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# CERTIFICATIONS & STUDIES

# Design for the Environment Safer Detergents Stewardship Initiative

**On this page:**



## What was the Safer Detergents Stewardship Initiative?

While no longer an active project within EPA's Design for the Environment (DfE) program, the Safer Detergents Stewardship Initiative (SDSI) recognized environmental leaders who voluntarily committed to the use of safer surfactants. Safer surfactants are surfactants that break down quickly to non-polluting compounds and help protect aquatic life in both fresh and salt water. Nonylphenol ethoxylates, commonly referred to as NPEs, are an example of a surfactant class that does not meet the definition of a safer surfactant.

The DfE program identified safer alternative surfactants through partnerships with industry and environmental advocates. CleanGredients® is a source of safer surfactants and can be found at www.CleanGredients.org EXIT <https://cleangredients.org/>.

## Who was eligible for recognition?

EPA's DfE program recognized businesses engaged in the production or use of surfactants, as well as those involved in the purchase or distribution of products containing surfactants. Others, including not-for-profit organizations, qualified through actively encouraging the use of safer surfactants.

## How businesses were recognized

Champion was the highest level of recognition offered under SDSI. At this level, the participant was listed on the EPA SDSI website as a Champion and was granted use of a special logo in their literature to help explain their participation in the program. The Partner category provided recognition of significant accomplishment towards the use of safer surfactants. Partners were listed on the EPA SDSI website and may have qualified for recognition as Champions in the future.

## SDSI Champions

### Champions: Chemical Manufacturers

Chemical Manufacturer SDSI Champions demonstrated the manufacture of only safer surfactants and documented a strategy for ensuring the manufacture of only safer surfactants.

- BASF Corporation
- Bio-Neat Inc.

### Champions: Product Formulators

Product Formulator SDSI Champions demonstrated the use of only safer surfactants in product and documented a strategy for ensuring that only safer surfactants were used in products.

- Ankem, Inc.
- Auto-Chlor System
- Barricade Fire Gel
- Big D Industries, Inc.
- Bissell Homecare Inc.

- Canberra Corporation
- Chemco Corporation
- ChemLink Laboratories LLC
- Clean Control Corporation
- Colgate-Palmolive Company
- Corporate Express, a Staples Company
- Earth Friendly Products
- Eco Concepts, Inc.
- EcoDiscoveries Tennant Company
- Environmental Manufacturing Solutions (EMS)
- GEMTEK Products LLC
- Georgia-Pacific Consumer Products LP
- Jaws International Ltd
- JohnsonDiversey, Inc.
- Klipper Group
- Method VASKA
- Momar, Inc.
- Multi-Clean Inc.
- Natural Soap Formulas
- Naturell
- PDQ Manufacturing
- Pure & Gentle Soap Inc.
- Racine Industries, Host and Von Schrader Divisions
- Reckitt Benckiser, Inc.
- RTX Scientifc, Inc.: DBA Refrigeration Technologies
- SafeWash Technologies
- S.C. Johnson & Son, Inc.
- Seventh Generation, Inc.
- Spurrier Chemical Companies Inc.
- State Chemical Solutions

- SYSCO Corporation
- The Dial Corporation, A Henkel Company
- The Procter & Gamble Company
- Transchem, Inc.
- US Formula Technology
- U.S. Polychemical Corporation
- Virox Technologies Inc.

## Champions: Retailers/Distributors

Retailer/Distributor SDSI Champions demonstrated that only safer surfactants were included in products intended for sale and documented a strategy for ensuring that only safer surfactants were included in products intended for sale.

- EPIC CleaningProducts
- Solutex, Inc.

## Champions: Institutional Purchasers

Institutional Purchaser SDSI Champions demonstrated the purchase of products containing only safer surfactants and documented a strategy for ensuring that purchased products contained only safer surfactants.

- Coverall Health-Based Cleaning System
- SRI / Surgical Express, Inc.

## Champions: Others

Other SDSI Champions demonstrated outstanding efforts to encourage the use of safer surfactants.

- GreenBlue
- ISSA -- The Worldwide Cleaning Industry Association
- Sierra Club
- Textile Rental Services Association of America and the Uniform and Textile Service Association
- Toxics Use Reduction Institute Laboratory

# SDSI Partners

Partners were recognized for significant accomplishments towards the use of safer surfactants.

## Partners: Product Formulators

Product Formulator SDSI Partners committed to manufacturing only safer surfactants by a date that was reasonable for the organization's circumstances and documented a strategy for ensuring the manufacture of only safer surfactants.

- 3M (Building and Commercial Services Division)
- Athea Laboratories
- Bullen Airx
- The Clorox Company
- Daley International
- Flexocleaners.com
- Gent-l-kleen Products, Inc.
- The Hain-Celestial Group (Martha Stewart Clean)
- HAZfree Inc.
- Hillyard Inc.
- Jelmar
- Mt. Hood Solutions
- National Chemical Laboratories, Inc.
- NILODOR, INC.
- Orange TKO Industries Inc.
- Racine Industries, Host and Von Schrader Divisions
- Rochester Midland Corporation
- Sanolite Corporation

## Partners: Retailers/Distributors

Retailer/Distributor SDSI Partners committed to using only safer surfactants in products by a date that was reasonable for the organization's circumstances and documented a strategy for ensuring that only safer surfactants would be used in products.

- The Hain-Celestial Group (Martha Stewart Clean)

## Partners: Institutional Purchasers

Institutional Purchaser SDSI Partners committed to using products containing only safer surfactants by a date that was reasonable for the organization's circumstance and documented the active encouragement of the use of safer surfactants.

- County of Riverside, Custodial Services Division
- Metropolitan Water Reclamation District of Greater Chicago

## Partners: Others

Other SDSI Partners documented the active encouragement of the use of safer surfactants.

- Clean Production Action


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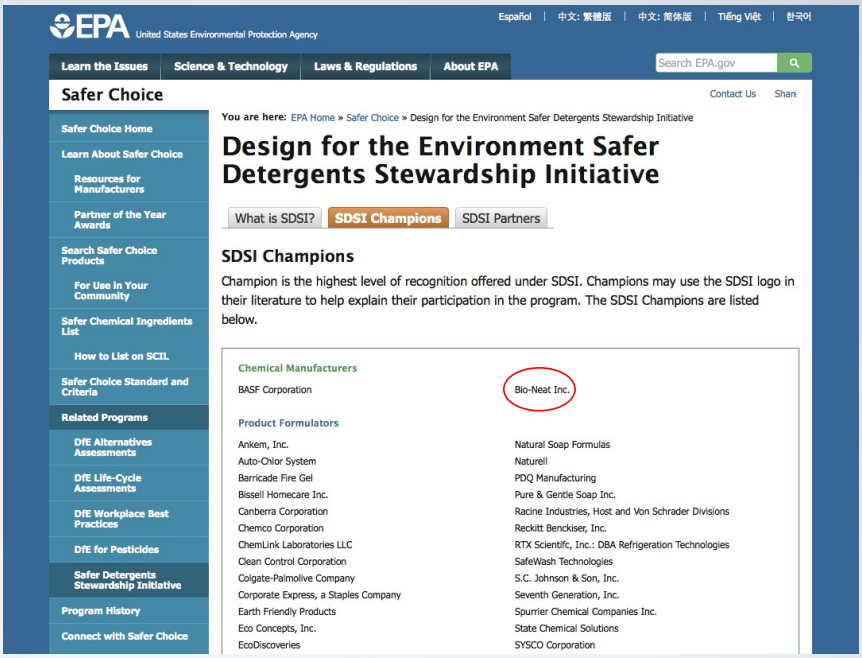
Design for the Environment U.S. EPA Safer Detergents Stewardship Initiative



EPA United States Environmental Protection Agency

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## Design for the Environment Safer Detergents Stewardship Initiative

What is SDSI? | SDSI Champions | SDSI Partners

### SDSI Champions

Champion is the highest level of recognition offered under SDSI. Champions may use the SDSI logo in their literature to help explain their participation in the program. The SDSI Champions are listed below.

**Chemical Manufacturers**

BASF Corporation

Bio-Neat Inc.

**Product Formulators**

Ankem, Inc.
Auto-Chlor System
Barricade Fire Gel
Bissell Homecare Inc.
Canberra Corporation
Chemco Corporation
ChemLink Laboratories LLC
Clean Control Corporation
Colgate-Palmolive Company
Corporate Express, a Staples Company
Earth Friendly Products
Eco Concepts, Inc.
EcoDiscoveries
Natural Soap Formulas
Naturell
PDQ Manufacturing
Pure & Gentle Soap Inc.
Racine Industries, Host and Von Schrader Divisions
Reckitt Benckiser, Inc.
RTX Scientifc, Inc.: DBA Refrigeration Technologies
SafeWash Technologies
S.C. Johnson & Son, Inc.
Seventh Generation, Inc.
Spurrier Chemical Companies Inc.
State Chemical Solutions
SYSCO Corporation

BIO-NEAT INC.

# Comprehensive Summary Report - Scientific Testing Performed on BioNeat NTS™

## Toxicity Testing

As requested by BIO-NEAT INC, I would like to interpret some scientific facts regarding BioNeat NTS™, the greenest, most effective cleaning product, based on their commercial laboratory test reports and my twelve years of scientific research experience as a colloid scientist with a doctorate (Ph.D.) in Chemistry.

BioNeat NTS™ is manufactured and distributed by BIO-NEAT INC. of Fort Lauderdale, Florida. It is formulated from all-natural, USEPA-satisfied, and USFDA-safe ingredients according to a proprietary process. The components of the BioNeat NTS™ product were completely analyzed by XENCO Laboratories (a NELAC-accredited testing laboratory) of Carrollton, Texas. The following analyses were performed:

1. Mercury by EPA 245.1
2. Metals by EPA 200.8
3. Pesticides and PCBs by EPA 608
4. VOA (Volatile Organic Analysis) GC/MS by EPA 624
5. Total Cyanide (Colorimetric, Automated UV) by SW-846 9012

For ALL substances tested (over 60 metals or volatile organic compounds), the amounts found were below reportable limits. No toxic heavy metals, organic solvents, PCBs or pesticides beyond the safety limit for everyday use were found. The contents of common heavy metals and chlorinated hydrocarbons were tested by Xenco Labs and their report indicates that BioNeat NTS™ is a completely safe product for humans and animals.

Table 1. Concentration of Heavy Metals and Chlorinated Hydrocarbons in BioNeat NTS™ (By Xenco Labs)

| COMPOUND | CONCENTRATION (mg/kg) |
|---|---|
| Arsenic | 0.0265 |
| Cadmium | <0.005 |
| Chromium | 0.0985 |
| Copper | <0.0150 |
| Lead | <0.0100 |
| Nickel | <0.0250 |
| Zinc | 0.2455 |
| Cyanide | <0.300 |
| Chlorinated Hydrocarbons | <5.0 |
| Mercury | <0.500 μg/L |

## Marine Toxicity Testing

Analytical results from Bio-Aquatic Testing Inc. (a NELAC-accredited testing laboratory) of Carrollton, Texas, demonstrate as follows: In comparison to the majority of conventional, market-branded cleaning products, which usually contain the effective surfactant Sodium Laurel Sulfate, BioNeat NTS is ***six to ten times less toxic*** when the marine invertebrate species, *Mysidopsis bahia* (*Americamysis bahia*) and the marine vertebrate species, *Menidia beryllina* were used as subjects in the tests. During their 48 hours and 96 hours survival experiment, 50% of the *Menidia beryllina* was killed in 96 hours in the presence of a 12.19 ppm solution of sodium laurel sulfate (LC50 or median lethal concentration), while the same survival rate was achieved at concentrations even as high as 136.12 ppm of BioNeat NTS™ in the solution. Bio-Aquatic Testing Inc.'s results show that BioNeat NTS™ can be ***up to 10 times less toxic*** than other common cleaning products containing Sodium Laurel Sulfate or similar surfactants as part of their formulation.

Table 2. Surface Washing Agent Toxicity (by Bio-Aquatic Testing Inc.)

| MATERIAL TESTED | SPECIES | LC50 (PPM) |
|---|---|---|
| BioNeat NTS™ | *Menidia beryllina*<br>*Mysidopsis bahia* | 136.1<br>70.7 |
| No. 2 Fuel Oil | *Menidia beryllina*<br>*Mysidopsis bahia* | 3.35<br>2.24 |
| Product & No. 2 Fuel Oil | *Menidia beryllina*<br>*Mysidopsis bahia* | 4.73<br>2.24 |
| Reference Toxicant: (Sodium Laurel Sulfate) | *Menidia beryllina*<br>*Mysidopsis bahia* | 12.19<br>10.53 |

BioNeat NTS™ is not only safe to be used for everyday cleaning, it is also a powerful, industrial-strength cleaning product that can even clean an oil-drilling rig or an oil spill. Its safety properties are highly related to its unique formulation. BioNeat NTS™ only contains FDA-approved (GRAS list) components and uses natural products in its formulation. Its superior cleaning capability is powered by modern bio-nanotechnology and science. BioNeat NTS™ forms particles four-nanometers in size when it is manufactured. This effective component of the BioNeat NTS™ formulation has extraordinary surface activity. These nanoparticles can surround oil drops, grease, and many other contaminants rapidly with their fatty tails and leaving their soluble heads outside. When water is applied, these oily contaminants can be easily rinsed away as a biodegradable biomass. Due to the extremely small size of these particles, BioNeat NTS™ is significantly more effective as a surfactant and emulsifier than other conventional soaps or detergents with larger-sized micelles.

In December of 2013, BIO-NEAT INC. was granted the Champion Award as a manufacturer of only safer surfactants by the U.S. EPA's Design for the Environment/Safer Detergents Stewardship Initiative Program. In April of 2014, BioNeat NTS™ was granted organic certification by the Organic Materials Review Institute (OMRI).

## Measurement of Micelle Size of BioNeat NTS™

**Testing and Report prepared by Bio-Neat Inc. Consulting Chemist Shaoyong Yu, Ph.D.**

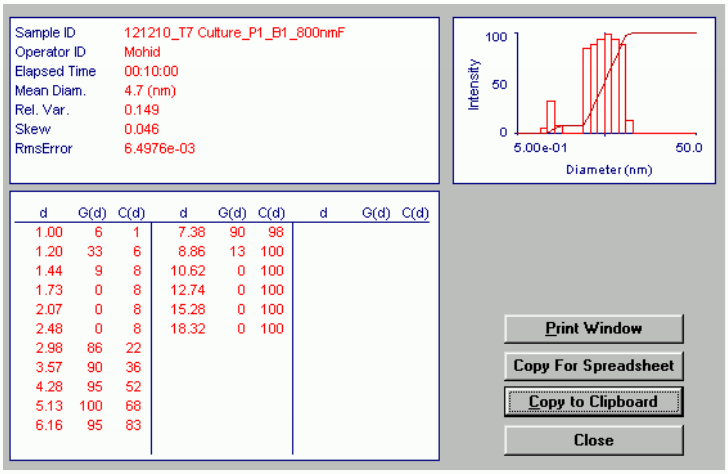
The micelle size of BioNeat NTS™ was obtained by Dynamic Light Scattering (DLS) Technique. DLS measurements were performed on a Proton Correlation Spectrometer with a BI9000 AT Digital Correlator (Brookhaven Instruments) equipped with a Compass 315M-150 Laser (Coherent Technologies), which provides a green light source (λ=532nm).

