TECHNICAL SUMMARY

Aquaox On-site Generated Disinfectants

PRODUCT EFFICACY

Aquaox On-site generated disinfectants are Hypochlorous Acid (HOCL) solutions produced electrochemically from Sodium Chloride and water. Using established ASTM standards and USP General Chapter 51 standards, a series of studies have been conducted to characterize the solutions' abilities to disinfect and reduce microorganisms through a one-step disinfecting mechanism. These studies are further discussed below.

1. USP 51 Antimicrobial Effectiveness Test

The USP 51 antimicrobial effectiveness test, also known as the *preservative efficacy test*, is performed to determine if the chosen preservative is appropriate for a product formulation. It is also carried out as part of a stability study, to ascertain whether a preservative system is still effective up to the expiration date of a product. Testing is performed according to compendial requirements in both **USP <51>** and **EP 5.1.3**.

2. ASTM E1052 Efficacy of Antimicrobial agenets against Viruses in Suspension

This laboratory test method is a suspension test used to evaluate the effectiveness of antimicrobial solutions against specific viruses. This test method may be employed with most viruses and is designed for cell culture host systems.

3. ASTM E1053 Method to Access Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces.

This test method is used to evaluate the virucidal efficacy of liquid, aerosol, or trigger-spray microbicides intended for use on inanimate, nonporous environmental surfaces.

4. ASTM E2315-03 Assessment of Antimicrobial Activity Using a Time-Kill Procedure

This basic method measures the changes of a population of aerobic microorganisms within a specified sampling time when tested against antimicrobial test materials in vitro.

TABLE 1. Efficacy Test Summary – Aquaox On-site Generated Disinfectant

Test Product	Study Type	Test Method	Challenge Organisms	Organism Type	Results	Lab
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	NAMSA
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	ATS Lab
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	ATS Lab
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	ATS Lab
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	ATS Lab
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.	ATS Lab
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.	ATS Lab
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.	ATS Lab
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	NAMSA
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.	NAMSA
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.	ATS Lab
Aquaox Disinfectant 250 ppm	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 30s.	ATS Lab

PRODUCT SAFETY

A nonclinical toxicology investigation has been done on the above products as following. The Aquaox On-site Generated Disinfectant products contains Hypochlorous Acid as the active ingredients. The only inactive ingredient in the product solution is residual Sodium Chloride from the electrolysis process. Sodium Chloride (CAS RN 8028-77-1) is listed as an inactive ingredient in FDA CDER database for use in approved drug products. Moreover, the Sodium Chloride used in Aquaox electrolysis process is NSF certified. Therefore, the presence of Sodium Chloride in the Aquaox On-site Generated Disinfectant products does not present a safety concern.

A series of non-clinical toxicology testing has been done on the product solutions to assess their potential local and systemic toxicity. The toxicology studies were conducted at NAMSA and IIT Research Institute (IITRI), both of which being AALAC approved facilities. All toxicology studies conducted were in compliance with Good Laboratory Practice (GLP) regulations.

The GLP toxicology testing program was based on ISO-10993 requirements on biocompatibility testing for a surface device with contact with breached or compromised surface. These studies, together with the study results, are listed in Table 1.

TABLE 2. *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

Exposure to L-929 cells *in vitro* to the product solutions produced a slight cell lysis, which was not considered cytotoxic per USP requirement. Product solutions were also not considered a primary dermal or ocular irritant, and did not show sensitization potential in the dermal and ocular irritation studies. Product was considered non-toxic in both the acute oral toxicity study and the single dose inhalation study when tested at the maximal feasible concentration. In a 28-day repeated dose toxicity study, topical application of the product to intact and wounded skin areas did not result in any treatment-related skin irritation or wound healing issues. Therefore, the results of the toxicology testing program confirmed the biocompatibility and safety profile of the product solutions for its intended use.