued product. Better disinfection and microbiological control measures are needed in almost every area of the food industry.

As a disinfectant, ozone has a tremendous ability to oxidize substances. It's thousands of times faster than chlorine and disinfects water three to four times more effectively. As it oxidizes a substance ozone will literally destroy the substance's molecule. It can oxidize organic substances such as bacteria and mildew, sterilize the air, and destroy odors and toxic fumes. Ozone has been used by industry for many years in numerous applications such as odor control, water purification, and as a disinfectant (Mork, 1993). Recent government approval of ozone for use with foods and food contact surfaces has opened the door to many more exciting possibilities for this technology.

In June 2001, the U.S. Food and Drug Administration approved the use of ozone as a sanitizer for food contact surfaces, as well as for direct application on food products. Prior to that time, chlorine was the most widely used sanitizer in the food industry. Ozone may be a better choice for disinfection of surfaces than chlorine. Chlorine is a halogen-based chemical that is corrosive to stainless steel and other metals used to make food-processing equipment. Chlorine can also be a significant health hazard to workers; when mixed with ammonia or acid cleaners, even in small amounts, a toxic gas can form.

Chlorine is a common disinfect used in meat processing and is effective and safe when used

at proper concentrations. However, chlorine is far less effective than ozone and can result in the production of chloroform, carbon tetrachloride, chloromethane, and tri-halomethanes. In contrast, ozone leaves no residual product upon its oxidative reaction.

An important advantage of using ozone in food processing is that the product can be called organic. An organic sanitizer must be registered as a food contact surface sanitizer with the U.S. Environmental Protection Agency (EPA). Ozone has such an EPA registration, and is approved by FDA as a sanitizer for food contact surfaces and for direct application on food products.

Ozone has become more accepted for use in food processing in recent years and is being used in more than just surface applications. A recent U.S. FDA recommendation (2004) stated that "ozone is a substance that can reduce levels of harmful microorganisms, including pathogenic *E. coli* strains and *Cryptosporidium*, in juice. Ozone is approved as a food additive that may be safely used as an antimicrobial agent in the treatment, storage, and processing of certain foods under the conditions of use prescribed in 21 CFR 173.368."

MATERIALS AND METHODS

Preparation of Cultures:

The following bacteria and fungi cultures were used for the study: *Bacillus globigii* (ATCC # 31028, 49822, 49760), *Staphylococcus aureus* (ATCC # 10832D, 25178, 11987), *Candida albicans* (ATCC # 96108, 96114, 96351), *Stachybotrys chartarum* (ATCC # 18843, 26303, 9182), *Pseudomonas aeruginosa* (ATCC# 12121, 23315, 260), *Escherichia coli* (ATCC# 27214, 19110, 67053), *Streptococcus pneumoniae* (ATCC# 27945, 29514, 10782), and *Staphylococcus aureus* - methicillin resistant (ATCC# 33591). Cultures were revived using ATCC recommended instructions.

Bacteria, yeast, and mold strains were individually grown in tripticase soy broth (TSB; Difco Laboratories, Sparks, MD) and YM broth (Difco Laboratories), respectively, to midexponential phase followed by a wash and resuspension in 0.1% peptone water. The cultures were combined by specie type to ca. 10⁸ CFU/ml.

Preparation of Samples and Ozone Treatment:

The microbial species used to validate the ozone generators were tested as microbial cocktails inoculated onto 6.3 x 1.8 cm on #8 finish stainless-steel coupons (17.64 cm² double sided area). Four stainless steel coupons were dipped per microbial inoculum and vortexed 15 sec to optimize microbial dispersion. Using sterile binder clips, stainless steel coupons were suspended on a cooling rack contained inside a laminar flow cabinet for 1 h to dry. The initial microbial populations attached to the stainless steel coupons ranged from 5 to 6 log CFU/cm². The inoculated stainless steel coupons were transferred to a controlled airflow test cabinet (Mini-Environmental Enclosure, Terra Universal, Anaheim, CA) at 26°C and 46% relative humidity (ambient conditions), and treated using the EcoQuest Radiant Catalytic Ionization Cell for 0, 2, 6, and 24 h. The EcoQuest Breeze AT Ozone generator was evaluated separately for treatment periods of 0, 2, 6 and 24 h. Ozone levels were monitored throughout the study (Model 500, Aeroqual, New Zealand).

Sampling:

At the end of the ozone contact time the coupons were vortexed for 30 sec in 30 ml of 0.1% peptone water. Samples inoculated with bacterial cultures were serially diluted, plated on tripticase soy agar (TSA; Difco Laboratories), and incubated for 24 h at 35°C. After preparing serial dilutions, samples inoculated with yeast were plated on potato dextrose agar (PDA; Difco Laboratories) and those inoculated with mold cultures were plated on commeal plates. Both PDA and commeal plates were incubated 30°C for 5 days. Following incubation, data for each microorganism were reported as colony-forming units per square centimeter (CFU/cm²).

RESULTS AND DISCUSSION

Reductions in microbial populations on #8 finish stainless steel coupons following 0, 2, 6, and 24 h exposure to the EcoQuest Radiant Catalytic Ionization Cell are presented in Figure 1. Exposure to ozone levels of 0.02 ppm for 2 h reduced all microbial populations tested by at least 0.7 log CFU/cm2. Longer exposure times resulted in greater reductions, with the greatest reductions found after 24 h exposure. After 24 h exposure, mean microbial reductions for each organism were as follows: S. aureus (1.85 log CFU/cm2), E. coli (1.81 log CFU/cm2), Bacillus spp. (2.38 log CFU/cm²), S. aureus met^e (2.98 log CFU/cm2), Streptococcus spp. (1.64 log CFU/cm2), P. aeruginosa (2.0 log CFU/cm2), L. monocytogenes (2.75 log CFU/cm2), C. albicans (3.22 log CFU/cm2), and S. chartarum (3.32 log CFU/cm²).

Reductions in microbial populations following treatment of stainless steel coupons with the EcoQuest Breeze AT Ozone generator are shown in Figure 2. Reductions of at least 0.2 and 0.4 log CFU/cm² were observed after 2 and 6 h of ozone exposure, respectively. After 24 h exposure, mean reductions for *C. albicans* and *S. chartarum* were 1.48 and 1.32 log CFU/cm², respectively.

The EcoQuest Radiant Catalytic Ionization Cell and EcoQuest Breeze AT Ozone generators reduced microbial populations on stainless steel surfaces within 2 h under ambient conditions, with greater reductions associated with longer exposure times. The Radiant Catalytic Ionization Cell was more effective than the Breeze AT Ozone Generator at reducing microbiological populations at shorter exposure times of 2 and 6 hours. This study demonstrated that ozone gas has the potential to be an effective surface disinfectant for use in food processing applications. Testing is currently ongoing to evaluate non-treated controls. Phase II of the project, scheduled to be completed by the end of this year, will evaluate the effectiveness of the system for eliminating airborne contamination using the same microorganisms and oxidative technologies.

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RESEARCH REPORT: *Control of Contaminants in Indoor Air*

SCIENTIFIC VALIDATION

Report Summary





Reduction of Airborne Contaminants

ActivePure Technology testing conducted by University of Cincinnati

Results based on laboratory testing

*Scientific testing has demonstrated the use of Beyond by Aerus' ActivePure technology to substantially reduce airborne pathogens Field results may vary based on environmental conditions. These results have not been evaluated by the FDA.

Environ. Sci. Technol. 2007, 41, 606-612

Control of Aerosol Contaminants in Indoor Air: Combining the Particle **Concentration Reduction with** Microbial Inactivation

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An indoor air purification technique, which combines unipolarion emission and photocatalytic oxidation (promoted by a specially designed RCI cell), was investigated in two test chambers, 2.75 m³ and 24.3 m³, using nonbiological and biological challenge aerosols. The reduction in particle concentration was measured size selectively in realtime, and the Air Cleaning Factor and the Clean Air Delivery Rate (CADR) were determined. While testing with virions and bacteria, bioaerosol samples were collected and analyzed, and the microorganism survival rate was determined as a function of exposure time. We observed that the aerosol concentration decreased ~10 to ~100 times more rapidly when the purifier operated as compared to the natural decay. The data suggest that the tested portable unit operating in ~25 m³ non-ventilated room is capable to provide CADR-values more than twice as great than the conventional closed-loop HVAC system with a rating 8 filter. The particle removal occurred due to unipolar ion emission, while the inactivation of viable airborne microorganisms was associated with photocatalytic oxidation. Approximately 90% of initially viable MS2 viruses were inactivated resulting from 10 to 60 min exposure to the photocatalytic oxidation. Approximately 75% of viable B. subtilis spores were inactivated in 10 min, and about 90% or greater after 30 min. The biological and chemical mechanisms that led to the inactivation of stress-resistant airborne viruses and bacterial spores were reviewed.

Introduction

Exposure to respirable airborne particles and microbial agents may cause various health problems. Numerous techniques have been developed to reduce the exposure to indoor particles. Aerosol control in confined, poorly ventilated spaces, when the air exchange with filtration cannot be successfully applied, represents a particular challenge.

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Another challenge is to decrease the indoor concentration of specific airborne contaminants, e.g., viable biological particles. While some indoor air purification techniques aim solely at the aerosol concentration reduction, others are designed to inactivate viable bioaerosols (e.g., viruses, bacteria, and fungi).

Some commercial air cleaners generate excessive ozone (either as a primary biocidal agent or as a bi-product); these devices have raised public health concerns (1). Among various guidelines for ozone exposures, the following thresholds have been specified for occupational environments: 0.2 ppm for 2 h (2), 0.05-0.10 for 8 h (2), 0.1 ppm for 8 h (3), and 0.05 ppm for instantaneous (no time limit specified) exposure (4). For comparison, the outdoor air standard is 0.08 ppm for 8 h (5). Ozone generators can inactivate viable microorganisms; however, the inactivation occurs at concentrations significantly exceeding health standards (6, 7).

Photooxidation involving UV radiation and TiO2 as a photocatalyst has been applied for gas-phase detoxification of organic contaminants (8, 9) and for inactivating microorganisms in water (10-12). Some effort has been made to explore its application for air cleaning inside a closed-loop system (13, 14). The investigators reported significant photocatalytic inactivation of stress-resistant Serratia marcesens that occurred when aerosolized bacteria circulated in a closed-loop duct equipped with a TiO2 filter for a relatively long period of time. Pal et al. (15) found similar effect for Escherichia coli, Microbacterium sp., and Bacillus subtilis; Keller et al. (16) reported considerable inactivation of airborne E. coli passing through a photoreactor coated with TiO₂ film. The biocidal effect of the photocatalytic oxidation can be attributed to photogenerated valence-band holes, hydroxyl radicals, hydrogen peroxide, and other reactive oxygen species. Lin and Li (17) tested the viability change in airborne bacteria and fungi exposed to photooxidation inside a small photoreactor for a very short time, on the order of a second. No significant decrease in the colony forming unit (CFU) count was observed during such a short time

To our knowledge, no data are available on the effectiveness of portable UV/TIO2-based air purifiers to inactivate viable airborne microorganisms in indoor air environments. These data are needed to assess the feasibility of photocatalytic oxidation for air purification in residential and occupational settings. Furthermore, for hybrid air purifiers, which involve several air cleaning mechanisms, no sufficient information is available to differentiate their particle removal efficiency and the biocidal capabilities, which both aim at reducing the bioaerosol exposure in indoor air.

In this study, we investigated a novel air purification technique that combines different aerosol/bioaerosol control mechanisms: unipolar ion emission and photocatalytic oxidation promoted by the "radiant catalytic ionization (RCI)" technique. Unipolar ion emission has been shown earlier to reduce the particle concentration in indoor air (18-20), but no scientific data are available on the efficiency of the hybridtype technique

Experimental Section

The indoor air purification process was investigated in the experimental facility shown in Figure 1. The particle removal was determined by measuring the concentration of challenge aerosols size-selectively in real-time. When testing with viable bioaerosols, the microorganism survival rate was also determined. The experimental protocols validated in our previous studies (18, 19, 21) were adopted. The experiments were conducted when a freestanding hybrid air purifier was

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FIGURE 1. Experimental setup.

operating inside the chamber and when it was turned off. The challenge aerosol was generated from a liquid suspension using a Collison nebulizer (BGI Inc., Waltham, MA) and charge-equilibrated by passing through a 10-mCl Kr⁴⁵ charge equilibrator (3M Company, St. Paul, MN). After being mixed with clean, HEPA-filtered air at a specific temperature (T = 24-26 °C) and relative humidity (RH = 21–30%), the aerosol entered the chamber. Following a 10–15-minute adjustment period established to achieve a uniform aerosol concentration pattern, the experiment began (t = 0).

In most of the tests, the aerosol concentration, *C*, and particle size distribution, $\Delta C \Delta \log(d)$, were measured with an electrical low-pressure impactor (ELPI, TSI Inc./Dekati Ltd, St. Paul, MN), which utilizes the cascade impaction principle and also has a direct-reading capability to determine the concentration of particles of different aerodynamic sizes in 12 channels (each channel = impaction stage), from 0.041 to 8.4 µm (midpoint). When the experiments were conducted with viral aerosol that included particles smaller than the lower limit of the ELPI, we used a wide-range particle spectrometer (WPS; MSP Inc., Shoreview, MN). The WPS is a high-resolution real-time instrument combining differential mobility analysis, condensation particle counting, and laser light scattering to measure the diameter and number concentration of aerosol particles ranging from 10 nm to 10 µm.

For every measured particle size, *d*, the aerosol concentration at t = 0 was set to exceed the background level (obtained before the challenge aerosol was generated) by about 100-fold. First, the natural concentration decay was characterized by recording *C_{nutural}* (*d*, *t*) every 10 s with the ELPI and every 2.5 min with the WPS. Subsequently, the test aerosol was generated and mixed in the chamber again to reach the same initial concentration level. At t = 0, the air purifier was turned on and the concentration C_{AP} (*d*, *t*) was monitored during and up to 120 min (or until the particle count decreased below the limit of detection). To quantify the efficiency of the particle removal exclusively due to the air purifier operation, the Air Cleaning Factor (ACF) was determined size-selectively as a function of time:

$$ACF(d, t) = \frac{C_{natural}(d, t)}{C_{AP}(d, t)}$$
(1)

In addition, the overall particle removal rate was calculated as

$$l, t) = \frac{1}{t} \ln \left[\frac{C(d, t = 0)}{C(d, t)} \right], \quad (2)$$

and the particle removal rate (exclusively due to air purifier) was defined following the first-order kinetics as

$$PRR(d, t) = \frac{1}{t} \ln \left[\frac{C_{AP}(d, t=0)}{C_{AP}(d, t)} \right] - \frac{1}{t} \ln \left[\frac{C_{natural}(d, t=0)}{C_{natural}(d, t)} \right]$$

In case $C_{AP}(d, t = 0) = C_{natural}(d, t = 0)$,

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$$PRR(d, t) = \frac{1}{t} \ln[ACF(d, t)] \qquad (4)$$

(3)

This was needed to determine the Clean Air Delivery Rate (CADR), which, according to the ANSI/AHAM (American National Standards Institute/Association of Home Appliance Manufacturers) standard, is defined as

$$CADR(d, t) = V \times PRR(d, t) [m^3/h]$$
 (5)

The CADR concept allows for comparison of air cleaning efficiencies of a freestanding air purifier and a closed- loop ventilation/air-filtration system in an air volume V (note that PRR is a function of V).

Two nonbiological challenge aerosols, NaCl and smoke, were used to study the particle removal by the air purifier. The generated particles were primarily in the size range of 0.02–2.0 µm, which includes ultrafine and fine fractions and represents most of the known viruses and bacteria. MS2 virus and *Bacillussubtilis* bacterial spores were the main biological challenge aerosols. Selected experiments were performed with *Pseudomonas fluorescens* bacteria.

MS2 bacteriophage, a 27 nm tailless non-enveloped icosahedral RNA-coliphage, relatively stable against environmental stress, has been used in the past as a simulant of most mammalian viruses, and it is known as an indicator for enteric viruses (22–26). Stock suspension of MS2 virus was prepared by adding 9 mL of Luria–Bertani broth to freezedried phage vial (ATCC 15597-B1). This suspension was

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filtered using a membrane filter of 0.2 μm porosity and serially diluted so that the nebulizer suspension had 10⁶-10⁹ PFU/ mL (PFU = plaque forming unit). MS2 phage titer was determined by following a modified plaque assay protocol of Adams (27); *Escherichia coli* (ATCC 15597, strain C3000) was used as the host organism.

B. subtilis is a gram-positive spore-forming bacterium with rod-shaped spores of approximately $0.7-0.8\,\mu m$ in width and 1.5-1.8 µm in length (28). B. subtilis spores have previously been used in laboratory studies as a surrogate of environmentally resistant, pathogenic bacteria (29-31). Freeze-dried bacterial spores of B. subtilis (obtained from the U.S. Army Edgewood Laboratories, Aberdeen Proving Ground, Maryland) were activated at 55-60 °C for 25 min and then washed two times with sterile deionized water by vortexing followed by centrifugation at 7000 rpm for 7 min at room temperature. The total bacterial concentration in suspension was adjusted to 108-109 per mL using a hemacytometer. The viable bacteria were enumerated by cultivating on trypicase soy agar (TSA) media at 30 °C for 18 h; the viable (culturable) concentration in the nebulizer suspension was of the same order of magnitude as the total concentration, i.e., $10^{6}-10^{9}$ CFU/mL (CFU = colony forming unit). P. fluorescens bacteria (used in selected tests) are relatively sensitive to environmental stresses. Prior to aerosolization, vegetative cells of P. fluorescens (ATCC 13525) were cultured in trypticase soy broth at 28 °C for 18 h and washed similarly as B. subitilis spores.

