AQUAOX™ Disinfectant 275 & 525 & 1650

Product Efficacy & Safety Test Summary

AQUAOX™ Hypochlorous Products: Efficacy and Safety Lab Test Documentation

Aquaox Disinfectant 275 | Aquaox Disinfectant 525 | Aquaox Disinfectant 1650

AQUAOX™ Disinfectant 275, 525, and 1650 are solutions made from a Sodium Chloride and water, using an electrochemical process to create Hypochlorous Acid (HOCl), a highly effective, ecologically sound antimicrobial pesticide. The product numbers indicate the products' respective HOCl concentrations (e.g., Aquaox 275 is a 275-parts-per-million solution of HOCl).

As EPA-approved disinfectants, they are suitable for hospitals and other medical facilities as well as for general use.

This document provides exhaustive details reported from efficacy and safety tests performed on the solutions, conducted following official standards to determine their effectiveness in killing microorganisms in one step as well as assuring usage safety.[1]

We provide this level of information from independent laboratory sources, as you will see, to assure you of the products' effectiveness as antimicrobial agents while simultaneously providing a cutting-edge, user-friendly option for your medical-facility disinfection needs.

This document will be divided into an Efficacy and a Safety section, each listing separate lab tests, their importance, procedures used, and the results in tabular form.

[1] All three products (275-ppm, 525-ppm, and 1650-mmp HOC, respectively) a r e Environmental Protection Agency (EPA) registered antimicrobial pesticides bearing a Hospital and a General/Broad Spectrum Disinfectant claims per Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Section 3(c)(5). Using established American Society for Testing and Materials (ASTM) standards, Association of Official Analytical Chemists - International (AOAC) methods and EPA guidelines.

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AQUAOX ELECTROSTATIC SPRAY SAFETY ASSESSMENT - DETAILS 1

PRODUCT EFFICACY

Tests Demonstrating Rapid Biocidal Disinfection

AOAC[2] Use-Dilution Method[3]

The AOAC Use-Dilution Test is a crucial, high-level test for disinfectants. On a pass-fail basis, it measures how effectively a disinfectant is in a short time, usually less than 10 minutes. If the disinfectant works well in this brief period, it passes the test.

Procedure

1. Bacteria from Table 1 are mixed with a substance to simulate a dirty surface.
2. This mix is cultured for 48 hours.
3. The 48-hour-old bacteria are placed on small steel surfaces, creating at least 10 "contaminated" samples.
4. Each sample is dipped in the disinfectant for 10 minutes at room temperature (20 – 25°C).
5. Afterwards, they're placed in a liquid that stops the disinfectant's effect.
6. This mix is left for 48 hours to see if the bacteria regrow.
7. The number of samples with regrown bacteria is then counted.

TABLE 1. Aquaox Disinfectants evaluated against Gram+ and Gram- Bacteria in the presence of 5% Organic Soil Load

Exposure Time: 10 minutes
Sample Dilution: Ready to Use (RTU)

Test Organism	Strain	Number of Positive Carriers per Number Tested	Test Result
Pseudomonas aeruginosa	ATCC 15442	0 / 10	Pass
Staphylococcus aureus	ATCC 6538	0 / 10	Pass
Staphylococcus aureus (HA-MRSA)	ATCC 33591	0 / 10	Pass
Salmonella enterica	ATCC 10708	0 / 60	Pass
Escherichia coli (NDM-1)	ATCC BAA-2469	0 / 10	Pass
Vancomydin Resistant Enterococcus feacalis (VRE)	ATCC 700221	0 / 10	Pass

Conclusion

When tested with 5% dirt, Aquaox Disinfectant 275 and 525 effectively killed the listed bacteria in 10 minutes at room temperature.

[2] AOAC = Association of Official Analytical Chemists
[3] AOAC 955.14, 955.15, 964.02

AOAC Tuberculocidal Activity of Disinfectants

The AOAC Tuberculocidal Activity test is a tough pass-fail standard for disinfectants. To pass, a disinfectant must effectively kill an EPA-approved bacterial surrogate for tuberculosis quickly, in less than 10 minutes.

Procedure

1. The surrogate bacteria are mixed with a 5% serum to simulate a "dirty" surface.
2. The bacteria is grown for 21 days.
3. It is then placed on several stainless-steel test surfaces.
4. Each surface is exposed to the *disinfectant* for 10 minutes at room temperature.
5. After the exposure, they are moved to a liquid that neutralizes the disinfectant's effect.
6. These neutralized surfaces are then placed in a growth environment.
7. They are incubated there for 60 days.
8. At the end of the incubation, a record is made of how many surfaces show bacterial growth.

TABLE 2. Aquaox Disinfectants evaluated against Mycobacterium bovis BCG in the presence of 5% Fetal Bovine Serum

Exposure Time: 10 minutes **Sample Dilution:** Ready to Use (RTU)		
Challenge Suspension Initial Population (CFU/mL)	**Number of Positive Carriers per Number Tested (All Media Types)**	**Test Result**
2.850 x 10^7	0 / 10	Pass
2.850 x 10^7	0 / 10	Pass

Conclusion

When tested with 5% dirt, Aquaox Disinfectant 275 and 525 effectively killed *Mycobacterium bovis BCG* in 10 minutes at room temperature.

Basic Tests Demonstrating Hard-Surface Virucidal Effectiveness

This series of tests is designed to provide a real-world analog demonstrating AQUAOX™ Disinfectant 275 and 525 virucidal performance on the kinds of surfaces simulating use in medical settings.

The four challenging pathogens designated by the ASTM E1053 certification method are: HIV-1, H1N1, Rhinovirus 16 and Murine Norovirus, again using a simulated dirty surface.

Procedure

1. Load the test virus with a 5% organic soil to simulate a "dirty" surface.
2. Spread the virus over a carrier and let it dry.
3. Inoculate the dried virus onto a 100 x 15 mm glass Petri dish.
4. Prepare two carriers for surrogate viruses and one for non-surrogate viruses.
5. Treat the virus films with the disinfectant for 10 minutes at room temperature.
6. Neutralize the films with a solution after contact time.
7. Scrape the films using a cell scraper.
8. Plate and culture the test suspensions.
9. Check for the virus's presence or absence

Results

TABLE 3.1. Aquaox Disinfectant evaluated against HIV-1 virus in the presence of 5% Organic Soil Load

Virus / Strain: *HIV-1/Mn (ZeptoMetrix #0810027CF)*
Exposure Time: 10 minutes
Sample Dilution: Ready to Use (RTU)

Dilution	Virus Control		After Exposure to Test Substance – Lot #1		After Exposure to Test Substance – Lot #2	
	Carrier 1	Carrier 2	Carrier 1	Carrier 2	Carrier 1	Carrier 2
10^{-2}	Not Tested		0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-3}	+ + + +	+ + + +	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-4}	+ + + +	+ + + +	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-5}	+ + + +	+ + + +	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-6}	0 0 0 +	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0

10^{-7}	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$ (log 10)	5.750	5.500	≤ 1.50	≤ 1.50	≤ 1.50	≤ 1.50
Average $TCID_{50}$ (log 10)	5.625		≤ 1.50		≤ 1.50	
Log 10 Reduction	N/A		≥ 4.125	≥ 4.125	≥ 4.125	≥ 4.125
Average Log 10 Reduction			≥ 4.125		≥ 4.125	
Percent Reduction			> 99.99	> 99.99	> 99.99	> 99.99
Average % Reduction			> 99.99		> 99.99	

Dilution refers to the fold of dilution from virus inoculum
(+) = Positive for the presence of test virus
(0) = No test virus recovered
(≤)= Indicates a viral titer at or below the limit of detection for this assay

TABLE 3.2. Aquaox Disinfectant evaluated against Swine Influenza A (H1N1) virus in the presence of 5% Fetal Bovine Serum – Virus Controls and Test Results

Virus / Strain: Swine Influenza A (H1N1) Virus, ATCC VR-333
Strain A / Swine / Iowa / 15 / 30
Exposure Time: 10 minutes
Sample Dilution: Ready to Use (RTU)

Dilution	Input Virus Control	Dried Virus Control	After Exposure to Test Substance
Cell Control	0 0	0 0 0 0	0 0 0 0
10^{-1}	+ +	+ + + +	0 0 0 0
10^{-2}	+ +	+ + + +	0 0 0 0
10^{-3}	+ +	+ + + +	0 0 0 0
10^{-4}	+ +	+ + + +	0 0 0 0
10^{-5}	+ +	+ + + +	0 0 0 0
10^{-6}	0 0	+ 0 + 0	0 0 0 0
10^{-7}	0 0	0 0 0 0	0 0 0 0
10^{-8}	0 0	0 0 0 0	0 0 0 0
$TCID_{50}$ (log 10) / 100uL	6.50	6.00	≤ 0.50
Log 10 Reduction	N/A		≥ 5.50

TABLE 3.3. Aquaox Disinfectant evaluated against Rhinovirus 16 (common cold agent) in the presence of 5% Fetal Bovine Serum – Virus Controls and Test Results

Virus / Strain: Rhinovirus 16 (Rhino 16), ATCC VR-283
Exposure Time: 10 minutes
Sample Dilution: Ready to Use (RTU)

Test Substance	Log 10 Infectious Units per Carrier	Log 10 Reduction after Exposure	Percent Reduction after Exposure
Control	5.80	N/A	N/A
After Exposure to Test Substance	≤ 1.80	≥ 4.00	≥ 99.99%

Viral stock enumeration demonstrated a titer of 7.00 log 10 per 0.1 mL

TABLE 3.4. Aquaox Disinfectant evaluated against Murine Norovirus (without 5% FBS) – Virus Controls and Test Results

Virus / Strain: Murine Norovirus, Strain MNV-G
Exposure Time: 10 minutes
Sample Dilution: Ready to Use (RTU)

