FINAL REPORT

Virucidal Hard-Surface Efficacy Test – Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)

Test Substance
EcoloxTech HOCl 200

Lot Numbers
1005
1006
1007

Test Organism
Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus), Strain: USA-WA1/2020; BEI Resources, NR-52281

Test Guidelines
EPA (2018) Guidelines 810.2000 and 810.2200 (G)

Author
Cameron Wilde

Study Completion Date
07/09/20

Performing Laboratory
Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

Laboratory Project Identification Number
974-107

Protocol Identification Number
974.1.04.30.20

Sponsor
IET, Inc. dba Ecolox Tech
102 NW 22 Ave
Miami, FL 33125

STATEMENT OF NO DATA CONFIDENTIALITY

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA sec.10(d)(1)(A), (B) or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10(g).

Submitter signature: _____ Date: _____
Printed Name of Signer: _____
Printed Name of Company: _____

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The following is a detailed description of all differences between the practices used in the study and those required by 40 CFR part § 160:

- Information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test substance resides with the sponsor of the study.

Study Director Signature: _____ Date: 02/09/20
Typed Name: Cameron Wilde
Typed Name of Laboratory: Microbac Laboratories, Inc.

Sponsor Signature: _____ Date: _____
Printed Name: _____
Printed Name of Company: _____

Submitter Signature: _____ Date: _____
Printed Name: _____
Printed Name of Company: _____

QUALITY ASSURANCE UNIT STATEMENT

The Quality Assurance Unit of Microbac has inspected Project Number 974-107 to be in compliance with current Good Laboratory Practice regulations, (40 CFR § 160).

The dates that inspections were made and the dates that findings were reported to management and to the study director are listed below.

Phase Inspected	Date of Inspection	Date Reported to Study Director	Date Reported to Management
Protocol	05/28/20	05/28/20	05/28/20
In Process (Incubation)	05/28/20	05/28/20	05/28/20
Final Report	06/22/20	06/22/20	06/22/20

Jeanne M. Anderegg RQAP-GLP
Quality Assurance Manager

07-09-2020
Date

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TEST SUBSTANCE CHARACTERIZATION

Test Substance characterization as to the identity, strength, purity, solubility and composition, as applicable, according to 40 CFR, Part 160, Subpart F [160.105] was documented prior to its use in the study. The Test Substance Certificate of Analysis Reports, provided by the sponsor, are found in Appendix II.

TEST SUMMARY

Study Title:	Virucidal Hard-Surface Efficacy Test – Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)
Project No.:	974-107
Protocol No.:	974.1.04.30.20
Test Method:	ASTM International E1053-11 “Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces”
Sponsor:	IET, Inc. dba Ecolox Tech 102 NW 22 Ave Miami, FL 33125
Testing Facility:	Microbac Laboratories, Inc. 105 Carpenter Drive Sterling, VA 20164
Study Objective:	This test was performed in order to substantiate virucidal efficacy claims for a test substance to be labeled as a virucide by determining the potential of the test substance to disinfect hard surfaces contaminated with Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus). This test was designed to simulate consumer use and was performed in conformance to EPA OCSPP 810.2000 (2018) and 810.2200 (2018) Product Performance Test Guidelines.
Study Dates:	Study Initiation: 05/27/20 Experimental Start: 05/27/20 Experimental End: 05/27/20 Study Completion: See page 1

TEST SUMMARY (continued)

Test Substance: EcoloxTech HOCl 200

- Lot No.: 1005, Received: 05/27/20, assigned DS No. K713
- Lot No.: 1006, Received: 05/27/20, assigned DS No. K714
- Lot No.: 1007, Received: 05/27/20, assigned DS No. K715
- Physical Description: Liquid
- Storage Condition: 1-10°C
- Active Ingredient: Hypochlorous Acid
- Dilution: Ready to Use
- Diluent: N/A

Test Conditions:

Organic Soil Load:	5.0% serum in viral inoculum
Contact Time:	5 minutes
Contact Temperature:	21°C
Contact Relative Humidity:	45%

Challenge Virus: Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)

- Strain: USA-WA1/2020
- Source: BEI Resources, NR-52281

Indicator Cells: Vero-E6 cells

- Source: ATCC CRL-1586

Incubation Time: 7 days

Incubation Temperature: 36 ± 2°C with 5 ± 3% CO_2

Dilution Medium (DM): Minimum Essential Medium (MEM) + 2% Newborn Calf Serum (NCS)

Neutralizer: MEM + 10% NCS + 0.5% $Na_2S_2O_3$

Other Reagents: N/A

Study Design: This study was performed according to the signed protocol and project sheet(s) issued by the Study Director (see Appendix I).

TEST SUMMARY (continued)

Study Personnel:	Cameron Wilde	Senior Scientist Study Director)
	Tanya Kapes	Scientist I
	Brandon G. Narvaez	Associate Scientist I

TEST PROCEDURES

Indicator Cells:

Vero-E6 cells were obtained from ATCC and maintained in cell culture at 36 ± 2°C with 5 ± 3% CO_2 prior to seeding. The indicator cell plates were prepared 12 – 30 hours prior to inoculation with test sample. The cells were seeded in 24-well plates at a density of 1.5 x 10^5 cells/mL at 1 mL per well.

Virus Inoculum:

Frozen viral stock was thawed on the day of the test. Stock virus contained an organic load of 5.0% NCS.

Test Substance:

The test substance was received ready to use.

Test Carriers:

0.4 mL of virus inoculum was spread uniformly over the bottom of a glass petri dish (carrier). The carriers were dried for 30 minutes at 21°C with 45-46% Relative Humidity (RH).