When testing with biological particles, air samples were collected using Button Samplers (SKC Inc., Eighty Four, PA) equipped with gelatin filters (SKC Inc.) and operated at a flow rate of 4 L/min for 5 min. Eight Button Samplers were utilized in each test generating one blank, one background sample, three samples taken at t = 0, and the other three taken at a specific time interval; four time intervals were tested: t = 10, 15, 30, and 60 min. Additional selected experiments were performed by using a BioSampler (SKC Inc. Eighty Four, PA) to collect *P. fluorescens* and *B. subtilis*. The BioSampler efficiently collects viable bacteria (29) while the liquid medium minimizes the desiccation stress. As its cutoff size is too high to efficiently sample small MS2 virions, the BioSampler was not used as an alternative to gelatin filters for collecting MS2 virus.

The samples were analyzed for viable airborne virions (PFU) and bacteria (CFU) to quantify the percentages of those survived over time t. These were obtained with and without operating the air purifier. Our preliminary tests showed that the air purifier's operation considerably reduces the total bioaerosol concentration in the chamber due to ion emission. Therefore, the ion emitter was temporarily disabled in the hybrid unit when testing virus and bacteria inactivation to ensure sufficient number of microorganisms for determining the viable count at the end of the test.

An aliquot of 200 µL of dissolved gelatin filter extract was used for plaque assay to determine the number of airborne active (viable) virions (PFU/cm³). Similarly, extract was cultivated on TSA plates to obtain the airborne concentration of viable bacteria (CFU/cm³).

Additional testing was initiated to examine whether the biocidal effect of the air purifier took place indeed in the aerosol phase (and not after microorganisms were collected on filters). For this purpose, aerosolized microorganisms were collected on eight gelatin filters during 5 min in the chamber without air purifier. Four filters were analyzed for viable microorganisms immediately after this test, while the other four were exposed to the air purifier in the chamber for 10, 15, 30, and 60 min and then analyzed. The comparison of two sets allowed examining if the microorganism inactivation occurred on filters during the collection process.

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The ozone level and the air ion concentration were monitored in real-time in the chamber using an ozone monitor (PCI Ozone & Control Systems, Inc., West Caldwell, NJ) and an air ion counter (AlphaLab Inc., Salt Lake City, UT), respectively. The air temperature in the test chamber was $24 \pm 2^{\circ0}$ cand the relative humidity ranged from $22 \pm 2\%$ to $28 \pm 2\%$ as monitored with a thermo/hygrometer pen (Fischer Scientific Co., Pittsburgh, PA).

The purifier prototype (Ecoquest International Inc., Greeneville, TN) used in the study utilized an ion emitter and a specially designed RCI cell. The former produces negative ions into indoor air, where they are acquired by aerosol particles. It is important to note that this method is different from air cleaning by charging particles at the entrance of the purifier and subsequently collecting them on metal electrodes by electrostatic precipitation. The RCI cell features a flow optimized target structure comprising matrices of elongated tubular elements made of polycarbonate and arranged in a parallel orientation on opposite sides or alternatively on four sides of a broad-spectrum UV light source. The UV lamp utilizes argon gas with mercury and carbide filaments with a spectral output between 100 and 367 nm. Besides, a coating was applied to the target structure of the cell comprising hydrophilic properties and containing the following grouping of materials: titanium dioxide, rhodium, silver, and copper. As a result, a photocatalytic oxidation forms reactive species, such as hydroxyl radicals, valence-band holes, superoxide ions, and hydrogen peroxides

The tests were conducted in two indoor test chambers including a large walk-in chamber (24.3 m3) that simulated a residential room and a smaller chamber (2.75 m³) that simulated a confined space (e.g., bathroom, small office area. or automobile cabin). The particle removal was investigated in both chambers, whereas the bioaerosol viability tests were performed in the smaller chamber that was made of stainless steel and allowed bio-decontamination. The air purifier was tested in non-ventilated chambers (no air exchange) as it is known that portable air cleaners are primarily beneficial in poorly ventilated spaces (20, 21). Air exchange was introduced only when testing the closed-loop ventilation/air-filtration system equipped with an HVAC filter to compare its performance to that of the portable air purifier in terms of CADR. The ventilation/air-filtration system was also deployed to clean the test chamber between experiments. In most of the tests, the air purifier operated in the corner of the chamber, facing the center. A separate experiment was carried out to examine whether its location and orientation affected the ACF.

Results and Discussion

Particle Removal from Air. Figure 2 shows the evolution of the concentration and particle size distribution of NaCl aerosol when the air purifier operated in the large test chamber. As seen from this example, the aerosol concentration of 0.1 µm particles decreased by a factor of 28 in 1 h and by a factor of about 250 in 2 h; the corresponding decreases for 1 µm particles were approximately 10- and 50-fold. When testing with smoke particles, the aerosol concentration decreased even more rapidly. The above levels of the aerosol concentration reduction are considerably greater than those predicted by either tranquil or stirred natural decay models (32). This result was obtained when both the air ion emitter and the RCI cell operated in the unit. Interestingly, statistically the same particle reduction effect (p > 0.05) was observed when the RCI cell was turned off and only the ion emitter operated. The latter finding provides the evidence that the particle removal was achieved as a result of unipolar ion emission but not due to photocatalytic reactions



FIGURE 2. Particle concentration and size distribution of NaCl aerosol as measured with the ELPI in the 24.3 m³ chamber with the air purifier operating facing the chamber's center at 1.7 m from the measurement point. No ventilation in the chamber. The initial total aerosol concentration = 1.50 × 10⁶ /cm².

This finding agrees with previously published data on the effect of unipolar air ionization on the airborne concentration (18-21). The air purification is particularly efficient at higher initial aerosol concentrations (>10⁶ particles/cm²) that ensure adequate interaction between the air ions and aerosol particles. As mentioned above, the effect is expected to be much more pronounced in non-ventilated environments than in ventilated ones.

The aerosol reduction was especially high for the particles of $d \le 0.3 \ \mu\text{m}$. E.g., when the air purifier with an ion output of $\sim 10^{12} \ e'$ sec continuously operated in a corner of the 24.3m³ chamber facing the center for 2 h, ACF reached $\sim 30-70$ for $d = 0.08-0.3 \ \mu\text{m}$ and $\sim 13-16$ for $d = 0.8-2 \ \mu\text{m}$ (in the tests conducted with NaCl and smoke as challenge aerosols). The same ACF levels may be achieved more rapidly in indoor environments of smaller volumes and slower in larger spaces. The experimental trends agree with the ion-induced aerosol removal model (20).

The ACF was found to depend not only on the operation time and the particle size but also on the location/orientation of the purifier in the chamber. For example, a corner location facing the center of the room was found preferable as opposite to the orientation facing the wall. The difference in ACF obtained for the center and corner locations was significant and increased with the operation time. The shaded area in Figure 3 presents the ion-induced Air Cleaning Factor when the particle size-selective data were integrated over the measured sizes of NaCl particle up to 2.5 μ m and averaged over the three selected locations/orientations in the 24.3-m³ chamber: in the corner facing the center, in the center, and at 80 cm from the wall facing it.

Figure 4 presents the CADR values achieved by operating the tested air purifier for five selected sizes of NaCl and smoke particles acting as aerosol contaminants in the non-ventilated 24.3 m² chamber. The CADR ranges approximately from 42.1 \pm 0.1 to 62.1 \pm 1.8 m³/h for NaCl particles of d = 0.04-1.99µm, and from 72.4 \pm 0.9 to 115.5 \pm 10.8 m³/h for smoke particles of the same size range. The difference may be attributed to different ability of NaCl and smoke particles to acquire electric charges from air ions, which results in their different mobilities and subsequently different migration velocities. The above explanation seems valid given that unipolar ion emission was shown to be the major mechanism causing the aerosol particle concentration reduction.







FIGURE 4. Clean Air Delivery Rate (CADR) determined for the NaCl and smoke aerosols as measured with the ELPI in the non-ventilated 24.3 m³ chamber. The performance of the air purifier is compared to that of a standard HVAC filter (ASHRAE rating = 8) installed in the closed-loop air exchange system of the chamber.

In addition, Figure 4 presents the CADR values achieved by the closed-loop air exchange system equipped with a standard ASHRAE rating 8 HVAC filter at two air exchange rates, 2.5 and 7.7 ACH. The data suggest that the tested portable air purifier operating in about 25 m³ non-ventilated room is capable to provide a CADR more than twice greater than the conventional central HVAC system with the rating 8 filter. Obviously, more efficient particulate filters provide more rapid reduction of aerosol contaminants and may perform better than the tested air purifier. For example, compared to the portable unit, HEPA filter installed in the closed-loop air exchange system of the 24.3 m³ chamber provided approximately 4- and 3-fold greater CADRs at 2.5 and 7.7 ACH, respectively, when challenged with NaCI particles, and 2.2- and 1.4-fold greater when challenged with smoke particles. However, HEPA filters are rarely used in residential central HVAC systems because of the highpressure drop and the loading effect on their performance.

The particle removal from indoor air by the hybrid air purification technique was also investigated in the smaller (2.75 m³) chamber, which otherwise was utilized primarily for assessing the viable microorganism inactivation. The CADR values obtained with MS2 virions from the WPS measurements were 73 \pm 5 m³/h, which is in the CADR-

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TABLE 1. Percentage of Airborne Microorganisms Survived over Time t in the 2.75 m³ Chamber with the RCi-cell Operating in it, as Measured via PFU Count (for MS2 Virus) or CFU Count (for *Bacillus subtilis* Endospores)^a

exposure	percentage (mean \pm SD) of airborne microorganisms survived in the chamber with air purifier operating during time t	
time, t (min)	MS2 virus, [PFU/cm ³] _r /[PFU/cm ³] _{r=0}	Bacillus subtilis endospores, [CFU/cm ³]/[CFU/cm ³] _{r=0}
10	9.3 ± 2.0 (n = 5)	24.1 ± 3.7 (n = 2)
15	9.2 ± 4.3 (n = 12)	15.7 ± 1.7 (n = 3)
30	$8.3 \pm 1.1 (n = 8)$	$7.9 \pm 1.1 (n = 3)$
60	10.3 ± 1.7 (n = 5)	$10.1 \pm 1.3 (n = 3)$

^a Bioaerosol sampling was conducted with the Button Sample equipped with gelatin filters. n = number of replicates.

range obtained for NaCl and smoke particles in the large chamber for the viral sizes. This suggests the feasibility of using nonbiological particles to determine the ion-induced aerosol reduction of bio-particles of the same size range. Furthermore, this finding implies that, at least for the particle size range representing MS2 virions, PRR due to ion emission in indoor air environment is inversely proportional to the air volume [see eq 5].

Ozone. In both test chambers (non-ventilated), the ozone concentration gradually increased as the purifier was continuously operating. In the 24.3-m³ chamber, it increased from 0.006 to 0.05 ppm in about 35 min, while in a smaller (2.75-m³) chamber the same increase occurred in approximately 5 min. However, once an air exchange was introduced (as low as 1 ACH), the ozone concentration in the 24.3-m³ chamber did not significantly increase as compared to the initial level (p > 0.05). Our monitoring data obtained with the tested unit operating in a non-ventilated room of ~100 m³ (not presented here) suggest that the ozone level can be kept below 0.05 ppm while the unit continuously operates for many hours.

Some air purifiers utilizing ion emission and, to a greater extent, the photocatalytic oxidation may cause greater increase of indoor ozone concentration than the tested one. The use of such devices in confined occupied air spaces may not be appropriate as their continuous operation may eventually lead to excessive ozone levels and, in the presence of certain chemical compounds, produce nanoparticles (33). Although the unipolar ion emission has a potential to suppress this effect, it seems important to keep the ozone level below existing thresholds. We believe that the solution can be found by implementing an intermittent regime (as an alternative to continuous one), which allows the air purifier operating until the ozone reaches a certain level, after which the ozone-generating element is automatically turned off to allow the ozone concentration to drop; then the cycle can be repeated.

Microbial Inactivation. Table 1 summarizes the microbial inactivation results. Only approximately 10% of initially viable MS2 virions survived 10-60 min exposure to the purifier in the chamber and about 90% were inactivated. When the natural concentration decay of aerosolized MS2 was monitored in the chamber (with no purifier operating), we found that the concentration of active viruses was relatively stable: the decrease did not exceed 20.3 \pm 0.9% during 1 h. The data suggest that the viral inactivation occurs rather quickly since the percent of survived virions did not show dependence on the exposure time for t = 10-60 min. Thus, a relatively short time may be sufficient to reduce the percent of viable viruses in an air volume by a factor of 10 while those that survived showed remarkable resistance to the continuing stress. When aerosolized virions are exposed to photocatalytic oxidation, the hydroxyl radicals can affect the protein capsid and binding sites, thus disabling the virus's subsequent interaction with

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the host and formation of PFUs (34). Additionally, the TiO_2 photocatalytic cell may produce oxidative damage to the virus capsid (35) and the radicals may cause alteration in the virus's genetic material (36, 37). Our findings suggest that the hybrid air purifier may be used continuously for short time intervals or in intermittent regime to achieve considerable virus inactivation rate. On the other hand, a prolonged operation of the air purifier is believed to be advantageous in environments with a continuous supply of "fresh" active virions.

Approximately 75% of airborne *B. subtilis* spores exposed to the air purifier were inactivated during the first 10 min, 85% during the first 15 min, and about 90% or greater after 30 min (Table 1). Between 30 and 60 min of exposure, we did not observe significant decrease in the number of survived spores (similar to the trend found for virions), which suggests a nonlinearity of the effect. The natural decay in the culturable count was not significant (p > 0.05) during 1 h, as measured using the Button Samplers equipped with gelatin filters. However, the overall standard deviation of the data obtained in these control tests was as high as 58% and the CFU counts from filters were close to the detection limit. To address this issue, we measured the natural decay of viable *B. subtilis* spores with the BioSampler at t = 0 and at t = 2 h. It was confirmed that the viability was constant within about $\pm 20\%$ in the absence of the air purifier.

In bacteria, the inactivation process by reactive hydroxyl radicals can proceed in five reaction pathways:

 •oxidation of coenzyme A causing inhibition of cell respiration and cell death (38);

 destruction of the outer membrane of bacterial cells (12);
 oxidation of unsaturated phospholipid in bacterial cell membrane (39);

leakage of intracellular K⁺ ions (11); and

detrimental effects on DNA and RNA (36, 37).

One reason that the inactivation of *B. subtilis* endospores was time-dependent is their thick membrane layer containing peptidoglycans. This is consistent with the study of Matsunaga et al. (40), who found that photooxidation of coenzyme A by the TiO₂ photocatalyst was not entirely effective against the algae *Chlorella vulgaris* in water because of its thicker cell wall. Some other self-defense mechanisms of bacteria against the oxidation stress, including synthesis of superoxide dismutase enzymes, can also slow down the inactivation process (41).

Although the time was a factor in the bacterial spore inactivation, the viability loss occurred relatively quickly for both the MS2 virus and B. subtilis. This can be attributed to rapid interaction of valence-band holes (h⁺) ($TiO_2 + hv \rightarrow$ + e⁻.) with the organic substances, which are present in the viral and bacterial outer walls or membranes. The abovementioned interaction likely occurs before considerable number of hydroxyl radicals (OH) is generated in the air volume. Although previous studies (11, 12) emphasized the role of hydroxyl radicals (H₂O + $h^+ \rightarrow$ OH + H^+), these radicals may not be the primary factor in microbial inactivation, particularly in the air. Furthermore, since our experiments were conducted in relatively dry air (RH < 30%), water molecules were not predominant species in contact with the catalyst, and thus the contribution of hydroxyl radicals was likely much lower than in liquids. Shang et al. (9) have concluded that in the gas phase, organic compounds, such as heptane, can readily interact with photogenerated holes while the interaction with water vapor molecules is not as prominent. Alberici and Jardim (8) have reported that the valence-band holes generated from TiO2 photooxidation are capable of oxidizing any organic compound. The process also produces hydrogen peroxide (O2 + e- O2-; O2- + H+ → HO₂, 2HO₂, → O₂ + H₂O₂), which can freely penetrate into cell membranes and walls and cause microbial inactivation

(42). Further biochemical studies on the role of gas-phase TiO2 oxidation on the airborne microorganisms as well as studies on the reaction kinetics at the aerosol phase seem worthwhile to further examine the above interpretations.

Experiments with P. fluorescens revealed CFU counts below the detection limit both in the test and control samples. In contrast to *B. subtilis* endospores, even a very short exposure to ambient air (RH < 30%) considerably decreased the viability of aerosolized P. fluorescens vegetative cells, which are known to be stress-sensitive. Perhaps, microorganisms sensitive to desiccation stress are more usable for this kind of test if the test is performed at higher relative humidity levels.