Dilution	Virus Stock Titer Control	Plate Recovery Control	After Exposure to Test Substance – Lot #1	After Exposure to Test Substance – Lot #2
10^{-2}	Not Tested	Not Tested	0 0 0 0	0 0 0 0
10^{-3}	Not Tested	+ + + +	0 0 0 0	0 0 0 0
10^{-4}	+ + + +	+ + + +	0 0 0 0	0 0 0 0
10^{-5}	+ + + +	+ + + +	0 0 0 0	0 0 0 0
10^{-6}	+ + + +	+ + + +	0 0 0 0	0 0 0 0
10^{-7}	0 0 + +	0 0 0 0	0 0 0 0	0 0 0 0
10^{-8}	0 0 0 0	0 0 0 0	Not Tested	Not Tested
10^{-9}	0 0 0 0	Not Tested	Not Tested	Not Tested
$TCID_{50}$ (log 10) / mL	7.00	6.50	≤ 1.50	≤ 1.50
$TCID_{50}$ (log 10) per Carrier (0.40mL Challenge)	N/A	6.10	≤ 1.10	≤ 1.10
Log 10 Reduction	N/A		≥ 5.00	≥ 5.00

Conclusion

In the presence of 5% organic soil, Aquaox Disinfectant 275 and 525 showed more than 99.99% (4-log) reduction in H1N1 and *Rhinovirus* 16 after 10 minutes at room temperature. It reduced HIV-1 Virus even further at 99.999% (5-log). Without the soil, it also reduced Murine Norovirus at more than 99.999%.

In short, Aquaox Disinfectant 275 and 525 successfully met the EPA standards for demonstrating virus-killing effectiveness.

TABLE 3.5. Aquaox 1650 2-minute evaluation against *Feline Calicivirus* in the presence of 5% Fetal Bovine Serum – Virus Controls and Test Results

Consistent with the series of tests described above, Aquaox Disinfectant 1650 was evaluated against Feline Calicivirus (an EPA-approved Human norovirus surrogate), strain F-9, ATCC 782, supplemented with a 5% Fetal Bovine Serum -FBS soil load with a 2-minute exposure time.

		Test Results – Lot: 03082021D2201	
		Replicate 1	Replicate 2
Dilution	10^{-1}	0 0 0 0	0 0 0 0
	10^{-2}	0 0 0 0	0 0 0 0
	10^{-3}	0 0 0 0	0 0 0 0
	10^{-4}	0 0 0 0	0 0 0 0
	10^{-5}	0 0 0 0	0 0 0 0
	10^{-6}	0 0 0 0	0 0 0 0
$TCID_{50}$/0.1 ml		$\leq 0.50 \log_{10}$	$\leq 0.50 \log_{10}$
$TCID_{50}$/Carrier		$\leq 0.80 \log_{10}$	$\leq 0.80 \log_{10}$
Avg. $TCID_{50}$/Carrier		$\leq 0.80 \log_{10}$	
Avg. Log_{10} Reduction/Carrier		$\geq 4.50 \log_{10}$	

Conclusion

At a contact time to two minutes, Aquaox Disinfectant 1650 exceeded the EPA Performance Guidelines with 99.995% kill rate for both of two exposures.

Further Proof of Efficacy, Including Using AQUAOX™ 1650

As would be expected, AQUAOX™ 1650, having 3 times the HOCl concentration as AQUAOX™ 550, handily passed the EPA requirements for registration as well as being included on EPA's List N for disinfectants that have been proven effective against the novel coronavirus and other emerging pathogens. The results of these and additional test summaries for the other two disinfectants are included in TABLE 4 (275), TABLE 5 (550), and TABLE 6 (AQUAOX™ 1650), below.

TABLE 4. Efficacy Test Summary - Aquaox Disinfectant 275

Test Product	Study Type	Test Method	Challenge Organisms	Organism Type	Results	Lab
Aquaox Disinfectant 275 (Tested at **10ppm** FAC)	Antimicrobial Effectiveness Study using a Time Kill Assay	USP<51> Guideline	*Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Serratia marcescens, Klebsiella pneumoniae, Proteus vulgaris, Acinetobacter baumannii*	All **Gram-Negative** Bacteria except for *Staphylococcus aureus* , which is **Gram-Positive**	Log reduction in **15 s**: S. aureus: > 5.25 P. aeruginosa: > 5.00 E. Coli: > 4.85 S. marcescens: > 4.88 K. pneumoniae: > 4.98 P. vulgaris: > 4.98 A. baumannii: > 5.12	NAMSA
Aquaox Disinfectant 275	Antimicrobial Effectiveness Study using a Time Kill Assay	ASTM Guideline E2315-03	*Acinetobacter baumannii* - Multi Drug Resistant, *Enterococcus faecium* - Multi Drug Resistant, Methicillin Resistant *Staphylococcus aureus* (MRSA), Vancomycin Resistant *Enterococcus faecalis* (VRE)	**Gram-Negative** Bacteria **Gram-Positive** Bacteria **Gram-Positive** Bacteria **Gram-Positive** Bacteria	Log reduction in **15 s**: A. baumannii: > 5.45 E. faecium: > 5.30 MRSA: > 5.36 VRE: > 5.56	ATS Lab
Aquaox Disinfectant 275	Antimicrobial Effectiveness Study using a Time Kill Assay	ASTM Guideline E2315-03	*Bacteroides fragilis, Haemophilus influenzae, Streptococcus pyogenes*	**Gram-Negative** Bacteria **Gram-Negative** Bacteria **Gram-Positive** Bacteria	Log reduction in **15 s**: B. fragilis: > 5.89 H. influenzae: > 4.44 S. pyogenes: > 5.79	ATS Lab
Aquaox Disinfectant 275	Antimicrobial Effectiveness Study using a Time Kill Assay	ASTM Guideline E2315-03	*Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus saprophyticus*	All **Gram-Positive** Bacteria and of the *Staphylococcus* genus	Log reduction in **15 s**: S. epidermidis: > 5.08 S. haemolyticus: > 5.01 S. hominis: > 5.32 S. saprophyticus: > 5.15	ATS Lab
Aquaox Disinfectant 275	Antimicrobial Effectiveness Study using a Time Kill Assay	ASTM Guideline E2315-03	*Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Micrococcus luteus, Proteus mirabilis, Serratia marcescens*	All **Gram-Negative** Bacteria except for *Micrococcus luteus* , which is **Gram-Positive to Gram-Variable**	Log reduction in **15 s**: E. aerogenes: > 5.88 E. coli: > 5.61 K. pneumoniae: > 5.42 M. luteus: > 4.46 P. mirabilis: > 5.92 S. marcescens: > 5.43	ATS Lab
Aquaox Disinfectant 275	Testing Disinfectant against *Pseudomonas aeruginosa* and *Staphylococcus aureus*	AOAC Official Method, 964.02, 955.15, Use-Dilution Method	*Staphylococcus aureus, Pseudomonas aeruginosa*	**Gram-Positive** Bacteria **Gram-Negative** Bacteria	Killed **10 out of 10** treated carriers in **5% organic soil** load in 10 minutes	Bioscience
Aquaox Disinfectant 275	Testing Disinfectant against *Salmonella enterica*	AOAC Official Method, 955.14, Use-Dilution Method	*Salmonella enterica*	**Gram-Negative** Bacteria	Killed **10 out of 10** treated carriers in **5% organic soil** load in 10 minutes	ATS Lab
Aquaox Disinfectant 275	Antimicrobial Effectiveness Study using a Time Kill Assay	ASTM Guideline E2315-03	*Mycobacterium bovis - BCG*	Bacteria that causes **Tuberculosis** in humans	> 5.21 log reduction in **60 s**	ATS Lab
Aquaox Disinfectant 275	Assessment of Microbicidal Activity against Viruses in Suspension	ASTM Guideline E1052, E1482	*Hepatitis B Virus*	**Virus**	> 5.25 log reduction in **30 s**	ATS Lab
Aquaox Disinfectant 275	Assessment of Microbicidal Activity against Viruses in Suspension	ASTM Guideline E1052, E1482	*Rhinovirus type 37*	**Virus**	> 3.75 log reduction in **60 s**	ATS Lab
Aquaox Disinfectant 275	Assessment of Microbicidal Activity against Viruses in Suspension	ASTM Guideline E1052, E1482	*Swine Influenza A (H1N1) Virus*	**Virus**	> 5.50 log reduction in 5% organic soil load in 10 minutes	ATS Lab
Aquaox Disinfectant 275	Assessment of Microbicidal Activity against Viruses in Suspension	ASTM Guideline E1052	*Murine Norovirus*	**Virus**	> 5.00 log reduction in 10 minutes	Microbac Lab
Aquaox Disinfectant 275	Antimicrobial Effectiveness Study using a Time Kill Assay	USP<51> Guideline	*Aspergillus Brasiliense*	**Fungus**	Log reduction in **15 s** A. Brasiliense: = 4.11	NAMSA
Aquaox Disinfectant 275 (Tested at **10ppm** FAC)	Antimicrobial Effectiveness Study using a Time Kill Assay	USP<51> Guideline	*Candida albicans*	**Fungus**	> 4.38 log reduction in **15 s**	NAMSA
Aquaox Disinfectant 275	Antimicrobial Effectiveness Study using a Time Kill Assay	ASTM Guideline E2315-03	*Candida albicans*	**Fungus**	> 5.31 log reduction in **15 s**	ATS Lab
Aquaox Disinfectant 275	Antimicrobial Effectiveness Study using a Time Kill Assay	ASTM Standard Guideline E2315-03, E2839-11	*Clostridium difficile* - spore form	**Spore**	> 5.35 log reduction in **30 s**	ATS Lab
Aquaox Disinfectant 275	Virucidal Activity of Liquid, Aerosol, Trigger-spray and Towelettes Disinfectants	ASTM Modified Protocol (E 1053-20) to Determine the virucidal Activity of Liquid	*Human Coronavirus*	**Virus**	> 5.25 log reduction in **5% organic soil load (fetal bovine serum)** in 10 minutes	CREMCO