Data obtained from DLS can be interpreted in different ways using different mathematic models. The figure below, which uses a Cumulant model, is the easiest and most common interpretation of the measurement.

| | Gamma ($s^{-1}$) | Diff. Coef. ($cm^2 s^{-1}$) | Eff. Diam. (nm) | Poly | Skew | Kurtosis |
|---|---|---|---|---|---|---|
| Linear: | 4.808e+04 | 9.685e-07 | 5.1 | | | |
| Quadratic: | 6.063e+04 | 1.221e-06 | 4.0 | 0.187 | | |
| Cubic: | 6.573e+04 | 1.324e-06 | 3.7 | 0.323 | 0.30 | |
| Quartic: | 6.957e+04 | 1.401e-06 | 3.5 | 0.453 | 0.62 | 3.20 |

Gamma is the average decay rate, and Diff. Coef. is the Diffusion Coefficient. Both of these values characterize the moving speed of the micelles in solution, i.e., Brownian movement or thermal diffusion. The larger the micelle, the lower the diffusion coefficient. The micelle size can be solved from the Cumulant model with four different orders of approximation. Each order provides a slightly different micelle size (Eff. Diam) in nanometer (nm) units, which is the DIAMETER of the micelles. Since the quadratic (4.0) or cubic (3.7) results are typically used, that result indicates that the BioNeat micelles are approximately 4 nm with polydispersity (Poly) index of about 0.2 to 0.3.

The following two figures are different interpretation of the measurement with more complicated models. CONTIN mode (below) is better when there is a polydispersed system.



| | |
|---|---|
| Sample ID | 121210_T7 Culture_P1_B1_800nmF |
| Operator ID | Mohid |
| Elapsed Time | 00:10:00 |
| Mean Diam. | 4.7 (nm) |
| Rel. Var. | 0.149 |
| Skew | 0.046 |
| RmsError | 6.4976e-03 |

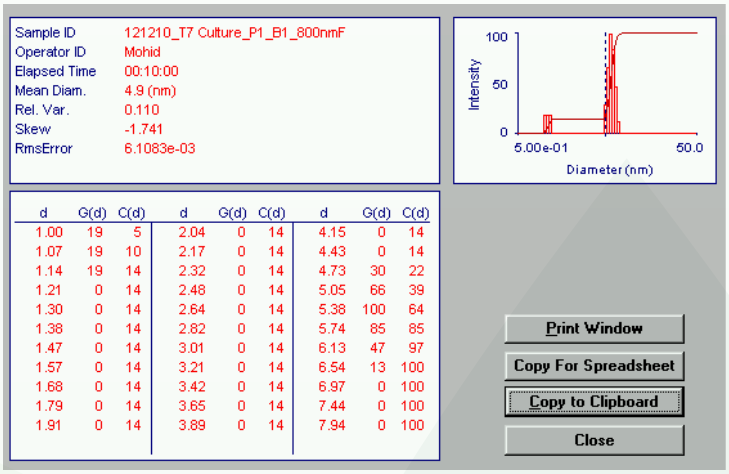


| d | G(d) | C(d) | d | G(d) | C(d) | d | G(d) | C(d) |
|---|---|---|---|---|---|---|---|---|
| 1.00 | 6 | 1 | 7.38 | 90 | 98 | | | |
| 1.20 | 33 | 6 | 8.86 | 13 | 100 | | | |
| 1.44 | 9 | 8 | 10.62 | 0 | 100 | | | |
| 1.73 | 0 | 8 | 12.74 | 0 | 100 | | | |
| 2.07 | 0 | 8 | 15.28 | 0 | 100 | | | |
| 2.48 | 0 | 8 | 18.32 | 0 | 100 | | | |
| 2.98 | 86 | 22 | | | | | | |
| 3.57 | 90 | 36 | | | | | | |
| 4.28 | 95 | 52 | | | | | | |
| 5.13 | 100 | 68 | | | | | | |
| 6.16 | 95 | 83 | | | | | | |



This chart indicates that BioNeat NTS™ micelles have two populations: one is about 1.2 nm and the other one is around 5nm, so the overall average (Mean Diam.) size from this model is 4.7 nm, which agrees well with the Cumulant result when polydispersity is considered.

NNLS (below) is another model to interpret the measurement, which gives a quite close value 4.9nm.



| | |
|---|---|
| Sample ID | 121210_T7 Culture_P1_B1_800nmF |
| Operator ID | Mohid |
| Elapsed Time | 00:10:00 |
| Mean Diam. | 4.9 (nm) |
| Rel. Var. | 0.110 |
| Skew | -1.741 |
| RmsError | 6.1083e-03 |



| d | G(d) | C(d) | d | G(d) | C(d) | d | G(d) | C(d) |
|---|---|---|---|---|---|---|---|---|
| 1.00 | 19 | 5 | 2.04 | 0 | 14 | 4.15 | 0 | 14 |
| 1.07 | 19 | 10 | 2.17 | 0 | 14 | 4.43 | 0 | 14 |
| 1.14 | 19 | 14 | 2.32 | 0 | 14 | 4.73 | 30 | 22 |
| 1.21 | 0 | 14 | 2.48 | 0 | 14 | 5.05 | 66 | 39 |
| 1.30 | 0 | 14 | 2.64 | 0 | 14 | 5.38 | 100 | 64 |
| 1.38 | 0 | 14 | 2.82 | 0 | 14 | 5.74 | 85 | 85 |
| 1.47 | 0 | 14 | 3.01 | 0 | 14 | 6.13 | 47 | 97 |
| 1.57 | 0 | 14 | 3.21 | 0 | 14 | 6.54 | 13 | 100 |
| 1.68 | 0 | 14 | 3.42 | 0 | 14 | 6.97 | 0 | 100 |
| 1.79 | 0 | 14 | 3.65 | 0 | 14 | 7.44 | 0 | 100 |
| 1.91 | 0 | 14 | 3.89 | 0 | 14 | 7.94 | 0 | 100 |



It is important to note that all of these results represent an average of statistical results, so the approximate level of 4-5 nm in all these results are sufficiently valid for all practical purposes.

## OECD 301B Ready/Ultimate Biodegradability Assessment

Performed by Respirtek Laboratories, Biloxi, MS

**Project ID: BIO-2413** **Date: November 19, 2014**

### Study Summary

The test substance, BioNeat NTS™, was evaluated for ready and ultimate biodegradability in an aqueous medium, when exposed to an inoculum source according to procedures detailed in the OECD 301B methodology.

Based on the test method employed, the maximum biodegradability of the test materials is as follows:

| Test Substance | Percent Biodegradation | Classification |
|---|---|---|
| BioNeat NTS™ | 61.7% | Ultimate |

This value is the highest observed during the 28-day test for each substance.

Based on the testing conducted in accordance with methods specified by OECD 301B Procedure, test product BioNeat NTS™ achieved 61.7% biodegradation. The product met method requirements for ***Ultimate Biodegradability*** classification.

RespirTek™
CONSULTING LABORATORY
12450 Shortcut Rd., Bldg F ♦ Biloxi, Mississippi 39532
Voice 228.392.7977 ♦ Fax 228.396.3984
www.respirtek.com

**Certificate of Biodegradability**

RespirTek, Inc., an ISO/IEC 17025:2005 certified environmental laboratory, has conducted biodegradability testing in accordance with *OECD 301B* using the product identified as BioNeat NTS™.

The listed product results are as follows:

***61.7% biodegradation***

The product BioNeat NTS™ meets requirements for

***Ultimate Biodegradability***.

Documentation is available from testing initiator at:

Bio-Neat, Inc.
101 SE 10th Street
Ft. Lauderdale, FL 33316

ilac-MRA
PJLA Testing
**Accreditation #69085**

Anthony Miranda **BIO-2413**
Technical Director
RespirTek, Inc.

## Antibacterial Efficacy Testing #1

Performed by BCS Laboratories Inc. 4609 NW 6th St. Bldg. A, Gainesville, FL 32609

We have completed the antibacterial efficacy study on the provided sample of BioNeat NTS™. The testing was done according to the protocol we regularly use to assess antimicrobial efficacy of disinfectants.

The disinfectant efficacy was determined against *Escherichia coli* and *Salmonella typhimurium* as models of gram-negative human bacterial pathogens that are transmissible through feces. According to observed results, the tested formula exhibited excellent antibacterial efficacy. It should effectively reduce and control the transmission of the tested pathogens when used as directed. Following you will find a summary of the results of our analysis.

Table 1: The inactivation of fecal bacterial pathogens by the BioNeat Natural Soap Formula at a 10% concentration and following 5 and 15 minute exposure to the diluted formula.

| Microorganism | Average colony forming units (cfu)/ml present initially. (untreated Control)# | Average cfu/ml following 5 minute exposure | Average cfu/ml following 15 minute exposure | Percent reduction |
|---|---|---|---|---|
| Escherichia coli | $3.4 \times 10^5$ | <1.0 | <1.0 | **>99.999%** |
| Salmonella Typhimurium | $1.8 \times 10^5$ | <1.0 | <1.0 | **>99.999%** |

**Conclusion: BioNeat NTS™ demonstrates average over 99% effectiveness in killing gram-negative pathogenic bacteria.**

## Antibacterial Efficacy Testing #2

Performed by BCS Laboratories Inc. 4609 NW 6th St. Bldg. A, Gainesville, FL 32609

We have conducted the exploratory study on the provided BioNeat NTS™ concentrate to determine the BioNeat NTS™ solution's potential efficacy on the reductions of *Listeria monocytogenes* from inoculated surfaces; BCS 1409054. The testing was conducted as per AOAC Method 961.02 (AOAC Official Methods of Analysis; 2005) and ASTM E2197-11 "Standard Quantitative Disk Carrier Test Methods for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal and Sporicidal Activities of Chemicals." Following, you will find a summary of the results of our analysis.

Table 1. The efficacy of a spray application of diluted BioNeat NTS™ concentrate on the reduction of *Listeria monocytogenes*; Method 961.02 Germicidal Spray Products as Disinfectants (2005) using a 10 minute contact time.

| Treatment Slide | Average microorganism recovered from positive control# | Average cfu/mL recovered from each slides sprayed* | Percent Reduction | $Log_{10}$ Reduction | Average Percent Reduction | Average $Log_{10}$ Reduction |
|---|---|---|---|---|---|---|
| 1 | $5.2 \times 10^3$ cfu/mL | <0.45 | <99.991% | >4.1 | <99.991% | >4.1 |
| 2 | $5.2 \times 10^3$ cfu/mL | <0.45 | <99.991% | >4.1 | <99.991% | >4.1 |
| 3 | $5.2 \times 10^3$ cfu/mL | <0.45 | <99.991% | >4.1 | <99.991% | >4.1 |

**Conclusion: BioNeat NTS™ demonstrates average over 99% effectiveness in killing gram-positive pathogenic bacteria.**

## Antiviral Efficacy Testing #1

Performed by BCS Laboratories Inc. 4609 NW 6th St. Bldg. A, Gainesville, FL 32609

**The virucidal efficacy of a spray application of diluted BioNeat NTS™ concentrate against Human poliovirus 1 (ATCC VR 1562) inoculated onto non-porous surfaces. Test was conducted as per adaptation of ASTM E1053 "Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces" and ASTM E2197-11 "Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals."**

| Microorganism | Number of Replicates Tested | Contact Time (Minutes) | Average Infectious Units Control Slides | Average Recovered From Each Sprayed Slide | Percent Reduction | Average Percent Reduction | $Log_{10}$ Reduction |
|---|---|---|---|---|---|---|---|
| Human Poliovirus 1 | 3 | 10 | $1.6 \times 10^4$ iu/ml | 790 iu/ml | 95.1% | **95.9%** | 1.4 |
| " | " | " | " | 940 iu/ml | 94.1% | " | " |
| " | " | " | " | 230 iu/ml | 98.6% | " | " |

**Conclusion: BioNeat NTS™ demonstrates average 95.9% effectiveness in killing Human Poliovirus.**

## Antiviral Efficacy Testing #2

Performed by BCS Laboratories Inc. 4609 NW 6th St. Bldg. A, Gainesville, FL 32609

**The virucidal efficacy of a spray application of diluted BioNeat NTS™ concentrate against Murine Norovirus 1 (MNV-1, PTA-5935) inoculated onto non-porous surfaces. Test was conducted as per adaptation of ASTM E1053 "Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces" and ASTM E2197-11 "Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals."**

| Microorganism | Number of Replicates Tested | Contact Time (Minutes) | Average Infectious Units Control Slides | Average Recovered From Each Sprayed Slide | Percent Reduction | Average Percent Reduction | $Log_{10}$ Reduction |
|---|---|---|---|---|---|---|---|
| Murine Norovirus 1 | 5 | 10 | 85,000 iu/ml | 1,640 iu/ml | 80.7% | **82.5%** | 0.76 |
| " | " | " | " | 1,520 iu/ml | 82.1% | " | " |
| " | " | " | " | 1,260 iu/ml | 85.2% | " | " |
| " | " | " | " | 1,480 iu/ml | 82.6% | " | " |
| " | " | " | " | 1,533 iu/ml | 82.0% | " | " |

**Conclusion: BioNeat NTS™ demonstrates average 82.5% effectiveness in killing Murine Norovirus.**

## Antifungal/Mold Efficacy Testing

Performed by BCS Laboratories Inc. 4609 NW 6th St. Bldg. A, Gainesville, FL 32609

We have conducted the fungicidal efficacy testing on the provided Bio-Neat NTS concentrate. The testing was conducted as per AOAC Method 961.02 (AOAC Official Methods of Analysis; 2005) and ASTM E2197-11 "Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals."