Additional control experiments were performed to investigate if the viability decrease found for MS2 virus and B. subtilis spores occurred in the aerosol phase or on the sampling filter. For MS2, we found that 1835 ± 270 PFU/mL and 1855 ± 325 PFU/mL developed when filter extracts were cultivated from unexposed and 10-min exposed gelatin filters, respectively. For B. subtilis, we observed 1770 ± 275 CFU/ mL and 1125 ± 410 CFU/mL in extracts taken from unexposed and 60-min exposed filters, respectively. No significant changes in either viral or bacterial viability occurred as a result of a non-aerosol exposure (p > 0.05). Thus, these findings confirm that the viral and bacterial inactivation observed in our tests indeed occurred in the aerosol phase and was not associated with the inactivation on filters

Combined Effect (Sample Calculation). It was concluded that the particle removal took place solely due to unipolar ion emission, while the inactivation of viable airborne MS2 virions and B. subtilis spores occurred due to the photocatalytic reaction promoted by the RCI cell. Both mechanisms working simultaneously in a hybrid type air purifier may result in considerable decrease of the exposure to pre-existing viable aerosol biocontaminants in indoor environment. Ozone produced by the RCI cell is not believed to cause significant microbial inactivation because its level was not sufficient. Tseng and Li (43) referred to 3.43 ppm as an appropriate level for airborne MS2 virus, and Li and Wang (44) did not observe any inactivation of airborne B. subtilis spores at O3 as high as 20 ppm.

The following estimate was made based on the experimental data obtained in this study. Assuming that the ion-induced air cleaning removes about 80% of viable airborne pathogens from a room air in 30 min and the RCI-induced photoxidation leaves only 10% of the remaining airborne microorganisms viable, the overall aerosol exposure to the viable pathogen in this room after 30 min is reduced by a factor of about 50.

The observed rapid inactivation of microorganisms makes unnecessary to run the RCI cell continuously. The data suggest that it can be used "part-time" for 10-30 min and "rest" for about 1-2 h until the background ozone level is reached (proposed above as an intermittent regime), while the ion emission can take place continuously to keep the aerosol concentration decreasing.

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RESEARCH REPORT: *Hepatitis A*

Report Summary

HEPATITIS A



LABORATORY: Kansas State University PRODUCT: FreshAir 2.0 TECHNOLOGY: Radiant Catalytic Ionization (RCI) EXPOSURE TIME: 0.5 Hour
Hepatitis A (HAV) : Kills It (Hepatitis)

Beyond by Aerus ActivePure Technology Kills Hepatitis A (HAV)



Hours of Treatment

Kansas State University • Inactivation of Picornaviruses

Inactivation of Picornaviruses using EcoQuest Radiant Catalytic Ionization

Introduction

The viral family *Picornaviridae*, which includes Hepatitis A virus, is characterized as including viruses which are non-enveloped with single stranded positive sensed RNA genomes known to be very resistant to physical and chemical means of inactivation (1). Hepatitis A virus (HAV) is known to spread predominantly through contaminated drinking water and food sources. During an outbreak, HAV can contaminate and remain infectious on various environmental surfaces. Standard disinfection processes are usually not effective for inactivating this virus due to the high resistance. Stringent disinfection and sanitation procedures of an affected environment in combination with stringent employee hygiene procedures are recommended for preventing HAV outbreaks in the food industry.

The purpose of this study was to validate the complete inactivation of HAV using a closely related enterovirus as a surrogate virus following exposure to the EcoQuest Radiant Catalytic Ionization CellTM (RCI-CellTM) system. The RCI-CellTM system is an advanced oxidation tool which combines UV inactivation in the presence of hydroxical radicals so that synergy between two highly effective inactivation technologies occurs. Efficacy of this technology was determined by inoculating stainless steel coupons with virus and allowing inoculum to dry. Control samples were taken at this time and then coupons were exposed to the RCI-CellTM for various times. Non-treated control samples were also evaluated to compare the reduction in infectious virus titer in a 24 hour period in controlled environments. Efficacy was determined by measuring any reduction in infectious titer using end-point titration in tissue culture for treated and coupons compared to non-treated positive control coupons.

Materials and Methods

Virus and cells. BEV-2 (ATCC VR-754, Manassas, VA) was propagated in Madin Darby Bovine Kidney (MDBK, ATCC CCL-22) cells. MDBK cells were propagated in minimum essential medium (Eagle) with 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, and 1.0 mM sodium pyruvate with 7% fetal bovine serum supplemented with 2.5 mg/L amphotericin B, 0.67 g/L streptomycin, and 0.3 g/L penicillin. MDBK cells were infected with BEV-2 without the addition of 10% FBS. Inoculum titer was assessed using tissue culture infective dose 50, TCID₅₀ and calculated by the Reed-Muench method (2).

Virus inactivation. Type 302 stainless steel (McMasterCarr, Altanta, GA) coupons (2 x 10 cm², thickness 0.8 mm) were sterilized by autoclaving for 15 min at 121° C. In a biosafety class II cabinet, 100 μ l of BEV-2 was added to each test coupon and spread to cover the entire surface using the pipette tip and allowed to dry completely for approximately 20 minutes. Then, the inoculated coupons were placed into a sterile transport container and transported to the test chamber. The test coupons were then placed within the test chamber and exposed to the RCI-CellTM system for a 24 hour period. As non-treatment controls, test coupons were also prepared as described above and added to a test chamber which would not be exposed to the RCI-CellTM system for a 24 hour period. One inoculated coupon was removed initially for both the RCI-CellTM chamber and the non-treated control chamber to be used as an initial measure of starting virus titer. The RCI-CellTM device was then turned on and samples were removed from both testing chambers after 2, 4, 8, 12, and 24 hours by removing a test coupon and preparing it for virus recovery as described below.

Kansas State University • Inactivation of Picornaviruses

Virus Recovery. BEV-2 was recovered from the stainless steel surfaces by adding the test coupon to a sterile 50 ml conical vial containing 5 ml of infectivity media. Tubes were then vortexed for 1 minute to release virus from the inoculuated coupon. Samples were titrated by infecting confluent MDBK wells in a 96-well format using $TCID_{50}$ endpoint titration. Plates were incubated at 37° C, 5% CO₂ for 48 hours. Cytopathic effect (CPE) typical for BEV-2 was determined for each well and viral titers were reported as $TCID_{50}/ml$.

Results

The average amount of BEV-2 virus recovered from the control stainless steel coupons in all experiments was 6.00 \log_{10} TCID₅₀/ml. Following treatment with the RHI-CellTM, the average log reductions of infectious BEV-2 virus were 3.87 and 4.87 \log_{10} TCID₅₀/ml following 2 and 4 hour treatments (Figure 1). No infectious BEV-2 was recovered following the 8, 12, or 24 hour exposure to the RCI-CellTM system. Infectious BEV-2 was recovered from all coupons sampled following 2, 4, 8, 12, or 24 hour periods in the chamber not receiving the RCI-CellTM treatment (Figure 1)



Figure 1: Infectious BEV recovered and reported as TCID₅₀/ml in MDBK cells following treatment to RCI-CellTM (yellow bars) or no treatment to RCI-CellTM (blue bars) following 2, 4, 8, 12, or 24 hour sampling times.

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RESEARCH REPORT: *Murine Norovirus*

Report Summary



Norovirus : Kills It

Beyond by Aerus ActivePure Technology Kills Norovirus



Survival of MNV following Low-Oxidation Treatment

Survival of MNV following Ozone-Free Treatment



Evaluation of the Efficacy of Ecoquest's Decontamination Systems in Reducing Murine Norovirus Titers Performed by Dr. Lela Riley, RADIL LLC, Columbia MO November 18, 2008

Introduction

Members of the genus *Norovirus* are nonenveloped viruses with a linear, positive-sense, single-stranded RNA genome. Noroviruses are in the family *Caliciviridae*, which also includes the genera *Sapovirus*, *Lagovirus*, and *Vesivirus*. Formerly known as "Norwalk-like viruses" or "small round structured viruses," noroviruses cause acute gastroenteritis in humans, typically lasting 24 to 48 h, and infect people of all ages.

Recently, the first murine norovirus, was isolated from mice. This newly described pathogen of mice can be grown in cell culture, providing the first example of a norovirus that can be cultured in vitro. In these studies, the efficacy of Ecoquest's decontamination platform has been evaluated against Murine norovirus (MNV), as a representative of the *Caliciviridae* family, using an in vitro culture system.

Experimental Design

Virus stock and culture

MNV-4 used in this study was maintained in RAW267.4 cells, a murine macrophage cell line. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. The virus was propagated, concentrated, and purified. Purified viral stocks were titered via plaque titration. Viral stocks were stored in a -80°C freezer.

Preparation of surfaces

To assess efficacy of the Ecoquest Ozone-free and Low-Oxidation decontamination systems for reducing MNV titers, virus-contaminated surfaces were exposed to the decontamination system for various time periods. Decontamination was evaluated on three types of surfaces: Stainless steel, carpet and cloth. Stainless stell cassettes measuring 1.5 inches by 1.25 inches were used as the stainless steel surface. Samples of carpet and cloth were cut to 1 inch squares. Prior to the experiment, all surfaces were sterilized in a steam autoclave. To contaminate the surfaces, 200 μ L of MNV viral stock (1 x 10⁷ PFU/mI) was pipetted onto the center of each surface, covering ~ 1-2 cm. The surfaces were allowed to air dry in a type II biosafety cabinet. At the end of the hour, the zero time point control samples were collected and the remaining inoculated surfaces were placed in a humidified 28^oC incubator for either low oxidation treatment or ozone free treatment. A set of four inoculated samples for each surface

After the specific times of exposure had been reached, the surfaces were immersed into 10 mls DMEM containing 10ug/ml ciprofloxacin. Stainless steel surfaces were scraped with a sterile cell scraper to remove virus from the cassette surface. Carpet and cloth samples were placed in a sterile bag and homogenized for 1 minute in a Stomacher Lab Blender. Samples were removed from the bag and placed in a 15 ml conical centrifuge tube and spun at 1000 x g for 10 minutes to remove residual carpet and cloth fragments. As controls, each surface was inoculated with an equivalent amount of virus and placed in a 28° incubator without treatment to serve as the 24 hour untreated controls. Each of the samples subjected to the decontamination system was tested in quadruplicate at each time point. Controls were also tested in quadruplicate. Data are expressed as an average of all data points.

Calculation of viral titer and viral reduction

After neutralization of the disinfectant in specified volumes of DMEM, stainless steel surfaces were thoroughly scraped with a sterile cell scraper to elute the virus into the DMEM. Carpet and cloth samples were suspended in sterile DMEM and homogenized using a Stomacher blender to release the virus. The viral titer of each eluate was determined

inoculating cell cultures with serial ten-fold dilutions of the eluates, and calculating the tissue culture infective dose 50 (TCID50) based on observations of characteristic cytopathic effects associated with MNV. The final titer was calculated by averaging the individual titers calculated from each replicate and the decrease in viral titer was then calculated.

Results

The following tables summarize the results of these experiments.

	Stainless steel			Carpet			Cloth		
Treatment time	Untreated (TCID ₅₀ /ml)	Treated (TCID ₅₀ /ml)	Percent decrease from t=0	Untreated (TCID ₅₀ /ml)	Treated (TCID ₅₀ /ml)	Percent decrease from t=0	Untreated (TCID ₅₀ /ml)	Treated (TCID ₅₀ /ml)	Percent decrease from t=0
0 hrs	1.2 x10 ⁶	2111	\leq	1.6 x10 ⁶	2111 în		4.0 x10 ⁵		
2 hrs	21112	3.5 x 10 ⁵	70.8		3.4 x 10 ⁵	78.8		2.1 x 10 ⁴	94.8
4 hrs		3.6 x10 ⁴	97.0		7.5 x 10 ⁴	95.3		1.7 x 10 ⁴	95.8
6 hrs		1 x 10 ²	99.9		<1 x10 ³	>99.9		<1 x10 ³	>99.8
24 hrs	1 x 10 ³	1 x10 ²	99.9	<1 x10 ³	<1 x10 ³	>99.9	8.6 x 10 ²	<1 x10 ³	>99.8

Table 1. Reduction in Murine Norovirus Titer Following Ecoquest's Ozone-free Treatment

Figure 1. Survival of MNV following Ozone-Free Treatment



	Stainless steel			Carpet			Cloth		
Treatment time	Untreated (TCID ₅₀ /ml)	Treated (TCID ₅₀ /ml)	Percent decrease from t=0	Untreated (TCID ₅₀ /ml)	Treated (TCID ₅₀ /ml)	Percent decrease from t=0	Untreated (TCID ₅₀ /ml)	Treated (TCID ₅₀ /ml)	Percent decrease from t=0
0 hrs	1.6 x 10 ⁵	\sim		2.8 x10 ⁵	\leq		2.5 x 10 ⁴	\leq	1112
2 hrs		9.03 X10 ³	94.4		9.5 x 10 ⁴	66.1		1.4 x10 ⁴	44.0
4 hrs	\leq	7.6 x10 ³	95.3		2.8 x 10 ⁴	90.0	ZIII]	8.6 x10 ³	65.6
6 hrs	111	<1 x 10 ²	>99.9	111	<1 x10 ³	>99. 9		<1 x10 ²	>99.6
24 hrs	9.3 x10 ³	<1 x 10 ²	>99.9	<1 x10 ³	<1 x10 ³	>99.9	<1 x10 ²	<1 x10 ²	>99.6

Table 2. Survival of Murine Norovirus following Low-oxidation Treatment

Figure 2. Survival of MNV following Low-Oxidation Treatment



This report prepared by:

Jue K. Niery

<u>November 19, 2008</u>

Lela K. Riley, PhD Managing Partner, RADIL LLC Date



MNV-4 Titers Following Two Hour Ozone Free Active Pure Cell Treatment

MNV-4 Titers Following Ozone Free Active Pure Cell Treatment



MNV-4 Titers Following Two Hour Lo Oxidation Active Pure Cell Treatment



MNV-4 Titers Following Low Oxidation Active Pure Cell Treatment



RESEARCH REPORT: *Avian Influenza*

Report Summary



Influenza Virus (H5N8) : Kills It (Avian-Bird Flu)

Beyond by Aerus ActivePure Technology Kills Influenza Virus (H5N8)



RCI Inactivation of Avian Influenza

INTRODUCTION

The influenza virus, a member of the viral family *Orthomyxoviridae*, is characterized as being an enveloped single stranded negative sensed RNA virus (6) that can result in yearly endemic outbreaks and more severe world-wide pandemic outbreaks. Influenza A commonly infects human, swine, equine, and avian isolates. In the case of a pandemic outbreak, highly pathogenic avian influenza (H5N1) is currently the greatest threat due to current epidemic status in Asia, Europe, and Africa and continued threat for pandemic spread. Reassortment of genomic information of the influenza virus can result in a more pathogenic and infectious isolate is heightened during ongoing outbreaks, which could result in a devastating human-to-human transmissibility. Influenza virus is typically spread via aerosols, large droplets, or contact with infectious secretions or fomites (4).

Rapid containment of an outbreak is important for preventing further spread and minimizing the potential for reassortment to occur. Influenza has been shown to survive on nonporous surfaces for up to 48 hours and on material surfaces such as cloth, paper, or tissue for up to 12 hours after being deposited at approximately a 10^5 TCID₅₀/ml level (1). In addition to surface sanitation and disinfection regimens, airborne inactivation of influenza virus is also vital to address predominant modes of transmission such as aerosol and large droplet (4). Environmental contamination with aerosolized droplets containing this pathogen can serve as a reservoir for infection and must be controlled by effective sanitation and disinfection protocols. Minimizing the degree of environmental contamination with highly effective decontamination measures would aid in the overall containment efforts of an outbreak.

The purpose of this study is to validate the complete inactivation of influenza A viruses using a low pathogenic avian influenza (H5N8) as a surrogate virus for the highly pathogenic avian influenza (H5N1) following exposure to the Radiant Catalytic Ionization-CellTM (RCI-CellTM) system. The RCI-CellTM system is an advanced oxidation tool which combines UV inactivation in the presence of hydroxical radicals so that synergy between two highly effective inactivation technologies occurs. Efficacy will be determined for dried inoculum on solid surfaces, in cell culture propagated inoculum, and nebulized in a controlled chamber. Efficacy will be determined by reduced or complete loss of infectivity in a cell culture system for treated samples compared to non-treated positive control samples.

MATERIALS AND METHODS

Virus and cells. Low pathogenic avian influenza H5N8 (H5N8, provided generously by the Centers for Disease Control and Prevention, Atlanta, GA) was propagated in 10 day embryonated hen eggs (Kansas State University Department of Poultry Science, Manhattan, KS) to approximately $10^7 \log_{10} \text{ TCID}_{50}$ (as determined in Madin Darby Canine Kidney, MDCK cells). Cells were maintained in Minimal Essential Medium with Earle's salts and L-glutamine (Invitrogen Corporation, Carlsbad, CA) and 2.2 g/L sodium

Kansas State University • Inactivation of Avian Influenza

bicarbonate (Fisher Scientific, Hampton, NH) collectively referred to as MEM containing 10% fetal bovine serum (FBS, Hyclone Laboratories, Logan, UT) supplemented with antibiotics [2.5 mg/L amphotericin B; 0.67 g/L streptomycin; and 0.3 g/L penicillin G (all from Fisher Scientific)]. Infectivity media was made by adding MEM with the addition of 0.1% TPCK treated trypsin (Fisher Scientific) and supplemented with antibiotics (2.5 mg/L amphotericin B; 0.67 g/L streptomycin; and 0.3 g/L penicillin G).