TABLE 5. Efficacy Test Summary - Aquaox Disinfectant 525

Test Product	Study Type	Test Method	Challenge Organisms	Organism Type	Results	Lab
Aquaox Disinfectant 525	Testing Disinfectant against *Pseudomonas aeruginosa* and *Staphylococcus aureus*	AOAC Official Method, 964.02, 955.15, Use-Dilution Method	*Staphylococcus aureus, Pseudomonas aeruginosa*	**Gram-Positive** Bacteria **Gram-Negative** Bacteria	Killed **10 out of 10** treated carriers in **5% organic soil** load in 10 minutes	Bioscience
Aquaox Disinfectant 525	Testing Disinfectant against Hospital Acquired Methicillin Resistant *Staphylococcus aureus* (HA-MRSA)	AOAC Official Method, 964.02, Use-Dilution Method	Hospital Acquired Methicillin Resistant *Staphylococcus aureus (HA-MRSA)*	**Gram-Positive** Bacteria	Killed **10 out of 10** treated carriers in **5% organic soil** load in 10 minutes	ATS Lab
Aquaox Disinfectant 525	Testing Disinfectant against *Salmonella enterica*	AOAC Official Method, 955.14, Use-Dilution Method	*Salmonella enterica*	**Gram-Negative** Bacteria	Killed **60 out of 60** treated carriers in **5% organic soil** load in 10 minutes	ATS Lab
Aquaox Disinfectant 525	Testing Disinfectant against NDM-1 *E.coli and VRE*	AOAC Official Method, 955.15, Use-Dilution Method	*NDM-1 Escherichia coli Vancomycin Resistant Enterococcus faecalis (VRE)*	**Gram-Negative** Bacteria **Gram-Positive** Bacteria	Killed **10 out of 10** treated carriers in **5% organic soil** load in 10 minutes	ATS Lab
Aquaox Disinfectant 525	AOAC Tuberculocidal Activity of Disinfectants	AOAC Official Method, 965.12, 960.09	*Mycobacterium bovis - BCG*	Bacteria that causes **Tuberculosis** in humans	Killed **10 out of 10** treated carriers in **5% organic soil** load in 10 minutes	Bioscience
Aquaox Disinfectant 525	Assessment of Virucidal Activity against Viruses in Suspension	ASTM Guideline E1053, E1482	*Swine Influenza A (H1N1) Virus*	**Virus**	> 5.50 log reduction in **5% organic soil load** in 10 minutes	ATS Lab
Aquaox Disinfectant 525	Assessment of Virucidal Activity against Viruses in Suspension	ASTM Guideline E1053	*Human Immunodeficiency Virus Type 1 (HIV-1)*	**Virus**	> 4.125 log reduction in **5% organic soil load** in 10 minutes	Bioscience
Aquaox Disinfectant 525	Assessment of Virucidal Activity against Viruses in Suspension	ASTM Guideline E1053	*Rhinovirus 16 (Common Cold Agent)*	**Virus**	> 4.000 log reduction in **5% organic soil load** in 10 minutes	ATL Lab
Aquaox Disinfectant 525	Standard Quantitative Disk Carrier Test Method for Determining Sporicidal Activities	ASTM Standard Guideline E2197-11, Standard Quantitative Disk Carrier Test Method	*Clostridium difficile* - spore form	**Spore**	> 5.96 log reduction in 10 minutes in the absence of organic soil load	ATS Lab
Aquaox Disinfectant 525	StandardPracticeto Assess Virucidal Activity of Chemicals for Disinfection of Inanimate, Nonporous Environmental Surfaces	ASTM Standard Guideline E1053-20, StandardPracticeto Assess Virucidal Activity	*Human Coronavirus*	**Virus**	> 4.50 log reduction in **5% organic soil load (fetal bovine serum)** in 10 minutes	ALG Lab
Aquaox Disinfectant 525	StandardPracticeto Assess Virucidal Activity of Chemicals for Disinfection of Inanimate, Nonporous Environmental Surfaces	ASTM Standard Guideline E1053-20, StandardPracticeto Assess Virucidal Activity	*Feline Calicivirus*	**Virus**	> 5.50 log reduction in **5% organic soil load (fetal bovine serum)** in 10 minutes	ALG Lab
Aquaox Disinfectant 525	StandardPracticeto Assess Virucidal Activity of Chemicals for Disinfection of Inanimate, Nonporous Environmental Surfaces	ASTM Standard Guideline E1053-20, StandardPracticeto Assess Virucidal Activity	*Murine Norovirus*	**Virus**	> 3.25 log reduction in **5% organic soil load (fetal bovine serum)** in 10 minutes	Microbac
Aquaox Disinfectant 525	StandardPracticeto Assess Virucidal Activity of Chemicals for Disinfection of Inanimate, Nonporous Environmental Surfaces	ASTM Standard Guideline E1053-20, StandardPracticeto Assess Virucidal Activity	*SARS-Cov-2 virus*	**Virus**	> 3.75 log reduction in **5% organic soil load (fetal bovine serum)** in 10 minutes	Microbac

TABLE 6. Efficacy Test Summary - Aquaox Disinfectant 1650

Test Product	Study Type	Test Method	Challenge Organisms	Organism Type	Results	Lab
Aquaox Disinfectant 1650	Testing Disinfectant against *Pseudomonas aeruginosa* and *Staphylococcus aureus*	AOAC Official Method, 964.02, 955.15, Use-Dilution Method	*Staphylococcus aureus, Pseudomonas aeruginosa*	**Gram-Positive** Bacteria **Gram-Negative** Bacteria	Killed **10 out of 10** treated carriers in **5% organic soil** load in 1 minute	Microchem
Aquaox Disinfectant 1650	Testing Disinfectant against *Salmonella aureus*	AOAC Official Method, 955.14, Use-Dilution Method	*Salmonella aureus*	**Gram-Positive** Bacteria	Killed **10 out of 10** treated carriers in **5% organic soil** load in 1 minute	Microchem
Aquaox Disinfectant 1650	Assessment of Virucidal Activity against Viruses in Suspension	ASTM Guideline E1053	*Rhinovirus 16 (Common Cold Agent)*	**Virus**	Killed **60 out of 60** treated carriers in **5% organic soil** load in 1 minute	Microchem
Aquaox Disinfectant 1650	Virucidal Activity of test substance for use on inanimate, nonporous surfaces	ASTM Guideline E1053	*Feline calicivirus ATCC-94)*	**Virus**	>5.3 Log reduction in **5% three-part soil** load in 2 minutes	Microchem
Aquaox Disinfectant 1650	Testing Disinfectant against spores of Clostridioides difficile, ATCC 43598	AOAC Official Method, 955.15, Use-Dilution Method	*Clostridium difficile* - spore form	**Spore**	>4.27 Log **reduction** in 5% three-part soil load in 10 minutes 1065ppm at 5.90	Microbac
Aquaox Disinfectant 1650	Testing Disinfectant against spores of Clostridioides difficile, ATCC 43598	AOAC Official Method, 955.15, Use-Dilution Method	*Clostridium difficile* - spore form	**Spore**	>4.77 Log **reduction** in 5% three-part soil load in 5 minutes 1305ppm at 5.5	Microbac
Aquaox Disinfectant 1650	Testing Disinfectant against spores of Clostridioides difficile, ATCC 43598	AOAC Official Method, 955.15, Use-Dilution Method	*Clostridium difficile* - spore form	**Spore**	>5.59 Log **reduction** in 5% three-part soil load in 10 minutes 1305ppm at 5.82	Microbac
Aquaox Disinfectant 1650	Testing Disinfectant against spores of Clostridioides difficile, ATCC 43598	AOAC Official Method, 955.15, Use-Dilution Method	*Clostridium difficile* - spore form	**Spore**	> Log reduction in 5% three-part soil load in 10 minutes 1500ppm at 6.5	Microbac
Aquaox Disinfectant 1650	Testing Disinfectant against Candida Auris, AR-BANK#0385 (resistant strain)	AOAC Official Method, 955.15, Use-Dilution Method	*Candida auris _AR BANK#0385*	**Fungi**	> Log reduction in 5% three-part soil load in 10 minutes 1500ppm at 6.5	Microbac