**Table 1. The fungicidal efficacy of a spray application of diluted Bio-Neat NTS™ concentrate on *Aspergillus niger;* Method 961.02 Germicidal Spray Products as Disinfectants (2005) using a 10-minute contact time.**

| Treatment Slide | Average microorganisms cfu/ml recovered from control | Average cfu/ml recovered from each of slides sprayed | Percent Reduction | $Log_{10}$ Reduction | Cumulative Percent Reduction | Cumulative $Log_{10}$ Reduction |
|---|---|---|---|---|---|---|
| 1 | $5.0 \times 10^3$ | 575 | 88.5% | 0.9 | **98.4%** | 1.9 |
| 2 | " | 24.1 | 99.5% | 2.3 | " | " |
| 3 | " | 7.3 | 99.9% | 2.8 | " | " |
| 4 | " | 20.9 | 99.6% | 2.4 | " | " |
| 5 | " | 19.1 | 99.6% | 2.4 | " | " |
| 6 | " | 20 | 99.6% | 2.4 | " | " |
| 7 | " | 37.3 | 99.3% | 2.1 | " | " |
| 8 | " | 19.1 | 99.6% | 2.4 | " | " |
| 9 | " | 45.6 | 99.1% | 2.1 | " | " |
| 10 | " | 40.9 | 99.2% | 2.1 | " | " |

**Conclusion: BioNeat NTS™ demonstrates average 98.4% effectiveness in killing fungus/mold spores.**

## Cytotoxicity Testing

Performed by BCS Laboratories Inc. 4609 NW 6th St. Bldg. A, Gainesville, FL 32609

We have conducted the ASTM F895-11 (Standard Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity) preliminary screening study as per your request. The analysis is equivalent to ISO 10993-5. The study was performed on the provided "BioNeat NTSTM Concentrate" sample (BCS # 1409054) received on September 09, 2014. In the following pages, you will find a brief description of the methodology used and the results of our analyses. Based on the observed results, the diluted solution exhibited **Negligible Toxicity** on the CCL-1 cell line tested as per the agar diffusion method.

**Conclusion: BioNeat NTS™ demonstrated to be of "Negligible Toxicity" to human and animal tissue.**

## Gas Chromatograph/Mass Spectrometer (GC/MS) Testing

Performed by Waters Agricultural Laboratories, 257 Newton Hwy. P.O. Box 382, Camilla, GA 31730

BioNeat NTS™ was tested using Gas Chromatography/Mass Spectrometer instrumentation to determine the presence of any contaminating compounds such as heavy metals, pesticides, VOCs, etc. Results indicate that NO contaminants of any kind were present in the sample tested.

**Waters Agricultural Laboratories, Inc.**

257 Newton Highway Post Office Box 382 Camilla, Georgia 31730-0382 (229) 336-7216 FAX (229) 336-7967
www.watersag.com eMail: info@watersag.com

### GC Mass Spectrometer Screen Analysis

**Ship To:**
BioNeat Inc-Jim Giorgi
101 SE 10th Street
Ft Lauderdale,FL 33316

**Report No:** GA15-3222PH-1
**Report Date:** 04/10/2015
**Method:** Proprietary
**Lab Number:** 3222

**Sample Date:** Unknown
**Received Date:** 04/08/2015
**Sample Description:** Surfactant
**Sample Number:** BioNeat NTS

**Grower:**
Jim Giorgi

| Compound | Level Detected (ppm) | MDL (ppm) |
|---|---|---|
| No Compounds Detected | U | N/A |

*Authorized by:*
Data Administrator

Comments:

**U=Undetectable** **T=Trace** **MDL=Method Detection Limit**

This document may be reproduced only in its entirety. As we have no control over the manner in which the sample was taken, the analysis is based solely on the sample as received. Lab liability is limited to the sample received and the fee assessed on same.

*Unless otherwise noted, sample was received in good condition.*

Page 1 of 1

This Comprehensive Scientific Summary Report was prepared by Yaoshong Yu, Ph.D., Consulting Chemist for BIONEAT INC. Copies of full laboratory reports are available upon request.

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Biobased and Biogenic Carbon Testing Laboratory
ISO/IEC 17025:2005 Accredited

Beta Analytic, Inc.
4985 SW 74 Court
Miami, FL 33155 USA
Tel: 305-667-5167
Fax: 305-663-0964
info@betalabservices.com
www.betalabservices.com

April 05, 2018

Robert Trachman
Bioneat, Inc.
101 SE 10TH ST
Fort Lauderdale, FL, 33316-1023
United States

Dear Mr. Trachman

Please find enclosed your radiocarbon (C14) report for the material recently submitted. The result is reported as “% Biobased Carbon”. This indicates the percentage carbon from “natural” (plant or animal by-product) sources versus “synthetic” (petrochemical) sources sources. For reference, 100 % Biobased Carbon indicates that a material is entirely sourced from plants or animal by-products and 0 % Biobased Carbon indicates that a material did not contain any carbon from plants or animal by-products. A value in between represents a mixture of natural and fossil sources.

The analytical measurement is cited as “percent modern carbon (pMC)”. This is the percentage of C14 measured in the sample relative to a modern reference standard (NIST 4990C). The % Biobased Carbon content is calculated from pMC by applying a small adjustment factor for C14 in carbon dioxide in air today. It is important to note is that all internationally recognized standards using C14 assume that the plant or biomass feedstocks were obtained from natural environments.

Reported results are accredited to ISO/IEC 17025:2005 Testing Accreditation PJLA #59423 standards and all chemistry was performed here in our laboratory and counted in our own accelerators in Miami, Florida.

The international standard method utilized for this analysis is cited on your report. The report also indicates if the result is relative to total carbon (TC) or only total organic carbon (TOC). When interpreting the results, please consider any communications you may have had with us regarding the analysis. If you have any questions please contact us. We welcome your inquiries.

Sincerely,

Darden Hood
President



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Biobased and Biogenic Carbon Testing Laboratory
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Beta Analytic, Inc.
4985 SW 74 Court
Miami, FL 33155 USA
Tel: 305-667-5167
Fax: 305-663-0964
info@betalabservices.com
www.betalabservices.com

**Summary of Results -** % Biobased Carbon Content
ASTM D6866-16 Method B (AMS)

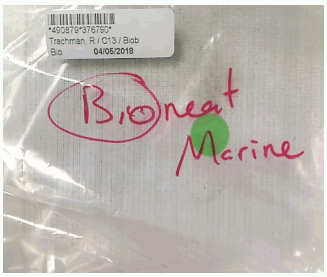
**Certificate Number:** 37679049087991028

**Validation:** Christopher Patrick, Deputy Director

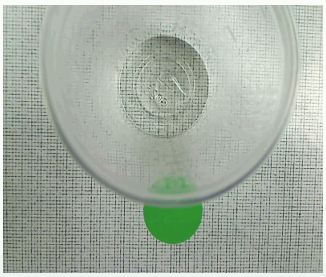
| | |
|---|---|
| **Submitter** | Robert Trachman |
| **Company** | Bioneat, Inc. |
| **Date Received** | March 29, 2018 |
| **Date Reported** | April 05, 2018 |
| **Submitter Label** | Bioneat Marine / (USDA Application# 6507) |

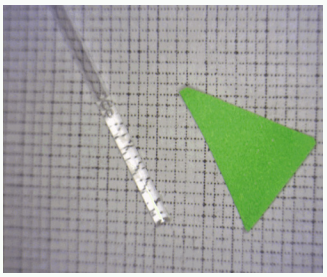
**RESULT:** 41 % Biobased Carbon Content (as a fraction of total organic carbon)

| | |
|---|---|
| **Laboratory Number** | Beta-490879 |
| **Percent modern carbon (pMC)** | 41.67 +/- 0.15 pMC |
| **Atmospheric adjustment factor (REF)** | 100.5; = pMC/1.005 |



Package received - labeling COC


Representative content (1mm x 1mm scale)


Representative sample analyzed (1mm x 1mm scale)

Disclosures: All work was done at Beta Analytic in its own chemistry lab and AMSs. No subcontractors were used. Beta's chemistry laboratory and AMS do not react or measure artificial C 14 used in biomedical and environmental AMS studies. Beta is a C14 tracer-free facility. Validating quality assurance is verified with a Quality Assurance report posted separately to the web library containing the PDF downloadable copy of this report.

Precision on the RESULT is cited as +/- 3% (absolute). The cited precision on the analytical measure (pMC) is 1 sigma (1 relative standard deviation). The reported result only applies to the analyzed material. The accuracy of the RESULT relies on the measured carbon in the analyzed material having been in recent equilibrium with $CO_2$ in the air and/or from fossil carbon (from living more than 40,000 years ago such as petroleum or coal. The RESULT only applies to relative carbon content, not to relative mass content. The RESULT is calculated by adjusting pMC by the applicable "Atmospheric adjustment factor (REF)" cited in this report.

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Beta Analytic, Inc.
4985 SW 74 Court
Miami, FL 33155 USA
Tel: 305-667-5167
Fax: 305-663-0964
info@betalabservices.com
www.betalabservices.com

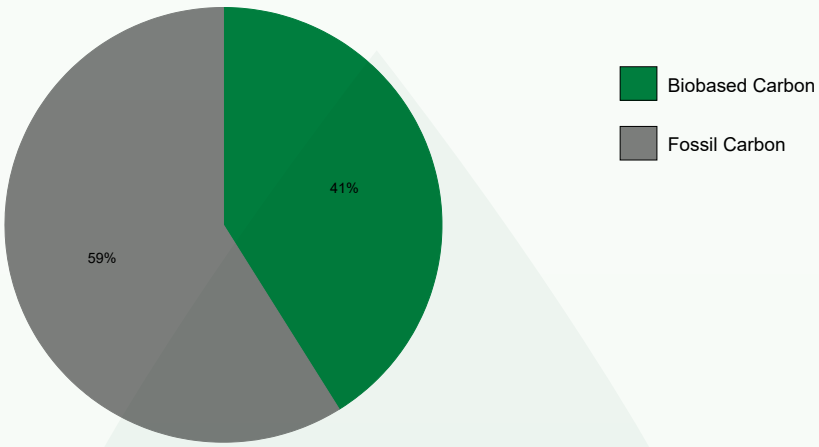
**Summary of Results -** % Biobased Carbon Content
ASTM D6866-16 Method B (AMS)

**Certificate Number:** 37679049087991028
**Validation:** Christopher Patrick, Deputy Director

| | |
|---|---|
| **Submitter** | Robert Trachman |
| **Company** | Bioneat, Inc. |
| **Date Received** | March 29, 2018 |
| **Date Reported** | April 05, 2018 |
| **Submitter Label** | Bioneat Marine / (USDA Application# 6507) |

**RESULT:** 41 % Biobased Carbon Content (as a fraction of total organic carbon)

| | |
|---|---|
| **Laboratory Number** | Beta-490879 |
| **Percent modern carbon (pMC)** | 41.67 +/- 0.15 pMC |
| **Atmospheric adjustment factor (REF)** | 100.5; = pMC/1.005 |




Precision on the RESULT is cited as +/- 3% (absolute). The cited precision on the analytical measure (pMC) is 1 sigma (1 relative standard deviation). The reported result only applies to the analyzed material. The accuracy of the RESULT relies on the measured carbon in the analyzed material having been in recent equilibrium with $CO_2$ in the air and/or from fossil carbon (from living more than 40,000 years ago such as petroleum or coal. The RESULT only applies to relative carbon content, not to relative mass content. The RESULT is calculated by adjusting pMC by the applicable "Atmospheric adjustment factor (REF)" cited in this report.

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Beta Analytic, Inc.
4985 SW 74 Court
Miami, FL 33155 USA
Tel: 305-667-5167
Fax: 305-663-0964
info@betalabservices.com
www.betalabservices.com

## % Biobased Carbon Content ASTM D6866-16 Method B (AMS)

### Explanation of Results

The result was obtained using the radiocarbon isotope (also known as Carbon-14, C14 or 14C), a naturally occurring isotope of carbon that is radioactive and decays in such a way that there is none left after about 45,000 years following the death of a plant or animal. Its most common use is radiocarbon dating by archaeologists. An industrial application was also developed to determine if consumer products and CO2 emissions were sourced from plants/biomass or from materials such as petroleum or coal (fossil-based). By 2003 there was growing demand for a standardized methodology for applying Carbon-14 testing within the regulatory environment. The first of these standards was ASTM D6866-04, which was written with the assistance of Beta Analytic. Since ASTM was largely viewed as a US standard, European stakeholders soon began demanding an equivalent CEN standard while global stakeholders called for ISO standardization.

The analytical procedures for measuring radiocarbon content using the different standards are identical. The only difference is the reporting format. Results are usually reported using the standardized terminology "% biobased carbon". Only ASTM D6866 uses the term "% biogenic carbon" when the result represents all carbon present (Total Carbon) rather than just the organic carbon (Total Organic Carbon). The terms "% biobased carbon" and "% biogenic carbon" are now the standard units in regulatory and industrial applications, replacing obscure units of measure historically reported by radiocarbon dating laboratories e.g. disintegrations per minute per gram (dpm/g) or radiocarbon age.

The result was obtained by measuring the ratio of radiocarbon in the material relative to a National Institute of Standards and Technology (NIST) modern reference standard (SRM 4990C). This ratio was calculated as a percentage and is reported as percent modern carbon (pMC). The value obtained relative to the NIST standard is normalized to the year 1950 AD so an adjustment was required to calculate a carbon source value relative to today. This factor is listed on the report sheet as the terminology "REF".

Interpretation and application of the results is straightforward. A value of 100% biobased or biogenic carbon would indicate that 100% of the carbon came from plants or animal by-products (biomass) living in the natural environment and a value of 0% would mean that all of the carbon was derived from petrochemicals, coal and other fossil sources. A value between 0-100% would indicate a mixture. The higher the value, the greater the proportion of naturally sourced components in the material.

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*OF NORTH FLORIDA, INC.*

November 21, 2014

Jim Giorgi
Bio-Neat Inc
101 SE 10th Street
Ft. Lauderdale, FL 33316
954-729-1220
jim@bioneat.com

RE: Study report of the fungicidal efficacy testing of Bio-Neat™ NTS concentrate; BCS 1409054

Dear Mr. Giorgi,

We have conducted the fungicidal efficacy testing on the provided Bio-Neat NTS concentrate. The testing was conducted as per AOAC Method 961.02 (AOAC Official Methods of Analysis; 2005) and ASTM E2197-11 "Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals."

In the following pages, you will find a summary of the methodology used and the results of our analysis. Should you have any questions or concerns, please do not hesitate to contact me.

