AQUAOX™ On-Site-Generated Disinfectants

TECHNICAL SUMMARY

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INTRODUCTION

AQUAOX™ on-site Generators electrolyze a solution of salt and water to produce Hypochlorous Acid (HOCl), an effective, ecologically sound antimicrobial for use on hard surfaces. Produced in batches of 275-ppm, 525-ppm, and 1650-ppm, the products retain effectiveness for 30 days. Their disinfectant properties have been tested by EPA-certified labs following Good Lab Practice (GLP) guidelines and recognized as compliant by the U.S. Food and Drug Association (FDA), the Environmental Protection Agency (EPA), and the European Chemicals Agency (ECHA).

ON-SITE GENERATOR REGULATORY STATUS

On-site generators producing hard-surface disinfectants like hypochlorous acid through electrolysis are classified as devices by the EPA. While the EPA doesn't mandate registration for these devices, they must still adhere to certain pesticide requirements under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the federal law establishes the basic U.S. system of pesticide regulation to protect applicators, consumers, and the environment.

FIFRA REQUIREMENTS

On-site generating devices must adhere to FIFRA provisions, including labeling standards and production in registered places. They are also subject to EPA's labeling requirements, which forbid any misleading information on the device's label.

Key label elements include the product's name, ingredients, effectiveness statements, hazards, and usage directions. These specifications are laid out under FIFRA section 2(q)(1) and section 12 as well as 40 CFR 152.500 and 156.10.

FIFRA emphasizes accurate composition, antimicrobial effectiveness, and safety statements. While devices don't need formal registration, manufacturers self-certify their performance and safety. Third-party data can validate pesticide claims for on-site generated disinfectants without a certified lab or EPA fees. The EPA requires device manufacturers to back their claims, expecting on-site generators with disinfecting claims to match registered pesticide standards. State regulations may also apply.

PRODUCT EFFICACY DETERMINATION

AQUAOX™ produces On-site generated Hypochlorous Acid (HOCL) disinfectants electrochemically from Sodium Chloride and water. Using the American Society for Testing and Materials (ASTM) and United States Pharmacopeia (USP) National Formulary General Chapter 51 standards, multiple studies have evaluated the solutions' disinfecting capabilities, validating AQUAOX™ claims.

1. **USP 51 - Antimicrobial Effectiveness Test**
   This test determines the appropriateness of a preservative for a product and its effectiveness throughout the product's declared shelf life. It meets both USP <51> and EP 5.1.3 requirements.

2. **ASTM E1052 - Efficacy of Antimicrobial agents against Viruses in Suspension**
   A suspension test assessing the antimicrobial solutions' potency against specific viruses, suitable for most viruses and designed for cell culture systems.

3. **ASTM E1053 - Method to Access Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces**.
   Evaluates the virucidal efficiency of microbicides for nonporous environmental surfaces in various forms.

1. 4. **ASTM E2315-03 - Assessment of Antimicrobial Activity Using a Time-Kill Procedure**
   This method gauges the impact on aerobic microorganism populations when exposed to antimicrobial test materials.

LABORATORY EFFICACY STUDIES

AQUAOX™ Hypochlorous Acid solutions sold and distributed in ready-to-use (spray) bottles – created equivalently to those generated on-site – underwent extensive testing on bacteria, spores, viruses, and fungi to validate their disinfectant properties. The ready-to-use solutions adhered to the Lowest Certified Limit Testing Guidelines (2013). The efficacy tests provided align with the contact times mentioned in AQUAOX™ EPA labels: AQUAOX™ Disinfectant 275, 525, and 1650.