TABLE 7: GENERAL USAGE COMPILATION TABLE FOR PATHOGENS

	Pathogen	Minimum Doses FAC required (ppm)	Minimum Contact time required (minutes)
	[Food Contact] Sanitizer Claims		
99764-1	*Staphylococcus aureus [*(ATCC 6538)]	338 ppm[5]	1 minute
99764-1	*Salmonella enterica [*(ATCC 6539)]	338 ppm[5]	1 minute
99764-1	*Escherichia coli (ATCC 11229)*	338 ppm[5]	1 minute
99764-1	*Listeria monocytogenes [*(ATCC 19117)]	338 ppm[5]	1 minute
	[Hard Surface] Disinfection Claims – bacteria		
93392-1	*Staphylococcus aureus* [(ATCC 6538)]	248 ppm[1]	10 minutes
93392-2		477 ppm[2]	10 minutes
93908-1		460 ppm[4]	10 minutes
93392-3		1490 ppm[3]	1 minute
93392-2	*Methicillin Resistant Staphylococcus aureus* – (MRSA) [(ATCC 33591)]	477 ppm[2]	10 minutes
93908-1		460 ppm[4]	10 minutes
93392-1	*Salmonella enterica* [(ATCC 10708)]	248 ppm[1]	10 minutes
93392-2		477 ppm[2]	10 minutes
93908-1		460 ppm[4]	10 minutes
93392-1	*Pseudomonas aeruginosa* [(ATCC 15442)]	248 ppm[1]	10 minutes
93392-2		477 ppm[2]	10 minutes
93809-1		460 ppm[4]	10 minutes
93392-3		1490 ppm[3]	1 minute
93392-2	*Escherichia coli* (NDM) [(ATCC BA-2469]	477 ppm[2]	10 minutes
93392-2	Vancomycin resistant *Enterococcus faecalis* (VRE) [(ATCC 700221)]	477 ppm[2]	10 minutes
93908-1	Vancomycin resistant *Enterococcus faecalis* (VRE) [(ATCC 51229)]	460 ppm[4]	10 minutes
93908-1	*Bordetella bronchiseptica* [Kennel cough] (ATCC 10580)	460 ppm[4]	10 minutes
93908-1	*Clostridium difficile* – spore (C.Diff or C. Difficile) (ATCC 43598)	460 ppm[4]	10 minutes
93908-1	Escheria coli (E coli) (ATCC 11229)	460 ppm[4]	10 minutes
93908-1	*Klebsiella pneumonia* New Delhi Metallo-Beta Lactamasa (NDM-1), Carbapenem resistant (CRE) *Klebsiella pneumoniae (NDM-1 [*(CRKP), CDC10002	460 ppm[4]	10 minutes
	Mycobactericidal Claims		
93392-2	*Mycobacterium bovis* (BCG) ([ATCC 35734)]	477 ppm[2]	10 minutes
	Virucidal Claims - Enveloped viruses		
93392-1	Swine Influenza Virus (H1N1)(ATCC VR-333)	248 ppm[1]	10 minutes
93392-2		477 ppm[2]	10 minutes
93908-1	Swine Flu Virus (H1N1) A/Swine/1976/31 (ATCC VR-99)	460 ppm[4]	10 minutes
93908-1	Canine distemper (ATCC VR-1587) [(Strain Snyder Hill)]	460 ppm[4]	10 minutes

TABLE 7: GENERAL USAGE COMPILATION TABLE FOR PATHOGENS – Page 2 of 2			
93908-1	Influenza A (H1N1) [(Strain A/Virginia/ATCC1/2009)][(ATCC VR-1736)][(representative for common flu virus)]	460 ppm[4]	2 minutes
93908-1	[Human] Hepatitis C [Virus] [(as bovine diarrhea virus)] [(HCV)] [Strain ADL] [(ATCCVR-1422)]	460 ppm[4]	2 minutes
93392-2	Human Coronavirus strain 229E [(ATCC VR-740)] [†]	477 ppm[2]	10 minutes
93908-1	Respiratory syncytial virus (RSV) (Strain A-2) [(ATCC VR-1540]	460 ppm[4]	10 minutes
93392-2	Human Immunodeficiency Virus Type 1 (HIV-1] [(Strain IIIB)] (Mn;zeptometrix #08110027CF)	477 ppm[2]	10 minutes
93908-1		460 ppm[4]	10 minutes
Virucidal Claims - Non-enveloped viruses			
93908-1	Adenovirus (1 of Type 1) (Strain 71) (ATCC VR-1)	460 ppm[4]	10 minutes
93392-3	Rhinovirus [Type 14] [(ATCC VR-283)]	1490 ppm[3]	1 minute
93908-1	Rhinovirus [Type 16] (Strain 11757) [(ATCC VR-283)]	460 ppm[4]	10 minutes
93908-1	Rotavirus (A or Group A) (Strain WA) (ATCC VR-2018) [(the virus that causes diarhea)]	460 ppm[4]	10 minutes
93392-2	Murine Norovirus	477 ppm[2]	10 minutes
93908-1	Norovirus or Norwalk Virus (as Feline Calicivirus) (Strain F-9) (ATCC VR-782)	460 ppm[4]	10 minutes
93392-2	Feline Calicivirus (ATCCVR-782)	477 ppm[2]	10 minutes
93392-2	SARS-CoV-2 Virus	477 ppm[2]	10 minutes
Virucidal Claims - Non-enveloped Parvo viruses			
93908-1	Canine parvovirus (ATCC VR-2016) (Strain Cornell)	460 ppm[4]	10 minutes
Yeast			
93908-1	Candida albicans (ATCC 10231)	460 ppm[4]	10 minutes
Bloodborne Pathogens			
93908-1	[Human] Hepatitis C [Virus] [(as bovine diarrhea virus)] [(HCV)] [Strain ADL] [(ATCCVR-1422)]	460 ppm[4]	2 minutes
93392-2	Human Immunodeficiency Virus Type 1 (HIV-1] [(Strain IIIB)] (Mn;zeptometrix #08110027CF)	477 ppm[2]	10 minutes
93908-1		460 ppm[4]	10 minutes
Fungicidal Claims			
93908-1	*Candida albicans [*(ATCC 10231)]	460 ppm[4]	10 minutes
	Sporicidal Claims		
93908-1	*Clostridioides difficile* spores *[formerly Clostridium difficile]* [(ATCC 43598)]	460 ppm[4]	10 minutes

[1] This product can be obtained by diluting AX-5000 to a >248ppm FAC solution whereas pH is between 6.3 and 7.2. See EPA product 93392-1
[2] This product can be obtained by diluting AX-5000 to a >477ppm FAC solution whereas pH is between 6.3 and 7.2. See EPA product 93392-2
[3] This product can be obtained by diluting AX-5000 to a >1490ppm FAC solution whereas pH is between 6.3 and 7.2. See EPA product 93392-3
[4] This product can be obtained by diluting AX-5000 to a >460ppm FAC solution whereas pH is between 6.3 and 7.2. See EPA product 93908-1
[5] This product can be obtained by diluting AX-5000 to a >338ppm FAC solution whereas pH is between 6.3 and 7.2. See EPA product 9

PRODUCT SAFETY

As indicated above, AQUAOX™ Disinfectant products contain Hypochlorous Acid as the active ingredient. The sole inactive ingredient, besides the water of the solution, is residual Sodium Chloride (pure salt) from the electrolysis process, which is FDA-approved (CAS RN 8028-77-1) and NSF-certified, ensuring its safety.

EPA-Required Toxicity Testing for AquaOx 275, 550, 1650

To evaluate potential toxicity, a series of tests were performed at the North American Science Associates (NAMSA) and Illinois Institute of Technology Research Institute (IIT RI), both Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-approved facilities, in line with Good Laboratory Practice (GLP).

The testing program adhered to ISO-10993 standards for biocompatibility of devices in contact with compromised surfaces. Detailed study results can be found in ***Table 8****.*

TABLE 8. Nonclinical Toxicity Testing Summary

Study Type	Test Species	Route	Result	Testing Facility
In vitro Cytotoxicity	L-929 Mouse Fibroblast Cells	In vitro	Not Cytotoxic / Meet USP Requirement	NAMSA
Repeated-Dose Toxicity	Rats	Dermal	No Local or Systemic Toxicity on Intact or Wounded skin	NAMSA
Maximization Sensitization	Guinea Pigs	Dermal	Not a Sensitizer (Does not induce allergic responses)	NAMSA
Acute Toxicity	Rats	Oral	Non-Toxic	NAMSA
Acute Toxicity	Rats	Inhalation / Nose	Non-Toxic	IIT RI
Skin Irritation	Rabbits	Dermal	Not a Skin Irritant on Intact or Abraded Skin	NAMSA
Eye Irritation	Rabbits	Ocular	Not an Eye Irritant	NAMSA

Conclusion

AQUAOX™ disinfectant products comply with the acceptable levels or criteria of safety and efficacy as defined by the United States Pharmacopeia. Exposure (USP). They didn't irritate the skin or eyes and weren't found to cause allergies. When tested at the highest possible concentration, the product wasn't toxic if ingested or inhaled. Additionally, using the product on skin, even on wounds, for 28 days didn't cause any irritation or wound healing problems. Overall, the tests confirmed the product is safe and biocompatible for its intended use.

NOTE: Aquaox 1650 was actually tested at a concentration of 2200-ppm HOCl using an onsite generation process. It still received a Category I rating (on a scale of I to IV), the lowest toxicity rating allowed by the EPA. The EPA does not acknowledge the possibility an antimicrobial disinfectant can be non-toxic. Still, it's the highest HOCl concentration registered by the EPA, underscoring the lack of any toxicity concerns for the lower concentration products.

Electrostatic Spray Safety Assessment

INTRODUCTION:

The typical practice with Aquaox disinfectant use is to spray a surface and then wipe with one of our microfiber cloths, which, not incidentally provide a more effective cleaning step than using a regular wipe would. However, there are other situations where this approach is not practical due, for instance, to the volume of surface involved or the inaccessibility of an area. In that circumstance, we suggest using our Electrostatic Spray option.

There are advantages to the spraying including scales of efficiency. We also want to mention some studies have been done demonstrating that consistent use of the spraying approach over time, even without any additional wiping step, results in a significant decrease in biofilm accumulation on environmental surfaces.

However, we want to assure users of the safety of our spraying approach. To that end we done extensive research on the issue to minimize any concerns.

A detailed version of findings will be offered in the Appendices of this document in case you want to go into more depth than provided in this summary.

I. Acute Inhalation Injury and Particle Size of Inhaled Substances

Our airways and lungs are continuously vulnerable to irritants through breathing. This is especially true in the case of cleaning agents, where users can be exposed to fumes like chlorine and ammonia. Another potential vulnerability factor involves the size of particles. Smaller particles can enter the lungs whereas larger ones tend to be trapped by the nose before they can cause any damage to the respiratory system.