Best Regards,

George Lukasik, Ph.D.
Laboratory Director


BCS LABORATORIES, INC. - GAINESVILLE
4609 NW 6TH STREET, STE A, GAINESVILLE, FLORIDA 32609
TEL. (352) 377-9272, FAX. (352) 377-5630
WWW.MICROBIOSERVICES.COM
FL DOH LABORATORY #E82924, EPA# FL01147



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**Fungal Culture Preparation**

*Aspergillus niger* (ATCC 6275) stock culture was obtained from Microbiologics (MN, USA) and maintained as per the supplier's recommendations. The culture was propagated on Sabouraud Dextrose Agar (SDA, Neogen, USA). For the preparation of the spore suspension, the culture was spread onto SDA plates and allowed to incubate for 14 days at 25.0°C. Following, the fungal growth on the plates was saturated PBW w/Tween 80 and the mycelial mats were removed using a sterile spatula and placed into a centrifuge tube. The culture was macerated and filtered through Whatman® #4 paper. The spore suspension was then centrifuged and suspended in PBW as per ASTM 2197-11. The suspension was enumerated by spread plating serial 1000-fold dilutions onto Sabouraud Dextrose Agar and incubating at 25.0ºC for 7-8 days. The suspension was used in the challenge study within 30 days of harvest.

**Test Article/Product: Bio-Neat NTS Concentrate**

On September 09, 2014, a bottle of Bio-Neat™ NTS concentrate solution was delivered to BCS Laboratories. The solution was issued BCS identifier 1409054. The solution's directions were followed based on the client's recommendations. The concentrate was diluted with distilled or demineralized water to recommended working solution. Briefly, a


BCS LABORATORIES, INC. - GAINESVILLE
4609 NW 6TH STREET, STE A, GAINESVILLE, FLORIDA 32609
TEL. (352) 377-9272, FAX. (352) 377-5630
WWW.MICROBIOSERVICES.COM
FL DOH LABORATORY #E82924, EPA# FL01147

1/1 dilution was made with 50ml of laboratory grade reagent water and 50 ml Concentrate. The solution was again diluted 1/1 with an additional 100ml of laboratory grade reagent water. The final diluted solution was placed in a sterile spray bottle and used within 30 minutes of preparation for the microbial spray studies. The temperature of the diluted solution prior to application and during the efficacy testing was maintained at 20-22°C. All tests were conducted in a Class II biological cabinet.

**Study Date for *Aspergillus niger*:** Study initiated November 14, 2014 and completed November 25, 2014.

**AOAC Official Method 961.02 Germicidal Spray Products as Disinfectants (2005)**

**Test Methodology Narrative:**

On the day of study, the cultures used were prepared as described previously and as per Method 961.02. The fungal population in each of the cultures was determined to be greater than $10^6$ cfu/mL.

An aliquots of the microbial suspension was spread onto each of 12 sterile 22 $mm^2$ glass slides (Propper Scientific, NY). The inoculum was allowed to dry in a covered chamber at 37°C for 50-60 minutes. Ten of the twelve inoculated slides were sprayed


BCS LABORATORIES, INC. - GAINESVILLE
4609 NW 6TH STREET, STE A, GAINESVILLE, FLORIDA 32609
TEL. (352) 377-9272, FAX. (352) 377-5630
WWW.MICROBIOSERVICES.COM
FL DOH LABORATORY #E82924, EPA# FL01147

for 10 seconds from a distance of approximately 12” with an even fine mist the prepared solution. The glass slides were completely saturated with solution. The slides were t allowed to incubate at 20-22°C for 10 minutes. Immediately following, each of the slides were picked up with sterile forceps, the excess liquid was allowed to run off, and the slide was placed into a sterile glass tube containing 10 ml of D/E neutralization buffer with tween (Neogen, USA). Additionally, un-inoculated slides were sprayed and incubated to serve as negative and neutralization controls. The remaining inoculated slides that were not exposed to the spray were used as positive recovery controls. The positive, neutralization, and negative controls slides were eluted in the same manner. The samples were agitated on a tabletop rotary shaker at a medium speed for 10 minutes. Following, the solution was assayed for fungal species by spread plating onto SDA as per Lab Standard Operating Procedure: SOP-1. Each of the treatment samples, neutralization control and negative control samples were analyzed by plating duplicate 0.1 ml and 1.0 ml samples of the solution directly. Positive controls were plated as described at 1/100 and 1/1000 dilutions. The Sabouraud Dextrose Agar plates were incubated at 25ºC ±1 for 10 days. Following incubation, the colonies on the respective plates were enumerated. Neutralization control recoveries confirmed the efficient neutralization of and antimicrobial residual.


BCS Laboratories, Inc. - Gainesville
4609 NW 6th Street, Ste A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
FL DOH Laboratory #E82924, EPA# FL01147

Study data are summarized in the provided table(s). Positive, negative, and process controls were performed as outlined in the method and as per Good Laboratory Practices. All analyses were performed in accordance with laboratory practices and procedures set-forth by our NELAP/TNI accreditation standards (ISO 17025) unless otherwise noted. BCS makes no express or implied warranty regarding the ownership, merchantability, safety or fitness for a particular purpose of any such property or product. The results presented pertain only to the study conducted on the test articles/samples provided by the client (or client representative). The study was authorized and commissioned by the client. The results presented pertain only to the samples analyzed and identifier number(s) indicated. The data provided is strictly representative of the study conducted using the material/samples/articles provided by the client (or client's representative) and it's (their) condition and homogeneity when received and at the time of test. Thus, the data may not be representative of the lot or batch number or other samples. Consequently, the data may not necessarily justify the acceptance or rejection of a lot or batch, a product recall, or support legal proceedings. It is the responsibility of the client to provide all information relevant to the analysis requested. The study and data obtained under the laboratory conditions may not be representative or indicative of a real-life process and/or application. This


BCS Laboratories, Inc. - Gainesville
4609 NW 6th Street, Ste A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
FL DOH Laboratory #E82924, EPA# FL01147

report does not imply that BCS Laboratories has been engaged to consult upon the consequences of the analysis and for any action that should be taken as a result of the analysis.


BCS Laboratories, Inc. - Gainesville
4609 NW 6th Street, Ste A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
FL DOH Laboratory #E82924, EPA# FL01147

**Table 1. The fungicidal efficacy of a spray application of diluted Bio-Neat™ NTS concentrate on *Aspergillus niger*; Method 961.02 Germicidal Spray Products as Disinfectants (2005) using a 10 minute contact time.**

| Treatment Slide | Average microorganism cfu/mL recovered from control # | Average cfu/mL recovered from each of slides sprayed* | Percent Reduction | $Log_{10}$ reduction | Cumulative Percent Reduction | Cumulative $Log_{10}$ reduction |
|---|---|---|---|---|---|---|
| 1 | $5.0 \times 10^3$ | 575 | 88.5% | 0.9 | 98.4% | 1.9 |
| 2 | | 24.1 | 99.5% | 2.3 | | |
| 3 | | 7.3 | 99.9% | 2.8 | | |
| 4 | | 20.9 | 99.6% | 2.4 | | |
| 5 | | 19.1 | 99.6% | 2.4 | | |
| 6 | | 20 | 99.6% | 2.4 | | |
| 7 | | 37.3 | 99.3% | 2.1 | | |
| 8 | | 19.1 | 99.6% | 2.4 | | |
| 9 | | 45.6 | 99.1% | 2.1 | | |
| 10 | | 40.9 | 99.2% | 2.1 | | |

# This number represents the average number of microorganisms (colony forming units/ milliliter) recovered from glass slides inoculated, dried, and not exposed to spray treatment (positive control).

* Glass slides were inoculated with the indicated microorganisms and allowed to dry. Slides were sprayed to saturation with the solution and allowed to incubate at 20-22.0°C for ten minutes. Slides were eluted and examined for growth as described in the methodology section.


BCS LABORATORIES, INC. - GAINESVILLE
4609 NW 6TH STREET, STE A, GAINESVILLE, FLORIDA 32609
TEL. (352) 377-9272, FAX. (352) 377-5630
WWW.MICROBIOSERVICES.COM
FL DOH LABORATORY #E82924, EPA# FL01147

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BIOLOGICAL CONSULTING SERVICES
*OF NORTH FLORIDA, INC.*

May 27, 2015

Jim Giorgi
Bio-Neat Inc
101 SE 10th Street
Ft. Lauderdale, FL 33316
954-729-1220
jim@bioneat.com

RE: Study report of the Norovirus virucidal efficacy testing of Bio-Neat™ NTS concentrate (BCS 1409054

Dear Mr. Giorgi,

We have conducted the norovirus virucidal efficacy testing on the provided Bio-Neat NTS concentrate. The testing was conducted as per ASTM E1053 "Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces," and ASTM E2197-11 "Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals." Murine Norovirus (MNV-1) was used in this study as a surrogate for human Norovirus.

In the following pages, you will find a summary of the methodology used and the results of our analysis. Should you have any questions or concerns, please do not hesitate to contact me.

Best Regards,

George Lukasik, Ph.D.
Laboratory Director



BCS LABORATORIES, INC. - GAINESVILLE
4609 NW 6TH STREET, STE. A, GAINESVILLE, FLORIDA 32609
TEL. (352) 377-9272, FAX. (352) 377-5630
WWW.MICROBIOSERVICES.COM
FL DOH LABORATORY #E82924, EPA# FL01147



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**AOAC Official Method 961.02 Germicidal Spray Products as Disinfectants (2005)**

Murine Norovirus 1 (MNV-1, PTA-5935) were propagated and enumerated as infectious units (iu) using EPA ICR Methodology (EPA 600/R-95/178; Total Culturable Viral Quantal Assay). Viruses were harvested by infecting cell monolayers and incubating at 36.5°C and 5% $CO_2$ until 90–95% of the cells demonstrated a cytopathic effect. The cells were frozen and thawed twice, followed by high speed centrifugation and filtration through a 0.1 µm filter. The supernatant was aliquoted as test virus suspension and stored at -80°C. For enumeration, aliquots containing virus were inoculated on freshly prepared monolayers of RAW 264.7 (ATCC TIB-71) cells. The cells were then supplemented with growth media, incubated at 36.5ºC and 5% carbon dioxide, and monitored for cytopathic effect development over a 10 day period. Infectious foci enumeration and cytopathic effect (CPE) grading followed by a Most Probable Number (MPN) calculation was used to determine the infectious units/ml in the samples analyzed.

For challenge studies, frozen viral stock (typically 1 x $10^8$ iu/ml) was thawed rapidly in a 35°C water bath on the day of the study. The virus stock was titered by performing serial ten-fold dilutions in PBS and was inoculated onto the respective cells as described above.



BCS Laboratories, Inc. - Gainesville
4609 NW 6th Street, Ste. A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
FL DOH Laboratory #E82924, EPA# FL01147

**Test Article/Product: Bio-Neat NTS Concentrate**

On September 09, 2014, a bottle of Bio-Neat™ NTS concentrate solution was delivered to BCS Laboratories. The solution was issued BCS identifier 1409054. The solution's directions were followed based on the client's recommendations. The concentrate was diluted with distilled or demineralized water to desired strength. Using 50ml of the concentrate, a 1/1 dilution was made with 50ml of laboratory grade reagent water. The 100ml diluted solution was again diluted 1/1 with an additional 100ml of laboratory grade reagent water. The final diluted solution was placed in a sterile spray bottle and used within 15 minutes of preparation for the microbial spray disinfection studies. The temperature of the diluted solution prior to application and during disinfection efficacy testing was maintained at 20-22°C. All tests were conducted in a Class II biological cabinet.

**Study Date for Murine Norovirus 1 virus:**

Study initiated May 11, 2015 and completed May 31, 2015.



BCS Laboratories, Inc. - Gainesville
4609 NW 6th Street, Ste. A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
FL DOH Laboratory #E82924, EPA# FL01147

**Test Methodology Narrative for Murine Norovirus 1 virus:**

On the day of study, viral stock concentrate was removed from -80º C, and thawed rapidly immediately prior to the study. Viral concentrate typically contain approximately $10^8$ infectious units/ ml.

Twenty-microliters of the homogenized microbial suspensions were placed and spread onto sterile 24x26 mm glass slides (Propper Scientific, NY). Nine slides were used for the viral study. Seven of the slides were inoculated and allowed to dry in a covered chamber at 25°C for 30 minutes. Five of the seven inoculated slides were sprayed for 6-7 seconds from a distance of approximately 12 inches with the diluted concentrate. The spray consisted of a fine steady mist. The glass slides were saturated with sprayed solution. The slides were incubated at 20-22°C for 10 minutes. Additionally, un-inoculated slides were sprayed and incubated to serve as negative and neutralization controls. The additional remaining inoculated slides that were not exposed to the spray disinfectant were used as a positive control. Immediately following 10 minutes, each of the slides were picked up with sterile forceps, the excess liquid was allowed to run off and the slides were placed each into sterile 50ml tubes (Corning,USA) containing 10 ml of D/E neutralization broth w/ tween. The recovered microorganisms from the positive control slide were used to determine the initial titer of



BCS Laboratories, Inc. - Gainesville
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Tel. (352) 377-9272, Fax. (352) 377-5630
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the microorganisms and thus the percent reductions. The tubes were placed onto a rotary shaker for 15 minutes to elute the viral particles.

The eluates were assayed for the presence of infectious virus particles using a MPN based assay onto RAW 264.7 cells monolayers. Positive, negative, cytotoxicity, and neutralization controls were performed as per ASTM recommendations and validated the test results. The samples were inoculated directly onto RAW 264.7 cells in sets of five at 0.1ml, and 0.01ml. The positive controls were diluted 1/100 in phosphate buffered saline (3M) and inoculated onto RAW 264.7 cells also in sets of five at 1.0, 0.1, and 0.1 ml. Cells were incubated at 36.5º C for 1 week.

Study data are summarized in the provided table(s). Positive, negative, and process controls were performed as outlined in the method and as per Good Laboratory Practices. All analyses were performed in accordance with laboratory practices and procedures set-forth by our NELAP/TNI accreditation standards (ISO 17025) unless otherwise noted. BCS makes no express or implied warranty regarding the ownership, merchantability, safety or fitness for a particular purpose of any such property or product. The results presented pertain only to the study conducted on the test articles/samples provided by the client (or client representative). The study was



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authorized and commissioned by the client. The results presented pertain only to the samples analyzed and identifier number(s) indicated. The data provided is strictly representative of the study conducted using the material/samples/articles provided by the client (or client's representative) and it's (their) condition and homogeneity when received and at the time of test. Thus, the data may not be representative of the lot or batch number or other samples. Consequently, the data may not necessarily justify the acceptance or rejection of a lot or batch, a product recall, or support legal proceedings. It is the responsibility of the client to provide all information relevant to the analysis requested. The study and data obtained under the laboratory conditions may not be representative or indicative of a real-life process and/or application. This report does not imply that BCS Laboratories has been engaged to consult upon the consequences of the analysis and for any action that should be taken as a result of the analysis.