Test Substance Application and Exposure Conditions:

2.0 mL of test substance was sprayed until thoroughly wet from a distance of 6-8” to the dried virus inoculum and held for the contact time of 5 minutes at 21°C with 45% RH.

Recovery of Samples:

After the contact time, the test substance was neutralized with 2.0 mL of neutralizer. The mixture was scraped from the surface of the carrier with a cell scraper. This post-neutralized sample (PNS) was considered the 10^{-1} dilution. An aliquot of the PNS was ten-fold serially diluted in DM.

TEST PROCEDURES (continued)

Infectivity Assay:

Selected dilutions of the sample were inoculated onto the plates at 1.0 mL per well, 4 wells per dilution, and incubated at 36 ± 2°C with 5 ± 3% CO_2. After 1 day, plates were aspirated, refed using fresh DM and then returned to the incubator for the remainder of the incubation period. After 7 days, the plates were removed from incubation, scored, and recorded for test-substance specific cytotoxic effects and/or virus-specific cytopathic effect (CPE).

Neutralizer Effectiveness and Viral Interference Control (NE/VI):

The control was performed to assess whether residual active ingredient was present after neutralization (Neutralizer Effectiveness) or if the neutralized test substance interferes with virus infectivity (Viral Interference). The NE/VI was prepared identically to the test sample except DM was used in lieu of virus inoculum to inoculate the carrier. After test substance application and neutralization, the PNS was divided into two portions, one for NE/VI and one for Cytotoxicity (see below). For the NE/VI, a 0.5 mL aliquot of the PNS was ten-fold serially diluted and 100 µL of virus stock (containing 1413 $TCID_{50}$ units per well) was added individually to selected dilutions and held for at least the contact time. Selected dilutions were inoculated onto indicator cell plates and incubated in an identical manner as the test samples.

Cytotoxicity Control (CT):

This control was performed to assess the cytotoxic effects of the test substance on indicator cells. The CT (obtained from the NE/VI) was prepared identically to the NE/VI except no virus was added to the selected dilutions inoculated onto indicator cells plates and incubated in an identical manner as the test samples.

Plate Recovery Control (PRC):

This control was performed to establish the input viral load to compare with the test substance results to evaluate the viral reduction by the test substance. The PRC was prepared identically to the test sample except DM was used in lieu of test substance to treat the dried virus inoculum during test substance application. Selected dilutions were inoculated onto indicator cell plates and incubated in an identical manner as the test samples.

TEST PROCEDURES (continued)

Cell Viability Control (CVC):

This control was performed to demonstrate that the indicator host cells remained viable and to confirm the sterility of the media employed throughout the incubation period. Indicator cell plates were aspirated, and 1.0 mL of DM was added to 4 wells of indicator cells and incubated in an identical manner as the test samples.

Virus Stock Titer Control (VST):

This control was performed to demonstrate that the titer of the stock virus was appropriate for use and that the viral infectivity assay was performed appropriately. An aliquot of the virus inoculum used in the study was ten-fold serially diluted in DM. Selected dilutions were inoculated onto indicator cell plates and incubated in an identical manner as the test samples.

PROTOCOL CHANGES

Protocol Amendments:

1. Protocol "Miscellaneous Information", Section A: The batch number, manufacturing date, and expiration date for the third batch of test substance was left blank. Per the Sponsor, they should be "1007", "May 11, 2020", and "June 11, 2020", respectively. This amendment serves to clarify the Protocol.

2. Protocol "Miscellaneous Information", Section A: The test substance storage conditions are listed as "☑ Ambient". Per the Sponsor, it should be "Refrigerated". This amendment serves to correct the Protocol.

Protocol Deviations:

No protocol deviations were present in this study.

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, VA 20164, from 05/27/20 to 06/03/20. The study director signed the protocol on 05/27/20. The study completion date is the date the study director signed the final report. The individual test dates are as follows:

- Testing started at 3:55 pm on 05/27/20 and ended at 3:38 pm on 06/03/20

All changes or revisions of the protocol were documented, signed by the study director, dated and maintained with the protocol.

RECORDS TO BE MAINTAINED

All testing data, protocol, protocol modifications, test substance records, the final report, and correspondence between Microbac and the sponsor will be stored in the archives at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, VA 20164, or at a controlled facility off site.

TEST ACCEPTANCE CRITERIA

The test was considered acceptable for test substance evaluation due to the criteria below being satisfied:

- The infectious virus recovered from the PRC was ≥ 4.8 Log_{10} $TCID_{50}$ units.
- Viral-induced CPE was distinguishable from test substance induced cytotoxicity (if any).
- Virus was recovered from dilutions of the NE/VI control not exhibiting cytotoxicity.
- The CVC did not exhibit CPE.

CALCULATIONS

Titer Calculation:

The 50% Tissue Culture Infectious Dose per mL ($TCID_{50}$/mL) was determined using the Spearman-Karber method using the following formula:

$$m = x_k + \left(\frac{d}{2}\right) - d\sum p_i$$

where: m = the logarithm of the dilution at which half of the wells are infected relative to the test volume
x_k = the logarithm of the smallest dosage which induces infection in all cultures
d = the logarithm of the dilution factor
p_i = the proportion of positive results at dilution i
$\sum p_i$ = the sum of p_i (starting with the highest dilution producing 100% infection)

The values were converted to $TCID_{50}$/mL using a sample inoculum of 1.0 mL.

Viral Load Calculation:

Load (Log_{10} $TCID_{50}$) per carrier = Titer (Log_{10} $TCID_{50}$/mL) + Log_{10} [volume per sample (mL)]

Viral Reduction Calculation:

Log_{10} Reduction = Initial Viral Load (Log_{10} $TCID_{50}$*) – Output Viral Load (Log_{10} $TCID_{50}$*)

* per assayed volume and per carrier

RESULTS

Results are presented in Tables 1 – 7.