Particle size in inhalation impacts where particles settle in our respiratory system. There are three main categories:

1. *Respirable fraction*: Particles ≤10µm. These can reach the deepest parts of our lungs.
2. *Extra-thoracic fraction*: Particles >25µm. These stay between the nostrils/mouth and the end of the larynx.
3. *Thoracic fraction*: Particles ≤25µm. These penetrate head airways, reaching the lungs.

Generally, larger particles (>25µm) are less concerning as they're often trapped and expelled through actions like sneezing.

Aquaox ESS Sprayer and Dispensed Droplet Size

The Aquaox ES 3001-5 is a portable electrostatic sprayer designed for water-based solutions and suitable for chemicals labeled for aerosol or mist use. It features a motor/blower, high-voltage power supply, charging components, and a precision metering valve to control flow rate and particle size. Particle output ranges from 10 to 50 µm Volume Mean Diameter (VMD), with size increasing alongside flow rate, adjustable through three valve settings.

Valve Position Settings, Approximate Flow Rates and Droplet Size

Position	Flow Rate	Droplet Size (µm VMD)
1	6 oz/min (177 mL/min)	10 – 20
2	7.5 oz/ min (222 mL/min)	20 – 30
3	9.5 oz/min (281 mL/min)	30 – 50

In response to the respiratory information, described above, Aquaox customized their ESS Sprayer by removing the metering valve and formulation tank, allowing unobstructed liquid flow above 9.5 oz/min producing particle sizes consistently larger than 30 µm VMD, as higher flow rates produce larger droplets. The sprayer is shown below:

Original Spray Head with Metering Valve	Modified Spray Head with No Metering Valve
	

The modified sprayer design limits respiratory concerns in three ways:

1. The output particle size: The generated particles are now too large to be inhaled deeply.
2. Falling time of particles: In accordance with the World Health Organization (WHO) table below, most if not all of them will have fallen to the floor by the end of the Aquaox recommended 10-minute wait before re-entry.
3. The effect of the electrostatic ion field: Particles leave the nozzle through the device's electrostatic ion field. The imparted charge means particles not removed by gravity will be attracted to nearby surfaces, removing them from circulation.

II. Chlorine Exposure Limits

OSHA has not yet implemented a standard regulating HOCl exposure limits nor a method for determining HOCl concentration, so Chlorine – the active component of HOCl – was measured to assure the spraying procedure rendered amounts that fell within the current Occupational Safety and Health Administration (OSHA) Permissible Exposure Limits (PEL). These regulations include a short-term exposure limit for up to a 15- minute

exposure not to exceed 1 ppm (2.9 mg/m3 where mg/m3 is defined as mg Chlorine per m3 of air), and a time-weighted average for up to 8 hours not to exceed 0.5 ppm (1.5 mg/m3).

PROCEDURE:

Study 1: Separately, spraying Aquaox 275 and 525, air samples from a controlled experimental room are evaluated for HOCl concentration using the NIOSH 7607 Method after using the Aquaox ESS. Samples are taken following a 5-minute spray and 10-minute wait and analyzed for short-term exposure (15 minutes) and time-weighted average levels (6 hours). The samples, drawn at 1 L/min into a specially prepared tube, are sent to ALS Environmental for ion chromatography analysis, focusing on chlorine compounds, excluding trichloramines.

Study 2: The experiment assesses short-term HOCl exposure using the OSHA ID-101 method in a small experimental room. After spraying for 5 minutes and allowing for a 10-minute settling period, air samples are collected for 15 minutes. Samples are drawn through a glass bubbler with an acid solution, and HOCl is measured by its reaction with DPD and potassium iodide, creating a red dye measured at 540nm. A standard curve is generated from known HOCl concentrations to determine the HOCl levels in the test samples.

Results: Chlorine concentrations were found to be 0.207ppm for AX275 and 0.262ppm for AX525 after spraying, both below OSHA's Chlorine limit of 1ppm.

Study 3: Chlorine gas levels are measured in air samples using the Draeger Chlorine 0.2/a System immediately after spraying and after a 10-minute wait. Draeger tubes react with chlorine to indicate concentration by color change, with results analyzed directly by Aquaox**.**

Results: Chlorine levels were below 1 ppm after using AX275 and AX525 solutions, with immediate readings of 0.2ppm and 0.5ppm, respectively, and both dropping to below 0.2ppm after 10 minutes.

CONCLUSION: No indications for concern were raised by these comprehensive measurements. For reference, the levels measured are not only well below the PEL, but they are also far below the mean air concentration measures of roughly 1.36 ppm found in a study of the air around a sample of indoor swimming pools. [4]

[4] https://doi.org/10.1016/j.gaceta.2013.02.002

Testing of Disinfectant Effects on Surfaces Using the BOEING Protocol for Interior-Cleaning Solutions

This BOEING D6-7127 protocol includes 11 tests, first listed below and then iteratively detailed with the respective results for Aquaox Disinfectants 275 and 525 provided at the end of each section.

This protocol provides the gold standard for rigorously evaluating cleaning and corrosion prevention products to ensure they are safe for Boeing aircraft and won't damage them.

These results, of course, can be generalized to surfaces in other settings like the medical arena.

Table listing properties of AquaOx Disinfectants 275 and 550:

Test Liquid Properties

Aquaox Disinfectant 275

TEST	ANALYSIS	UNITS
Free Available Chlorine	302	ppm
pH	6.72	n/a
Conductivity	2544	µS/cm
ORP	878	mV

Aquaox Disinfectant 525

TEST	ANALYSIS	UNITS
Free Available Chlorine	546	ppm
pH	6.86	n/a
Conductivity	2099	µS/cm
ORP	913	mV

Summary of Procedure – Tests Performed: [5]

A. Sandwich Corrosion Test
B. Immersion Corrosion Test
C. Rubber Test
D. Sealant Test
E. Painted Surface Test
F. Tedlar Surface Test
G. Vinyl Surface Test
H. Fabric and Carpet Test
I. Leather and Naugahyde Test
J. Polycarbonate Crazing Test
K. Flash Point Test

[5] Boeing D6-7127 Rev P incorporating PDD 6-8 – Cleaning Interiors of Commercial Transport Aircraft - Category: Disinfectants

A. SANDWICH CORROSION TEST (Reference: ASTM F1110)

This test method checks if chemicals – in this case a disinfectant - can corrode or damage aircraft aluminum alloys.

Procedure

This test employs a significant challenge through assessing the disinfectants' effect on how the chemicals might corrode when trapped between aluminum alloy surfaces. [6]

The test results are judged by comparing the appearance of surfaces from three coupon sets. One set is exposed only to water to set a standard. If the surfaces exposed to test chemicals show more corrosion than the water-exposed set, they are deemed non-conforming. The relative corrosion severity rating system below is used to allow for a numerical classification of the test results.

Relative corrosion severity rating system:

0—No visible corrosion and no discoloration present
1—Very slight corrosion or very slight discoloration, and/or up to 5 % of area corroded [7]
2—Discoloration and/or up to 10 % of area corroded
3—Discoloration and/or up to 25 % of area corroded
4—Discoloration and/or more than 25 % of area corroded, and/or pitting present

Aquaox Test Results:

Test Chemical	Clad 7075-T6 Aluminum Alloy	Bare 7075-T6 Aluminum Alloy	Test Result
Aquaox Disinfectant 275	1	1	Conforms
Test Control	1	1	

Conclusion

- Test results of Aquaox Disinfectant 275 conform for all test surfaces on all test criteria.
- Test result of Aquaox Disinfectant 525 does not conform on the Aluminum Alloy surface because corrosion caused by the test chemical is in excess of that caused by the test control, which means, if left in constant, sandwiched contact with Aluminum, some discoloration or corrosion is possible.

B. IMMERSION CORROSION TEST (Reference: ASTM F483)

This method measures how chemicals affect aircraft metals (Aluminum) over time by weighing the metals after they've been fully soaked in the disinfectant.

Procedure

Small pieces of the materials are treated with the test chemical and dried. Their weight is checked before and after this process. The chemical shouldn't corrode the materials or change their weight by more than ± 10mg in

[6] The test uses Clad 7075T6 Aluminum Alloy and Bare 7075-T6 Aluminum Alloy, anodized per MILA-8625 Type I, as testing materials
7 "Area" refers to area where the test material was applied.

24 hours for each 1" x 2" piece.

Aquaox 525 Test Results:

Test Chemical	Test Panel	Weight Loss in mg (per 1" x 2" panel)	Test Result
Aquaox Disinfectant 525	Clad 2024-T3 Aluminum (QQ-A-250/5)	0.1	Conforms
	Bare 2024-T3 Aluminum (QQ-A-250/4) alodined per MIL-C-5541	2.3	Conforms
	Bare 2024-T3 Aluminum (QQ-A-250/4) anodized per MIL-A-8625 Type I	0.3	Conforms
	Bare 7178-T6 Aluminum (QQ-A-250/14) anodized per MIL-A-8625 Type I	2.9	Conforms

Conclusion

The Aquaox Disinfectant 525 conformed on all test panels for all test criteria, that is, no concerns regarding corrosion during immersion arose. If this is true for the 525 concentration, it will hold for the lower 275 concentration.

C. RUBBER TEST (Reference: ASTM D471)

This test evaluates the comparative ability of rubber and rubber-like compositions to withstand the effect of the disinfectants.

Procedure

Rubber specimens (vulcanized rubber sheets, vulcanized-rubber-coated fabric, and articles finished with rubber) are immersed in the most concentrated version of the disinfectant for 24 hours and are evaluated based on the criteria in the table below:

Aquaox Test Results:

Test Chemical	Property	Maximum Change Allowed	Test Result
Aquaox Disinfectant 525	Tensile Strength	25 % Loss	< 5 %
	Elongation	25 % Loss	< 5 %
	Volume	± 15 % Loss	< 5 %

Conclusion

Aquaox 525 conforms on all test specimens for all test criteria.