H5N8 inactivation. Type 302 stainless steel (McMasterCarr, Altanta, GA) coupons (2 x 10 cm², thickness 0.8 mm) were sterilized by autoclaving for 15 min at 121 C. In a biosafety class II cabinet, 100 μ l of egg propagated H5N8 was added to each test coupon and spread to cover the entire surface using the pipette tip and allowed to dry completely for approximately 10-15 min. Then, the inoculated coupons were placed into a sterile transport container and transported to the test chamber. The test coupons were then attached to clips within the test chamber so that all sides of the coupon would be exposed to the RCI-CellTM treatment. One coupon was removed prior to starting the RCI-CellTM treatment to be used as the initial control sample. The RCI-CellTM device was then turned on and samples were taken at various intervals (2, 4, 8, 12, 24 hours) by removing a test coupon and preparing it for virus recovery as described below.

Virus Recovery. H5N8 virus was recovered from the stainless steel surfaces by adding the test coupon to a sterile 50 ml conical vial (Fisher Scientific) containing 5 ml infectivity media. Tubes were then vortexed for 1 min. Endpoint dilution titration was conducted in MDCK cells by adding 220 μ l from the 5 ml infectivity media containing any suspended virus to the first dilution well in a minimum of 6 wells of a 96 well microtiter plate containing confluent MDCK cells. Then, serial 1:10 dilutions were prepared by adding 20 μ l from the first well into the next 6 wells each containing 180 μ l infectivity media. The final well contained only 200 μ l infectivity media to serve as a negative cellular control. Plates were incubated at 37 C, 5% CO2 for 48 hours. Cytopathic effect (CPE) was determined for each well and viral counts were reported as TCID50/ml as calculated by Reed and Muench (3).

Real-Time Reverse Transcription Polymerase Chain Reaction (*r***RT-PCR**). Viral RNA was recovered using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA). Quantitative detection of the extracted influenza RNA was conducted using *r*RT-PCR using a fluorescently labeled TaqMan probe. The *r*RT-PCR primer and probe sequences were provided generously by the Molecular Genetics Influenza Branch, Centers for Disease Control and Prevention in Atlanta, GA. The detection threshold for successfully detecting influenza RNA was a FAM fluorescence signal \geq 3 using the SmartCycler.

RESULTS

The average amount of H5N8 recovered from the stainless steel coupons in all experiments was $5.35 \log_{10} \text{TCID}_{50}/\text{ml}$. Following treatment with the RCI-CellTM, the average log reductions of the H5N8 virus were 1.85, 2.79, 4.16, 5.35, and 5.35 $\log_{10} \text{TCID}_{50}/\text{ml}$ following 2, 4, 8, 12, and 24 hour treatments (Figure 1) based on the recovery of infectious virus.

Kansas State University • Inactivation of Avian Influenza



Figure 1: Recovery of H5N8 post-treatment with RCI-Cell[™] based on TCID₅₀/ml in MDCK cells.

The average amount of viral H5N8 RNA recovered from the stainless steel coupons in all experiments was 4.00 \log_{10} based on a quantitative RT-PCR available for influenza A viruses. Following treatment with the RCI-CellTM, the average log reductions of the H5N8 virus based on the amount of RNA recovered varied between 0.23 to 0.54 \log_{10} following all exposure times (2, 4, 8, 12, and 24 hour) indicating that the mechanism of action for loss of infectivity was more likely due to disruption of the lipid envelope or structural proteins than with degradation of the viral nucleic acid (Figure 2).



Figure 2: Recovery of H5N8 RNA post-treatment with RCI-Cell™ based on quantitative RT-PCR.

DISCUSSION

In an effort to better understand the inactivation of the influenza virus using the RCI-CellTM, the efficacy was evaluated using a low pathogenic avian influenza isolate, H5N8 inoculated onto stainless steel surfaces. Inactivation efficacy was determined following

the current EPA guidelines for determining virus disinfection (2) which allows the recovery of treated virus as endpoint dilution including a $TCID_{50}$ recovery assay of infectious virus. In addition to the recovery of infectious virus, we wanted to determine if any disruption of viral RNA was occurring by using a quantitative RT-PCR assay specific for influenza A viruses in our experiments.

Based on the current EPA guidelines to achieve $a > 4.0 \log 10$ reduction in starting virus titer (2), RCI-CellTM treatment for 8 hours or more resulted in the successful inactivation of the H5N8 isolate (Figure 1) for a starting contamination level of 5.35 log₁₀ TCID₅₀/ml. Additional testing would be required to determine if lower exposure times would result in complete inactivation for contamination levels lower than 5.35 log₁₀ TCID₅₀/ml, which might be more representative in a real outbreak (1, 5).

The quantitative RT-PCR results indicate that degradation of viral RNA (Figure 2) was not the major mechanism for viral inactivation, as the levels of RNA recovered after each treatment time were not significantly different from each other, P > 0.05. Other possible viral targets include the lipid envelope and structural proteins which were likely affected by the RCI-CellTM treatment. The oxidative mechanism of this treatment likely disrupted the relatively susceptible envelope and could have resulted in denaturing the surface structural proteins of the influenza virus necessary for successful attachment and entry mechanism vital for infectivity.

The results obtained in this research experiment show that exposure to the RCI-CellTM system for 8 hours results in the required level of inactivation of an avian influenza isolate, H5N8 which was used as a safe surrogate for the highly pathogenic H5N1 isolate. The mechanism of action of this technology is likely due to the oxidative chemistry resulting in both disruption of the lipid envelope and the denaturing effect on the structural viral proteins necessary for virus replication.

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RESEARCH REPORT: Influenza A H1N1

Report Summary



LABORATORY: Kansas State University PRODUCT: FreshAir 2.1 TECHNOLOGY: Radiant Catalytic Ionization (RCI) EXPOSURE TIME: 8 Hours

H1N1 : Kills It (Swine Flu)

Beyond by Aerus ActivePure Technology Kills H1N1



Hours of Treatment



October 14, 2009

To: Joe Urso – Aerus Corporation

From: James Marsden, Ph.D.

Subject: Inactivation of Influenza A H1N1 using Radiant Catalytic Ionization (RCITM)

Interim Progress Report

This is an interim progress report on the evaluation of the RCITM system for inactivating Influenza H1N1 on environmental surfaces. Additional research is planned to fully evaluate the effect of the reactive oxygen species (ROS) produced by the RCITM system on Influenza H1N1 under controlled laboratory conditions.

The results of this study are preliminary and should not be used to position the RCI TM as a medical device or as a means of reducing the risk of H1N1 infections.

I recommend that Aerus Corporation meet with FDA officials to obtain guidance on future research and how best to position the RCI technology to consumers.

Background

Novel influenza A (H1N1) is a new flu virus of swine origin that first caused illness in Mexico and the United States in March and April, 2009. H1N1 is an acute and highly contagious respiratory virus similar to seasonal flu but affecting a younger age group. Less immunity exists to this novel strain of the flu than to seasonal flu. H1N1 influenza in humans can vary in severity from mild to severe. The H1N1 virus is thought to spread in the same way seasonal flu is spread: from person to person through droplets produced by coughs and sneezes, or from touching contaminated surfaces and then touching your mouth, nose or eyes. Novel H1N1 infection has been reported to cause a wide range of flu-like symptoms, including fever, cough, sore throat, body aches, headache, chills and fatigue. In addition, many people also have reported nausea,

vomiting and/or diarrhea. The virus can stay alive on surfaces and your hands and body for at least two hours.

The first novel H1N1 patient in the United States was confirmed by laboratory testing at CDC on April 15, 2009. The second patient was confirmed on April 17, 2009. It was quickly determined that the virus was spreading from person-to-person. On April 22, CDC activated its Emergency Operations Center to better coordinate the public health response. On April 26, 2009, the United States Government declared a public health emergency and has been actively and aggressively implementing the nation's pandemic response plan

By June 19, 2009, all 50 states in the United States, the District of Columbia, Puerto Rico, and the U.S. Virgin Islands have reported novel H1N1 infection. While nationwide U.S. influenza surveillance systems indicate that overall influenza activity is decreasing in the country at this time, novel H1N1 outbreaks are ongoing in parts of the U.S., in some cases with intense activity.

Preliminary Experiment – Inactivation of Influenza A H1N1 on Inoculated Stainless Steel Surfaces

Influenza A *H1N1* (ATCC # VR-333) was evaluated in this study. The procedures for maintaining the virus culture and enumerating the virus prior to and after treatment were obtained from Dr. Rick Falkenberg – FSPT. The virus culture was maintained on ATCC complete growth medium and minimum essential medium (ATCC, Manassas, VA., USA) with 2 μ M L-glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 μ M non-essential amino acids, and 1.0 μ M sodium pyruvate, 90%; fetal bovine serum, 10% and cultured in Trypticase Soy Agar with added; sodium bicarbonate, non-essential amino acids, and combination of sodium pyruvate and fetal bovine serum, in aerobic growth conditions at 37.0°C and *Influenza A* at 33-35° C.1 Cells from both of the above (approx. 1x107 CFU/ml) from a 24 hour static culture incubated at 37.0°C and *Influenza A* at 33-35° C were used to inoculate various 5 cm x 3 cm stainless steel coupons. The inoculum suspensions were enumerated by surface plating in duplicate samples on TSA after serial dilution in 0.1% peptone solution. The plates were incubated for 24 hour at 37.0°C.

A 100 µl droplet from the initial inoculum suspension of each of the bacteria/viruses was used to inoculate the external surface (6.3 cm x 1.8 cm) on # 8 stainless steel coupons. This resulted in a final inoculum level of approximately 7.0-log CFU/5 g sample. The inoculated samples were air dried for 1 hour at 22.0°C prior to RCI TM treatment being initiated. The 1 hour drying allows the inoculated cells to attach to the surface host and minimize the growth of inoculated cells during drying. Four stainless steel coupons were used for each sampling time.

A biocontainment chamber was equipped with an RCITM cell (obtained from Aerus Corporation) and allowed to equilibrate for a period of two hours prior to placement of 12 inoculated coupons inside the chamber. The effect of the RCITM treatment was measured at 0, 1,

2, 4, 6, 8, 12 and 24 hours. A control study was conducted in the same chamber without the presence of the RCI cell. Temperature, relative humidity, and ambient Ozone levels and Hydrogen Peroxide levels were monitored in the chamber.

After treatment, each of the 5 cm x 3 cm coupons were transferred into a 400 ml stomacher bag (Fisher Scientific Inc., PA., USA) combined with 50 ml sterile 0.1% peptone solution, and then blended with a AES Easy Mix Stomacher (AES Laboratories, Princeton, NJ., USA) for 2 min at normal speed. Wash fluid was serially diluted, followed by surface plating for enumeration. A centrifugation method was used to recover low populations of ROS injured bacteria and viruses. The centrifugation method (Mossel and others 1991) was modified and used to concentrate the bacterial and virus populations in the wash fluid so that less than 250 CFU/ml of bacteria can be enumerated by the surface plating.

Results and Discussion

Table 1. Average recoveries (Log CFU/cm²) of *Influenza A* H1N1on inoculated stainless steel coupons treated using an RCI TM Cell for periods of 1, 2, 4, 6, 8, 12 and 24 Hours

Sample	Influenza A H1N1 Treated with RCI Cell	<i>Influenza A</i> H1N1 Control
Initial – 0 Time	6.8	6.9
1 Hour	4.1	6.7
2 Hours	3.4	6.4
4 Hours	1.2	6.1
6 Hours	BDL	5.5
8 Hours	BDL	5.7
12 Hours	BDL	5.7
24 Hours	BDL	5.7

Table 1 summarizes the results of the preliminary study. The study demonstrated the effectiveness of the reactive oxygen species produced by the RCI^{TM} cell in the inactivation of Influenza A – H1N1. After 6 hours of treatment, levels of the H1N1 virus on inoculated stainless steel coupons were below the detection limit. No recovery was observed at 8, 12, or 24 hours.

The ambient ozone levels in the chamber ranged from 0.02 - 0.04 PPM. Levels of vaporized Hydrogen Peroxide ranged from 0.06 - 0.09 PPM. The relative humidity ranged from 45 - 57% and the temperature from 70 - 73 degrees F.

Based on the results of this preliminary study, it appears that the reactive oxygen species produced by the RCI TM cell are effective at inactivating *Influenza A* H1N1 virus on inoculated stainless coupons under the conditions of these tests. Additional testing is recommended to evaluate other strains of the virus and other environmental surfaces and application parameters.

RESEARCH REPORT: *Clostridium difficile spores*

Report Summary



C. diff endospores over 99% Reduction

Beyond by Aerus ActivePure Technology Reduces C. diff endospores





STUDY REPORT

<u>Study Title</u> Antibacterial Activity and Sanitizing Efficacy of Aerus' Device

Test Method

ASTM International Method E1153 Modified for Devices Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces

Study Identification Number

Study Sponsor

Andy Eide Aerus 300 East Valley Drive Bristol, VA 24201 (214) 378-4090 Aeide@aerusonline.com

Test Facility

Microchem Laboratory 1304 W. Industrial Blvd Round Rock, TX 78681 (512) 310-8378 Testing performed by: M. Cash





ASTM E1153: General Information

ASTM International, formerly the American Society for Testing and Materials (ASTM), is an internationally recognized organization that develops and publishes product and testing standards. ASTM E1153 is a quantitative test method designed to evaluate the antimicrobial efficacy of sanitizers on pre-cleaned inanimate, nonporous, non-food contact surfaces. The method is typically used with a high contact time, during which the sanitizer reduces the concentration of viable test microorganisms. ASTM E1153 utilizes non-antimicrobial agents as controls to establish baselines for microbial reductions. The ASTM E1153 method is a benchmark method for non-food contact surface sanitizers and is recognized by several regulatory agencies as an approved method for claim substantiation. See study modifications for changes made to the study method to accommodate a device.

Laboratory Qualifications Specific to ASTM E1153

Microchem Laboratory began conducting the ASTM E1153 test method in 2007. Since then, the laboratory has performed hundreds of ASTM E1153 tests on a broad array of test substances, against a myriad of bacterial and fungal species. The laboratory is also experienced with regard to modifying the test method as needed in order to accommodate customer needs. Every ASTM E1153 test at Microchem Laboratory is performed in a manner appropriate for the test substances submitted by the Study Sponsor, while maintaining the integrity of the method.

Study Timeline Enumeratior Surface Culture Surfaces Surfaces Report Freatment Plates Initiated Inoculated Harvested Delivered Initiated Evaluated C. difficile ATCC 43598 (Endospores) Contact time: 8 hours 18 NOV 2016 21 NOV 2016 01 DEC 2016 N/A Spore Stock 18 NOV 2016 18 NOV 2016 Contact time: 24 hours N/A Spore Stock 16 NOV 2016 16 NOV 2016 17 NOV 2016 21 NOV 2016 01 DEC 2016 Contact time: 48 hours N/A Spore Stock 16 NOV 2016 16 NOV 2016 18 NOV 2016 21 NOV 2016 01 DEC 2016 Page 2 of 10



Test Device Information

The test device containing active pure cells was received on 07 NOV 2016

Test Microorganism Information



The test microorganism(s) selected for this test:

Clostridium difficile 43598 (endospores)

This bacteria is a Gram-positive, rod shaped, endospore generating obligate anaerobe. *Clostridium* species are part of the normal human gut flora that produce spores which are highly resistant to chemical and environmental conditions. *C. diff* is commonly associated with hospital acquired infections and is know to cause antibiotic assisted colitis. Because of it's high resistance to antimicrobials, *C. difficile* is a benchmark bacteria for sporicidal and sterilant activity of chemicals.

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Summary of the Procedure

- The test microorganism is prepared, usually by growth in liquid culture medium or on an appropriate agar plate.
- The test culture may be supplemented with an artificial soil load, such as horse or fetal bovine serum, for one-step cleaner/sanitizer claims.
- Sterilized carriers are inoculated with a volume of the test culture. Inoculated slides are dried. Only completely dried carriers are used in the test.
- Test carriers are treated with the test device and incubated for the predetermined contact time.
- Control carriers are harvested at appropriate intervals to accurately represent any reduction during the contact time.
- At the conclusion of the contact time, test and control carriers are chemically neutralized.
- Dilutions of the neutralized test substance are evaluated using appropriate growth media to determine the surviving microorganisms at the respective contact time.
- The effect of the test substance is compared to the effect of the control substance in order to determine microbial reductions.

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Criteria for Scientific Defensibility of an ASTM E1153 Study

For Microchem Laboratory to consider an ASTM E1153 study to be scientifically defensible, the following criteria must be met:

- 1. Ordinary consistency between replicates must be observed for the control carriers.
- 2. Positive/Growth controls must demonstrate growth of appropriate test microorganism.
- 3. Negative/Purity controls must demonstrate no growth of test microorganism.