For the latest up-to-date EPA labels, we refer to the following links:

- AQUAOX™ Disinfectant 275: https://ordspub.epa.gov/ords/pesticides/f?p=PPLS:8:17335217651098::NO::P8_PUID,P8_RINUM:514 876,93392-1
- AQUAOX™ Disinfectant 525: https://ordspub.epa.gov/ords/pesticides/f?p=PPLS:8:17335217651098::NO::P8_PUID,P8_RINUM:514 953,93392-2
- AQUAOX™ Disinfectant 1650 https://ordspub.epa.gov/ords/pesticides/f?p=PPLS:8:17335217651098::NO::P8_PUID,P8_RINUM:539 899,93392-3

Further GLP tests with varied active chlorine levels were executed for contact times of ≤ 1 and ≤ 5 minutes and are detailed in TABLES 2 and 3, respectively.

In addition, the ECHA has approved active chlorine from hypochlorous acid for use in certain biocidal products. Active chlorine released from hypochlorous acid is approved as an active substance for use in biocidal products of product- types 2, 3, 4 and 5 subject to the specifications and conditions set out in the list below, in Table 1, immediately below:

TABLE 1 – ECHA APPROVED HYPOCHLOROUS APPLICATIONS

BPF2; Surface disinfection	PT2	#4a	**Instrument disinfection by immersion or filling (Hard surfaces / instruments / equipment disinfection)**	**550 ppm – pH = 5.5-7.7 / 5 minutes 20°C Bacteria, Yeast, Virus and fungal spores.**
	PT2	#4b	**Hard surfaces Health care (Hard surfaces / instruments / equipment disinfection)**	**236 ppm – pH = 6.3-6.4 / 5 minutes 20°C Bacteria, Yeast.**
	PT2	#4c	**Hard surfaces non-Health care (Hard surfaces / instruments / equipment disinfection)**	**224 ppm – pH = 6.4 / 5 minutes 20°C Bacteria**
	PT3	#18	**Instrument/equipment disinfection**	**550 ppm – pH = 5.5-7.7 / 5 minutes 20°C Bacteria, Yeast, Virus and fungal spores.**
	PT3	#28a	Surfaces veterinary (Products used for disinfection of the materials and surfaces associated with the housing or transportation of animals)	499 ppm – pH = 3.7-6.4 / 30 minutes 10°C Bacteria and yeast (pending on porous surfaces result EN 16437)
	PT3	#28b	Hard surfaces and transport vehicles (Products used for disinfection of the materials and surfaces associated with the housing or transportation of animals)	499 ppm – pH = 3.7-6.4 / 30 minutes 10°C Bacteria, Yeast and viruses (pending on viruses result EN 14675)
	PT4	#30a	Instrument disinfection by immersion or filling (Hard surfaces / instruments / equipment disinfection)	236 ppm – pH = 6.3 - 5 minutes 20°C Bacteria and Yeast
	PT4	#30b	Hard surfaces food (Hard surfaces / instruments / equipment disinfection)	236 ppm – pH = 6.3 - 5 minutes 20°C Bacteria and Yeast
	PT1	#1a	Hygienic handrub non-medical use	550 ppm – pH = 4.5-5.0 / 60 sec. Bacteria, Virus and Yeast