Key (for all tables):

T/y =	Cytotoxicity observed in y wells inoculated; viral cytopathic effects (CPE) could not be determined
X/y =	X wells out of y wells inoculated exhibited positive viral cytopathic effect
0/y =	0 out of y wells inoculated exhibited positive viral CPE; no cytotoxicity or bacterial contamination was observed in any of the wells inoculated

RESULTS (continued)

Table 1
Plate Recovery Control (PRC)

Dilution*	PRC
10^{-3}	4/4
10^{-4}	4/4
10^{-5}	4/4
10^{-6}	0/4
10^{-7}	0/4
10^{-8}	0/4
Titer (Log_{10} $TCID_{50}$/mL)	5.50
Load (Log_{10} $TCID_{50}$)**	5.10

*Dilution refers to the fold of dilution from the virus inoculum.
**Per carrier (0.4 mL of Undilute [10^0])

Table 2
Test Substance

Dilution*	EcoloxTech HOCl 200		
	Lot No. 1005	Lot No. 1006	Lot No. 1007
10^{-2}	0/4	0/4	0/4
10^{-3}	0/4	0/4	0/4
10^{-4}	0/4	0/4	0/4
10^{-5}	0/4	0/4	0/4
10^{-6}	0/4	0/4	0/4
10^{-7}	0/4	0/4	0/4
Titer (Log_{10} $TCID_{50}$/mL)	≤ 1.50	≤ 1.50	≤ 1.50
Load (Log_{10} $TCID_{50}$)**	≤ 1.10	≤ 1.10	≤ 1.10
Log_{10} Reduction***	≥ 4.00	≥ 4.00	≥ 4.00

*Dilution refers to the fold of dilution from the virus inoculum.
**Per carrier (0.4 mL of Undilute [10^0])
***Per assayed volume and per carrier

RESULTS (continued)

Table 3
Neutralizer Effectiveness/Viral Interference (NE/VI) and Cytotoxicity (CT) Controls

Dilution*	EcoloxTech HOCl 200 Lot Nos. 1005	
	NE/VI	CT
10^{-2}	4/4	0/4
10^{-3}	4/4	0/4
10^{-4}	4/4	0/4

*Dilution refers to the fold of dilution from the mock inoculum.

Table 4
Neutralizer Effectiveness/Viral Interference (NE/VI) and Cytotoxicity (CT) Controls

Dilution*	EcoloxTech HOCl 200 Lot Nos. 1006	
	NE/VI	CT
10^{-2}	4/4	0/4
10^{-3}	4/4	0/4
10^{-4}	4/4	0/4

*Dilution refers to the fold of dilution from the mock inoculum.

Table 5
Neutralizer Effectiveness/Viral Interference (NE/VI) and Cytotoxicity (CT) Controls

Dilution*	EcoloxTech HOCl 200 Lot Nos. 1007	
	NE/VI	CT
10^{-2}	4/4	0/4
10^{-3}	4/4	0/4
10^{-4}	4/4	0/4

*Dilution refers to the fold of dilution from the mock inoculum.

Table 6
Cell Viability Control (CVC)

CVC
0/4
Cells were viable; media was sterile

Table 7
Virus Stock Titer Control (VST)

Dilution*	VST
10^{-4}	4/4
10^{-5}	4/4
10^{-6}	4/4
10^{-7}	2/4
10^{-8}	0/4
10^{-9}	0/4
Titer (Log_{10} $TCID_{50}$/mL)	7.00

*Dilution refers to the fold of dilution from the virus inoculum.

TEST SUBSTANCE EVALUATION CRITERIA

According to the US Environmental Protection Agency, the test substance passes the test if the following criteria are met:

- The test substance must demonstrate a ≥ 3 Log_{10} reduction on each surface in the presence or absence of cytotoxicity; and
- If cytotoxicity is present, the virus control titer should be increased to demonstrate a ≥ 3 Log_{10} reduction in viral titer on each surface beyond the cytotoxic level.

CONCLUSIONS

When tested as described, EcoloxTech HOCl 200, Lot Nos. 1005, 1006, and 1007 passed the Virucidal Hard-Surface Efficacy Test when Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus), containing 5.0% Newborn Calf Serum, was exposed to the test substance for 5 minutes at 21°C and 45% RH.

All controls met the criteria for a valid test. These conclusions are based on observed data.

REFERENCES

1. ASTM E1053-11, Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, ASTM International, West Conshohocken, PA, 2011.

2. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces, Guidance for Efficacy Testing, February 2018.

3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides, Guidance for Efficacy Testing, February 2018.

4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Frequently Asked Questions (FAQ) for OCSPP 810.2000, 810.2100, and 810.2200.

APPENDIX I

MICROBAC®

Microbac Protocol

VIRUCIDAL HARD-SURFACE EFFICACY TEST -

Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)

Testing Facility
Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA20164

Prepared for
IET, Inc. dba Ecolox Tech
102 NW 22 Ave
Miami, FL 33125

April 30, 2020

Microbac Protocol: 974.1.04.30.20

Microbac Project: 974-107

OBJECTIVE:

This test is designed to substantiate virucidal effectiveness claims for a test substance to be labeled as a virucide. It determines the potential of the test substance to disinfect hard surfaces contaminated with the test virus. The test is designed to simulate consumer use and conforms to EPA OCSPP 810.2000 (2018) and 810.2200 (2018) Product Performance Test Guidelines, Frequently Asked Questions (FAQ) for OCSPP 810.2000, 810.2100, and 810.2200, and follows the procedure outlined in the ASTM International test method designated E1053-11, "Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces".