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**Table 1. The virucidal efficacy of a spray application of diluted Bio-Neat™ NTS concentrate against Murine Norovirus 1 inoculated onto non porous surfaces. Test was conducted as per adaptation of ASTM E1053 "Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces" and ASTM E2197-11 "Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals."**

| Microorganism | Number of Replicates Tested | Contact Time (Minutes) | Average Infectious Units Control Slides # | Average Recovered From Each Sprayed Slide* | Percent Reduction | Average Percent Reduction | $Log_{10}$ reduction |
|---|---|---|---|---|---|---|---|
| Murine Norovirus 1 | 5 | 10 | 8,500 iu/ml | 1,640 iu/mL | 80.7% | 82.5% | 0.76 |
| | | | | 1,520 iu/mL | 82.1% | | |
| | | | | 1,260 iu/mL | 85.2% | | |
| | | | | 1,480 iu/mL | 82.6% | | |
| | | | | 1,533 iu/mL | 82.0% | | |

# This number represents the average number of infectious virus particles (units) recovered from glass slides inoculated, dried, and not exposed to treatment (positive control).
* Glass slides were inoculated and allowed to dry. Slides were sprayed to saturation with the solution and allowed to incubate for 10 minutes. Slides were eluted and enumerated for infectious viral particles on RAW cell monolayers as described.



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October 30, 2014

Jim Giorgi
Bio-Neat Inc
101 SE 10th Street
Ft. Lauderdale, FL 33316
954-729-1220
jim@bioneat.com

RE: Study report of the virucidal efficacy testing of Bio-Neat™ NTS concentrate; BCS 1409054

Dear Mr. Giorgi,

We have conducted the virucidal efficacy testing on the provided Bio-Neat NTS concentrate. The testing was conducted as per ASTM E1053 "Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces," and ASTM E2197-11 "Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals."

In the following pages, you will find a summary of the methodology used and the results of our analysis. Should you have any questions or concerns, please do not hesitate to contact me.

Best Regards,

George Lukasik, Ph.D.
Laboratory Director



BCS LABORATORIES, INC. - GAINESVILLE
4609 NW 6TH STREET, STE. A, GAINESVILLE, FLORIDA 32609
TEL. (352) 377-9272, FAX. (352) 377-5630
WWW.MICROBIOSERVICES.COM
FL DOH LABORATORY #E82924, EPA# FL01147



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**AOAC Official Method 961.02 Germicidal Spray Products as Disinfectants (2005)**

Human poliovirus 1- strain CHAT virus (ATCC VR-1562) was propagated and enumerated as infectious units (iu) using EPA ICR Methodology (EPA 600/R-95/178). Viruses were harvested by infecting cell monolayers and incubating at 36.5°C and 5% $CO_2$ until 90–95% of the cells demonstrated a cytopathic effect. The cells were frozen and thawed twice, followed by high speed centrifugation and filtration through a 0.1 µm filter. The supernatant was aliquoted as test virus suspension and stored at -80°C. For enumeration, aliquots containing herpes virus were inoculated on freshly prepared monolayers of Buffalo Green Monkey (BGM) kidney cells. The cells were supplemented with growth media and incubated at 36.5ºC and 5% carbon dioxide and monitored for cytopathic effect development over a 10 day period. Infectious foci and cytopathic effects (CPE) was determined as per methodology outlined in EPA 600/R9-95/178 and a Most Probable Number (MPN) assay was used to determine the infectious units/ml in the samples analyzed.

For challenge studies, frozen viral stock (typically 1 x $10^8$ iu/ml) was thawed rapidly in a 35°C water bath on the day of the study. The virus stock was titered by performing



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4609 NW 6th Street, Ste. A, Gainesville, Florida 32609
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serial ten-fold dilutions in PBS and was inoculated onto the respective cells as described above.

**Test Article/Product: Bio-Neat NTS Concentrate**

On September 09, 2014, a bottle of Bio-Neat™ NTS concentrate solution was delivered to BCS Laboratories. The solution was issued BCS identifier 1409054. The solution's directions were followed based on the client's recommendations. The concentrate was diluted with distilled or demineralized water to desired strength. Using 50ml of the concentrate, a 1/1 dilution was made with 50ml of laboratory grade reagent water. The 100ml diluted solution was again diluted 1/1 with an additional 100ml of laboratory grade reagent water. The final diluted solution was placed in a sterile spray bottle and used within 15 minutes of preparation for the microbial spray disinfection studies. The temperature of the diluted solution prior to application and during disinfection efficacy testing was maintained at 20-22°C. All tests were conducted in a Class II biological cabinet.

**Study Date for Human poliovirus 1- strain CHATt virus:**

Study initiated October 17, 2014 and completed October 29, 2014.



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4609 NW 6th Street, Ste. A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
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**Test Methodology Narrative for Human poliovirus 1- strain CHAT virus:**

On the day of study, viral stock concentrate was removed from -80º C, and thawed rapidly immediately prior to the study. Viral concentrate typically contain approximately $10^8$ infectious units/ ml.

Twenty-microliters of the homogenized microbial suspensions were placed and spread onto sterile 24x26 mm glass slides (Propper Scientific, NY). Seven slides were used for the viral study. Five of the slides were inoculated and allowed to dry in a covered chamber at 37°C for 50-60 minutes. Three of the five inoculated slides were then sprayed for 5 seconds from a distance of approximately 12 inches with the diluted concentrate. The spray consisted of a fine steady mist. The glass slides were saturated with sprayed solution. The slides were then incubated at 20-22°C for 10 minutes. Additionally, un-inoculated slides were sprayed and incubated to serve as negative and neutralization controls. The additional remaining inoculated slides that were not exposed to the spray disinfectant were used as a positive control. Immediately following 10 minutes, each of the slides were picked up with sterile forceps, the excess liquid was allowed to run off and the slides were placed into sterile 50ml tubes



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(Corning,USA) containing 10 ml of D/E neutralization broth w/ tween. The recovered microorganisms from the positive control slide were used to determine the initial titer of the microorganisms and thus the percent reductions. The tubes were placed onto a rotary shaker for 15 minutes to elute the viral particles.

The eluates were assayed for the presence of infectious virus particles using a MPN based assay onto BGM cell monolayers. Positive, negative, cytotoxicity, and neutralization controls were performed as per ASTM recommendations and validated the test results. The samples were diluted 1/100 and inoculated onto BGM cells in sets of five at 1.0ml, 0.1ml, and 0.01ml. The positive controls were diluted 1/1000 in phosphate buffered saline (Weber) and inoculated onto BGM cells also in sets of five at 1.0, 0.1, and 0.1 ml. Cells were incubated at 36.5º C for 2 weeks.

Study data are summarized in the provided table(s). Positive, negative, and process controls were performed as outlined in the method and as per Good Laboratory Practices. All analyses were performed in accordance with laboratory practices and procedures set-forth by our NELAP/TNI accreditation standards (ISO 17025) unless otherwise noted. BCS makes no express or implied warranty regarding the ownership, merchantability, safety or fitness for a particular purpose of any such property or



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product. The results presented pertain only to the study conducted on the test articles/samples provided by the client (or client representative). The study was authorized and commissioned by the client. The results presented pertain only to the samples analyzed and identifier number(s) indicated. The data provided is strictly representative of the study conducted using the material/samples/articles provided by the client (or client's representative) and it's (their) condition and homogeneity when received and at the time of test. Thus, the data may not be representative of the lot or batch number or other samples. Consequently, the data may not necessarily justify the acceptance or rejection of a lot or batch, a product recall, or support legal proceedings. It is the responsibility of the client to provide all information relevant to the analysis requested. The study and data obtained under the laboratory conditions may not be representative or indicative of a real-life process and/or application. This report does not imply that BCS Laboratories has been engaged to consult upon the consequences of the analysis and for any action that should be taken as a result of the analysis.



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Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
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**Table 1. The virucidal efficacy of a spray application of diluted Bio-Neat™ NTS concentrate against Human poliovirus 1 (ATCC VR 1562) inoculated onto non porous surfaces. Test was conducted as per adaptation of ASTM E1053 "Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces" and ASTM E2197-11 "Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals."**

| Microorganism | Number of Replicates Tested | Contact Time (Minutes) | Average Infectious Units Control Slides # | Average Recovered From Each Sprayed Slide* | Percent Reduction | Average Percent Reduction | $Log_{10}$ reduction |
|---|---|---|---|---|---|---|---|
| Human Poliovirus 1 | 3 | 10 | $1.6 \times 10^4$ iu/ml | 790 iu/mL | 95.1% | 95.9% | 1.4 |
| | | | | 940 iu/mL | 94.1% | | |
| | | | | 230 iu/mL | 98.6% | | |

# This number represents the average number of infectious virus particles (units) recovered from glass slides inoculated, dried, and not exposed to treatment (positive control).
* Glass slides were inoculated and allowed to dry. Slides were sprayed to saturation with the solution and allowed to incubate for 10 minutes. Slides were eluted and enumerated for infectious viral particles on Buffalo Green Monkey cell monolayers as described.



BCS Laboratories, Inc. - Gainesville
4609 NW 6th Street, Ste. A, Gainesville, Florida 32609
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April 30, 2015

Jim Giorgi
Bio-Neat Inc
101 SE 10th Street
Ft. Lauderdale, FL 33316
954-729-1220
jim@bioneat.com

RE: Study report of Bio-Neat™ NTS solution's potential efficacy on the reduction of *Listeria monocytogenes* from inoculated surface; BCS 1409054

Dear Mr. Giorgi,

We have conducted the exploratory study on the provided Bio-Neat NTS concentrate as requested. The testing was conducted as per AOAC Method 961.02 (AOAC Official Methods of Analysis; 2005) and ASTM E2197-11 "Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals."

In the following pages, you will find a summary of the methodology used and the results of our analysis. Should you have any questions or concerns, please do not hesitate to contact me.

Best Regards,

George Lukasik, Ph.D.
Laboratory Director


BCS LABORATORIES, INC. - GAINESVILLE
4609 NW 6TH STREET, STE A, GAINESVILLE, FLORIDA 32609
TEL. (352) 377-9272, FAX. (352) 377-5630
WWW.MICROBIOSERVICES.COM
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**Bacterial Culture Preparation**

*Listeria monocytogenes* (ATCC 15313) stock cultures were obtained from Microbiologics Inc (USA) and maintained as per suppliers' recommendations. Working cultures of bacteria were propagated from pure stocks onto Tryptic Soy Agar (TSA, Neogen, MI). For the challenge study, a single colony from a pure plate stock was transferred to 10 ml of Tryptic Soy Broth (TSB, Neogen). Cultures were incubated at 36.5 ºC for 48 hours. A colony of culture was transferred to a fresh tube of TSB and grown at 36.5 ºC for 24 hours. The overnight culture was transferred to a fresh tube of TSB broth the next day. This step was repeated again the following day. Two days prior to the study, a final transfer was made. An aliquot of the culture was transferred to a fresh tube of TSB and grown for an additional 48 hours at 36.5±1°C prior to the day of the challenge study. On the day of the challenge, the broth culture was mixed at high speed (Vortex Mixer) until homogenous. This suspension was used for the challenge studies following settling for 10 minutes.


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4609 NW 6th Street, Ste A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
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**Test Article/Product: Bio-Neat NTS Concentrate**

On September 09, 2014, a bottle of Bio-Neat™ NTS concentrate solution was delivered to BCS Laboratories. The solution was issued BCS identifier 1409054. The solution's directions were followed based on the client's recommendations. On the day of the study, the concentrate was diluted with distilled or demineralized water to recommended working solution. Briefly, a 1/1 dilution was made with 50ml of laboratory grade reagent water and 50 ml Concentrate. The solution was again diluted 1/1 with an additional 100ml of laboratory grade reagent water. The final diluted solution was placed in a sterile spray bottle and used within 15 minutes of preparation for the microbial spray studies. The temperature of the diluted solution prior to application and during the efficacy testing was maintained at 20-22°C. All tests were conducted in a Class II biological cabinet.

**Study Date**

Study initiated April 27, 2015 and completed April 30, 2015.


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4609 NW 6th Street, Ste A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
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**AOAC Official Method 961.02 Germicidal Spray Products as Disinfectants (2005)**

**Test Methodology Narrative**

On the day of study, the culture used was prepared as described previously and as per Method 961.02. The bacterial and population in each of the cultures was determined to be greater than $10^7$ cfu/mL

Twenty microliters of the microbial suspension was spread onto five sterile 22 $mm^2$ glass slides (Propper Scientific, NY). The inoculum was allowed to dry in a covered chamber at 25°C for 30 minutes. Three of the five inoculated slides were sprayed for 10 seconds from a distance of approximately 12" with an even fine mist the prepared solution. The glass slides were completely saturated with solution. The slides were to allowed to incubate at 20-22°C for 10 minutes. Immediately following, each of the slides were picked up with sterile forceps, the excess liquid was allowed to run off, and the slide was placed into a sterile glass tube containing 10 ml of D/E neutralization buffer with tween (Neogen, USA). Additionally, un-inoculated slides were sprayed and incubated to serve as negative and neutralization controls. The remaining inoculated slides that were not exposed to the spray were used as positive recovery controls. The positive, neutralization, and negative controls slides were eluted in the same manner.


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The samples were agitated on a tabletop rotary shaker at a medium speed for 10 minutes. Following, the solution was assayed for microbial species by spread plating onto TSA as per Lab Standard Operating Procedure: SOP-1. Each of the treatment samples, neutralization control and negative control samples were analyzed by plating duplicate 0.1 ml and 1.0 ml samples of the solution directly. Positive controls were plated as described at 1/1000 dilutions. The tryptic soy agar plates were incubated at 36.5ºC ±1 for 48 hours. Following incubation, the colonies on the respective plates were enumerated. Neutralization control recoveries confirmed the efficient neutralization of and antimicrobial residual.

Study data are summarized in the provided table(s). Positive, negative, and process controls were performed as outlined in the method and as per Good Laboratory Practices. All analyses were performed in accordance with laboratory practices and procedures set-forth by our NELAP/TNI accreditation standards (ISO 17025) unless otherwise noted. BCS makes no express or implied warranty regarding the ownership, merchantability, safety or fitness for a particular purpose of any such property or product. The results presented pertain only to the study conducted on the test articles/samples provided by the client (or client representative). The study was authorized and commissioned by the client. The results presented pertain only to the


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samples analyzed and identifier number(s) indicated. The data provided is strictly representative of the study conducted using the material/samples/articles provided by the client (or client's representative) and it's (their) condition and homogeneity when received and at the time of test. Thus, the data may not be representative of the lot or batch number or other samples. Consequently, the data may not necessarily justify the acceptance or rejection of a lot or batch, a product recall, or support legal proceedings. It is the responsibility of the client to provide all information relevant to the analysis requested. The study and data obtained under the laboratory conditions may not be representative or indicative of a real-life process and/or application. This report does not imply that BCS Laboratories has been engaged to consult upon the consequences of the analysis and for any action that should be taken as a result of the analysis.