Passing Criteria

Due to the modified nature of testing, the study sponsor may determine success criteria.

Testing Parameters used in this Study

	c. annene	43370		
Carrier size and type	rrier size and type 1" x 3" Stainless Steel Coupons		Triple (3)	
Culture growth media	BHIY-HT Agar	Incubation time	48-72 hours	
Culture dilution media	PBS	Culture Supplement	5% Tri-Part Soil	
Target concentration	~1 x 10 ⁶ CFU/Carrier	Inoculum volume	0.010ml	
Contact time(s)	8 hours, 24 hours, and 48 hours	Contact temperature	Ambient	
Carrier distances	12 inches	Neutralizer (Vol.)	Dey Engley Broth (20 ml)	

Study Notes

Carriers were dried at ambient (room) temperature (~23°C) for approximately 20 minutes until visibly dry. Carriers were aseptically placed onto the carrier holder immediately after drying. The device operated on a light switch that was outside of the test chamber.

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Control Results

Neutralization Method: Not Applicable Media Sterility: Sterile – No Growth Growth Confirmation: Confirmed – Target Microorganism

<u>Calculations</u>

Percent Reduction =
$$\left(\frac{B-A}{B}\right) \times 100$$

Where:

B = Number of viable test microorganisms on the control carriers after the contact time A = Number of viable test microorganisms on the test carriers after the contact time

$$Log_{10}Reduction = Log(\frac{B}{A})$$

Where:

B = Number of viable test microorganisms on the control carriers after the contact time A = Number of viable test microorganisms on the test carriers after the contact time

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Results of the Study – 8 Hour Contact Time

Test Microorganism	Time Point	Run Type	Replicate	Replicate CFU/carrier	Average CFU/carrier	Average Percent Reduction vs. Parallel Control	Average Log ₁₀ Reduction vs. Parallel Control
C. difficile ATCC 43598			1	1.90E+06	2.40E+06		
	Time Zero	Control	2	3.20E+06		N/A	
			3	2.10E+06			
	8 hours	Control	1	1.30E+06		N/A	
			2	3.50E+06	2.37E+06		
			3	2.30E+06			
		Test	1	5.00E+05	5.67E+05	76.06%	
			2	7.00E+05			0.62
			3	5.00E+05			



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RESULTS

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Results of the Study – 24 Hour Contact Time

Test Microorganism	Time Point	Run Type	Replicate	Replicate CFU/carrier	Average CFU/carrier	Average Percent Reduction vs. Parallel Control	Average Log ₁₀ Reduction vs. Parallel Control
			1	1.30E+06	2.17E+06	N/A	
C. difficile ATCC 43598	Time Zero	Control	2	2.50E+06			
			3	2.70E+06			
	24 hours	Control	1	2.70E+06		N/A	
			2	2.00E+06	2.20E+06		
			3	1.90E+06			
		Test	1	1.24E+05	1.46E+05	93.35%	
			2	1.27E+05			1.18
			3	1.88E+05			



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RESULTS



Results of the Study – 48 Hour Contact Time

Test Microorganism	Time Point	Run Type	Replicate	Replicate CFU/carrier	Average CFU/carrier	Average Percent Reduction vs. Parallel Control	Average Log ₁₀ Reduction vs. Parallel Control
C. difficile ATCC 43598			1	1.30E+06		N/A	
	Time Zero	Control	2	2.50E+06	2.17E+06		
			3	2.70E+06			
	48 hours	Control	1	1.90E+06		N/A	
			2	1.90E+06	1.73E+06		
			3	1.40E+06			
		Test	1	1.00E+04	1.20E+04	99.31%	
			2	1.30E+04			2.16
			3	1.30E+04			



RESULTS

The results of this study apply to the tested substances(s) only. Extrapolation of findings to related materials is the responsibility of the Sponsor.

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RESEARCH REPORT: Legionella pneumophila

Report Summary



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Legionella pneumophila : Kills It

Beyond by Aerus ActivePure Technology Kills Legionella pneumophila



Hours of Treatment



3040 E. Comwallis Road = PO Box 12194 = Research Triangle Park. NC 27709-2194 = USA Telephone 919.541.6000 = Fax 919.541.5985 = www.rti.org

Testing the Inactivation and Surface Kill of *Legionella* pneumophila Using Fresh Air with ActivePure by Aerus/Vollara

Test Report

Purchase Order Number: 0000024544 RTI Project No.: 0212534.111

Prepared by: Jean H. Kim, Ph.D. RTI International P.O. Box 12194 Research Triangle Park, NC 27709 (919) 541-8087 jeankim@rti.org

> Prepared for: Andrew Eide Aerus LLC 5420 LBJ Freeway Suite 1010 Dallas, TX 75240 (214) 378-4090 AEide@aerusonline.com

> > November 2, 2015

turning knowledge into practice

RTI International is a trade name of Research Triangle Institute.

Testing the Inactivation and Surface Kill of Legionella pneumophila Using Fresh Air with ActivePure by Aerus/Vollara

1. Introduction

Under Purchase Order with Aerus/Vollara, RTI performed inactivation and surface kill testing of *Legionella pneumophila* inoculated on 1" x 1.5" stainless steel coupons using the Fresh Air with ActivePure device provided by Aerus/Vollara. The objective for this study was to determine the kill efficiency by assessing the survivability of the bacteria following exposure to the airborne oxidizers from the device. This was accomplished via plate counts for colony forming units per milliliter. This report covers the statement of work for this Purchase Order.

2. Procedures

The Fresh Air with ActivePure device was placed in a class II biosafety cabinet (BSC) throughout the testing process. *L. pneumophila* were cultured on buffered charcoal yeast extract agar (BYCE) plates. The cultures were harvested and suspended in 10 mL of sterile saline until it measured an optical density at 600 nm (OD₆₀₀) of 1.9 - 2.0. The suspended cells were further concentrated by centrifugation, and the resulting pellet was resuspended in 1.4 mL of sterile saline making up the inoculum. Fifteen stainless steel coupons were sterilized in the autoclave and inoculated with 50 µL of the inoculum. A pipette tip was used to spread the bacteria on the surface of each coupon, then the coupons were allowed to dry. At time zero hour, three of the coupons were placed into specimen containers with 10 mL of Phosphate Buffer Saline + Tween 20 (PBST). These were shaken in the wrist action shaker for 10 min and plated. The remaining coupons were divided into unexposed controls and the device-exposed samples for the 4 and 6

hour time points. The device-exposed samples were placed upright in front of the grill. For each time point, the coupons were processed in the same manner as the time zero control samples and plated. The plates were counted to determine colony forming units per milliliter of PBST (CFU/mL). In order to determine the percent reduction at a given time point, Equation 1 was used.

A = Concentration of L. pneumophila from control coupons at the time point B = Concentration of L. pneumophila from sample coupons at the time point

3. Results

Table 1 shows the data for the test. The data are averages of the three coupons at each time point.

	Control	Device				
Time (hr)	CFU/mL	CFU/mL	% Reduction			
0	1.60E+05	-	-			
4	< 2.5E+03	5.42E+02	>92.3			
6	< 2.5E+03	< 2.5E+02	>91.3			
1						

Table 1. Summary of Results.
Estimated concentrations were reported for samples with colony counts that were below the acceptable range. Overall, there was a loss of *Legionella* following exposure to the device. By four hours, there was a percent reduction of >92.3%. By six hours, the percent reduction was similar (>91.3%) to the four hour time point due to a greater loss of *Legionella* from the unexposed control coupons because of desiccation.

Conclusion: The Fresh Air with ActivePure device reduced the level of *Legionella* on the coupons by more than 91% for both the 4 and 6 hour exposure times.

OHIO SCHOOL REDUCTION

in Cases of Staph

Report Summary



OHIO SCHOOL REDUCTION IN CASES OF STAPH

LABORATORY: N/A PRODUCT: AP3000 TECHNOLOGY: Radiant Catalytic Ionization (RCI) EXPOSURE TIME: 3 Years

Ohio School • Reduction in Cases of Staph

100% Reduction in Cases of Staph

(ActivePure Technology Installed in Ohio School Sports Facilities)



INDIANA SCHOOL REDUCTION

in Flu Related Dismissals

Report Summary



Indiana School • Reduction in Flu Related Dismissals



80% + Reduction in Number of Flu Related Dismissals

(ActivePure Technology Installed in Indiana School Facility)

PENNSYLVANIA PRESCHOOL:

Reduction in Absenteeism

Report Summary



Pennsylvania Preschool • Reduction in Absenteeism

80% + Reduction in Preschool Absenteeism

(ActivePure Technology Installed in Pennsylvania Preschool Facilities)



HOTEL INSTALLATION Testing Results

Report Summary



HOTEL INSTALLATION TESTING RESULTS

Beyond by Aerus Process Test Results

Pre- and Post-Installation Testing

To demonstrate the overall effectiveness of the Beyond by Aerus process, testing was conducted on multiple rooms at an internationally recognized hotel brand, just prior to and 30 days after installation. Samples were carefully collected in each room, from six separate areas, then tested by an independent laboratory for both bacteria and fungi. The air in each room was also tested for particle counts before and after installation. The following information summarizes the results of those tests.

Testing Process

Sample Collection

Six room touch point locations were selected based on hospitality studies performed by microbiologists at the University of Houston and University of California, Davis for bacteria and mold contamination in hotel rooms. These locations are the inside door handle, main room light switch, TV remote control (specifically the on/off, channel and volume buttons), working area of the desk/table, carpeting next to the bed and the HVAC control knob or button. The telephone was considered but discarded as today most travelers use personal cell phones.

Each sample collected followed a protocol to prevent outside contamination during the collection process. The finished samples are shipped overnight to the lab for analysis.

Independent Third Party Lab Testing

Analysis of collected samples is performed by an independent third party lab that operates under Current Good Laboratory Practice Standards, which are US government standards that must be met by laboratories generating data for the EPA or FDA.

Testing Summary

Before Beyond by Aerus Treatment

Samples were collected so an initial baseline bacteria and fungi contamination count can be established. The results below show an excessive total number of Colony Forming Units (CFU) in the rooms prior to the Beyond by Aerus^{**}treatment.

Bacteria Count – 3 Rooms	Before - CFU Count	Fungi Count - 3 Rooms	Before - CFU Count
TV Remote Control Buttons	4285	TV Remote Control Buttons	920
Desk Surface	460	Desk Surface	520
Carpet Next to Bed	290	Carpet Next to Bed	345
HVAC Control Knob	250	HVAC Control Knob	200
Main Light Switch	160	Main Light Switch	105
Inside Main Door Handle	75	Inside Main Door Handle	55

Combined Total Air Particle Count for 3 rooms: 15,300

30 Days After Beyond by Aerus Treatment

Samples were collected after the treatment to verify the bacteria and fungi counts are as expected. As shown in the tables below, the Beyond by Aerus process significantly decreased bacteria and fungi in the treated areas of the hotel rooms.

Bacteria Count – 3 Rooms	After - CFU Count	Fungi Count - 3 Rooms	After - CFU Count
TV Remote Control Buttons	0	TV Remote Control Buttons	0
Desk Surface	5	Desk Surface	0
Carpet Next to Bed	5	Carpet Next to Bed	0
HVAC Control Knob	5	HVAC Control Knob	0
Main Light Switch	25	Main Light Switch	0
Inside Main Door Handle	5	Inside Main Door Handle	0

Combined Total Air Particle Count for 3 rooms after treatment: 0

Summary report prepared by Beyond by Aerus 11.10.13

Testing Results: Bacteria





Bacteria Colony Forming Unit (CFU) Count



Bacteria Colony Forming Unit (CFU) Count



Testing Results: Fungi

Fungi Colony Forming Unit (CFU) Count

Combined Results From Three Rooms









Fungi Colony Forming Unit (CFU) Count



Testing Results: Air Particles

Air Particle Count

Particles down to 1 micron or smaller per cubic foot



MAJOR LEAGUE BASEBALL

Texas Rangers Testing Results

Report Summary



Executive Summary

To evaluate and measure the continued efficacy of the Beyond by Aerus process, one year post-treatment field samples of air and surface areas were collected from the Texas Rangers Baseball Stadium Locker Room and Conditioning Facility. The purpose of the study was to determine if, and to what degree, bacteria and fungi (including mold and yeast), air particles and MRSA exist within this environment one year post-treatment.

The **one year post-treatment** testing results indicated the continued **virtual elimination** of contaminants in the areas of the Texas Rangers Locker Room and Conditioning Facility which were treated with the Beyond by Aerus patent pending solution. **Bacteria**, including **MRSA**, in most instances remained reduced to zero, as was **fungi**. **Air quality** and purity continued to be **massively improved**.

When used as suggested the Beyond by Aerus treatment maintains its effectiveness which results in several significant benefits including:

- Reduction in the number of common cold and flu outbreaks and sick days due to illness
- Protection from allergy and asthma triggering contaminants and the resulting illness this can cause
- Reduction in respiratory issues which may lead to additional health problems
- Protection from MRSA and E. coli
- Improved productivity from healthier, cleaner air and surfaces
- Protection from illness causing bacteria and viruses

One Year Post-Treatment Protocols, Methods, and Test Results

To evaluate and measure the specific risks and levels of contaminants, 10 individual surface locations inside the Texas Rangers Baseball Stadium Locker Room and Conditioning Facility in Arlington, Texas were selected for sample collection. The selected locations focused on areas where players, coaches, trainers and other staff routinely make contact and have significant risks of contamination. In addition to testing surface areas, air quality sampling was performed in 25 locations throughout the facility including the locker rooms, meeting space and conditioning space. Sampled areas were selected to provide the best overall representation of bacteria and fungi conditions on surfaces and air particulate counts in the facility.

Each surface sample was collected according to appropriate protocols established by an independent third party lab that operates under Current Good Laboratory Practice Standards, which are US government standards that must be met by laboratories generating data for the EPA or FDA. Each sample collected followed a protocol to prevent outside contamination during the collection process. All of the samples were then shipped overnight to the lab for analysis.

Air samples were collected in real time in 25 separate locations within the Locker Room and Conditioning Facility. The air samples were collected and analyzed using an environmental laser particle counter specifically designed for counting air particles as scientifically calibrated by the manufacturer. The laser counter also determined the particle size as it passed through the laser. The sample air particle count, which is based on an average over six seconds, was stabilized and then recorded on an air particulate sample form. The particulate counter does not determine the type of contaminate contained in the particles, only the presence of particles.

One Year Post-Treatment Test Results Summary

Bacteria and Fungi

The testing of selected surfaces revealed the **<u>continued decreased</u>** presence of bacteria and fungi at Colony Forming Unit (CFU) levels in the majority of the treated areas.

MRSA

Select areas were also specifically tested for MRSA. The One Year Post-Treatment testing results showed continued <u>non-existent levels of MRSA</u> on the tested surfaces.

Airborne Particulates

Testing of the 25 air samples found particle counts that continued to be at **exceptionally pure levels** which ranged from 1,000 to 4,000 at 1.0um or smaller per cubic feet of air. One of the 25 areas showed increased air particulate levels due to the Beyond Guardian Air having been turned off by the facility.

Pre- and Post-Installation Testing including 30-day Testing



Player A Locker

Pre- and Post-Installation Testing including 30-day Testing



Player B Locker

INDICATES VALUE OUTSIDE OF CHART RANGE

Pre- and Post-Installation Testing including 30-day Testing

Fungi MRSA Bacteria 100,000 50,000 15,050 10,000 Colony Forming Unit (CFU) Count NOTE: Environmental conditions of water leakage 5,000 in the ceiling caused abnormal results. 1,620 1,000 500 105 90 100 75 60 50 25 5 10 0 0 0 0 0 0 0 0 0 0 0 **Before Treatment** After Treatment 30 Days After Treatment 120 Days After Treatment 150 Days After Treatment **1 Year After Treatment**

Sofa In Locker Room

▲ INDICATES VALUE OUTSIDE OF CHART RANGE

Pre- and Post-Installation Testing including 30-day Testing

Unhealthy air within weight and training rooms is compounded by inhalation rates while exercising



Air Particle Count : Clubhouse

One year of decreased bacteria and fungi on treated and tested surfaces

One year of MRSA reduced to zero on treated and tested surfaces

One year of exceptionally pure air particulate levels

MAJOR HOSPITAL OPERATING ROOM Testing Results

Report Summary



Executive Summary

Post-surgical site infections are of major concern to hospitals, doctors and patients. In a recent Center for Disease Control (CDC) report, an estimated 16 million operative procedures were performed in acute care hospitals in the US. Studies found that Surgical Site Infections (SSI) were the most common healthcare associated infection. A CDC survey found an estimated 157,500 or 1.0% infection rate associated with surgeries in 2011. The two week average stay for patients who contract a Surgical Site Infection has doubled the readmission rate and increased healthcare costs by 300+%. Even a small reduction in SSI occurrences can translate into significantly improved overall patient care and lower medical costs.