TESTING CONDITIONS:

Virus will be dried on a suitable sterile hard surface at ambient temperature. One test substance (liquid spray), three batches (lots), will be tested at one contact time and one replicate (N=1). The test substance will be used to treat the dried virus on a glass Petri dish carrier. After a defined exposure period as specified by the sponsor, the test substance-virus mixture will be neutralized, scraped off from the surface, collected, and tested for the presence of infectious virions.

MATERIALS:

A. Test, control and reference substances will be supplied by the Sponsor of the study. Microbac will append the Sponsor-provided Certificate(s) of Analysis (CoA) to this study report, as per CFR 40.160.105:

- The identity, strength, purity, and composition, or other characteristics which will appropriately define the test, control, or reference substance shall be determined and shall be documented by the sponsor before its use in a study. Methods of synthesis, fabrication, or derivation of the test, control, or reference substance shall be documented and retained by the sponsor.

- When relevant to the conduct of the study the solubility of each test, control, or reference substance shall be determined by the sponsor before the experimental start date. The stability of the test, control, or reference substance shall be determined by the sponsor before the experimental start date or concomitantly according to written standard operating procedures, which provide for periodic analysis.

The test substance will be tested as supplied by the sponsor unless directed otherwise. All operations performed on the test substance such as dilution or specialized storage conditions must be specified by the sponsor before initiation of testing.

The sponsor assures Microbac testing facility management that the test substance has been appropriately tested for identity, strength, purity, stability, and uniformity as applicable.

Microbac will retain all unused test substances for a period of one year upon completion of the test, and then discard them in a manner that meets the approval of the safety officer or return them to the Sponsor. The test materials and the paper records will be retained in accordance to FIFRA. Microbac will contact the Study Sponsor to arrange for transfer of records when/if the test substance is returned to the Sponsor.

B. Materials supplied by Microbac, including, but not limited to:

1. Challenge virus (requested by the sponsor of the study): Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus), Strain: USA-WA1/2020, Source: BEI Resources, NR-52281

2. Host cell line: Vero E6 cells, ATCC CRL-1586

3. Laboratory equipment and supplies.

4. Media and reagents:

   Media and reagents relevant to the virus-host system and test substance being tested will be documented in the first project sheet and data pack.

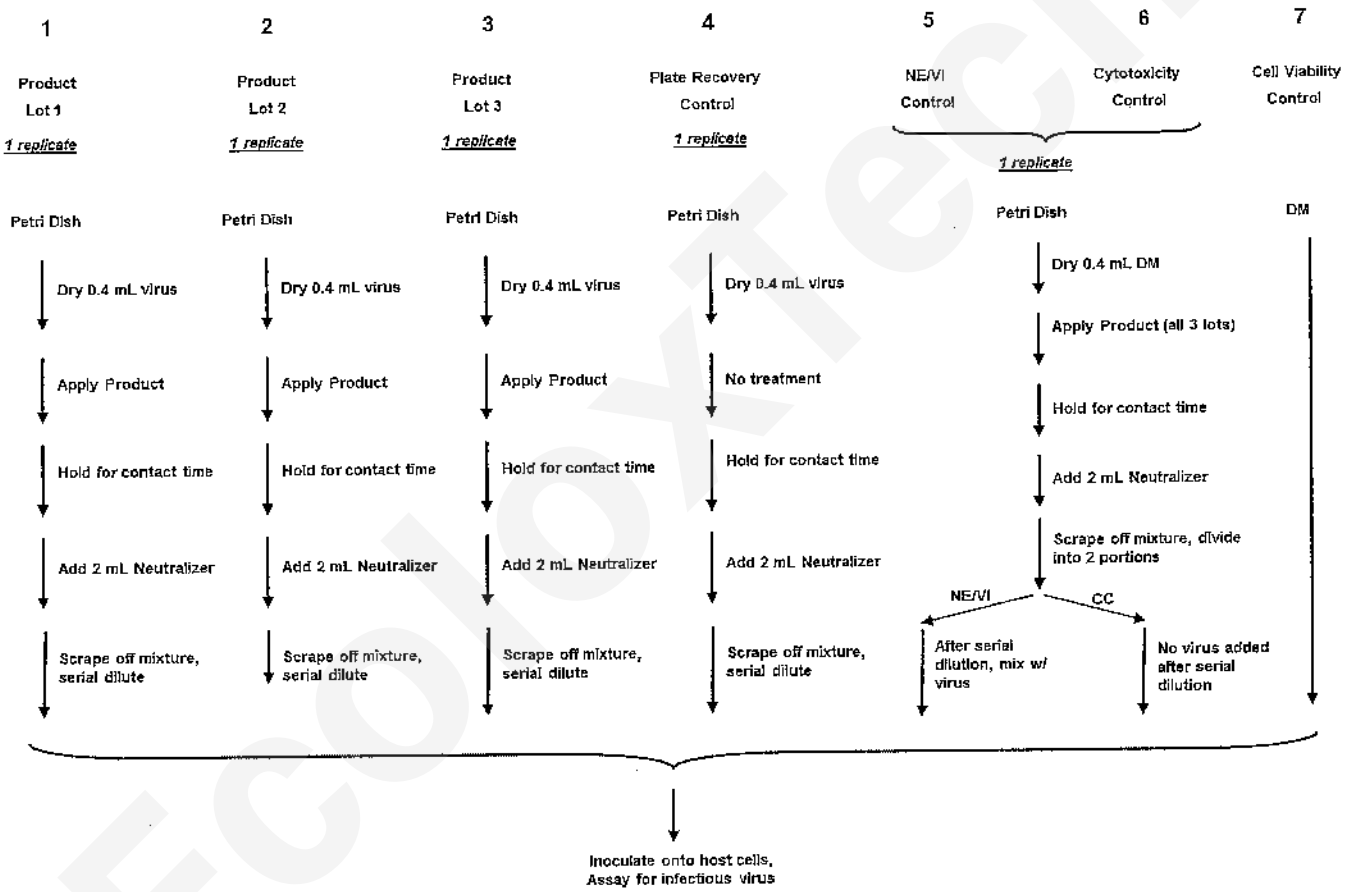
TEST SYSTEM IDENTIFICATION:

All Petri dishes, dilution tube racks, and host-containing apparatus will be appropriately labeled with the following information: virus, host, and test substance and/or project number.