D. SEALANT TEST

This test evaluates whether the most concentrated disinfectant solution affects a sealed surface.

Procedure

A paint-primed Aluminum surface is smeared with a sealant. This treated surface is submerged in a test fluid for approximately 70 hours at a temperature of around 120 degrees Fahrenheit. The expected result is that there should be no signs of the sealant lifting or detaching from the surface after the immersion test.

Aquaox Test Results:

Test Chemical	Test Result
Aquaox Disinfectant 525	Sealant did not lift at edges or lose adhesion.
Test Control	No lifting or loss of adhesion when pried away from edge.

Conclusion

Aquaox Disinfectant 525 conforms on all test surfaces for all test criteria.

E. PAINTED SURFACE TEST (Reference: ASTM F502)

This test determines the effect of the more concentrated disinfectant on painted aircraft surfaces.

Painted aluminum alloy samples are subjected to a durability test using heated 525 disinfectant (~149). The test checks for any surface damage, such as streaking, color changes, or blistering, and evaluates the paint's softening by identifying the weakest pencil that can penetrate the paint. The paint must not visibly change color or soften by more than two levels of pencil hardness to pass the test.

Aquaox Test Results:

Test Chemical	Property	Test Result
Aquaox Disinfectant 525	Pencil Hardness Change	0
	Color Change	None

Conclusion

Aquaox Disinfectant 525 conformed on all test specimens for all test criteria.

F. TEDLAR SURFACE TEST

This test determines whether Aquaox 525 leaves any scratching, color change or staining on Tedlar, a polyvinyl fluoride film.

Procedure

Surfaces are observed for scratches or stains needing polish after being exposed to a test liquid at room temperature and rinsed. They shouldn't display scratching, significant color change, or staining.

Aquaox Test Results:

Test Chemical	Test Result
Aquaox Disinfectant 525	No Scratching, Color Change or Staining of specimens is observed.

Conclusion

Test results of Aquaox Disinfectant 525 conform on all test specimens for all test criteria.

G. VINYL SURFACE TEST

This test ensures exposure to Aquaox 525 does not leave any cracking, brittleness, color change or staining on the test vinyl surfaces (poly vinyl chloride – generally considered less durable than Tedlar).

Procedure

Test surfaces are exposed to Aquaox 525 room temperature and then rinsed and examined.

Aquaox Test Results:

Test Chemical	Test Result
Aquaox Disinfectant 525	No Scratching, Color Change or Staining of specimens is observed.

Conclusion

Test results of Aquaox Disinfectant 525 conform on all test specimens for all test criteria.

H. FABRIC AND CARPET TEST

This test determines if exposure to Aquaox 525 causes any color change or staining on the test fabric or carpet and also whether exposure increases flammability.

Procedure

Fabric and carpet surfaces are dipped in test liquid, rinsed, and checked for color changes or stains. For flammability, fabric and carpet samples are soaked in the liquid, dried, and then set on fire for 12 seconds. Burn length, self-extinguishing time, and drip extinguish time are then measured and compared to set standards.

Aquaox Test Results:

Test Chemical	Test Surface	Property		Maximum Value	Test Result
Aquaox Disinfectant 525	**Upholstery**	Color Change		N/A	None
		Staining		N/A	None
		Flammability	Extinguishing Time	15 seconds	< 3 seconds
			Burn Length	8 inches	7 inches
			Drip Extinguish Time	5 seconds	< 3 seconds
	Carpet	Color Change		N/A	None
		Staining		N/A	None
		Flammability	Extinguishing Time	15 seconds	< 3 seconds
			Burn Length	8 inches	4 inches
			Drip Extinguish Time	5 seconds	< 3 seconds

Conclusion

Test results of Aquaox Disinfectant 525 conform on all test specimens for all test criteria.

I. LEATHER AND NAUGAHYDE TEST

This test evaluates the compatibility of Aquaox Disinfectant 525 leather and Naugahyde surfaces.

Procedure

Leather and Naugahyde samples are soaked in Aquaox Disinfectant 525 and examined for any signs of crackling, brittleness, color change, or staining.

Aquaox Test Results:

Test Chemical	Property	Test Result
Aquaox Disinfectant 525	Cracking or Brittleness	None
	Color Change or Staining	None

Conclusion

Aquaox Disinfectant 525 conforms on all test specimens for all test criteria.

J. POLYCARBONATE CRAZING TEST (Reference: ASTM F484)

This test method covers the procedure for determining the crazing effect (i.e., the creation of fine spider-web-like surface cracks) caused by the Aquaox products when exposed test materials are then subjected to the stress of bending.

Procedure

Common aircraft plastics are bent slightly and soaked in a the Aquaox 525 liquid for 10 minutes. Afterward, they're checked visually for any cracks or surface damage.

Aquaox Test Result / Conclusion:

Test Chemical	Test Surface	Test Result
Aquaox Disinfectant 525	Lexan 9600	No cracking or crazing
	BMS8-400 BAC 70913	No cracking or crazing

Conclusion

Aquaox Disinfectant 525 results conform on all test specimens for all test criteria.

K. FLASH POINT TEST (Reference: ASTM D93)

This test is done for information only. The flash point of the test liquid is determined following the ASTM D93 method, all cleaning candidates having a flash point not lower than 212°F must then be approved by the Fire Protection Engineering before they can be evaluated to be used.

Conclusion

As expected, no flash point for the Aquaox Disinfectant is observed to 212°F for the test liquid.

SUMMARY OF CONCLUSIONS FOR ALL TESTS ON SURFACE EFFECTS[8]

Test results of Aquaox Disinfectant 525 (hence by implication for the lower-concentration product Aquaox 275) conform for all test criteria on all the tests included in the Boeing D6-7127 Protocol except for the Clad 7075 T6 Aluminum Alloy surface of the Sandwich Corrosion Test, where a mild effect occurred with Aquaox 525. By comparison, Aquaox Disinfectant 275 passed the Sandwich Corrosion Test.

[8] **References**

- SMI Test Report, Boeing D6-7127 Protocol, Aquaox Disinfectant 525, SMI/REF # 1412-370
- SMI Test Report, Boeing D6-7127 Protocol, Aquaox Disinfectant 275, SMI/REF # 1503-629
- Aquaox Certificate of Analysis, Aquaox Disinfectant 525, dated 01/14/15
- Aquaox Certificate of Analysis, Aquaox Disinfectant 275, dated 03/27/15

APPENDIX

AQUAOX ELECTROSTATIC SPRAY SAFETY ASSESSMENT - DETAILS

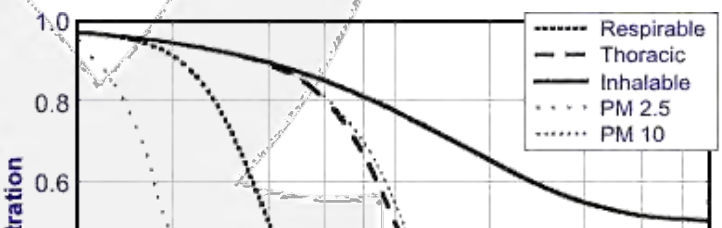
I. Particle Size

Acute Inhalation Injury and Particle Size of Inhaled Substances

The airways and lungs receive continuous first-pass exposure to non-toxic and irritant or toxic gases via inhalation. Smoke, chlorine, phosgene, sulfur dioxide, hydrogen chloride, hydrogen sulfide, nitrogen dioxide, ozone, and ammonia are common irritants. Damage can be widespread due to the gaseous nature of these elements. Acute inhalation injury may result from frequent and widespread inhalation of these elements, which are commonly caused by the use of household cleaning agents and industrial gases including chlorine and ammonia[1].

Inhaled substances may affect the respiratory system at various levels according to various factors, an important factor of which is the particle size of that substance. Bigger particles have enough mass and inertia to be trapped in the airway between the nostril and bronchi when inhaled, while smaller particles are drawn deeper into the lungs. Particularly large particles tend to become trapped in the nose, and are expelled by sneezing or blowing the nose. Therefore, the bigger the particle of the inhaled substance, the less likely they are to cause any damage to the respiratory system [2].

Particle size in inhalation toxicity is usually defined by their mass median aerodynamic diameter (MNAD) and aerodynamic equivalent diameter (AED) in micrometers (μm). According to Human Health Risk Assessment of Inhaled Materials, the term inhalable fraction refers to the mass fraction of particles capable of entering into the respiratory system. Among the inhalable fraction there are three categories, extrathoracic fraction, the thoracic fraction, and the respirable fraction. Particles of >25μm AED generally fall into the extrathoracic fraction, the fraction of the inhalable particles that can deposit in the area of the respiratory tract lying between the nostrils/ mouth and the distal end of the larynx. Particles of ≤25μm AED fall into the thoracic fraction, fraction of inhalable particles that can penetrate the head airways and enter the airways of the lung. Particles of ≤10μm AED fall into the respirable fraction, fraction of particles capable of penetrating the respiratory tract to the level of the on-ciliated airways and gas-exchange regions of the lungs. Figure 1 below shows the relationship between the % penetration into the respiratory system vs the particle size of the inhaled particles. Tree pollens have a particle size of 10 – 100 μm and atmospheric dust has a particle size of 0.001 - 40 μm whereas viruses have a particle size of 0.002 – 0.03 μm. The US EPA generally controls substances with a particle size of less than 10μm. The SEHSC recommends using 30μm MMAD (Mass Median Aerodynamic Diameter) with no more than 1% of particles having an AED of ≤10μm as the cutoff when considering a consumer aerosol application to ensure all aerosol particles to be trapped in the nasopharyngeal region[3]. In general, inhaled particles with larger particle size of >25μm are of a less concern because they tend to be trapped in the nasopharyngeal region and be expelled through sneezing and talking, thus less likely to harm the respiratory system.