This study was conducted to determine whether the Beyond by Aerus ActivePure® Technology could materially reduce or eliminate microorganisms and particulate matter from the air and on surfaces in a hospital operating room. Surface and airborne microorganisms are a major contributor of SSIs, with bacteria Staphylococcus and Methicillin Resistant Staphylococcus as the most common. Specific measures were taken of bacteria (including MRSA, fungi and airborne particles) before and after the installation of the Beyond Guardian Air units.

The results show material reduction in airborne particles, bacteria, MRSA and fungi after the installation of the Beyond Guardian Air units; significantly reducing the risk of infection from pathogens to those exposed in the operating room. Reductions ranged from 83% to over 95%.

Materials & Method

The selected locations focused on areas most likely to have routine physical contact before, during and after a surgical procedure. In addition to surface testing, petri dishes were exposed in three locations to collect airborne contaminants. A laser calibrated particle counter was used to independently measure airborne particulates of 1.0 um and smaller.

Pre-Treatment Protocols

On January 5th and 6th, 2015 surface and air samples were collected from a medical operating room to determine if and to what degree bacteria (including MRSA) and fungi exist within that environment. Normal operating room conditions were in use, as were all standard hospital protocols to eliminate air and surface pathogens.

To evaluate and measure the levels of contaminants, five individual surface locations inside the medical operating room were selected for sample collection. These were the upper and lower surgical light handles, anesthesiologist work area, lower surgical light bar and the fluid warming stand with orange cord.

Surface samples were collected according to appropriate protocols established by an independent third party lab that operates under Current Good Laboratory Practice Standards, which are US government standards that must be met by laboratories generating data for the EPA or FDA. The samples were then sent overnight to the lab for testing.

Air samples were collected in real time in three locations within the operating room. The air samples were collected and analyzed using a laser particle counter specifically designed for counting air particles as calibrated by the manufacturer. The laser counter reported in real time the number of particles analyzed 1.0 um and smaller in size per cubic meter of air. The particle counter does not determine the type of contaminant which makes up the count, only the number of particles.

Petri dishes were placed in the same locations the airborne particle counts were taken. An inoculum mixture was poured into a specially coated dish which would promote the growth of bacteria and fungi. Petri dishes remained exposed to the air for one hour except when operating room schedules did not permit a full hour of exposure. Collected plates were incubated for 120 hours at 30°± 2°C. The special coating and inoculum used permit growth of bacteria, mold and yeast on the same plate. Bacteria detected appears as red colonies, molds are white and "fuzzy", while yeasts are white and smooth.

Materials & Method Continued

Treatment Protocols

Upon completion of the January 6th sampling, two Beyond Guardian Air units were placed inside the operating room. These units utilize several technologies, most notably a better-than-HEPA filter media and ActivePure Technology, which generates safe hydroxyls that move through the air and onto surfaces, remediating pathogens. ActivePure is the only technology certified as Space Technology by NASA in its class and is derived from technology used on the International Space Station. The two air and surface purification units were turned on and allowed to operate continuously for six days, and normal hospital protocols for remediation of air and surface pathogens also continued. On January 12th and 13th, 2015, surface and air samples were again collected from the medical operating room using the same protocols as used in pre-treatment. Measurements were taken to determine whether, and to what degree, the use of the Beyond Guardian Air units reduced contamination from air particles, bacteria (including MRSA) and fungi within the operating room environment.

ActivePure® Technology utilizes a proprietary hydrophilic photo catalytic coating, consisting of non-nano titanium dioxide with a proprietary combination of additional transition elements to enhance efficacy. Activated by a specific wavelength of ultraviolet light, oxygen and humidity are extracted from the air to create a host of powerful hydroxyl oxidizers that target air and surface pathogens. No ozone is produced. These oxidizers are extremely effective at destroying bacteria, viruses, fungi, volatile organic compounds (VOCs) and other environmental contaminants.

Most significantly, they are not harmful to humans, pets and plants, and are completely safe for indoor use in occupied spaces.

The difference between the before samples and the samples after running the Beyond Guardian Air units for six days is very substantial and significant.

Airborne Particulates

When we look at the particle counts recorded each day for late afternoon which was between 2–3 PM (depending on the usage of Operating Room #11) the combined average particle count for test period January 5th and January 6th was **461,187** particles 1.0 um or smaller. This was prior to installing the Beyond Guardian Air units.

During the same test period time on January 12th and 13th, 2015, six days after installing the Beyond Guardian Air units and allowing them to operate continually, the combined average particle count for the late afternoon was reduced to **45,748** particles 1.0 um or smaller. This equates to a reduction in airborne particulates of **90.1%**.



Combined Average Particulate Counts – 90.1% Reduction (1.0um and Smaller) Mid-Afternoon Results

Total Bacteria

During the four sampling periods on January 5th and January 6th a combined total of **620,215** CFUs of bacteria were discovered as a result of the surface sampling.

During the same test period time on January 12th and 13th, 2015, six days after installing the Beyond Guardian Air units, the combined total bacteria count was reduced to **102,940**. This equates to a reduction in total bacteria of **83.4%**.



Day 1 and Day 2 Combined

Total Bacteria Count - 83.4% Reduction

Methicillin Resistant Staphylococcus (MRSA)

During the four sampling periods on January 5th and January 6th a combined total of **580** CFUs of MRSA were discovered as a result of the surface sampling.

During the same test period time on January 12th and 13th, 2015, six days after installing the Beyond Guardian Air units and allowing them to operate continually, the combined MRSA count for the late afternoon was reduced to **25**. This equates to a reduction in MRSA bacteria of **95.7%**.



Petri Dish Bacteria and Fungi Results

The petri dishes from January 5th and 6th showed a combined total of **477** CFUs. These dishes were only exposed for a short time. The petri dishes from January 12th and 13th showed a combined total of **1** CFU indicating that the air was materially less contaminated with bacteria and fungi as a result of the Beyond Guardian Air units.



Conclusion

Testing results indicate that the Beyond by Aerus ActivePure Technology materially reduces or eliminates microorganisms and particulate matter from the air and on surfaces in a hospital operating room. Results showed that airborne particulates were reduced over 90% during the test period. Bacteria was reduced over 83% and Methicillin Resistant Staphylococcus (MRSA) was reduced over 95% on surfaces during the test period. Moreover, petri dish results show dramatic reductions in bacteria and fungi carried in the air even for short measurement periods.

The Beyond by Aerus ActivePure Technology was very effective in eliminating bacteria (including MRSA) upon contact on all surfaces, as well as provide ongoing protection of the surfaces and air against future contamination.

It is possible that placement of a third Beyond Guardian Air unit could result in even greater reductions than just two units alone.

PORTER RANCH / ALISO CANYON GAS LEAK

Proof in Action

Report Summary



ActivePure Technology Resolves Indoor Air Quality

Problems in Porter Ranch Community Homes / Aliso Canyon Gas Leak

On October 23, 2015 a massive natural gas leak was discovered by SO CAL GAS employees at their Aliso Canyon underground storage facility in the Santa Suzanna mountain range above the Porter Ranch Community in Los Angeles, Ca. In addition to methane, the leak contained a sulfur based volatile organic compound (VOC) known as mercaptan. Natural gas is odorless so mercaptan is introduced into natural gas supply by utility companies to give it a "rotten egg" smell to alert consumers of a potential gas leak. The residents of Porter Ranch began to complain to the utility company about the air quality in their homes and community. It was determined by SoCalGas that these complaints were mainly driven by the resident's reaction to the mercaptan.

As SoCalGas worked shut down the gas leak, they simultaneously began to offer assistance to the residents of Porter Ranch in the form of temporary relocation and or the installation of air treatment products in their homes. Thru the efforts of our local HVAC professional, SoCalGas was introduced to the Air Scrubber Plus air purification system featuring ActivePure Certified Space Technology. After a few Air Scrubber unit installations, the people at SoCalGas were convinced that the Air Scrubber Plus was able to effectively address the issue at hand and purify the air in the homes to the customer's satisfaction when it was installed. In mid-December 2015 SoCalGas was put in contact with the President of Aerus, the manufacturer of the Air Scrubber Plus and owner of the ActivePure Technology. Aerus was able to by SoCalGas to produce and deliver over 10,000 Air Scrubber Plus units for installation in the Porter Ranch Community. Aerus was able to ramp up its supply chain and manufacturing capabilities to deliver the units needed between then and the time the gas leak was stopped in mid-February 2016. Over 10,000 ActivePure Technology units were installed.

The introduction of the Air Scrubber Plus with ActivePure Certified Space Technology allowed the residents of the Porter Ranch Community to remain in or return to their homes earlier than anticipated while enjoying clean air provided by ActivePure Technology. To our knowledge, every Porter Ranch customer is satisfied with the positive results that the Air Scrubber Plus Technology had on the air quality in their homes.



H_2O_2 AND O_3 EXPOSURE LIMITS

H₂O₂ and O₃ Exposure Limits Summary

SECTION A

The Occupational Safety and Health Administration (OSHA) is the responsible US governmental agency which sets human exposure limits for the workplace environment. The OSHA document Section 1910.1000, subsection Z, addresses Toxic & Hazardous air contaminants. Table Z-1 lists Hydrogen Peroxide (H2O2) and Ozone (O3) workplace exposure limits. The Hydrogen Peroxide 8 hour exposure limit is found on page A6 and the Ozone exposure limit on page A8 (OSHA and EPA have not established a 24 hour exposure limit). Hydrogen Peroxide limit is 1ppm and Ozone is 0.1ppm.

SECTION B

Independent laboratory testing validated our proprietary ActivePure Technology create a buildup Hydrogen Peroxide in the indoor work environment over time. We are well below 1 ppm.

SECTION C

The Beyond Guardian Air with our proprietary ActivePure Technology has been Independent laboratory tested for Ozone emissions. Intertek tested to UL 867, section 40 which requires maximum ozone of 0.050ppm. Testing shown our maximum was 0.001ppm. Well below the UL 867 standard and OSHA's 0.1ppm permitted in the indoor work environment.

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PART 1910—OCCUPATIONAL SAFETY AND HEALTH STANDARDS

Subpart Z—Toxic and Hazardous Substances

Sec 1910 1000 Air contaminants 1910.1001 Asbestos. 1910.1002 Coal tar pitch volatiles; interpretation of term. 1910.1003 13 Carcinogens (4-Nitrobiphenyl, etc.). 1910,1004 alpha-Naphthylamine. 1910.1005 [Reserved] 1910.1006 Methyl chloromethyl ether. 1910.1007 3,'-Dichlorobenzidine (and its salts). 1910.1008 bis-Chloromethyl ether. 1910.1009 beta-Naphthylamine 1910.1010 Benzidine. 1910.1011 4-Aminodiphenyl 1910.1012 Ethyleneimine. 1910.1013 beta-Propiolactone 1910.1014 2-Acetylaminofluorene. 1910.1015 4-Dimethylaminoazobenzene. 1910.1016 N-Nitrosodimethylamine. 1910.1017 Vinyl chloride. 1910.1018 Inorganic arsenic. 1910.1020 Access to employee exposure and medical records. 1910.1025 Lead. 1910.1027 Cadmium 1910.1028 Benzene. 1910.1029 Coke oven emissions. 1910.1030 Bloodborne pathogens. 1910.1043 Cotton dust. 1910.1044 1.2-dibromo-3-chloropropane. 1910.1045 Acrylonitrile. 1910.1047 Ethylene oxide. 1910.1048 Formaldehyde 1910.1050 Methylenedianiline 1910.1051 1,3-Butadiene. 1910.1052 Methylene Chloride. 1910.1096 Ionizing radiation. 1910.1200 Hazard communication

- 1910.1201 Retention of DOT markings, placards and labels. 1910.1450 Occupational exposure to hazardous chemicals in
- laboratories. SUBJECT INDEX FOR 29 CFR PART 1910—OCCUPATIONAL SAFETY AND HEALTH STANDARDS

Subpart Z—Toxic and Hazardous Substances

AUTHORITY: Sections 4, 6, and 8 of the Occupational Safety and Health Act of 1970 (29 U.S.C. 653, 655, and 657); Secretary of Labor's Order No. 12–71 (36 FR 8754), 8–76 (41 FR 25059), 9–83 (48 FR 35736), 1–90 (55 FR 9033), 6–96 (62 FR 111), and 3–2000 (65 FR 50017), as applicable, and 29 CFR part 1911.

All of subpart Z issued under section 6(b) of the Occupational Safety and Health Act of 1970 (29 U.S.C 653), except those substances that have exposure limits in Tables Z-1, Z-2, and Z-3 of 29 CFR 1910.1000. Section 1910.1000 also issued under section (6)(a) of the Act (29 U.S.C. 655(a)). Section 1910.1000, Tables Z-1, Z-2, and Z-3 also issued under 5 U.S.C. 553, but not under 29 CFR part 1911, except for the inorganic arsenic, benzene, and cotton dust listings.

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Section 1910.1001 also issued under section 107 of the Contract Work Hours and Safety Standards Act (40 U.S.C. 333) and 5 U.S.C. 553.

Section 1910.1002 also issued under 5 U.S.C. 553, but not under 29 U.S.C. 655 or 29 CFR part 1911.

Sections 1910.1018, 1910.1029, and 1910.1200 also issued under 29 U.S.C. 653.

SOURCE: 39 FR. 23502, June 27, 1974, unless otherwise noted. Redesignated at 40 FR 23072, May 28, 1975.

§ 1910.1000 Air contaminants.

An employee's exposure to any substance listed in Tables Z-1, Z-2, or Z-3 of this section shall be limited in accordance with the requirements of the following paragraphs of this section.

(a) Table Z-1--(1) Substances with limits preceded by "C"-Ceiling Values. An employee's exposure to any substance in Table Z-1, the exposure limit of which is preceded by a "C", shall at no time exceed the exposure limit given for that substance. If instantaneous monitoring is not feasible, then the ceiling shall be assessed as a 15-minute time weighted average exposure which shall not be exceeded at any time during the working day.

(2) Other substances—8-hour Time Weighted Averages. An employee's exposure to any substance in Table Z-1, the exposure limit of which is not preceded by a "C", shall not exceed the 8-hour Time Weighted Average given for that substance in any 8-hour work shift of a 40-hour work week.

(b) Table Z-2. An employee's exposure to any substance listed in Table Z-2 shall not exceed the exposure limits specified as follows:

(1) 8-hour time weighted averages. An employee's exposure to any substance listed in Table Z-2, in any 8-hour work shift of a 40-hour work week, shall not exceed the 8-hour time weighted average limit given for that substance in Table Z-2.

(2) Acceptable ceiling concentrations. An employee's exposure to a substance listed in Table Z-2 shall not exceed at any time during an 8-hour shift the acceptable ceiling concentration limit given for the substance in the table, except for a time period, and up to a concentration not exceeding the maximum duration and concentration allowed in the column under "acceptable maximum peak above the acceptable ceiling concentration for an 8-hour shift."

(3) Example. During an 8-hour work shift, an employee may be exposed to a concentration of Substance A (with a 10 ppm TWA, 25 ppm ceiling and 50 ppm peak) above 25 ppm (but never above 50 ppm) only for a maximum period of 10 minutes. Such exposure must be compensated by exposures to concentrations less than 10 ppm so that the cumulative exposure for the entire 8-hour work shift does not exceed a weighted average of 10 ppm.

(c) Table Z-3. An employee's exposure to any substance listed in Table Z-3, in any 8-hour work shift of a 40-hour work week, shall not exceed the 8-hour time weighted average limit given for that substance in the table.

(d) Computation formulae. The computation formula which shall apply to employee exposure to more than one substance for which 8-hour time weighted averages are listed in subpart Z of 29 CFR part 1910 in order to determine whether an employee is exposed over the regulatory limit is as follows:

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(1)(i) The cumulative exposure for an 8-hour work shift shall be computed as follows:

E = (Ca Ta+Cb Tb+...Cn Tn)+8

Where:

E is the equivalent exposure for the working shift.

C is the concentration during any period of time T where the concentration remains constant.

T is the duration in hours of the exposure at the concentration C.

The value of E shall not exceed the 8-hour time weighted average specified in subpart Z of 29 CFR part 1910 for the substance involved.

(ii) To illustrate the formula prescribed in paragraph (d)(1)(i) of this section, assume that Substance A has an 8-hour time weighted average limit of 100 ppm noted in Table Z-1. Assume that an employee is subject to the following exposure:

Two hours exposure at 150 ppm Two hours exposure at 75 ppm Four hours exposure at 50 ppm

Substituting this information in the formula, we have $(2 \times 150+2 \times 75+4 \times 50)$; 8=81.25 ppm

Since 81.25 ppm is less than 100 ppm, the 8- hour time weighted average limit, the exposure is acceptable.

(2)(i) In case of a mixture of air contaminants an employer shall compute the equivalent exposure as follows:

 $E_m = (C1 \div L1 + C2 \div L2) + \dots (Cn \div Ln)$

Where:

- E_m is the equivalent exposure for the mixture.
- C is the concentration of a particular contaminant.
- L is the exposure limit for that substance specified in subpart Z

of 29 CFR part 1910.

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The value of E_m shall not exceed unity (1).