EXPERIMENTAL DESIGN:

All of the procedures involved in performance of this study are described in a detailed series of SOPs that are maintained at Microbac. SOPs and Logs are referred to in the raw data and are required as part of GLP regulations. The study flow diagram is shown in Figure 1, with details described in the following sections.

FIGURE 1



DM: Dilution Medium
NE/VI: Neutralizer Effectiveness/Viral Interference control
CT: Cytotoxicity Control

Note: One test substance, three lots, will be tested at one exposure (contact) time and one replicate (N=1). The NE/VI and CT controls will be performed at one replicate per lot.

A. Inoculum preparation:

Viral stocks are purchased from reputable sources that identify them by scientifically accepted methods and may have been propagated at Microbac. Records are maintained that demonstrate the origin of the virus. The virus stocks are stored at an ultra-low temperature.

Frozen viral stocks will be thawed on the day of the test. Serum will be added to viral stock to achieve an organic load of 5.0% (if not already 5.0%), unless otherwise directed by the Sponsor and pre-agreed by Microbac. If the challenge virus culture is standardized by concentration or dilution, or if a column is used, these manipulations must be documented and reported.

Note: a level of approximately 4.8 – 6.8 Log_{10} virus challenge (as indicated by the plate recovery control load) when there is no cytotoxicity associated with the test substance, or approximately 3.0 – 5.0 Log_{10} beyond the level of cytotoxicity when present, should be achieved whenever possible.

B. Carrier preparation:

For each lot of the test substance, an aliquot of 0.4 mL of stock virus will be spread over the bottom of pre-sterilized glass Petri dishes. This volume will remain consistent among all test and control runs. Then the virus will be allowed to dry at ambient temperature. The drying time, temperature, and relative humidity will be recorded and reported.

One carrier will be prepared for each lot of the test substance using virus. One carrier will be prepared for the plate recovery control using virus. Additionally, one carrier will be prepared for each lot of test substance for the neutralizer effectiveness/viral interference and cytotoxicity controls using media in lieu of virus as the inoculum.

C. Test substance preparation:

Note: Information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test substance resides with the sponsor of the study.

The test substance will be prepared exactly according to the sponsor's directions (if provided). If the sponsor requests dilution of the test substance, the diluted test substance will be used for testing within three hours of preparation. The prepared test

substance, if not within the stipulated test temperature range, will be pre-equilibrated to the test temperature prior to use in the study as applicable.

D. Test:

Three lots of the test substance (liquid spray) will be tested at one contact time and one replicate (N=1). Note: The temperature and relative humidity during the exposure period will be recorded and reported.

For direct liquid application test substance, for each run, after the inoculum has dried, 2.0 mL of the test substance will be added. The dried virus film must be completely covered by the test substance. The plates will remain at the temperature and for the time specified by the sponsor. After the contact period, the test agent will be neutralized with 2.0 mL of appropriate neutralizer and the mixture will be scraped from the surface of the dish with a cell scraper. This post-neutralized sample (PNS) will be considered approximately a 10^{-1} dilution.

For spray type test substance, an aliquot of the test substance, ready-to-use, will be dispensed into a sterilized spray bottle. The spray bottle will then be shaken 2 – 3 times to ensure homogeneity and sprayed to charge the spray bottle. A mock spray action will be performed by applying the test substance as the sponsor directs onto at least two blank Petri dishes. Then the volume dispensed onto each dish will be measured and averaged. This averaged volume from the mock spray runs will be used for the neutralizer for all applicable runs and for the Plate recovery control runs. Then the test substance will be sprayed onto the virus carriers in a horizontal position until thoroughly wet from a distance of 6" – 8". Each carrier will be held in a horizontal position for the exposure time as specified by the sponsor. After the contact period, the test substance will be neutralized with an appropriate neutralizer using the averaged volume from the mock spray runs; and the mixture will be scraped off from the surface of the dish with a cell scraper. This post-neutralized sample (PNS) will be considered approximately a 10^{-1} dilution.

If Sephacryl columns are used to aid in the neutralization and to further reduce the cytotoxicity, each inoculum/test substance/neutralizer mixture sample will be loaded onto a pre-spun Sephacryl column. Following the passage through columns, the eluates will be aseptically collected and serially ten-fold diluted in DM. If columns are not used, serial ten-fold dilutions of the inoculum/test substance/neutralizer mixture will directly be prepared in DM.

E. Infectivity assay:

The residual infectious virus in all test and control samples will be detected by viral-induced cytopathic effect (CPE).

Selected dilutions of the neutralized inoculum/test substance mixture (test samples) and control samples will be added to cultured host cells (at least four wells per dilution, per reaction mixture) and incubated at 36±2°C with 5±3% CO_2 for total 4 – 9 days. The host cells may be washed twice with phosphate buffered saline prior to inoculation. The inoculated culture will be observed and refed with fresh media as necessary, during the incubation period. These activities, if applicable, will be recorded. The host cells will then be examined microscopically for presence of infectious virions. The resulting virus-specific CPE and test substance-specific cytotoxic effects will be scored by examining all test and control samples. These observations will be recorded.