Background of Aquaox ESS Sprayer and Dispensed Droplet Size

The Aquaox Electrostatic Sprayer features the ES 3001-5 Model. The device is a portable electrostatic aerosol applicator that utilizes a 3-nozzle air-assist design. The device is intended for applications of water-based formulations and is useful for dispensing most chemicals which are labeled aerosol or mist applications.

The device consists mainly of a motor/blower assembly, a high voltage power supply, a charging ring and electrode, a blower housing, a nozzle, a formulation tank and a metering valve. The flow rate of the liquid to be dispensed is regulated by a one-turn precision metering valve and determines the output particle sizes. The particle sizes of the dispensed particles typically range from 10 to 50 µm VMD (Volume Mean Diameter). There are three positions on the metering valve, positions 1, 2, and 3. Each position designates a different flow rate, which results in a different particle size range. Table 1 below shows the approximate flow rate and resulting droplet size of each position setting. Generally, the output droplet size increases with increasing flow rate.

Table 1: Valve Position Settings, Approximate Flow Rates and Droplet Size

Position	Flow Rate	Droplet Size (µm VMD)
1	6 oz/min (177 mL/min)	10 – 20
2	7.5 oz/ min (222 mL/min)	20 – 30
3	9.5 oz/min (281 mL/min)	30 – 50

Aquaox's Modification and Droplet Size of the Aquaox ESS

The metering valve mentioned above functions as an obstruction to the liquid being delivered to the device, and thus regulates the liquid flow rate. Aquaox has removed this metering valve and the formulation tank as part of the customization of this device. The removal of the metering valve results in no obstruction of the liquid flow into the device, thus the liquid is delivered to the device with a flow rate of above 9.5 oz/min. According to Table 1 above, the flow rate of 9.5 oz/min correlates to a droplet size range of 30 – 50 µm VMD. Since increasing flow rate associates with increasing particle size, the particle size will definitely be larger than 30 µm VMD as a result of the removal of the metering valve.

Original Spray Head with Metering Valve	Modified Spray Head with No Metering Valve
	

Aquaox ESS and Effect of Sprayed Droplets on Respiratory System

The dispensed particles of the Aquaox Electrostatic Sprayer should not cause any harm to the respiratory system due to the following reasons, 1) the output particle size, 2) falling time of particles, and 3) electrostatic ion field. These rationales are further explained below.

The output droplet size of the Aquaox ESS should always be larger than 30 µm VMD due to the modification of the device. As explained in the previous page, inhaled substances with a particle size of larger than 30 µm tend to be trapped in the nasopharyngeal region and thus expelled through sneezing, thus less likely to harm the respiratory system, according to previous research literatures. Therefore, the droplet size of the Aquaox ESS should be large enough not to cause any respiratory issues.

Secondly, according to the WHO Pesticide Evaluation Scheme (Table 2 below), the time it takes for a droplet size ranging from 20 – 50 µm VMD to fall 10 meters ranges from 14 minutes to 135 seconds. Aquaox implements a 10-minute dwelling time before reentry into the sprayed area as part of Aquaox instructions in the operation of the sprayer. Therefore, by the time one reenters the sprayed area, most, if not all, particles should have fallen and deposited on the floor, and thus not likely to be inhaled.

Table 2: Time required for a droplet to fall 10 meters (WHO Pesticide Evaluation Scheme)

Droplet Size (µm VMD)	Time to fall 10 meters	Droplet Density (no/cm^3)
1	93.7 hours	19120.0
5	3.7 hours	152.0
10	56 minutes	19.2
20	14 minutes	2.38
50	135 seconds	0.150
100	36 seconds	0.0192

Last but not least, the device features "electrostatic ion field," which the dispensed droplets pass through as they exit the nozzles. This results in electrostatically charged droplets which gravitate to neutral objects to form a uniform coverage. Particles of the smaller particle size range will be attracted to the nearby surfaces as a result of the electrostatic charge. Thus, upon completion of the dwelling time, larger particles will have deposited onto the floor due to gravity while smaller particles will be attracted to nearby surfaces due to electrostatic charge. This results in very few, if not none, air particles floating in the air and to be inhaled when someone reenters the room after the dwelling time.

In conclusion, the output particle size of the Aquaox ESS should be above 30 µm VMD, which should be big enough to be trapped in the nasopharyngeal region and not likely to harm the respiratory system. Furthermore, large particles should have deposited onto the floor due to gravity and small particles should have attached onto nearby surfaces due to electrostatic charge upon collapse of the dwell time, which results in theoretically no particles floating in the air and being inhaled by someone reentering the sprayed area. All the above rationales support that the Aquaox ESS should not cause any harm to the respiratory system if used following the Aquaox protocol.

II. Chlorine Exposure Limits

OSHA Standards on Chlorine Exposure Limits

The solutions that the Aquaox ESS dispenses include Aquaox Disinfectant 275 (AX275) and Aquaox Disinfectant 525 (AX525). The former solution contains 275 ppm Hypochlorous Acid (HOCl) while the latter contains 525 ppm HOCl as active ingredient. OSHA has not yet implemented a standard regulating HOCl exposure limits nor a method for determining HOCl concentration. Therefore, the standards for Chlorine have been adopted when concerning the safety of sprayed particles of the Aquaox ESS. Current OSHA permissible exposure limits (PEL) for Chlorine include a short-term exposure limit for up to a 15-minute exposure not to exceed 1 ppm (2.9 mg/m^3 where mg/m^3 is defined as mg Chlorine per m^3 of air), and a time-weighted average for up to 8 hours not to exceed 0.5 ppm (1.5 mg/m^3). Two experiments have been conducted internally to verify that the Aquaox ESS complies with the above required limits.

Experiment 1: Assessment of HOCl Concentration in Air Samples following NIOSH 7607 Method

The HOCl concentration in air samples in a sprayed area is assessed via the NIOSH 7607 Method. The Aquaox ESS is operated following the Aquaox ICS protocol in a 12ft x 18ft experimental room that has been constructed to mimic an average patient room in a hospital. Air samples are collected from the experimental room after a 5-minute spraying time and a 10-minute dwelling time. Upon completion of the dwelling time, air samples are collected for a period of 15-minutes (for determining the short term exposure level) and a period of 6 hours (for determining the time-weighted average level). Air sample is pumped into

a pre-coated sample collection tube via a calibrated AirChek sample pump at a rate of 1 L/minute. Samples are collected internally at Aquaox and sent out to ALS Environmental at Salt Lake City for analysis.

The sample collection tubes are prepared and the analysis is done following the NIOSH 7607 method. The sample collection tube is a tube of silica gel coated with sulfumic acid and potassium iodide. The collection tubes do not contain the filter cassettes because we do not intend to analyze trichloramines in our samples. The treated silica gel is nonspecific and traps soluble chlorine compounds including mono- and di-chloramines, hypochlorous acid, hypochlorites, and chlorine. The reaction of these chlorine compounds with potassium iodide in an acid medium yields chloride ion, which are then analyzed. Mobile Phase Ion Chromatography with suppressed conductivity detection is the technique used in the analysis. The analysis results should only capture hypochlorous acid, hypochlorites, and chlorine because there are no nitrogenous compounds in our samples to form any chloramines.

Specifications of Test Solutions:

1st Trial:
Air Sample Collection Date: October 5, 2015 (AX275)
October 6, 2015 (AX525)

Specifications of Test Solutions:

	Aquaox Disinfectant 275	**Aquaox Disinfectant 525**
FAC (ppm)	300	546
pH	6.79	6.58
ORP	832	873
Conductivity	2323	3250

2nd Trial:
Air Sample Collection Date: October 8, 2015 (AX275)
October 9, 2015 (AX525)

	Aquaox Disinfectant 275	**Aquaox Disinfectant 525**
FAC (ppm)	281	556
pH	6.78	6.52
ORP	842	877
Conductivity	2405	4832

Interpretation:

Experiment 2: Assessment of HOCl Concentration in Air Samples following the OSHA ID-101 Method

The above experiment is repeated internally following the OSHA ID-101 method and only short-term exposure is determined. The Aquaox ESS is operated following the Aquaox ICS protocol in a 12in x 18in experimental room as described above. Air samples are analyzed from the experimental room after a 5-minute spraying time and a 10-minute dwelling time. Upon completion of the dwelling time, air samples are collected for a period of 15-minutes for determining the short term exposure level. Air sample is pumped into a midget fritted glass bubbler containing 0.1% sulfumic acid solution using a calibrated AirChek sample pump at a rate of 1 L/minute. The collected sample then reacts with DPD (N,N-diethyl-p-phenylenediamine) in the presence of potassium iodide to yield a red-colored product that absorbs at a wavelength of 540nm. The concentration of HOCl in the original air sample can be determined by determining the absorbance at 540nm.

The experiment is done as described below. A standard solution with a known HOCl concentration is first generated. The absorbances of different volumes of this standard solution will then be determined and that will correspond to the different HOCl concentrations at different volumes of standard solutions (Graphs 1 and 2). The absorbances of different weights of the standard solutions will then be plotted against the corresponding HOCl concentrations (Graph 3) to generate a standard curve. The HOCl concentration of the test sample can be determined by comparing the absorbance of the test sample to the standard curve.