TABLE 2 – EFFICACY TEST SUMMARY AQUAOX™ DISINFECTANT WITH A CONTACT TIME OF 1 MINUTE OR LESS

Test Product	Study Type	Test Method	Challenge Organisms	Organism Type	Results
Aquaox Disinfectant tested at 10 ppm 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 K. pneumoniae:> 4.98 P. Vulgaris:>4.98 A. baumannii:>5.12
Aquaox Disinfectant 250 ppm	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
Aquaox Disinfectant 250 ppm	Antimicrobial Effectiveness Study Using a Time Kill Assay	ASTM Guideline E2315-03	*Bacteroides fragilis* *Haemmophilus influenza* *Streptococcus pyogenes*	Gram-Negative Bacteria Gram-Positive Bacteria Gram-Positive Bacteria	Log reduction in 15 s. B. fragilis:> 5.89 H. influenza:> 4.44 S. pyogenes:> 5.79
Aquaox Disinfectant 250 ppm	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. marcescens:> 5.43
Aquaox Disinfectant 250 ppm	Antimicrobial Effectiveness Study Using a Time Kill Assay	ASTM Guideline E2315-03	*Enterbacter 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
Aquaox Disinfectant 250 ppm	Antimicrobial Effectiveness Study Using a Time Kill Assay	ASTM Guideline E2315-03	*Myobacterium bovis – BCG*	Bacteria that causes Tuberculosis in humans	>5.21 log reduction in 60 s.
Aquaox Disinfectant 250 ppm	Assessment of Microbicidal Activity against Viruses in suspension	ASTM Guideline E1052, E1482	*Hepatitis B Virus*	Virus	>5.25 log reduction in 30 s.
Aquaox Disinfectant 250 ppm	Assessment of Microbicidal Activity against Viruses in suspension	ASTM Guideline E1052, E1482	*Rhinovirus type 37*	Virus	>3.75 log reduction in 60 s.
Aquaox Disinfectant 250 ppm	Antimicrobial Effectiveness Study Using a Time Kill Assay	USP<51> Guideline	*Aspergillus brasiliensis*	Fungus	Log reduction in 15 s. A. Brasiliensis: = 4.11
Aquaox Disinfectant (Tested at 10 ppm FAC)	Antimicrobial Effectiveness Study Using a Time Kill Assay	USP<51> Guideline	*Candida albicans*	Fungus	> 4.38 log reduction in 15 s.
Aquaox Disinfectant 250 ppm	Antimicrobial Effectiveness Study Using a Time Kill Assay	ASTM Guideline E2315-03	*Candida albicans*	Fungus	> 5.31 log reduction in 15 s.
Aquaox Disinfectant 250 ppm	Antimicrobial Effectiveness Study Using a Time Kill Assay	ASTM Guideline E2315-03, E2839-11	*Clostridium difficile* – spore form	Spore	> 5.35 log reduction in 30s.

TABLE 3 – EFFICACY TEST SUMMARY AQUAOX™ DISINFECTANT WITH A CONTACT TIME OF 5 MINUTES OR LESS

Pathogen	Required Doses FAC (ppm)	Required Contact time (minutes)
[No Rinse] [Food Contact] Sanitizer Claims		
Staphylococcus aureus [(ATCC 6538)]	140 ppm[1]	**1 minute**
Salmonella enterica [(ATCC 6539)]	140 ppm[1]	**1 minute**
Escheria coli (ATCC 11229)	140 ppm[1]	**1 minute**
Listeria monocytogenes [(ATCC 19117)]	140 ppm[1]	**1 minute**
Soft Non-Food Contact Surfaces Sanitizer Claims (Natural or Cotton Fabrics)		
Klebsiella aerogenes	477 ppm[2]	2 minutes
Staphylococcus aureus	477 ppm[2]	2 minutes
[Hard Surface] Disinfection Claims – bacteria		
Staphylococcus aureus [(ATCC 6538)]	248 ppm[1]	5 minutes
	477 ppm[2]	2 minutes
	1490 ppm[3]	**1 minute**
Methicillin Resistant Staphylococcus aureus – (MRSA) [(ATCC 33591)]	477 ppm[2]	5 minutes
Salmonella enterica [(ATCC 10708)]	248 ppm[1]	2 minutes
	477 ppm[2]	**1 minute**
Pseudomonas aeruginosa [(ATCC 15442)]	248 ppm[1]	5 minutes
	477 ppm[2]	2 minutes
	1490 ppm[3]	**1 minute**
Escherichia coli (NDM) [(ATCC BA-2469]	477 ppm[2]	5 minutes
Vancomycin resistant *Enterococcus faecalis* (VRE) [(ATCC 700221)]	477 ppm[2]	5 minutes
Vancomycin resistant *Enterococcus faecalis* (VRE) [(ATCC 51229)]	477 ppm[2]	5 minutes
Bordetella bronchiseptica [Kennel cough] (ATCC 10580)	477 ppm[2]	5 minutes
Clostridium difficile – spore (C.Diff or C. Difficile) (ATCC 43598)	477 ppm[2]	5 minutes
Escheria coli (E coli) (ATCC 11229)	477 ppm[2]	5 minutes
Klebsiella pneumonia New Delhi Metallo-Beta Lactamasa (NDM-1), Carbapenem resistant (CRE) *Klebsiella pneumoniae (NDM-1 [*(CRKP), CDC10002	477 ppm[2]	5 minutes