F. Controls:

1. Plate recovery control (PRC):

This control will be performed in a single run, concurrently with the test substance runs.

The virus inoculum will be spread over the surface of a sterile glass Petri dish and left to dry at ambient temperature. A volume of DM equivalent to that of the test substance will be added to the dried virus. Post-contact time, virus will be subjected to the identical neutralization procedure as the test substance. This control will determine the relative loss in virus infectivity resulting from drying and neutralization alone.

The results from this control will be compared with the test results to confirm recovery of at least 4.8-Log_{10} per carrier of infectious virus in this control following drying and neutralization. Its titer will be used to compare with the titers of the test results to reach the acceptable test criteria (see below).

2. Neutralizer effectiveness/Viral interference control (NE/VI):

This control will determine if residual active ingredient is present after neutralization and if the neutralized test substance interferes with the virus

infection system. This control will be performed for each lot of test substance at one replicate.

The test substance will be processed exactly as the test procedure but in lieu of virus inoculum, dried DM will be exposed to the test substance and assayed as previously described. Post-treatment and neutralization, the neutralized DM/test substance mixture will be divided into two portions, one for cytotoxicity control and the other for neutralizer effectiveness/viral interference control and processed as the test.

If columns are used, each portion will be passed through individual columns and the eluate will be serially diluted ten-fold in DM. If columns are not used, each portion will be directly diluted using serial ten-fold dilutions in DM.

The neutralizer effectiveness/viral interference control sample will be diluted as follows: using dilution test tubes and appropriate pipette, an aliquot of the PNS will be used for making serial 10-fold dilutions in DM (for example, 0.5 mL sample + 4.5 mL DM). Following serial dilution, 0.1 mL of a low titered virus, containing approximately 1,000 – 5,000 infectious units of virus, will be added to 4.5 mL of each dilution and held for a period of no shorter than the contact time. Then these samples will be used to inoculate host cells as described for the test procedure.

Selected dilutions of the sample will be added to cultured cell monolayers at a minimum of four wells per dilution per sample, as described in the "Infectivity Assay" section.

3. Cytotoxicity control (CT):

This control will be performed for each lot of test substance at one replicate.

The cytotoxicity sample, acquired from the neutralizer effectiveness/viral interference control run, will be diluted and have no virus added. Selected dilutions will be inoculated and incubated in the same manner as the rest of the test and control samples. These effects are distinct from virus-induced cytopathic effects, which will be evident in the plate recovery control cultures.

4. Column titer control (to be performed only if a Sephacryl column is used):

   This control will be performed to determine any affect the columns may have on infectious virus titer. It will be performed in a single run.

   The sample for this control will be acquired from a portion of the PRC, prior to passing through the columns and will be serially diluted in DM, then processed in the same manner as the test.

5. Cell viability control:

   This control will be performed in a single run. It will demonstrate that cells remain viable throughout the course of the assay period. In addition, it will confirm the sterility of the DM employed throughout the assay period. At least four wells of cells will receive only DM and will be incubated and processed with both test and other controls. This will serve as the negative control.

6. Virus Stock Titer control (VST)

   This control will be performed in a single run. An aliquot of the virus used in the study will be directly serially diluted and inoculated onto the host cells to confirm the titer of the stock virus. This control will demonstrate that the titer of the stock virus is appropriate for use and that the viral infectivity assay is performed appropriately.

G. Calculation:

The 50% tissue culture infective dose per mL ($TCID_{50}$/mL) will be determined using the method of Spearman-Karber (Kärber G., Arch. Exp. Pathol. Pharmakol. 1931, 162: 480-483) or other appropriate methods such as Reed and Muench (Am. J. of Hyg. 1938, 27:493). The $TCID_{50}$/carrier, i.e., the viral load per carrier, will be calculated as follows. These analyses will be described in detail in the final report. The test results will be reported as reduction of the virus titer post treatment with the test substance expressed as log_{10}.

The Virus Load ($TCID_{50}$/carrier) will be calculated in the following manner:
Virus Load (Log_{10} $TCID_{50}$) = Virus Titer (Log_{10} $TCID_{50}$/mL) + Log_{10} [Volume per sample (mL)]

The Log_{10} Reduction Factor (LRF) will be calculated in the following manner:
Log_{10} Reduction Factor = Initial viral load (Log_{10} $TCID_{50}$, per assayed volume and per carrier) – Output viral load (Log_{10} $TCID_{50}$, per assayed volume and per carrier)

TEST ACCEPTANCE CRITERIA:

The test will be acceptable for evaluation of the test results if the criteria listed below are satisfied. The study director may consider other causes that may affect test reliability and acceptance.

- The infectious virus recovered from the PRC control must be ≥ 4.8-log_{10} $TCID_{50}$ units.
- Viral-induced cytopathic effect must be distinguishable from test substance induced cytotoxic effects (if any).
- Virus must be recovered from the neutralizer effectiveness/viral interference control (not exhibiting cytotoxicity).
- The Cell Viability Control (assay negative control) must not exhibit virus.

TEST SUBSTANCE EVALUATION CRITERIA:

According to the US Environmental Protection Agency, the test substance passes the test if the following are met:

- The product must demonstrate a ≥ 3 log_{10} reduction on each surface in the presence or absence of cytotoxicity; and
- If cytotoxicity is present, the virus control titer should be increased to demonstrate a ≥ 3 log_{10} reduction in viral titer on each surface beyond the cytotoxic level.

PERSONNEL AND TESTING FACILITIES:

A study director will be assigned prior to initiation of the test. Resumes are maintained and are available on request. This study will be conducted at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, Virginia 20164.