Test Result:

Air Sample Collection Date: October 7, 2015 (AX275)
October 7, 2015 (AX525)

Specifications of Test Spray Solutions:

	Aquaox Disinfectant 275	**Aquaox Disinfectant 525**
FAC (ppm)	287	525
pH	6.82 / 25.7C	6.57 / 25.7C
ORP	852	878
Conductivity	2420	3162

Specifications of Standard Solution:

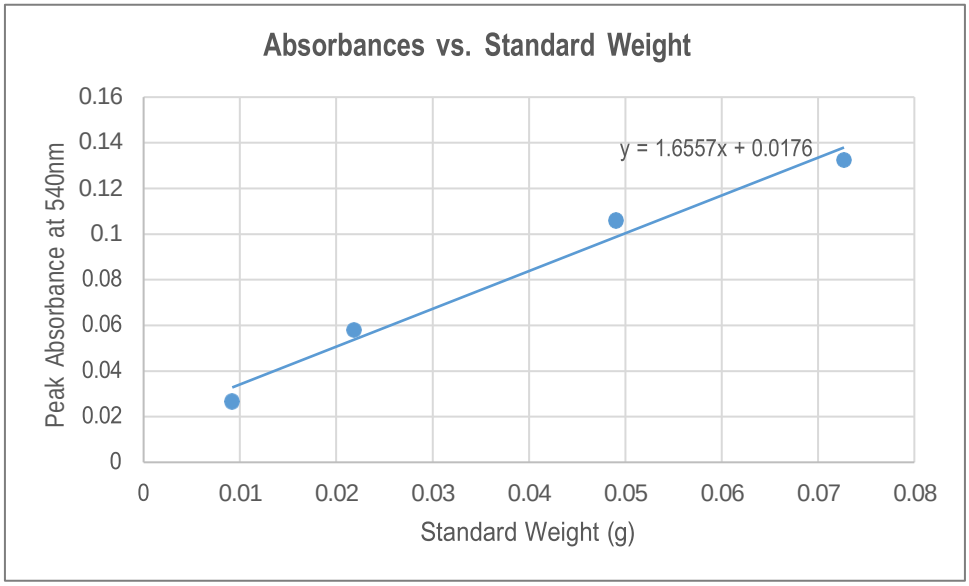
FAC (ppm)	295
pH	6.90 / 25.7C

Aquaox Disinfectant 275:

Absorbances of Standard and Collected Sample:

		Peak Absorbance at 540nm
295ppm Standard	0.0092g (10uL)	0.0266
	0.0218g (25uL)	0.0580
	0.0490g (50uL)	0.1060
	0.0727g (75uL)	0.1325
Collected Sample		**0.0550**

Standard Curve:



Calculations:

Absorbance of the Collected Sample:	0.0550
Corresponding Standard Weight: (determined using the linear equation y = 1.6557x + 0.0176)	0.0226g
HOCl Concentration of Standard:	295ppm
Corresponding Weight of HOCl in Air Sample:	0.0226g x 295ppm = 6.667ug
Volume of Air Sample Collected:	15L
Molecular Weight of HOCl:	52.46 g/mol
* HOCl Concentration of Air Sample, mg/m^3:	**0.444mg/m^3**
* HOCl Concentration of Air Sample, ppm:	**0.207ppm**

** The formulae for direct comparison with OSHA PEL for gas and aerosol is used according to NIOSH Manual of Analytical Methods as explained below.*

Physical Form of Substance Sampled	Unit of Air Concentration	Formula for Direct Comparison With OSHA PEL Table
Gas	ppm	$C_v = \frac{m \cdot 10^3}{V} \cdot \frac{24.46}{MW}$
Gas	mg/m3	$C = \frac{m \cdot 10^3}{V}$
Aerosol	mg/m^3	$C = \frac{m \cdot 10^3}{V}$

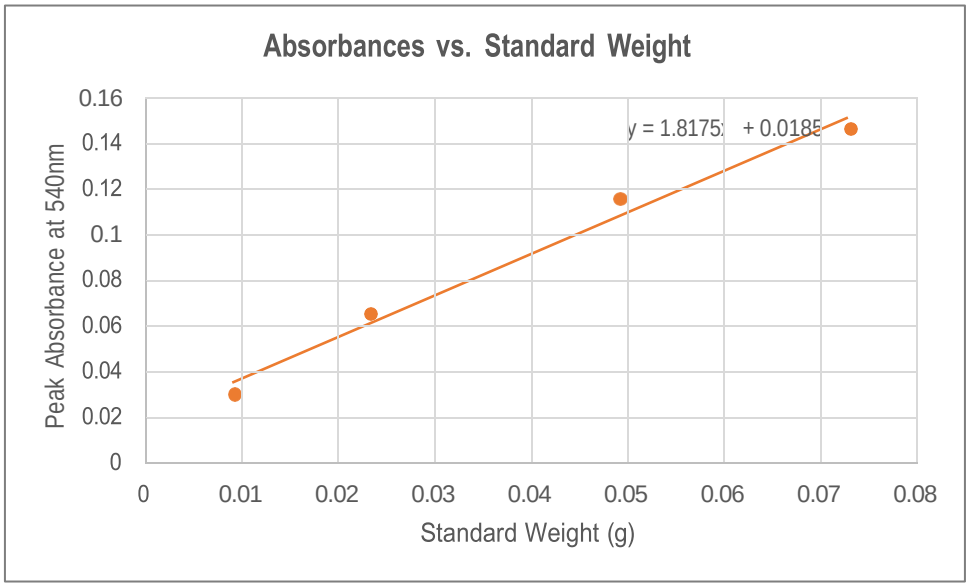
where: m = actual mass of substance, in mg, found on the sampling device
V = air volume, L, taken at the sampling site, ambient temperature and pressure
24.46 = the volume (L) of 1 mole of gas at 25 °C and 760 mm Hg
C_v = air concentration, ppm by volume, at 25 °C and 760 mm Hg
C = air concentration, mg/m^3
MW = molecular weight, grams/mole

Aquaox Disinfectant 525

Absorbances of Standard and Collected Sample:

		Peak Absorbance at 540nm
295ppm Standard	0.0092g (10uL)	0.0295
	0.0233g (25uL)	0.0648
	0.0492g (50uL)	0.1152
	0.0731g (75uL)	0.1460
Collected Sample		**0.0704**

Standard Curve:



Calculations:

Absorbance of the Collected Sample:	0.0704
Corresponding Standard Weight: (determined using the linear equation y = 1.8175x + 0.0185)	0.0286g
HOCl Concentration of Standard:	295ppm
Corresponding Weight of HOCl in Air Sample:	0.0286g x 295ppm = 8.424ug
Volume of Air Sample Collected:	15L
Molecular Weight of HOCl:	52.46 g/mol
* HOCl Concentration of Air Sample, mg/m^3:	**0.562mg/m^3**
* HOCl Concentration of Air Sample, ppm:	**0.262ppm**

Interpretation:

After a 5-minute spray time and a 10-minute dwell time using the AX275 solution, the HOCl concentration in the air sample collected in a 15-minute collection time is 0.444mg/m^3 (0.444mg HOCl per m^3 of air volume or 0.207ppm). For the AX525 solution, the HOCl concentration i is 0.562mg/m^3 (0.562mg HOCl per m^3 of air volume or 0.262ppm). Since there is not a standard level established for Hypochlorous Acid, the current OSHA PEL for Chlorine, 1 ppm (or 2.9 mg/m^3), is used. The HOCl levels in the collected air samples when using both test solutions are well below the OSHA PEL for Chlorine.

Experiment 3: Immediate Assessment of Chlorine Gas Concentration in Air Samples via the Draeger System

Chlorine gas concentration in immediate air samples is assessed via the Draeger Chlorine 0.2/a System. This system has a measurement range of 0.2 – 3 ppm for Chlorine gas and is widely used for detecting gases and vapors in industrial workspaces. The system contains the Draeger accuro pump and the Draeger tubes. The Aquaox ESS is operated following the Aquaox ICS protocol in the 12in x 18in experimental room as discussed above. Air samples are collected from the experimental room at two different time frames, 1) immediately after the 5-minute spray time and 2) upon completion of the 10-minute dwelling time after the spray time. Air samples are collected into the Draeger tube and test results are interpreted by the length of color change in the tube. Samples are collected and analyzed internally at Aquaox.

The chemistry behind this method is further explained below. The Draeger tubes are glass vials filled with a chemical reagent that reacts to the target chemical (or family of chemicals) to be measured. In this case, the tubes contain the chemical o-tolidine, which when reacts with Chlorine will yield a yellow orange reaction product. The pump draws a calibrated 100 mL of air sample into the tube with each stroke, and 10 strokes are performed for each experiment. Any Chlorine in the air sample will react with the reagent and yield a yellow orange reaction product. The length of the color change in the tube indicates the amount of reaction product, and thus the Chlorine gas concentration in the original air sample.

Test Result:

Aquaox Disinfectant 275		**Aquaox Disinfectant 525**	
Immediate after Spray Time	10 Minutes after Spray Time	Immediate after Spray Time	10 Minutes after Spray Time
			

	Aquaox Disinfectant 275	**Aquaox Disinfectant 525**
Test Date	October 8, 2015	October 7, 2015
Test Time	10:05am / 10:15am	9:30am / 9:40am
Immediately after Spray Time	0.2ppm	0.5ppm
10-Minute after Spray Time	<0.2ppm	0.2ppm

Specifications of Test Solutions:

	Aquaox Disinfectant 275	**Aquaox Disinfectant 525**
FAC (ppm)	281	525
pH	6.78	6.57
ORP	842	878
Conductivity	2405	3162

Interpretation:

After spraying with the AX275 solution for 5 minutes, a residual Chlorine of 0.2ppm immediately after the spray time and less than 0.2ppm was detected 10 minutes after the spray time. For the AX525 solution, a residual Chlorine of 0.5ppm immediately after the spray time and 0.2ppm was detected 10 minutes after the spray time. As a result, the residual Chlorine level in the air is always below the regulated concentration of 1 ppm under both scenarios (immediately or 10 minutes after),