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Tel. (352) 377-9272, Fax. (352) 377-5630
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**Table 1. The efficacy of a spray application of diluted Bio-Neat™ NTS concentrate on the reduction of *Listeria monocytogenes*; Method 961.02 Germicidal Spray Products as Disinfectants (2005) using a 10 minute contact time.**

| Treatment Slide | Average microorganism recovered from positive control # | Average cfu/mL recovered from each of slides sprayed* | Percent Reduction | $Log_{10}$ reduction | Average Percent Reduction | Average $Log_{10}$ reduction |
|---|---|---|---|---|---|---|
| 1 | $5.2 \times 10^3$ cfu/mL | <0.45 | >99.991% | >4.1 | >99.991% | >4.1 |
| 2 | | <0.45 | >99.991% | >4.1 | | |
| 3 | | <0.45 | >99.991% | >4.1 | | |

# This number represents the average number of microorganisms (colony forming units/ milliliter) recovered from glass slides inoculated, dried, and not exposed to spray treatment (positive control).
* Glass slides were inoculated with the indicated microorganisms and allowed to dry. Slides were sprayed to saturation with the solution and allowed to incubate at 20-22.0°C for ten minutes. Slides were eluted and examined for growth as described in the methodology section.


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WWW.MICROBIOSERVICES.COM
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December 15, 2016

Vincenzo Gizzi
Bio-Neat Inc
101 SE 10th Street
Ft. Lauderdale FL 33316
954-729-1220
Vince@bioneat.com
Client ID: BioNeat NTS, BioNeat NTS, BioNeat NTS

BCS ID: 1611253, 1611254, 1612119

Project Name: BioNeat NTS 1:3 Solution Efficacy Testing

Dear Vincenzo Gizzi,

We have completed the filtration efficacy study on the submitted units as outlined below. The contaminant species, study conditions, and water parameters utilized were based on client's request and adaptation of the guidance documents and protocols listed below:

| |
|---|
| AOAC Method 961.02. Germicidal Spray Protocol (NOT ISO17025 Accredited) |

Following, you will find our report on the results of the study conducted on the referenced samples. Should you have any questions, please do not hesitate to contact me.

Sincerely,

George Lukasik, Ph.D.
Laboratory Director


BCS LABORATORIES, INC. – GAINESVILLE
4609 NW 6TH STREET. STE. A. GAINESVILLE. FLORIDA 32609
TEL. (352) 377-9272, FAX. (352) 377-5630
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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Analysis: *S. Aureus Bacteria Reduction Efficacy* | | | | | Test Carrier: Glass Slide 25mm | | |
| Application Method: Spray | | | | | | Temp: 22.3 | |
| Conformance of Study Validation Data: Negative Control: Yes | | | Positive Control: Yes | | Neutralizer Control: Yes | | |
| Start Conc: | 7.60E+05 cfu/mL | | | Contact Time: 10 minutes | | | |
| BCS Sample ID 1 | 1611253 | Client ID 1 | BioNeat NTS | | | | |
| End Conc 1: | 7.30E+04 cfu/mL | | % Reduct 1: | 90.3 | Log10 Reduct 1: | 1 | |
| BCS Sample ID 2 | 1611254 | Client ID 2 | BioNeat NTS | | | | |
| End Conc 2: | 7.50E+04 cfu/mL | | % Reduct 2: | 90.1 | Log10 Reduct 2: | 1 | |
| BCS Sample ID 3 | 1612119 | Client ID 3 | BioNeat NTS | | | | |
| End Conc 3: | 7.40E+04 cfu/mL | | % Reduct 3: | 90.2 | Log10 Reduct 3: | 1 | |
| Test Notes: | | | | | | | |


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4609 NW 6th Street. Ste. A. Gainesville. Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com

Project: BioNeat NTS 1:3 Solution Efficacy Testing

Date Received: November 22, 2016 16:30 Analyst: David Sekora, M.S.

Test Start Date: December 08, 2016 Test End Date: December 09, 2016 Qualifier:

Report Notes:

20uL of the bacteria was applied directly to the center of sterile glass slides. The slides were allowed to incubate in a laminar follow hood for 30 minutes with the blower on. After 30 minutes the positive control slides were tranfered to tubes containing 10mL of D/E neutrilizeing buffer (BD, USA). A 1:3 dilution of the provided solution was prepared using laboratory grade reagent water and a set of three 25mm glass slides were sprayed at adistance of 10" over the course of 7 seconds. The slides were completely saturated with the prepared solution following application and given a 10 minute contact time before being tranfered to tubes containing 10mL of neutrilizing buffer. The samples were homogenized on an orbital shaker for 10 minutes to elute the microorganisms. The positive controls were diluted 1/1000 and plated in 0.1 and 1.0 mL duplicates. Negative controls were preformed for the microorganism and plated in 0.1mL and 1.0mL duplicates. Samples were plated onto TSA in 0.1 and 1.0mL duplicates. The plates were incubated for 24 hours at 36.5°C.


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4609 NW 6th Street. Ste. A. Gainesville. Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
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*I certify that I have examined  I am familiar with the information submitted herein. The results pertain only to the sample(s) analyzed  associated identifier #(s). Based on my inquiry of the individuals responsible for the analysis, I believe the data to be true, accurate, and complete.  Unit descriptions and names were obtained from the submitted documents.  The analysis was authorized and commissioned by the client or client's representative. The resulting data are representative of the analysis conducted on the collected samples and it's/their condition at the time of analysis. The data provided is strictly representative of the study conducted under laboratory conditions using the material/samples/articles provided by the client (or client's representative) and it's (their) condition at the time of test. The data obtained may not be representative or indicative of a real-life process and/or application. The sample(s) were analyzed in accordance with the appropriate method, however due to the inherent limitations of methods, microorganisms may avoid detection.  BCS Laboratories offers no express or implied warranties concerning the quality, safety, and/or purity of any sample, batch, source, or the process they are derived from.  Quality assurance controls were performed as outlined in the method and as per Good Laboratory Practices. Analyses were performed in accordance with laboratory practices and procedures set-forth by ISO 17025-2005 and NELAP/TNI accreditation standards unless otherwise noted. BCS makes no express or implied warranty regarding the ownership, merchantability, safety or fitness for a particular purpose of any such property or product.

Signature of Laboratory Director/Authorized Rep.  Date: December 15, 2016


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4609 NW 6th Street. Ste. A. Gainesville. Florida 32609
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| DATA QUALIFIER CODES | |
|---|---|
| SYMBOL | MEANING |
| D | Measurement was made in the field. |
| I | The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit. |
| J1 | The sample matrix interfered with the ability to make any accurate determination. |
| J2 | No Quality Control criteria exist for the component. |
| ^ | analysis conducted outside the Laboratory's scope of accreditation |
| L | Off scale high. Actual value is known to be greater than value given. |
| O | Sampled, but analysis not performed. |
| Q | Sample held beyond the accepted holding time. |
| U | Indicates that the compound was analyzed for but not detected. The reported value is the method detection limit. |
| V | Analyte was detected in both sample and associated method blank. Data may not be accurate. |
| Y | The laboratory analysis was from an improperly preserved sample. The data may not be accurate. |
| Z | Too many colonies present (TNTC); the numeric value given represents the upper end of the value that can be determined based on the volume. |
| ? | Data are rejected and should not be used. QC data did not meet acceptance criteria. |
| ** | Analysis of analyte submitted to an accredited sub-contract laboratory. |
| ! | Data deviate from historically established concentration range. |
| # | BCS Lab specific qualifier. See laboratory analysis notes. |


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4609 NW 6th Street. Ste. A. Gainesville. Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
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**Biological Consulting Services**
*of North Florida, Inc.*

June 28, 2011

Vincenzo Gizzi
Bio-Neat Inc.
13765 NW 22nd Street
Sunrise FL, 33323

Tel: 954 553 0069
vince@bioneat.com

Re: Antibacterial efficacy testing of the provided Bio-Neat Natural Shampoo/Soap Formula.

Dear Mr. Gizzi:

We have completed the antibacterial efficacy study on the provided shampoo/soap sample. The testing was done according to the protocol we regularly use to assess antimicrobial efficacy of disinfectants.

The disinfectant efficacy was determined against *Escherichia coli* and *Salmonella typhimurium* as models of human bacterial pathogens that are transmissible through feces. According to observed results, the tested formula exhibited excellent antibacterial efficacy. It should effectively reduce and control the transmission of the tested pathogens when used as directed.

In the following pages, you will find a summary of the methodology used and the results of our analysis. Should you have any questions please do not hesitate to contact me.

Best Regards,

George Lukasik, Ph.D.
Director



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4609 NW 6th Street, Bldg A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
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**Challenge Bacterial Culture Preparation and Enumeration**

*Salmonella Typhimurium* (ATCC 19585) stock culture was obtained from American Type Culture Collection (ATCC) and was maintained at -80°C. Working cultures were both kept and propagated on Tryptic Soy Agar (TSA, Beckton Dickinson, MD).

*Escherichia coli* (ATCC 15597) stock cultures were obtained from ATCC and were maintained at -80°C. Working cultures were kept and propagated on Tryptic Soy Agar (TSA, Beckton Dickinson, MD).

For challenge experiments, an overnight culture from colony purified plate stock was grown in 10 ml of Tryptic Soy Broth (TSB, Beckton Dickinson, MD) at 36 ºC prior to the date of the experiments. On the day of challenge, the broth culture was centrifuged at 3K x G for 5 minutes and suspended in 10 ml of phosphate buffered saline (PBS, Fisher scientific, PA).

The number of viable bacterial species was enumerated as colony forming units (cfu) using spread plating onto Tryptic Soy Agar. Plates were incubated at 36.5º C for 24 hours. The resulting colonies were enumerated and the cfu/ml was determined.



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4609 NW 6TH Street, Bldg A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
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**Provided Sample:**

On June 11, 2011 a bottle containing the Bio-Neat Natural Soap/Shampoo formula was received. The sample was issued BCS # 1106042. Sample was store at room temperature (23-27°C) till use.

**Challenge Study (June 16, 2011):**

The supplied formula was tested to evaluate the effect on the inactivation of bacterial fecal pathogens. The study was conducted to simulate exposure of potential pathogens to diluted product in an aqueous environment. This would resemble a bathing or washing scenario. The temperature of the liquids in the study was maintained at 20-22°. One-hundred microliters of the microbial suspension was added to 90 ml of Deionized Water (DI, Class I ASTM water). This was done for each microbial species. To the inoculated DI, 10 ml of the provided formula was added and agitated at a slow speed to ensure dispersion. The flasks were labeled “Treated”. Controls were also done by inoculating 100 µl of the bacterial suspension into 100 ml DI; the flasks were labeled “Untreated Control”. Samples from the inoculated “Treated” and “Untreated Control” flasks were removed at 0 minutes, 5 minutes, and 15 minutes



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4609 NW 6th Street, Bldg A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
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following addition of formula. The number of viable microbial species in each of the samples was enumerated as described. Analysis for each sample was conducted in duplicate

The results of the above study are summarized in the following Table. The results presented pertain only to the samples analyzed and the tested unit indicated in the condition at the time of testing. They are not representative nor are they indicative of a process. Positive and negative controls were performed as outlined in the Method and as per Good Laboratory Practices. All analyses were performed in accordance to NELAC accreditation standards (ISO 17025) unless otherwise noted.



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4609 NW 6th Street, Bldg A, Gainesville, Florida 32609
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**Table 1. The inactivation of fecal bacterial pathogens by the Bio-Neat Natural Shampoo/Soap Formula at a 10% concentration and following 5 and 15 minute exposure to the diluted formula.**

| Microorganism | Average colony forming units (cfu) / ml present initially (untreated Control)[#] | Average cfu/ml following 5 minute exposure | Average cfu/ml following 15 minute exposure | Percent Reduction |
|---|---|---|---|---|
| *Escherichia coli* | $3.4 \times 10^5$ | <1.0 | <1.0 | >99.999% |
| *Salmonella Typhimurium* | $1.8 \times 10^5$ | <1.0 | <1.0 | >99.999% |

[#] This number represents the average number of microorganisms present in the deionized water prior to addition of the formula. This number remained the same through out the study as evident by the number recovered from "Untreated Control".

* This number represents the average number of microorganisms present at the indicated time point in the deionized water adjusted to 10% formula. Samples were taken from the inoculated flasks following the addition of the formula and agitation at a slow speed



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4609 NW 6th Street, Bldg A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
FL DOH Laboratory #E82924, EPA# FL01147

# STUDY REPORT

**Study Title**
Virucidal Efficacy of a Test Substance For Use on Inanimate, Nonporous Surfaces

**Product Identity**
Bioneat
(BIO-1001)

**Test Microorganism**
Human coronavirus, Strain 229E, ATCC VR-740

**Study Identification Number**
NG15491

**Author**
Emily Cox, B.S.

**Study Completion Date**
05AUG2020

**Testing Facility**
Microchem Laboratory
1304 W. Industrial Blvd.
Round Rock, Texas 78681

**Study Sponsor**
United Food Products
Jeff Kaufman
11555 Heron Bay Blvd., Ste 200
Parkland, FL 33076

## STUDY REPORT SUMMARY

General Study Information

| | |
|---|---|
| Study Title: | ASTM E1053 Method<br>Virucidal Efficacy of a Test Substance For Use on Inanimate, Nonporous Surfaces |
| Study Identification Number: | NG15491 |

Test System

| | |
|---|---|
| Test Microorganism: | Human coronavirus, Strain 229E, ATCC VR-740 |
| Host Cell: | MRC-5 (CCL-171) |
| Test Substance: | Bioneat (BIO-1001) |
| Test Substance Receipt Date: | 22MAY2020 |

Test Parameters

| | |
|---|---|
| Test Substance Dilution: | Ready to use liquid test substance |
| Test Substance Application: | 2.0 ml delivered by serological pipette |
| Organic Soil Load: | No additional soil load incorporated into the inoculum |
| Number of Replicates Per Lot: | Double |
| Contact Time: | 10 minutes |
| Exposure Temperature: | Ambient room temperature (23.4 – 23.5°C) and 42% Relative Humidity (RH) |
| Neutralization Method: | Sephadex LH-20 gel filtration columns |

Study Dates

| | |
|---|---|
| Experimental Start Date/Time: | 17JUL2020 / 1135 |
| Experimental Termination Date/Time: | 24JUL2020 / 1545 |
| Study Completion Date: | 05AUG2020 |

## TEST PROCEDURE

Summary

- Stock virus was thawed and was not supplemented with an organic soil load.
- Sterile glass Petri dish carriers (100 x 15 mm) were inoculated with a volume of virus suspension. A sufficient number of test and control carriers were prepared.
- Inoculated carriers were dried at room temperature under laminar flow conditions.
- The test substance was prepared according to the Study Sponsor's instructions as requested, and applied to the test carriers using a serological pipette.
- The treated carriers were held for the predetermined contact time(s), and then neutralized in a manner appropriate for the test substance (e.g. dilution and/or gel filtration).
- The control carrier was held covered for the contact time then harvested and neutralized in the same manner as the test.
- Following neutralization of test and control carriers, the viral suspensions were quantified to determine the levels of infectious virus using standard cell culture (e.g. $TCID_{50}$) or plaque assay techniques.
- Assay trays/plates were incubated for the period most suitable for the virus-host cell system (e.g. 7 days).
- After the incubation period, the assay was scored for the presence/absence of test virus and cytotoxic effects. The appropriate calculations were performed (e.g. Spearman-Karber) to determine viral titers and levels of test substance cytotoxicity, where applicable.
- $Log_{10}$ and percent reductions were calculated for viral films exposed to the test product relative to the titer obtained for the study control carrier(s), and reported to the Study Sponsor.