REGULATORY COMPLIANCE AND QUALITY ASSURANCE (GLP studies only):

This study will be performed in compliance with the US Environmental Protection Agency's Good Laboratory Practices (GLP) regulations, 40 CFR 160 (note: information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test substance resides with the sponsor of the study unless otherwise stated).

The Quality Assurance Unit of Microbac will inspect the conduct of the study for GLP compliance. The dates of the inspections and the dates that findings are reported to the study management and study director will be included in the final report.

PROTOCOL AMENDMENTS AND DEVIATIONS:

Any protocol amendment(s) and protocol deviation(s) identified will be reported in project sheet(s) and included in the final report.

REPORT FORMAT:

This report will contain all items required by 40 CFR Part 160.185 and EPA 810.2000 and be in compliance with EPA PR Notice 2011-3. Microbac employs a standard report format for each test design. Each final report will provide at least the following information:

- Sponsor identification
- Test substance identification
- Type of assay and project number
- Study start and end time (clock time)
- Interpretation of results and conclusions
- Test results presented in tabular form
- Methods and evaluation criteria, if applicable
- Dates of study initiation and completion (GLP studies only)
- Signed Quality Assurance and Compliance Statements (GLP studies only)
- Certificate of Analysis (for GLP studies only; if provided by the Sponsor)
- List of personnel involved in the study

RECORDS TO BE MAINTAINED:

For all GLP studies, the original signed final report or an electronic copy will be sent to the Sponsor. The original signed final report, or a copy thereof, will be maintained in the study file. If requested, a draft report will be provided to the Sponsor for review prior to finalization of the report.

All raw data, protocol, protocol modifications, test substance records, the final report (or copy thereof), and correspondence between Microbac and the sponsor will be stored in the archives at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, Virginia 20164 or in a controlled facility off site.

All changes or revisions to this approved protocol will be documented, signed by the study director, dated and maintained with this protocol. The sponsor will be notified of any change, resolution, and impact on the study as soon as practical.

The proposed experimental start and termination dates; additional information about the test substance; challenge virus and host cell line monolayers used and the type of neutralizers employed in the test will be addressed in a project sheet issued separately for each study. The date the study director signs the protocol will be the initiation date. All project sheets issued will be forwarded to the study sponsor for appropriate action.

REFERENCES

1. ASTM E1053-11, Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, ASTM International, West Conshohocken, PA, 2011.

2. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces, Guidance for Efficacy Testing, February 2018.

3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides, Guidance for Efficacy Testing, February 2018.

4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Frequently Asked Questions (FAQ) for OCSPP 810.2000, 810.2100, and 810.2200.

MISCELLANEOUS INFORMATION:

The following information is to be completed by the sponsor prior to initiation of the study (please check all applicable open boxes):

A. Test substance information:

Test substance name	Ecolox tech HOCl 200.		
Test substance batch numbers	1005	1006	
Manufacture Date	May 11, 2020	May 11, 2020	
Expiration Date	June 11, 2020	June 11, 2020	
Active ingredient(s)	Hypochlorous Acid		
Test substance storage conditions	☑ Ambient ☐ Refrigerated ☐ Other:_____		
Level of active ingredients in testing	■ Lower Certified Limit (LCL)[1] ☐ At or below nominal		
MSDS provided	☑ Yes ☐ No	C of A provided	☑ Yes ☐ No
Dilution	Ready-to-use		
Diluent	Not applicable		
Contact time	5 minutes		
Contact temperature	■ Room Temperature (20±1°C) ☐ Other :_____		
Organic Load	■ 5.0% serum in viral inoculum ☐ Other: _____		
Test substance application	☐ Apply directly to dried virus via pipetting ■ Spray from 6-8 inches until thoroughly wet		
Study conduct	■ GLP ☐ Non-GLP		
Report submission	■ EPA ☐ Health Canada ☐ Other: _____		

[1] US EPA stipulates that 3 lots of test substance be tested at or below LCL for COVID-19

PROTOCOL APPROVAL BY SPONSOR:

Sponsor Signature: _____ Date: 5/18/2020

Printed Name: Scott Hartnett

PROTOCOL APPROVAL BY STUDY DIRECTOR (Microbac):

Study Director Signature: _____ Date: 05/27/2020

Printed Name: Cameron Wilde

Date Issued: 05/27/20 Project Sheet No. 1 Page No. 1 Laboratory Project Identification No. 974-107

STUDY TITLE: VIRUCIDAL HARD-SURFACE EFFICACY TEST – Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)

STUDY DIRECTOR: Cameron Wilde

Signature 05/27/2020 Date

TEST MATERIAL(S):
Ecolox Tech HOCl 200

LOT NO.	DATE RECEIVED:	DS NO.
1005	05/27/20	K713
1006	05/27/20	K714
1007	05/27/20	K715

PERFORMING DEPARTMENT(S):
Virology and Toxicology

STORAGE CONDITIONS:
Location: MBT# 1377
☐ Dark ☐ Ambient Room Temperature
☐ Desiccator ☐ Freezer ■ Refrigerator ☐ Other:

PROTECTIVE PRECAUTION REQUIRED: MSDS ■ Yes / ☐ No

PHYSICAL DESCRIPTION: ☐ Solid ■ Liquid ☐ Aerosol

PURPOSE: See attached protocol. **AUTHORIZATION:** See client signature.