Acinetobacter baumannii [(ATCC BAA-1709)]	1490 ppm[3]	**1 minute**
Mycobacterium bovis (BCG) ([ATCC 35734)]	477 ppm[2]	5 minutes
Swine Influenza Virus (H1N1)(ATCC VR-333)	248 ppm[1]	5 minutes
	477 ppm[2]	2 minutes
Swine Flu Virus (H1N1) A/Swine/1976/31 (ATCC VR-99)	477 ppm[3]	2 minutes
Canine distemper (ATCC VR-1587) [(Strain Snyder Hill)]	477 ppm[3]	2 minutes
Influenza A (H1N1) [(Strain A/Virginia/ATCC1/2009)][(ATCC VR-1736)] [(representative for common flu virus)]	477 ppm[2]	2 minutes
[Human] Hepatitis C [Virus] [(as bovine diarrhea virus)] [(HCV)] [Strain ADL] [(ATCCVR-1422)]	477 ppm[2]	2 minutes
Human Coronavirus strain 229E [(ATCC VR-740)] [†]	477 ppm[2]	2 minutes
Respiratory syncytial virus (RSV) (Strain A-2) [(ATCC VR-1540]	477 ppm[3]	2 minutes
Human Immunodeficiency Virus Type 1 (HIV-1] [(Strain IIIB)] (Mn;zeptometrix #08110027CF)	477 ppm[2]	2 minutes
	1490 ppm[3]	**1 minute**
Adenovirus (1 of Type 1) (Strain 71) (ATCC VR-1)	477 ppm[3]	2 minutes
Rhinovirus [Type 14] [(ATCC VR-283)]	1490 ppm[3]	**1 minute**
Rhinovirus [Type 16] (Strain 11757) [(ATCC VR-283)]	477 ppm[3]	2 minutes
Rotavirus (A or Group A) (Strain WA) (ATCC VR-2018) [(the virus that causes diarhea)]	477 ppm[3]	2 minutes
Murine Norovirus	477 ppm[2]	2 minutes
Norovirus or Norwalk Virus (as Feline Calicivirus) (Strain F-9) (ATCC VR-782)	477 ppm[2]	2 minutes
Feline Calicivirus (ATCCVR-782)	477 ppm[2]	2 minutes
SARS-CoV-2 Virus	477 ppm[2]	2 minutes
Canine parvovirus (ATCC VR-2016) (Strain Cornell)	477 ppm[2]	2 minutes
Candida albicans (ATCC 10231)	477 ppm[2]	5 minutes
[Human] Hepatitis C [Virus] [(HCV)] [Strain ADL] [(ATCCVR-1422)]	477 ppm[4]	2 minutes
Human Immunodeficiency Virus Type 1 (HIV-1] [(Strain IIIB)] (Mn;zeptometrix #08110027CF)	477 ppm[2]	5 minutes

PRODUCT SAFETY

A nonclinical toxicology assessment was conducted on AQUAOX™ On-site Generated Disinfectant products containing Hypochlorous Acid as the active ingredient. The sole inactive ingredient, besides the water of the solution, is residual Sodium Chloride from the electrolysis process, which is FDA-approved CAS RN 8028-77-1) and NSF-certified, ensuring its safety.

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 4.

TABLE 4 – 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

SAFETY CONCLUSION

In vitro exposure of L-929 cells to the product solutions resulted in minor cell lysis, but it was not deemed cytotoxic per USP standards. The solutions weren't primary skin or eye irritants and displayed no sensitization potential. They were considered non-toxic in acute oral and inhalation studies at the highest feasible concentration. A 28-day test showed no adverse effects on skin or wound healing.

Thus, toxicology tests affirm the product's biocompatibility and safety for its intended use.