

**Final Report submitted to Virox Technologies Inc.
Oakville, Ontario**

**ASSESSMENT OF THE MICROBICIDAL
ACTIVITY OF AN ACCELERATED HYDROGEN
PEROXIDE-BASED BROAD SPECTRUM
CLEANER AND NO-RINSE SANITIZER (AHP-
BSC)**

Syed A. Sattar, Ph.D.

Director

Centre for Research on Environmental Microbiology (CREM)

Faculty of Medicine, University of Ottawa

Ottawa, Ontario, Canada

K1H 8M5

Phone: (613) 562-5800 ext. 8314; *Fax:* (613) 562-5452

E. mail: ssattar@uottawa.ca

October 2004

TABLE OF CONTENTS

	Page No.
OBJECTIVE	3
MATERIALS AND METHODS	3
The Product	3
Soil Load	3
Neutralizer, Microbial Diluent and Filter Rinse	3
Standard Hard Water	3
Test Organisms	3
1. <i>E. coli</i> O157:H7 (MEA Isolate)	3
2. <i>S. choleraesuis</i> (ATCC 10708)	3
THE TEST METHODOLOGY	3
Suspension Test	3
Controls	4
Recovery Media and Detection of Viable Organisms	4
Neutralization Verification	4
PRODUCT PERFORMANCE CRITERIA	4
RESULTS	4
Activity of the AHP-BSC against <i>E. coli</i> O157:H7	4
Activity of the AHP-BSC against <i>S. choleraesuis</i>	4
Neutralization Verification Results to Arrest Activity of AHP	5
CONCLUDING REMARKS	5

OBJECTIVE

The main objective of this study was to determine the effectiveness of AHP-BSC as a sanitizer against *Escherichia coli* and *Salmonella choleraesuis* using a suspension test method.

MATERIALS AND METHODS

The Product:

One lot of the product was provided for testing in this study. Upon arrival in our laboratory, the bottle was stored at room temperature in a place with restricted access. The product was tested at a dilution of 1:128.

Soil Load:

No soil load was used in this testing.

Neutralizer, Microbial Diluent and Filter Rinse:

Lethen Broth (with 1% sodium thiosulfate pentahydrate) was used as the neutralizer and to rinse the membrane filters and the filter holder unit. Normal saline was used to make dilutions of the bacterial suspensions and as the final rinse of the carrier vials and the filter holder unit to aid in rinsing off the froth created by the Lethen broth.

Standard Hard Water:

Water with 200 ppm as calcium carbonate (CaCO₃) was used as the diluent for the product.

Test Organisms:

The organisms used and their specific strains were:

1. *Salmonella choleraesuis* (ATCC 10708)
2. *Escherichia coli* O157:H7 (MEA Isolate)

Stock suspensions of the bacteria were prepared by culturing them in tryptic soy broth (TSB; Difco) for 24 hours at 37°C.

THE TEST METHODOLOGY

The Suspension Test:

The test was carried out by adding 100 µL of the bacterial suspension with no soil load to 900 µL of the test product in a 2 mL-capacity cryovial, vortexed to mix and allowed to sit for the required contact time at room temperature. At the end of the contact time, the reaction mixture received 9.0 mL of the neutralizer and vortexed. This mixture was passed through a membrane filter and the vial was rinsed 2x with 10.0 mL of saline.

Controls:

Controls were tested in the same manner as the test by adding 100 µL of bacterial suspension to 900 µL saline instead of the disinfectant.

Recovery Media and Detection of Viable Organisms:

The control suspensions and the eluates tested were passed through 47 mm diameter membrane filters (Millipore; 0.22µm pore diameter). The filters were then placed on TSA plates, incubated at 37°C, and the colony forming units (CFU) recorded at 24 hour intervals for a total of 5 days.

Neutralization Verification:

Bactericidal Test:

One part of the use-dilution of the product was mixed with 9 parts of the neutralizer. The test organism was added to the neutralized solution. The neutralizer alone was used as the control solution. At the end of a contact time of 5 minutes at room temperature, the mixture was passed through a membrane filter to capture the bacteria. The filters were placed on the appropriate recovery medium. The plates were incubated and the colonies counted.

The time of 5 minutes was selected in these experiments because it is the maximum delay that may occur between the initial dilution of the product in the vial and the last lot of rinse passed through the membrane filter.

PRODUCT PERFORMANCE CRITERIA

The number of repeats in the suspension test was six. The test also included three control repeats. The results are reported as log₁₀ reductions in viability in reference to the control.

For a product to be considered bactericidal it was expected to reduce the viability titre of each test organism by at least 5 log₁₀ under the conditions of this test.

RESULTS

Activity of AHP-BSC against *E. coli* O157:H7: Table 1 summarizes the result of the suspension test. The product was able to bring about a >6 log₁₀ reduction in the viability titre of *E. coli* in a contact time of 30 seconds at room temperature indicating bactericidal activity against this organism.

Table 1: The activity of a 1:128 dilution of AHP-BSC against *E. coli* O157:H7

Date of Expt.	Dilution	Lot Number	# Of Repeats	Contact Time	CFU/control	CFU/test	Log ₁₀ Reduction
21/07/04	1:128	3811	6	30 sec	1.23X 10 ⁶	0	6.08

Activity of AHP-BSC against *S. choleraesuis*: Table 2 summarizes the result of the suspension test. The product was able to bring about a >6log₁₀ reduction in the viability titre of *S. choleraesuis* in a contact time of 30 seconds at room temperature indicating bactericidal activity against this organism.

Table 2: The activity of a 1:128 dilution of AHP-BSC against *S. choleraesuis*

Date of Expt.	Dilution	Lot Number	# Of Repeats	Contact Time	CFU/control	CFU/test	Log ₁₀ Reduction
---------------	----------	------------	--------------	--------------	-------------	----------	-----------------------------

27/07/01	1:128	3811	6	30 sec	1.07X 10 ⁶	0	6.03
----------	-------	------	---	--------	-----------------------	---	------

Neutralization Verification results of the product: Table 3 summarizes the results of the neutralization test of the product. The absence of any significant difference in the number of colonies of the test organism in the test and control was taken to mean that a 1:10 dilution of the Product in the neutralizer was sufficient to arrest its germicidal activity.

Table 3: Neutralization Verification of AHP-BSC

Test Organism	Product dilution used in testing	Number of colonies on plates after exposure to a 10-fold dilution of the test solution in the neutralizer	Number of colonies on plates after exposure to the neutralizer
<i>S. choleraesuis</i>	1:128 dilution	161/182	116/134
<i>E. coli</i>	1:128 dilution	104/105	141/135

CONCLUDING REMARKS

The AHP-BSC formulation tested was able to meet the product performance criteria under the conditions of the testing carried out in this study.