PROPOSED EXPERIMENTAL START DATE: 05/27/20 **TERMINATION DATE:** 06/05/20

CONDUCT OF STUDY: ☐ FDA ■ EPA ☐ R&D ■GLP ☐ GCP ☐Other:

SPONSOR: IET, Inc. dba EcoloxTech
102 NW 22 Ave
Miami, FL 33125

CONTACT PERSON: Scott Hartnett
Email: scott@ecolox.com

TEST CONDITIONS:

Challenge organisms:	Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus), Strain USA-WA1/2020; BEI Resources, NR-52281
Host:	Vero E6 cells, ATCC CRL-1586
Organic load:	5.0% serum in viral inoculum
Disinfectant application:	Spray from 6-8 inches until thoroughly wet.
Active Ingredient:	Hypochlorous Acid
Dilution medium:	Minimum Essential Medium (MEM) + 2% Newborn Calf Serum (NCS)
Dilution:	Ready to use
Diluent:	N/A
Neutralizer(s):	MEM + 10% NCS + 0.5% $Na_2S_2O_3$
Contact time:	5 minutes
Contact temperature:	Ambient room temperature (20±1°C)
Incubation time(s):	4 – 9 days
Incubation temperature(s):	36±2C in 5±3% CO_2

Date Issued: 06/22/20 Project Sheet No. 2 Page No. 1 Laboratory Project Identification No. 974-107

STUDY TITLE: VIRUCIDAL HARD-SURFACE EFFICACY TEST – Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2) (COVID-19 Virus)

STUDY DIRECTOR: Cameron Wilde

Signature 06/29/2020 Date

TEST MATERIAL(S):
Ecolox Tech HOCl 200

LOT NO.	DATE RECEIVED:	DS NO.
1005	05/27/20	K713
1006	05/27/20	K714
1007	05/27/20	K715

PERFORMING DEPARTMENT(S):
Virology and Toxicology

STORAGE CONDITIONS:
Location: MBT# 1377
☐ Dark ☐ Ambient Room Temperature
☐ Desiccator ☐ Freezer ■ Refrigerator ☐ Other:

PROTECTIVE PRECAUTION REQUIRED: MSDS ■ Yes / ☐ No

PHYSICAL DESCRIPTION: ☐ Solid ■ Liquid ☐ Aerosol

PURPOSE: See attached protocol. **AUTHORIZATION:** See client signature.

PROPOSED EXPERIMENTAL START DATE: 05/27/20 **TERMINATION DATE:** 06/05/20

CONDUCT OF STUDY: ☐ FDA ■ EPA ☐ R&D ■GLP ☐ GCP ☐Other:

SPONSOR: IET, Inc. dba EcoloxTech
102 NW 22 Ave
Miami, FL 33125

CONTACT PERSON: Scott Hartnett
Email: scott@ecolox.com

PROTOCOL AMENDMENT(S):

1. Protocol page 14, Section A: The batch number, manufacturing date, and expiration date for the third batch of test substance was left blank. Per the Sponsor, they should be "1007", "May 11, 2020", and "June 11, 2020", respectively. This amendment serves to clarify the Protocol.

2. Protocol page 14, Section A: The test substance storage conditions are listed as "☑ Ambient". Per the Sponsor, it should be "Refrigerated". This amendment serves to correct the Protocol.

APPENDIX II

Case Laboratories, Inc.

622 Route Ten · Whippeny, NJ 07981 · Tel: 973.428.9666 · Fax: 973.887.4419
info@case-labs.com · www.case-labs.com

Certificate of Analysis

This analysis was conducted in compliance with 40 CFR 160 as part of Case Laboratories, Inc. Study Number 6610-05.

Name:	EcoloxTech HOCl 200
Lot Number:	1005
Date of Manufacture:	May 11, 2020
Date of Analysis:	May 18, 2020
Supplied By:	IET, Inc. dba EcoloxTech

Test	Result
Hypochlorous Acid Assay	133 ppm
pH (25°C)	3.78

Brian A. Roe
Study Director
Case Laboratories, Inc.

6/17/2020
Date

John Connors
Quality Assurance Manager
Case Laboratories, Inc.

06/17/2020
Date

The raw data generated during analysis has been reviewed by the Quality Assurance Unit. The raw data confirm the results as listed above.

622 Route Ten · Whippany, NJ 07981 · Tel: 973.428.9686 · Fax: 973.887.4419
info@case-labs.com · www.case-labs.com

Certificate of Analysis

This analysis was conducted in compliance with 40 CFR 160 as part of Case Laboratories, Inc. Study Number 6610-05.

Name:	EcoloxTech HOCl 200
Lot Number:	1006
Date of Manufacture:	May 11, 2020
Date of Analysis:	May 18, 2020
Supplied By:	IET, Inc. dba EcoloxTech

Test	Result
Hypochlorous Acid Assay	128 ppm
pH (25°C)	3.83

Brian A. Roe
Study Director
Case Laboratories, Inc.

6/17/2020
Date

John Connors
Quality Assurance Manager
Case Laboratories, Inc.

06/17/2020
Date

The raw data generated during analysis has been reviewed by the Quality Assurance Unit. The raw data confirm the results as listed above.

622 Route Ten · Whippany, NJ 07981 · Tel: 973.428.9666 · Fax: 973.887.4419
info@case-labs.com · www.case-labs.com

Certificate of Analysis

This analysis was conducted in compliance with 40 CFR 160 as part of Case Laboratories, Inc. Study Number 6610-05.

Name:	EcoloxTech HOCl 200
Lot Number:	1007
Date of Manufacture:	May 11, 2020
Dates of Analysis:	May 18 and 20, 2020
Supplied By:	IET, Inc. dba EcoloxTech

Test	Result
Hypochlorous Acid Assay	123 ppm
pH (25°C)	3.82

Brian A. Roe
Study Director
Case Laboratories, Inc.

6/17/2020
Date

John Connors
Quality Assurance Manager
Case Laboratories, Inc.

06/17/2020
Date

The raw data generated during analysis has been reviewed by the Quality Assurance Unit. The raw data confirm the results as listed above.