(ii) To illustrate the formula prescribed in paragraph (d)(2)(i) of this section, consider the following exposures:

Substance	Actual con- centration of 8-hour exposure (ppm)	8-hour TWA PEL (ppm)	
B	500	1,000	
C	45	200	
D	40	200	

Substituting in the formula, we have:

E_m=500+1,000+45+200+40+200

E_ =0.500+0.225+0.200

E_ =0.925

Since E_m is less than unity (1), the exposure combination is within acceptable limits.

(e) To achieve compliance with paragraphs (a) through (d) of this section, administrative or engineering controls must first be determined and implemented whenever feasible. When such controls are not feasible to achieve full compliance, protective equipment or any other protective measures shall be used to keep the exposure of employees to air contaminants within the limits prescribed in this section. Any equipment and/or technical measures used for this purpose must be approved for each particular use by a competent industrial hygienist or other technically qualified person. Whenever respirators are used, their use shall comply with 1910.134.

(f) Effective dates. The exposure limits specified have been in effect with the method of compliance specified in paragraph (e) of this section since May 29, 1971.

TABLE Z-1-LIMITS FOR AIR CONTAMINANTS

Substance	CAS No. (c)	ppm (a)1	mg/m ³ (b) ¹	Skin Designation
Acetaldehvde	75-07-0	200	360	
Acetic acid	64-19-7	10	25	
Acetic anhydride	108-24-7	5	20	
Acetone	67-64-1	1000	2400	
Acetonitrile	75-05-8	40	70	
2-Acetylaminofluorine; see 1910.1014	53-96-3			
Acetylene dichloride; see 1,2-Dichloroethylene.				
Acetylene tetrabromide	79-27-6	1	14	
Acrolein	107-02-8	0.1	0.25	
Acrylamide	79-06-1		0.3	X
Acrylonitrile; see 1910.1045	107-13-1			
Aldrin	309-00-2		0.25	X
Allyl alcohol	107-18-6	2	5	X
Allyl chloride	107-05-1	1	3	
Allyl glycidyl ether (AGE)	106-92-3	(C)10	(C)45	
Allyl propyl disulfide	2179-59-1	2	12	
alpha-Alumina	1344-28-1			
Total dust			15	
Respirable fraction			5	
Aluminum, metal (as Al)	7429-90-5			
Total dust			15	
Respirable fraction			5	
4-Aminodiphenyl; see 1910.1011	92-67-1			
2-Aminoethanol; see Ethanolamine.				
2-Aminopyridine	504-29-0	0.5	2	
Ammonia	7664-41-7	50	35	
Ammonium sulfamate	7773-06-0			
Total dust			15	
Respirable fraction			5	
n-Amyl acetate	628-63-7	100	525	

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TABLE Z-1—LIMITS FOR AIR CONTAMINANTS—Continued				
Substance	CAS No. (c)	ppm (a)1	mg/m ³ (b) ¹	Skin Designation
sec-Amvl acetate	626-38-0	125	650	
Aniline and homologs	62-53-3	5	19	x
Anisidine (o-, p-isomers)	29191-52-4		0.5	X
Antimony and compounds (as Sb)	7440-36-0		0.5	
ANTU (alpha Naphthylthiourea)	86-88-4		0.3	
Arsenic, inorganic compounds (as As); see 1910.1018	7440-38-2			
Arsenic, organic compounds (as As)	7440-38-2		0.5	
Arsine	7784-42-1	0.05	0.2	
Asbestos; see 1910.1001	e 50 0			~
Parkum, coluble compounds (as Pa)	7440_30_3		0.2	^
Barium sulfate	7727-43-7		0.5	
Total dust			15	
Respirable fraction			5	
Benomyl	17804-35-2			
Total dust			15	
Respirable fraction			5	
Benzene; see 1910.1028	71-43-2			
See Table Z–2 for the limits applicable in the operations or sectors excluded in 1910.1028 d				
Republications 1010 1010	02.97.5			
p-Benzouloope: see Ouloope	52-07-5			
Benzo(a)nvrene: see Coal tar nitch volatiles.				
Benzoyl peroxide	94-36-0		5	
Benzyl chloride	100-44-7	1	5	
Beryllium and beryllium compounds (as Be)	7440-41-7		٢	
Biphenyl; see Diphenyl.				
Bismuth telluride, Undoped	1304-82-1			
Pacairable fraction			15	
Boron oxide	1303-86-2		,	
Total dust			15	
Boron trifluoride	7637-07-2	(C)1	(C)3	
Bromine	7726-95-6	0.1	0.7	
Bromoform	75-25-2	0.5	5	X
Butadiene (1,3-Butadiene); See 29 CFR 1910.1051; 29 CFR 1910.19(I).	106-99-0	1 ppm/5 ppm STEL		
Butanethiol; see Butyl mercaptan.				
2-Butanone (Methyl ethyl ketone)	78-93-3	200	590	
2-Butoxyethanol	111-76-2	50	240	x
n-Butyl-acetate	123-86-4	150	710	
set-butyl acetate	540, 99, 5	200	950	
n-Butyl alcohol	71-36-3	100	300	
sec-Butyl alcohol	78-92-2	150	450	
tert-Butyl alcohol	75-65-0	100	300	
Butylamine	109-73-9	(C)5	(C)15	X
tert-Butyl chromate (as CrO3)	1189-85-1		(C)0.1	x
n-Butyl glycidyl ether (BGE)	2426-08-6	50	270	
Butyl mercaptan	109-79-5	10	35	
p-tert-Butyttoluene	98-51-1	10	60	
Cadmium (as Cd); see 1910.1027	/440-43-9			
Total dust	1317-03-3		15	
Respirable fraction			5	
Calcium hydroxide	1305-62-0		-	
Total dust			15	
Respirable fraction			5	
Calcium oxide	1305-78-8		5	
Calcium silicate	1344-95-2			
Total dust		•••••	15	
Caldum sulfate	7778-18-0		2	
Total dust	1110-10-9		15	
Respirable fraction			5	
Camphor, synthetic	76-22-2		2	
Carbaryl (Sevin)	63-25-2		5	
Carbon black	1333-86-4		3.5	
Carbon Gloville	124-38-9	5000	9000	
Carbon monoxide	630-08-0	50	55	
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TABLE Z-1-LIN	ITS FOR AIR CONTAMINANTS-Continued	
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Substance	CAS No. (c)	ppm (a)1	mg/m ³ (b) ¹	Skin Designation
Carbon tetrachloride	56-23-5		O	
Cellulose	9004-34-6			
Total dust			15	
Respirable fraction			5	
Chlordane	57-74-9		0.5	
Chlorinated camphene	8001-35-2		0.5	
Chlorinated diphenyl oxide	55/20-99-5	(0)1	0.5	
Chlorine dioxide	10049-04-4	0.1	(C)3	
Chlorine triffuoride	7790-91-2	(C)0.1	(0).4	
Chloroacetaldehvde	107-20-0	(C)1	(C)3	
a-Chloroacetophenone (Phenacyl chloride)	532-27-4	ò.ó5	0.3	
Chiorobenzene	108-90-7	75	350	
o-Chlorobenzylidene malononitrile	2698-41-1	0.05	0.4	
Chlorobromomethane	74-97-5	200	1050	
2-Chloro-1,3-butadiene; see beta-Chloroprene.	52460.21.0			v .
Chlorodiphenyl (54% Chlorine) (PCB)	11007-69-1		0.5	Ç
1-Chloro-2.3-enoxypronane: see Epichlorohydrin.	11057 05 1		0.0	<u>^</u>
2-Chloroethanol; see Ethylene chlorohydrin.				
Chloroethylene; see Vinyl chloride.				
Chloroform (Trichloromethane)	67-66-3	(C)50	(C)240	
bis(Chloromethyl) ether; see 1910.1008	542-88-1			
Chloromethyl methyl ether; see 1910.1006	107-30-2	20	100	
Chloropictia	76-06-2	0.1	0.7	
beta-Chioroprene	126-99-8	25	90	x
2-Chloro-6-(trichloromethyl) pyridine	1929-82-4			
Total dust			15	
Respirable fraction			5	
Chromic acid and chromates (as CrO3)	(*)		(?)	
Chromium (11) compounds.	7440 47 2		0.5	
(ds C)	/10-1/-5		0.5	
(as Cr)	7440-47-3		0.5	
Chromium metal and insol. salts (as Cr)	7440-47-3		1	
Chrysene; see Coal tar pitch volatiles.				
Clopidol	2971-90-6			
Total dust			15	
Coal dust (less than 5% SiO2), respirable fraction			å	
Coal dust (greater than or equal to 5% SIO2), respirable			ĕ	
fraction.				
Coal tar pitch volatiles (benzene soluble fraction), anthracene,	65966-93-2		0.2	
BaP, phenanthrene, acridine, chrysene, pyrene.				
Color metal, dust, and turne (as Co)	/440-40-4		0.1	
Conter over emissions, see 1910.1029.	7440-50-8			
Fume (as Qu)			0.1	
Dusts and mists (as Cu)			1	
Cotton dust e; see 1910.1043			1	
Crag herbicide (Sesone)	136-78-7			
Total dust		•••••	15	
Cresol all isomers	1310-77-3	5	22	x
Crotonaldehyde	123-73-9:	2	6	<u>^</u>
	4170-30-3	_	-	
Cumene	98-82-8	50	245	x
Cyanides (as CN)	(*)		5	X
Cyclohexane	110-82-7	300	1050	
Cyclonexañol	108-93-0	50	200	
Ovclobevene	110-91-1	300	1015	
Cyclopentadiene	542-92-7	75	200	
2,4-D (Dichlorophenoxyacetic acid)	94-75-7		10	
Decaborane	17702-41-9	0.05	0.3	x
Demeton (Systox)	8065-48-3		0.1	x
Diacetone alcohol (4-Hydroxy-4-methyl-2-pentanone)	123-42-2	50	240	
1,2-Diaminoethane; see Ethylenediamine.	374 00 3	0.3	0.4	
Diborane	19287-45-7	0.1	0.1	
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Cubatranas	CAC No. (c)	ana (a)]	material (ball	Chip Decigoation
Substance	C/IS NO. (C)	ppm (a)-	ing/iir (b)-	akin besignation
1,2-Dibromo-3-chloropropane (DBCP); see 1910.1044	96-12-8			
1,2-Dibromoethane; see Ethylene dibromide.			_	
Dibutyl phosphate	107-66-4	1	5	
Dibutyl phthalate	89-/9-2	(0)50	(0300	
n-Dichlombenzene	106-46-7	75	450	
3.'-Dichlorobenzidine: see 1910.1007	91-94-1			
Dichlorodifluoromethane	75-71-8	1000	4950	
1,3-Dichloro-5,5-dimethyl hydantoin	118-52-5		0.2	
Dichlorodiphenyltrichloroethane (DDT)	50-29-3		1	X
1,1-Dichloroethane	75-34-3	100	400	
1,2-Dichloroethane; see Ethylene dichloride.	E40 E0 0	200	700	
1,2-Dichloroethyl ether	540-59-0	200	(000	v .
Dichloromethane: see Methylene chloride.	111-11-1	(C)15	(0)50	^ I
Dichloromonofluoromethane	75-43-4	1000	4200	
1,1-Dichloro-1-nitroethane	594-72-9	(C)10	(C)60	
1,2-Dichloropropane; see Propylene dichloride.				
Dichlorotetrafluoroethane	76-14-2	1000	7000	
Dichlorvos (DDVP)	62-73-7		1	x
Dicyclopentadienyl iron	102-54-5		15	
Respirable fraction			5	
Dieldrin	60-57-1		0.25	x
Diethylamine	109-89-7	25	75	
2-Diethylaminoethanol	100-37-8	10	50	x
Diethyl ether; see Ethyl ether.				
Difluorodibromomethane	75-61-6	100	860	
Diglycidyl ether (DGE)	2238-07-5	(C)0.5	(C)2.8	
Dinydroxybenzene; see Hydroquinone.	109-93-9	50	290	
Disopropulamine	108-18-9	5	20	x
4-Dimethylaminoazobenzene; see 1910.1015	60-11-7	-		
Dimethoxymethane; see Methylal.				
Dimethyl acetamide	127-19-5	10	35	x
Dimethylamine	124-40-3	10	18	
Dimethylaminobenzene; see Xylidine.	121 62 7	-	25	
Dimethylaniline (N,N-Dimethylaniline)	121-69-7	2	25	×
Dimethyl-1.2-dibromo-2.2-dichloroethyl phosphate	300-76-5		3	
Dimethylformamide	68-12-2	10	30	x
2,6-Dimethyl-4-heptanone; see Disobutyl ketone.				
1,1-Dimethylhydrazine	57-14-7	0.5	1	X
Dimethylphthalate	131-11-3		5	
Dimethyl sulfate	77-78-1	1	5	, X
(ortho)	528-20-0		•	^ I
(meta)	99-65-0			
(para)	100-25-4			
Dinitro-o-cresol	534-52-1		0.2	x
Dinitrotoluene	25321-14-6		1.5	X
Dioxane (Diethylene dioxide)	123-91-1	100	360	x
Diphenyin (Biphenyi)	92-52-4	0.2	1	
isocyanate				
Dipropylene glycol methyl ether	34590-94-8	100	600	x
Di-sec octyl phthalate (Di-(2-ethylhexyl) phthalate)	117-81-7		5	
Emery	12415-34-8			
Total dust			15	
Respirable fraction			5	
Endrin	/2-20-8		0.1	X X
EPN	2104-64-5	3	0.5	Î Î
1.2-Epoxypropane; see Propylene oxide.				
2,3-Epoxy-1-propanol; see Glycidol.				
Ethanethiol; see Ethyl mercaptan.				
Ethanolamine	141-43-5	3	6	-
2-Ethoxyethanol (Cellosolve)	110-80-5	200	740	L X
Ethyl acetate	141-78-6	400	1400	^
Ethyl acrylate	140-88-5	25	100	x

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TABLE Z-1—LIMITS FOR AIR CONTAMINANTS—Continued				
Substance	CAS No. (c)	ppm (a)1	mg/m ³ (b) ¹	Skin Designation
Ethyl alcohol (Ethanol)	64-17-5	1000	1900	
Ethylamine	75-04-7	10	18	
Ethyl amyl ketone (5-Methyl-3-heptanone)	541-85-5	25	130	
Ethyl benzene	100-41-4	100	435	
Ethyl bromide	106.35.4	200	890	
Ethyl olicyide	75-00-3	1000	250	
Ethyl ether	60-29-7	400	1200	
Ethyl formate	109-94-4	100	300	
Ethyl mercaptan	75-08-1	(C)10	(C)25	
Ethyl silicate	78-10-4	100	850	
Ethylene chlorohydrin	107-07-3	5	16	x
Ethylenediamine	107-15-3	10	25	
Ethylene dichloride (1.2 Dichloroethane)	107-05-2			
Ethylene olycol dinitrate	628-96-6	(C)0.2	(01	x
Ethylene glycol methyl acetate; see Methyl cellosolve acetate		(-)	(-/-	
Ethyleneimine; see 1910.1012	151-56-4			
Ethylene oxide; see 1910.1047	75-21-8			
Ethylidene chloride; see 1,1-Dichloroethane.				
N-Ethylmorpholine	100-74-3	20	94	x
Terbal dut	14484-64-1			
Ferrovanadium dust	12604-58-0	•••••	15	
Fluorides (as F)	(1)		2.5	
Fluorine	7782-41-4	0.1	0.2	
Fluorotrichloromethane (Trichlorofluoromethane)	75-69-4	1000	5600	
Formaldehyde; see 1910.1048	50-00-0			
Formic acid	64-18-6	5	9	
Furfural	98-01-1	5	20	x
Crain dust (ast wheat barley)	98-00-0	50	200	
Given (mist)	56-81-5		10	
Total dust	30 01 3		15	
Respirable fraction			5	
Glycidol	556-52-5	50	150	
Glycol monoethyl ether; see 2-Ethoxyethanol.				
Graphite, natural, respirable dust	7782-42-5		C	
Total dust			15	
Respirable fraction			5	
Guthion: see Azinphos methyl.			-	
Gypsum	13397-24-5			
Total dust			15	
Respirable fraction	7440 50 6		5	
Hamum	7440-58-6	•••••	0.5	~
Hentane (n-Hentane)	142-92-5	500	2000	^
Hexachloroethane	67-72-1	1	10	x
Hexachloronaphthalene	1335-87-1	-	0.2	x
n-Hexane	110-54-3	500	1800	
2-Hexanone (Methyl n-butyl ketone)	591-78-6	100	410	
Hexone (Methyl isobutyl ketone)	108-10-1	100	410	
sec-nexyl acetate	108-84-9	50	300	×
Hydrogen bromide	10035-10-6	3	10	^
Hydrogen chloride	7647-01-0	(05	(C)7	
Hydrogen cyanide	74-90-8	10	11	x
Hydrogen fluoride (as F)	7664-39-3		O	
Hydrogen peroxide	7722-84-1	1	1.4	
Hydrogen selenide (as Se)	7783-07-5	0.05	0.2	
Hydrogen Sumoe	122 21 0		2	
Information in the second se	7553-56-2	(C)0.1	(01	
Iron oxide fume	1309-37-1	(0)011	10	
Isoamyl acetate	123-92-2	100	525	
Isoamyl alcohol (primary and secondary)	123-51-3	100	360	
Isobutyl acetate	110-19-0	150	700	
Isobutyl alcohol	78-83-1	100	300	
Isophorone	78-59-1	25	140	
Isopropyi acetate	108-21-4	250	950	l