## SUCCESS CRITERIA

The following measures are met to ensure the acceptability of virucidal efficacy data:

- A minimum of 4.80 $\log_{10}$ infective units/control carrier is recovered from each plate recovery control film(s).
- The virus titer control demonstrate obvious and or typical cytopathic effects on the monolayers unless a detection method other than cytopathic effect is used.
- Neutralization of the test substance with a low titer (e.g. 1000-5000 infective units) of the test virus is demonstrated.
- Quantification of the test and control parameters are conducted at a minimum of four determinations per dilution.

The product performance criteria follows:

- In the presence or absence of cytotoxicity, the product should demonstrate a ≥3.00 $\log_{10}$ reduction in viral titer on each surface.
- If cytotoxicity is present, the virus control titer should be increased if necessary to demonstrate a ≥3.00 $\log_{10}$ reduction in viral titer on each surface beyond the cytotoxicity level.

## CALCULATIONS AND STATISTICAL ANALYSIS

The $TCID_{50}$ (Tissue Culture Infectivity Dose) represents the endpoint dilution where 50% of the cell cultures exhibit cytopathic effects due to infection by the test virus. The endpoint dilution at which 50% of the host cell monolayers exhibit cytotoxicity is termed the Tissue Culture Dose ($TCD_{50}$). The $TCID_{50}$, and $TCD_{50}$ was determined using the Spearman-Kärber method and calculated as follows:

Negative logarithm of endpoint titer =

[– Log of first dilution inoculated] – [((sum of % mortality at each dilution/100) – 0.5) x Logarithm of dilution]

The result of this calculation is expressed as $TCID_{50}$/0.1 ml (or volume of dilution inoculated) for the test, virus control, and neutralization control and $TCD_{50}$/0.1 ml (or volume of dilution inoculated) for the cytotoxicity control.

Calculation of the Log Reduction

The log reduction in viral titer was calculated as follows:

Plate Recovery Control $Log_{10}$ $TCID_{50}$ – Virus-Test Substance $Log_{10}$ $TCID_{50}$

Calculation of the Percent Reduction

The percent reduction in viral titer was calculated as follows:

Percent Reduction = 1- (C/B) x 100, where:
B = Average $TCID_{50}$ of virus in control suspensions.
C = Average $TCID_{50}$ of virus in virus-test suspensions.

The presence of any test substance cytotoxicity were taken into account when calculating the log and percent reductions in viral titer.

If multiple virus control and test replicates were performed, the average $TCID_{50}$ of each parameter was calculated and the average result used to calculate the log reductions in viral titer.

## RESULTS

**Table 1: Virus Titer and Plate Recovery Control Results**

| | | Virus Titer | Virus Plate Recovery Control Replicate #1 | Virus Plate Recovery Control Replicate #2 |
|---|---|---|---|---|
| Cell Control | | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| Dilution | $10^{-1}$ | + + + + | + + + + | + + + + |
| | $10^{-2}$ | + + + + | + + + + | + + + + |
| | $10^{-3}$ | + + + + | + + + + | + + + + |
| | $10^{-4}$ | + + + + | + + + + | + 0 + + |
| | $10^{-5}$ | 0 0 + + | 0 0 + 0 | 0 0 0 0 |
| | $10^{-6}$ | 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 |
| $TCID_{50}$ per 0.1 ml | | 5.00 $Log_{10}$ | 4.75 $Log_{10}$ | 4.25 $Log_{10}$ |
| $TCID_{50}$ per Carrier | | N/A | 5.05 $Log_{10}$ | 4.55 $Log_{10}$ |
| Average $TCID_{50}$ per Carrier | | 4.80 | | |

**Key:** + = Virus recovered; 0 = Virus not recovered and/or no cytotoxicity observed; T = Cytotoxicity observed

**Table 2: Test Results**

| | | Bioneat (BIO-1001) Replicate #1 | Bioneat (BIO-1001) Replicate #2 |
|---|---|---|---|
| Cell Control | | 0 0 0 0 | 0 0 0 0 |
| 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 |
| | $10^{-7}$ | 0 0 0 0 | 0 0 0 0 |
| $TCID_{50}$ per 0.1 ml | | $\leq 0.50$ $Log_{10}$ | $\leq 0.50$ $Log_{10}$ |
| $TCID_{50}$ per Carrier | | $\leq 0.80$ $Log_{10}$ | $\leq 0.80$ $Log_{10}$ |
| Average $TCID_{50}$ per Carrier | | $\leq 0.80$ $Log_{10}$ | |
| Average $Log_{10}$ Reduction | | $\geq 3.00$* | |
| Average Percent Reduction | | $\geq 99.90\%$ | |

**Key:** + = Virus recovered; 0 = Virus not recovered and/or no cytotoxicity observed; T = Cytotoxicity observed
*Log Reduction calculations performed taking cytotoxicity into account

**Table 3: Cytotoxicity and Neutralization Control Results**

| | | Cytotoxicity | Neutralization |
|---|---|---|---|
| Cell Control | | 0 0 0 0 | 0 0 0 0 |
| Dilution | $10^{-1}$ | T T T T | T T T T |
| | $10^{-2}$ | 0 0 0 0 | + + + + |
| | $10^{-3}$ | 0 0 0 0 | + + + + |
| $TCID_{50}$ per 0.1 ml | | 1.50 $Log_{10}$ | 1.50 $Log_{10}$ |

**Key:** + = Virus recovered; 0 = Virus not recovered and/or no cytotoxicity observed; T = Cytotoxicity observed

## STUDY CONCLUSION

The purpose of the study was to determine the virucidal efficacy of Bioneat (BIO-1001) against Human coronavirus Strain 229E, with no additional soil load incorporated into the inoculum, at a contact time of 10 minutes, and at an exposure temperature of room temperature (23.4 – 23.5°C) and 42% RH.

The Plate Recovery Control demonstrated an average viral titer of 4.80 $Log_{10}$ $TCID_{50}$ per carrier.

Test Substance cytotoxicity was detected in the lot of test substance assayed at 1.50 $Log_{10}$.

The Test Substance Neutralization Control demonstrated that the test substance was neutralized at 1.50 $Log_{10}$ for the lot assayed.

Taking the cytotoxicity and neutralization control results into consideration, the evaluated test substance, Bioneat (BIO-1001), demonstrated an average ≥3.00 $Log_{10}$ reduction in viral titer (≥99.90%) at a contact time of 10 minutes.

*The test substance will be disposed of 30 days after the completion of this study, unless otherwise requested by the Study Sponsor.*

*The results of this study apply to the tested substances(s) only. Extrapolation of findings to related materials is the responsibility of the Sponsor.*

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BIOLOGICAL CONSULTING SERVICES
OF NORTH FLORIDA, INC.

October 27, 2014

Jim Giorgi
President
Bio-Neat Inc.

Dear Mr Giorgi;

We have conducted the ASTM F895-11 (Standard Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity) preliminary screening study as per your request. The analysis is equivalent to ISO 10993-5. The study was performed on the provided "BioNeat NTS™ Concentrate" sample (BCS # 1409054) received on September 09, 2014. In the following pages, you will find a brief description of the methodology used and the results of our analyses. Based on the observed results, the diluted solution exhibited negligible toxicity on the CCL-1 cell line tested as per the agar diffusion method.

Should you have any questions or concerns, please do not hesitate to contact me.

Best Regards;

George Lukasik, Ph.D.
Laboratory Director



BCS LABORATORIES, INC. -GAINESVILLE
4609 NW 6TH STREET, SUITE A,
GAINESVILLE, FLORIDA 32609
TEL. (352) 377-9272, FAX. (352) 377-5630
WWW.MICROBIOSERVICES.COM
FL DOH LABORATORY #E82924, EPA# FL01147

**Cytotoxicity evaluation of the supplied liquid sample "BioNeat™ NTS Concentrate" (BCS# 1409054). ASTM F895-11 test method conducted on March 16, 2012.**

The Sample was analyzed as described in ASTM F895-11 and ISO 10993-5. Briefly, monolayers of L929 cells were grown in six-well cell culture plates (Corning, USA). Following 24 hours to cell passage, the plates were over-layed with agar supplemented dMEM/FBS media (MediaTech, VA) as described by the ASTM method. Once the agar solidified, sterile 13 mm cellulosic filters (Millipore, USA) were placed onto the surface of the agar. The provided concentrate was diluted as per clients request 1:16 in laboratory grade reagent water (Deionized water). Fifty microliters of the diluted concentrate was added to the center of each of 6 discs. Two discs received phosphate buffered saline as they served as negative controls. Additionally, 2 filters inoculated with 50µl of 0.5% phenol solution were used as positive controls. The cells were then incubated in 5% Carbon dioxide at 36.5 °C ±1 for 24 hours. The cells were then incubated in 5% carbon dioxide at 36.5 °C ±1 for 24 hours. The plates and cells were evaluated macro and microscopically for signs of malformation, degeneration, sloughing, or lysis of the cells within the zone directly beneath and surrounding the discs. Cells were then re-incubated for an additional 24 hours at the conditions described above and evaluated again. The size of the zone surrounding the inoculated area was evaluated for signs of cell growth inhibition and/or cell lysis. The numerical evaluation is presented in the following tables. Table 1 contains the numerical values describing the size of zone of lysis surrounding the inoculums as per the ASTM standards. Table 2 contains the relative numerical value describing the number of cells affected within the zone of inhibition or toxicity. Each sample observed is assigned a numerical value based on cellular degradation and/or cell death. The interpretation of the assigned numerical values is presented in Table 3 and Table 4; Briefly, number 0= unchanged/no evident cytotoxicity; 5= complete monolayer destruction and severe cytotoxicity. The ASTM and ISO 10993-5 consider a scoring of 3 or higher as toxic.



BCS Laboratories, Inc. -Gainesville
4609 NW 6th Street, Suite A,
Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
FL DOH Laboratory #E82924, EPA# FL01147

Study data are summarized in the provided table(s). Positive, negative, and process controls were performed as outlined in the method and as per Good Laboratory Practices. All analyses were performed in accordance with laboratory practices and procedures set-forth by our NELAP/TNI accreditation standards (ISO 17025) unless otherwise noted. BCS makes no express or implied warranty regarding the ownership, merchantability, safety or fitness for a particular purpose of any such property or product. The results presented pertain only to the study conducted on the test articles/samples provided by the client (or client representative). The study was authorized and commissioned by the client. The results presented pertain only to the samples analyzed and identifier number(s) indicated. The data provided is strictly representative of the study conducted using the material/samples/articles provided by the client (or client's representative) and it's (their) condition and homogeneity when received and at the time of test. Thus, the data may not be representative of the lot or batch number or other samples. Consequently, the data may not necessarily justify the acceptance or rejection of a lot or batch, a product recall, or support legal proceedings. It is the responsibility of the client to provide all information relevant to the analysis requested. The study and data obtained under the laboratory conditions may not be representative or indicative of a real-life process and/or application. This report does not imply that BCS Laboratories has been engaged to consult upon the consequences of the analysis and for any action that should be taken as a result of the analysis.



BCS Laboratories, Inc. -Gainesville
4609 NW 6th Street, Suite A,
Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
FL DOH Laboratory #E82924, EPA# FL01147

**Table 1. Description of the size of zone of lysis surrounding the filter disks[1] inoculated with dilution of BioNeat NTS™ Concentrate.**

| Sample | Zone Description Following 24 Hour Incubation* | | | Zone Description Following 48 Hour Incubation* | | |
|---|---|---|---|---|---|---|
| **Filter disks with 1:16 dilution[1]** | 1 | 1 | 1 | 2 | 2 | 1 |
| **(+) Control (0.5 %Phenol)** | 4 | | | 5 | | |
| **(-) CONTROL** | 0 | | | 0 | | |

*0= unchanged; 5= complete monolayer destruction and severe cytotoxicity.

[1] Dilutions of K BioNeat NTS™ Concentrate (BCS 1409054) were performed in sterile deionized laboratory water (Class I ASTM water). Cellulosic 13 mm filter discs were inoculated 50µl of the solution and were allowed to air dry prior to placing onto the agar overlaid cell monolayers.



BCS Laboratories, Inc. -Gainesville
4609 NW 6th Street, Suite A,
Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
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**Table 2. Description of the condition of cells within the zone of lysis surrounding the filter disks[1] as per the ASTM standards**

| Sample | Cell Description Following 24 Hour Incubation* | | | Cell Description Following 48 Hour Incubation* | | |
|---|---|---|---|---|---|---|
| **Filter disks with 1:16 dilution[1]** | 1 | 1 | 1 | 2 | 2 | 2 |
| **(+) Control (0.5 %Phenol)** | 5 | | | 5 | | |
| **(-) CONTROL** | 0 | | | 0 | | |

*0= unchanged; 5= complete monolayer destruction and severe cytotoxicity.

[1] Dilutions of BioNeat NTS™ Concentrate (BCS 1409054) were performed in sterile deionized laboratory water (Class I ASTM water). Cellulosic 13 mm filter discs were inoculated 50µl of the solution and were allowed to air dry prior to placing onto the agar overlaid cell monolayers.