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TABLE Z-1-LIMITS FOR AIR CONTAMINANTS-Continued

Substance	CAS No. (c)	ppm (a)1	mg/m ³ (b) ¹	Skin Designation
Isonrowd alcohol	67-63-0	400	980	
Isopropyl activity in an international inter	75-31-0	5	12	
Isopropylamine	/3-31-0	500	12	
Isopropyl ether	108-20-3	500	2100	
Isopropyl glycidyl ether (IGE)	4016-14-2	50	240	
Naolin	1332-58-7			
Total dust			15	
Respirable fraction			5	
Ketene	463-51-4	0.5	0.9	
Lead, inorganic (as Pb); see 1910.1025	7439-92-1			
Limestone	1317-65-3			
Total dust			15	
Respirable fraction			5	
Lindane	58-89-9		0.5	x
Lithium hydride	7580-67-8		0.025	
L.P.G. (Liquefied petroleum gas)	68476-85-7	1000	1800	
Magnesite	546-93-0			
Total dust			15	
Respirable fraction			5	
Magnesium gyide fume	1300-48-4		-	
Total particulate	1303 10 1		15	
Malathion	121-75-5			
Tabl dust	121-73-3		15	~
Total oust	100 31 6	0.35	15	· ·
Maleic annyoride	108-31-6	0.25	1	
Manganese compounds (as Mn)	/439-90-5		(C)5	
Manganese tume (as Mn)	7439-96-5		(C)5	
Marble	1317-65-3			
Total dust			15	
Respirable fraction			5	
Mercury (aryl and inorganic) (as Hg)	7439-97-6		O	
Mercury (organo) alkyl compounds (as Hg)	7439-97-6		O	
Mercury (vapor) (as Hg)	7439-97-6		(?)	
Mesityl oxide	141-79-7	25	100	
Methanethiol; see Methyl mercaptan.				
Methoxychlor	72-43-5			
Total dust			15	
2-Methoxyethanol (Methyl cellosolve)	109-86-4	25	80	X
2-Methoxyethyl acetate (Methyl cellosolye acetate)	110-49-6	25	120	x
Methyl acetate	79-20-9	200	610	
Methyl acetylene (Procyne)	74-99-7	1000	1650	
Methyl acetylene-propadiene mixture (MAPP)		1000	1800	
Methyl acrylate	96-33-3	10	35	x
Methylal (Dimethyny-methane)	109-87-5	1000	3100	^
Methyl alcohol	67-56-1	200	260	
Mathylamina	74-90-5	10	12	
Mathul and alcoholi cao Mathul isobutul carbinol	71-05-5	10	**	
Methyl anyl alcohol, see Meuryl Isobutyl Carbinol.	110 43 0	100	465	
Method beenide	74 93 0	100	(000	~
Method hadra hadra and 2 Management	/+-03-9	(C)20	(0)00	^ I
Methyl butyl ketone; see 2-nexanone.				
Methyl cellosolve; see 2-Methoxyethanol.				
Methyl cellosolve acetate; see 2-Methoxyethyl acetate.			~	
Methyl chloride	74-87-3		(*)	
Methyl chloroform (1,1,1-Trichloroethane)	71-55-6	350	1900	
Methylcyclohexane	108-87-2	500	2000	
Methylcyclohexanol	25639-42-3	100	470	
o-Methylcyclohexanone	583-60-8	100	460	x
Methylene chloride	75-09-2		O	
Methyl ethyl ketone (MEK); see 2-Butanone.				
Methyl formate	107-31-3	100	250	
Methyl hydrazine (Monomethyl hydrazine)	60-34-4	(C)0.2	(C)0.35	X
Methyl iodide	74-88-4	5	28	X
Methyl isoamyl ketone	110-12-3	100	475	
Methyl isobutyl carbinol	108-11-2	25	100	X
Methyl isobutyl ketone; see Hexone.				
Methyl isocyanate	624-83-9	0.02	0.05	X
Methyl mercaptan	74-93-1	(C)10	(C)20	^
Methyl methacrylate	80-62-6	100	410	
Methyl monyl ketone: see 7-Dentanone.	00 02 0		1.00	
alnha-Malhul chwana	09.93.0	((3100	(0)490	
Methylene bisnbenyl isocyanate (MDT)	101-68-9	(C)0.02	(C)0.2	
Mira: caa Cilinatae	101-00-0	(0)0.02	(c)oiz	
rina, see siludies.	1	1	1	1

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TABLE 2-1-LIMITS FOR	RAIRCONTAMIN	ANTS-Continue	ed	
Substance	CAS No. (c)	ppm (a)1	mg/m ³ (b) ¹	Skin Designation
MoMpdenum (as Mo)	7439-98-7			
Soluble compounds			5	
Insoluble compounds.				
Total dust			15	
Monomethyl aniline	100-61-8	2	9	x
Monomethyl hydrazine; see Methyl hydrazine.	110-01-9	20	70	×
Nanhtha (Coal tar)	8030-30-6	100	400	^ I
Naphthalene	91-20-3	10	50	
alpha-Naphthylamine; see 1910.1004	134-32-7			
beta-Naphthylamine; see 1910.1009	91-59-8			
Nickel carbonyl (as Ni)	13463-39-3	0.001	0.007	
Nickel, metal and insoluble compounds (as NI) Nickel, soluble compounds (as NI)	7440-02-0		1	
Nicotine	54-11-5		0.5	x
Nitric acid	7697-37-2	2	5	
Nitric oxide	10102-43-9	25	30	
p-Nitroaniline	100-01-6	1	6	X
Nitrobenzene	98-95-3	1	5	X
p-Nitrochiorobenzene	100-00-5		1	x
4-Nitrodipnenyi; see 1910.1003	92-93-3	100	310	
Nitroen dioxide	10102-44-0	(C)5	(C)9	
Nitrogen trifluoride	7783-54-2	10	29	
Nitroglycerin	55-63-0	(C)0.2	(C)2	x
Nitromethane	75-52-5	100	250	
1-Nitropropane	108-03-2	25	90	
2-Nitropropane	79-46-9	25	90	
N-Nitrosodimethylamine; see 1910.1016. Nitrotoluono (all icomerc)		5	30	×
o-isomer	88-72-2	2	30	^ I
m-isomer	99-08-1			
p-isomer	99-99-0			
Nitrotrichloromethane; see Chloropicrin.				
Octachloronaphthalene	2234-13-1		0.1	x
Octane	111-65-9	500	2350	
Ormium tetrovide /ac Oc)	20816-12-0		0.002	
Oxalic acid	144-62-7		1	
Oxygen difluoride	7783-41-7	0.05	0.1	
Ozone	10028-15-6	0.1	0.2	
Paraquat, respirable dust	4685-14-7;		0.5	x
	1910-42-5;			
Darathion	20/4-50-2		0.1	×
Particulates not otherwise regulated (PNOR) f.	30-30-2		0.1	^
Total dust			15	
Respirable fraction			5	
PCB; see Chlorodiphenyl (42% and 54% chlorine).		0.000		
Peritaborane	19624-22-7	0.005	0.01	
Pentachioronaprinalene	97-96-5		0.5	•
Pentaerythritol	115-77-5		0.5	<u>^</u>
Total dust			15	
Respirable fraction			5	
Pentane	109-66-0	1000	2950	
2-Pentanone (Methyl propyl ketone)	107-87-9	200	700	
Perchloroethylene (Tetrachloroethylene)	594-42-3	0.1	0.8	
Perchlorol fluoride	7616-94-6	3	13.5	
Petroleum distillates (Naphtha) (Rubber Solvent)		500	2000	
Phenol	108-95-2	5	19	x
p-Phenylene diamine	106-50-3		0.1	x
Phenyl ether, vapor	101-84-8	1	2	
Phenylethelene: see Sharee		1		
Phenyleutylene; see Styrene. Dhanyl obridul athar (DGE)	122-60-1	10	60	
Phenylhydrazine	100-63-0	5	22	x
Phosdrin (Mevinphos)	7786-34-7		0.1	x x
Phosgene (Carbonyl chloride)	75-44-5	0.1	0.4	
Phosphine	7803-51-2	0.3	0.4	

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Determination of Aerosolized Hydrogen Peroxide Accumulation produced by the Active Pure Technology

Test Report

Purchase Order No.: 0000023564 RTI Project No.: 12534.111

Prepared by: Kathleen Owen RTI International P.O. Box 12194 Research Triangle Park, NC 27709 (919) 541-6941 mko@rti.org

> Prepared for: AERUS, LLC. 300 East Valley Drive Bristol, VA 24201

> > April 27, 2015

turning knowledge into practice

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Test Procedure

Device to be tested: Vollara Branded Fresh Air Surround / AP3000, an active pure technology device of about 1 cubic foot designed to inactivate microorganisms on surfaces. The device was provided by Aerus, sister company to Vollara.

Humidity (RH) planned for the test: ~70 to ~80% RH.

Temperature (T): standard room temperatures, expected to be in the approximately 70-75F range.

Sampling and Analysis method: Draeger tubes for H_2O_2 sampling for 0.2 to 3 ppm (Draeger Tube for Hydrogen Peroxide). These tubes operate with a hand-held pump. A set number of compressions give a standard volume for a sample. A change in color indicates the presence of H_2O_2 in the air.

Test Chamber: A standard sealed stainless steel and plexiglass Bio-chamber with ports to allow T, RH, and H₂O₂ sampling.

Testing planned:

- Install device in T/RH controlled Bio-chamber
- Establish T/RH, record values
- Measure H₂O₂ in room (as background level) and in chamber, record values
- Use the port closest to the center front of the device for sampling
- Turn on unit
- Measure H₂O₂ after ~1 hour, record value, T and RH
- Measure and record H₂O₂, T, RH again at 24 h and at 48 h
- 7) Turn off unit and T/RH, vent chamber as needed/possible
- Tabulate and report data.

Test Results

During testing we had difficulty controlling the RH. However, the problem was high humidity as opposed to low humidity -- low humidity can affect H₂O₂ production below 20%.

For devices, such as the Active Pure Technology, that produce low levels of H_2O_2 , definitive measurements are difficult. None of the H_2O_2 samples showed a change in color indicating H_2O_2 build up in the Bio-chamber did not occur over time. For the samples in this study, lack of discoloration was seen indicating that the concentration of the H_2O_2 was at or below the MDL of 0.2 ppm. However, to be scientifically correct, these values are reported simply as no color change.

Date	Time	Tempera	Relative	H_2O_2	Notes
		ture (°F)	Humidity (%)	determination	
4/23/15	7:30 am		74.6		Checking RH stability
4/23/15	8:00 am	71.9	74.5	no color change	
4/23/15	8:12 am			no color change	Reading for lab room
4/23/15	8:14 am				Unit turned ON
4/23/15	9:14 am	88.8	73.0	no color change	
4/23/15	1:58 pm	82.0	81.4	no color change	
4/23/15	5:30 pm	78.9	101.8	no color change	No visible condensation
					in chamber
4/24/15	9:55 am	79.9	101.8	no color change	Slight condensation on
					the plexiglass door
4/24/15	10:30 am	78.2	77.1	no color change	Readings shortly after
					chamber door was
					opened to vent excess
					humidity
4/24/15	11:25 am	82.1	76.7	no color change	
4/24/15	3:30 pm			no color change	Opened door to
					chamber, took reading
					with tube ~1mm from
					center of unit grill

Conclusion

Under the conditions of this test, the Vollara Fresh Air Surround / AP 3000 unit with active pure technology did not create hydrogen peroxide levels higher than 0.2 ppm, the MDL of the Draeger tubes over time.



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20 September 2013

Model Number: F159E ARB Number: 1605

Limitation Statement:	The test data and results contained in this report are provided for client information and evaluation. No conclusions are drawn by Intertek.
Authorization:	The tests were authorized by signed quote #, dated 8/22/2013.
Standard Used:	UL Standard for Safety for Electrostatic Air Cleaners, UL 867, Section 40, Ozone Test, Fifth Edition, August 4, 2011.
Report Content:	

ability are my loss, y. Any use of

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1. Unit Under Test Information

MODEL

Manufacturer:	Aerus	Pre-Filter:	NA
Model Number:	F159E	HEPA Filter:	
Fan Speeds:	4	Ti02, Polypropylene, Carbon Filter:	Removable
O3/Voltage Settings:	64 -	UV Light:	YES
O3 Monitor:	-	lonizer:	Yes
Model Notes:			

FIRST SAMPLE

		Run-in Start:	1455 8/30/2013
Serial Number:	NA	Run-in End:	0752 9/3/2013
Manufacture Date:	7/23/2013	Run-in Temperature:	77 ± 4 degF
Receive Date:	8/27/2013		
Received Status:	OK		
Sample Notes:			

SECOND SAMPLE

		Run-in Start:	1455 8/30/2013
Serial Number:	NA	Run-in End:	0752 9/3/2013
Manufacture Date:	7/23/2013	Run-in Temperature:	77 ± 4 degF
Receive Date:	8/27/2013		
Received Status:	OK		
Sample Notes:			



2. Peak Ozone Test Results

		Date of Test:	9/6/2013
Grill Height:	15"	Air Periphery Height:	15"
Grill Width:	7"	Air Periphery Width:	7"
Estimated Grill Area:	105" sq	Est. Air Periphery Area:	105"sq
110100.	Fites and the fites of the	tests, the unit one much combin	radon and die i oo





PEAK OZONE CONCENTRATIONS

Location	With Filter(s)		Without Filter(s)		1.1	100 A 11
	Highest	Lowest	Highest	1		
1	0.0006	0.0003	0.0008	1		
2	0.0002	0.0000	0.0005	()	1	
3	0.0003	0.0008	0.0007	10 31	M + = =	- 1 km - 1
4	0.0004	0.0002	0.0005		— = 1 (* 12)	212
5	0.0001	0.0001	0.0003			121 122 124
6	0.0005	0.0003	0.0000			
7	0.0005	0.0002	0.0003			
8	0.0005	0.0004	0.0007	1		
9	0.0004	0.0005	0.0001	1	Let U the	1.1.2

Notes:
Ozone Concentrations less background level; in units of PPM.
Peak concentration for each iteration is in BOLD.





3. Max Ozone Test Results







		reat mini o(t) oo.	40.1.2	1,400	0.000	0.000	0.001	0.001	[PPiii]
		Test 5min C(t) O3:	40.1.2	PASS	0.000	0.000	0.000	0.000	[ppm]
1.0	Chamber Temperature:		40.4.2	PASS	77	76	77	1	[degF]
	Chamber Humidity:		40.4.2	PASS	50	49	51	2	[%RH]
	C	hamber Static Pressure:	-	PASS	0.02	0.01	0.02	0.01	["H2O]
	Ch	hamber Supply Air Flow:		· · · ·	20	20	20	0	[SCFM]
	Requi	red to Test 2nd Sample:	40.1.1	NO			1.1.1.1.1	1	
-		Test Duration:	*40.4.6	8 hours			1	1	
	Notes:	Max Ozone Reading	was 0.001	0ppm					



Start Date of Test:	9/13/2013	
Sample:	1 of 2	
Fan Speed:	High	
Filter(s):	No. All Filter(s) were Removed, UV light and Ionizer ON	

	UL Ref.	Pass/Fail	Mean	Min	Max	Delta	Units
Background C(t) O3:	40.4.3	PASS	0.000	0.000	0.000	0.000	[ppm]
Test 1min C(t) O3:	40.1.2	PASS	0.000	0.000	0.001	0.001	[ppm]
Test 5min C(t) O3:	40.1.2	PASS	0.000	0.000	0.000	0.000	[ppm]
Chamber Temperature:	40.4.2	PASS	77	76	78	1	[degF]
Chamber Humidity:	40.4.2	PASS	50	50	51	1	[%RH]
Chamber Static Pressure:		PASS	0.02	0.01	0.04	0.03	["H2O]
Chamber Supply Air Flow:		A. 11	20	20	20	0	[SCFM]
Required to Test 2nd Sample:	40.1.1	NO					
Test Duration:	*40.4.6	8 hours	I				



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Chamber Equipment Information

Instrument	Model	Intertek Ctrl #	Cal Due Date
			·
			•
		-	-
			-



4. Summary/Signatures

The test sample(s) documented in this report were tested in accordance to the standard(s) referenced in the first page of this report.

The representative sample(s) have been tested, investigated, and found to comply with the requirements of the UL Standard 867 Section 40, criteria of emitting a maximum ozone concentration of less than 0.050 ppm. Furthermore a second sample was not required to be tested as the first sample's maximum emissions were less than 0.030 ppm to satisfy the exception in the Section 40.1.1.



Fan Speed	Filter(s)	O3/Voltage Setting	C(t)max [ppm]		
High	YES	-	0.001		
Low	YES	-	0.001		
High	NO	-	0.001		

Completed by:	Philip R. Armstrong	Reviewed by:	Eric Dunay
Title:	Assoc. Engineer	Title:	Engineer
	Aberting)		ERCDOND
Signature:		Signature	/
Date	20 September 2013	Date:	20 September 2013



5. Appendix















ACTIVEPURE INSTALLATIONS

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