BCS Laboratories, Inc. -Gainesville
4609 NW 6th Street, Suite A,
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Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
FL DOH Laboratory #E82924, EPA# FL01147

**Table 3. Zone Description (for data presented in Table 1)**

| Zone index | Description of Zone |
|---|---|
| 0 | No detectable zone around or under specimen |
| 1 | Zone limited to area under specimen |
| 2 | Zone extends less than 0.5 cm beyond specimen |
| 3 | Zone extends 0.5 to 1.0 cm beyond specimen |
| 4 | Zone extends greater than 1.0 cm beyond specimen but does not involve entire dish |
| 5 | Zone involves entire dish |

**Table 4. Lysis Description (for data presented in Table 2)**

| Zone index | Description of Zone |
|---|---|
| 0 | No observable cytotoxicity |
| 1 | Less than 20 % of zone affected |
| 2 | 20 to 39 % of zone affected |
| 3 | 40 to 59 % of zone affected |
| 4 | 60 to 80 % of zone affected |
| 5 | Greater than 80 % of zone affected |



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4609 NW 6th Street, Suite A,
Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
FL DOH Laboratory #E82924, EPA# FL01147

RespirTek™
CONSULTING LABORATORY

# OECD 301B Ready/Ultimate Biodegradability Assessment

**Date of Final Report: November 19, 2014**

**Total Number of Pages: 14**

Report Prepared For:
Bio-Neat, Inc.
101 SE 10th Street
Ft. Lauderdale, FL 33316
954-462-2225

Report Prepared By:
RespirTek, Inc.
12450 Shortcut Rd. Bldg F
Biloxi, MS 39532
228-392-7977

## Table of Contents

## Study Summary

The Test Substance, BioNeat NTS™, was evaluated for ready and ultimate biodegradability in an aqueous medium, when exposed to an inoculum source according to the procedures detailed in the OECD 301B methodology.

Based on the test method employed, the maximum biodegradability of the test materials are as follows:

| Test Substance | Percent Biodegradation | Classification |
|---|---|---|
| BioNeat NTS™ | 61.7% | Ultimate |

This value is the highest observed during the 28 day test for each test substance.

**Project ID: BIO-2413**
**Date: November 19, 2014**
**Quality Assurance Unit Statement**

The purpose of the Quality Assurance Unit is to monitor the conduct and reporting of laboratory studies. Enclosed is the final report data for project ID BIO-2413. All analyses were conducted following procedures set forth by the ISO/IEC 17025:2005 accreditation program standards. A copy of RespirTek's ISO/IEC 17025:2005 certification and scope is attached at the end of this report. Quality assurance systems and quality control criteria have been reviewed for the data collected, either internally or externally by one of RespirTek's affiliate laboratories, and the data review generated the following response:

**QA/QC criteria met for all analyses**

Anthony Miranda, M.S.
Technical Director
RespirTek, Inc.
Phone: (228) 392-7977
Fax: (228) 396-3984
www.respirtek.com

Ryan Vandermeulen
Quality Manager
RespirTek, Inc.
Phone: (228) 392-7977
Fax: (228) 396-3984
www.respirtek.com

PJLA Testing
Accreditation #69085

**Client:** BIO-2413

**Test Product(s):** BioNeat NTS™

**Test Method:** OECD 301B - $CO_2$ Evolution Test

**Report Date:** November 19, 2014

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## 1.0 Laboratory

Testing as presented in this report was conducted by RespirTek, Inc (RespirTek). The testing facility is located at 12450 Shortcut Rd., Bldg F, Biloxi, MS 39532.

## 2.0 Sample Receipt

Sample receipt was recorded on September 10, 2014 at the RespirTek testing facility. One box was received from FedEx and contained1 product for testing. The sample material was securely wrapped and the lid was sealed. The sample was labeled as below and given the following laboratory identification:

- BioNeat NTS™ - BIO-TC1

The sample was received at ambient temperature in good condition with no evidence of damage or contamination. No temperature preservation was required.

## 3.0 Summary of Method

The OECD 301B biodegradability testing monitors the degree of activity of microorganisms exposed to a material that is being tested for a biodegradable status. In the test, if the microorganisms recognize the material as a food source, then an increase in biological activity is observed through data collection specifically designed to assess the biological conversion of organic carbon to inorganic carbon. If the material is not a recognizable food source or is toxic or inhibitory, then there is no measurable increase in biological activity or, in some cases, there is a marked decrease in activity relative to a biodegradable control.

## 4.0 Project Preparation

Prior to test setup the appropriate number of 5 L Pyrex reactor bottles was washed and rinsed with tap water. The bottles were then rinsed three times with distilled water (DI $H_2O$) and allowed to dry.

The mineral salt stock solutions for the project was prepared in media bottles using the appropriate chemicals and DI $H_2O$. The chemicals were weighed out using an analytical balance, and the DI $H_2O$ was measured out using several 1000 mL or 100 mL volumetric flasks. Individual solutions were made up as follows:

Solution 1: The following compounds were added to 1000 mL of DI $H_2O$:
- 8.50 g of $KH_2PO_4$
- 21.75 g of $K_2HPO_4$
- 33.40 g of $Na_2HPO_4 \bullet 2\ H_2O$
- 0.50 g of $NH_4Cl$

The pH of the solution was then adjusted to 7.4.

Solution 2: 36.40 g of $CaCl_2 \bullet 2\ H_2O$ was added to 1000 mL of DI $H_2O$.

Solution 3: 22.50 g of $MgSO_4 \bullet 7\ H_2O$ was added to 1000 mL of DI $H_2O$.

Solution 4: The following compounds were added to 1000 mL of DI $H_2O$:
- 0.25 g of $FeCl_3 \bullet 6\ H_2O$
- 1 drop of concentrated HCl

All mineral salt stock solutions were kept in cold storage at 4°C chiller until used. A record of all chemical lot numbers and expiration dates are maintained in the laboratory Quality Standards Log.

## 5.0 Inoculum Collection and Conditioning

The Inoculum was collected from the Escawtapa, Mississippi POTW on October 03, 2014. This inoculum was immediately taken to the lab, homogenized, then placed into a 6 L Erlenmeyer flask. A Teflon stir bar was then added to the flask. The inoculum was placed on a magnetic stir plate. A $CO_2$-free aeration system, which uses a $CO_2$ scrubber system consisting of KOH, was used to purge the inoculum. The inoculum continued stirring and aerating, uninterrupted, throughout the 5 day conditioning period.

## 6.0 Procedure

On October 07, 2014, a mineral stock solution was made up, as follows, according to OECD method 301B specifications:

| | |
|---|---|
| DI water: | 59,220 mL |
| Solution 1: | 600 mL |
| Solution 2: | 60 mL |
| Solution 3: | 60 mL |
| Solution 4: | 60 mL |
| For a total of: | 60 L |

Then, 2400 mL of the homogenized mineral stock solution was added to each 5 L reactor bottle. A Teflon stir bar was added to each reactor, which was then placed on a stir plate and connected to a $CO_2$ scrubber system consisting of series of soda lime and 10N NaOH scrubbers. Air flow to the system was confirmed using a Restek ProFlow 6000 Flowmeter to ensure air flows were within the 30–100 mL/min range that is stated within the method. The remaining nutrient solution was connected to a $CO_2$ scrubber overnight.

A Total Suspended Solids test was performed on the inoculum using a Hach Lange DR5000. The test was performed on a 1:10 dilution of inoculum to DI $H_20$ in triplicate. The average TSS was calculated to be 2,199mg/L.

The 301B method requires 30 mg of TSS to be added per liter of nutrient solution for a total of 3 L of nutrient biomass solution. Therefore 41 mL of inoculum was added to each reactor bottle already containing the mineral medium.

The nutrient - inoculum solution (2400 mL nutrient solution + 41 mL Inoculum) remained in the 5 L reactor bottles on a stir plate and hooked to the $CO_2$ scrubber system for 24 hrs.

On October 07, 2014 RespirTek, Inc. prepared stock solutions for the reference and test material, and performed an analysis of the test and reference materials to obtain Total Organic Carbon (TOC) values.

The TOC concentration values obtained during the preparation of the test and reference material concentrated stock solutions are tabulated below:

| Sample ID | TOC |
|---|---|
| BioNeat NTS™<br>BIO-TC1 | 253.1 mg/L |
| Sodium Benzoate<br>(PC) | 328 mg/L |

Using the concentrated stock solution TOC values, appropriate test chemicals, and positive control additions were made to obtain a final reactor TOC value of 10 mg/L for both the PC and TC.

The total amount of product to be added to the nutrient inoculum solution was added to enough mineral stock solution (the remaining solution that scrubbed overnight) to obtain a final total reactor composition of 3 L.

- **BioNeat NTS™ (BIO-TC1):** 118 mL BIO-TC1 test material stock solution + 2400 mL $CO_2$ Free Mineral Stock Solution+ 41 mL biomass + 441 mL DI water.
- **Sodium Benzoate (PC):** 91 mL Sodium Benzoate PC Stock Solution + 2400 mL $CO_2$ Free Mineral Stock Solution + 41 mL biomass + 468 DI water.
- **Blank (B)**: 2400 mL $CO_2$ Free Mineral Stock Solution + 41 mL biomass + 559 mL DI water only.

All reactors were delivered $CO_2$-free air by passing compressed air through several soda lime and 10N NaOH scrubbers. The reactors were then continually stirred, kept in diffuse light and allowed to vent into a three-series 0.05N NaOH scrubber solution. Each scrubber solution was analyzed for TIC (Total Inorganic Carbon) concentrations periodically throughout the extent of the test to determine concentrations of $CO_2$ produced by each reactor. Scrubber solutions were periodically refreshed to ensure adequate absorption of $CO_2$ was maintained. TIC analyses were performed on a Shimadzu TOC-V CSH instrument, which was calibrated prior to test initiation and periodically throughout the duration of the

test. Test reactors were setup in duplicate for statistical validation of results, and a total of 9 sampling events was executed.

## 7.0 Results and Conclusions

Based on the testing conducted in accordance with the specified method above, test product, BioNeat NTS™ achieved 61.7% biodegradation. The product met method requirements for *Ultimate Biodegradability* classification.

## 8.0 Records

Original raw data are archived at RespirTek, Inc. A copy of the final report and any report amendments are archived at RespirTek, Inc. The original final report, and any protocol amendments or deviations, is forwarded to the client.

All used and unused test substance shall be disposed of by RespirTek 6 months following test termination.

## 9.0 Confidentiality

Per corporate policy, confidentially shall be maintained in general, and in specific accordance with any relevant agreement specifically executed between RespirTek and the Client.

**Project Number: BIO-2413**
**Final Report Date: November 19, 2014**
**Project Initiation Date: October 07, 2014**
**Test Method: OECD 301B $CO_2$ Evolution Test**

| Test Chemical | Biodegradation (%) | Classification |
|---|---|---|
| BioNeat NTS™ | 61.7 | Ultimate |

**Prepared for Bio-Neat, Inc.**

**Prepared by RespirTek, Inc.**
**12450 Shortcut Road**
**Building F**
**Biloxi, MS 39532**

The enclosed data relates only to those samples received by the laboratory.

**BIO-2413 Data and Calculations**

**Test Compound BioNeat NTS™: TC1**
**Positive Control Sodium Benzoate: PC**

| | Analytical |
|---|---|
| Positive TOC | 10.00 |
| TC 1-1 TOC | 10.00 |
| TC 1-2 TOC | 10.00 |
| Positive ThCO2 | 110.00 |
| TC 1-1 ThCO2 | 110.00 |
| TC 1-2 ThCO2 | 110.00 |

Day 0 is a reference point for graphical illustrations only. No samples were collected on Day 0.

**TIC (mg/L)**

| | Day 0 | Day 2 | Day 5 | Day 8 | Day 12 | Day 15 | Day 19 | Day 23 | Day 26 | Day 28 |
|---|---|---|---|---|---|---|---|---|---|---|
| **Blank-1** | 0.0 | 12.5 | 17.1 | 16.6 | 21.8 | 15.3 | 20.6 | 20.3 | 18.5 | 28.1 |
| **Blank-2** | 0.0 | 13.6 | 15.4 | 17.9 | 21.4 | 15.1 | 20.7 | 22.6 | 19.9 | 21.8 |
| **PC-1** | 0.0 | 36.5 | 47.5 | 35.6 | 36.7 | 21.1 | 24.6 | 26.0 | 26.6 | 25.1 |
| **PC-2** | 0.0 | 40.8 | 47.2 | 36.9 | 39.4 | 18.9 | 27.4 | 25.5 | 25.3 | 26.2 |
| **TC1-1** | 0.0 | 19.7 | 35.9 | 37.6 | 44.6 | 32.1 | 32.1 | 25.5 | 19.0 | 23.1 |
| **TC1-2** | 0.0 | 18.0 | 38.1 | 39.6 | 36.2 | 24.3 | 29.5 | 22.8 | 18.7 | 19.6 |

**Total CO2 (mg)**

| | Day 0 | Day 2 | Day 5 | Day 8 | Day 12 | Day 15 | Day 19 | Day 23 | Day 26 | Day 28 |
|---|---|---|---|---|---|---|---|---|---|---|
| **Blank-1** | N/A | 9.1 | 12.5 | 12.1 | 16.0 | 11.3 | 15.1 | 14.9 | 13.6 | 20.6 |
| **Blank-2** | N/A | 10.0 | 11.3 | 13.2 | 15.7 | 11.1 | 15.2 | 16.6 | 14.6 | 16.0 |
| **PC-1** | N/A | 26.8 | 34.9 | 26.1 | 26.9 | 15.5 | 18.1 | 19.1 | 19.5 | 18.5 |
| **PC-2** | N/A | 29.9 | 34.6 | 27.1 | 28.9 | 13.9 | 20.1 | 18.7 | 18.5 | 19.2 |
| **TC1-1** | N/A | 14.4 | 26.4 | 27.6 | 32.7 | 23.6 | 23.6 | 18.7 | 13.9 | 16.9 |
| **TC1-2** | N/A | 13.2 | 27.9 | 29.0 | 26.6 | 17.9 | 21.7 | 16.8 | 13.7 | 14.4 |

**Cumulative CO2 (mg)*****

| | Day 0 | Day 2 | Day 5 | Day 8 | Day 12 | Day 15 | Day 19 | Day 23 | Day 26 | Day 28 |
|---|---|---|---|---|---|---|---|---|---|---|
| **Blank-1** | N/A | 9.1 | 21.7 | 33.8 | 49.8 | 61.1 | 76.2 | 91.1 | 104.7 | 125.3 |
| **Blank-2** | N/A | 10.0 | 21.3 | 34.5 | 50.2 | 61.3 | 76.5 | 93.1 | 107.7 | 123.7 |
| **PC-1** | N/A | 26.8 | 61.6 | 87.7 | 114.6 | 130.1 | 148.2 | 167.3 | 186.8 | 205.2 |
| **PC-2** | N/A | 29.9 | 64.5 | 91.6 | 120.5 | 134.4 | 154.5 | 173.3 | 191.8 | 211.0 |
| **TC1-1** | N/A | 14.4 | 40.8 | 68.4 | 101.1 | 124.7 | 148.3 | 167.0 | 180.9 | 197.9 |
| **TC1-2** | N/A | 13.2 | 41.1 | 70.2 | 96.8 | 114.6 | 136.3 | 153.0 | 166.7 | 181.1 |

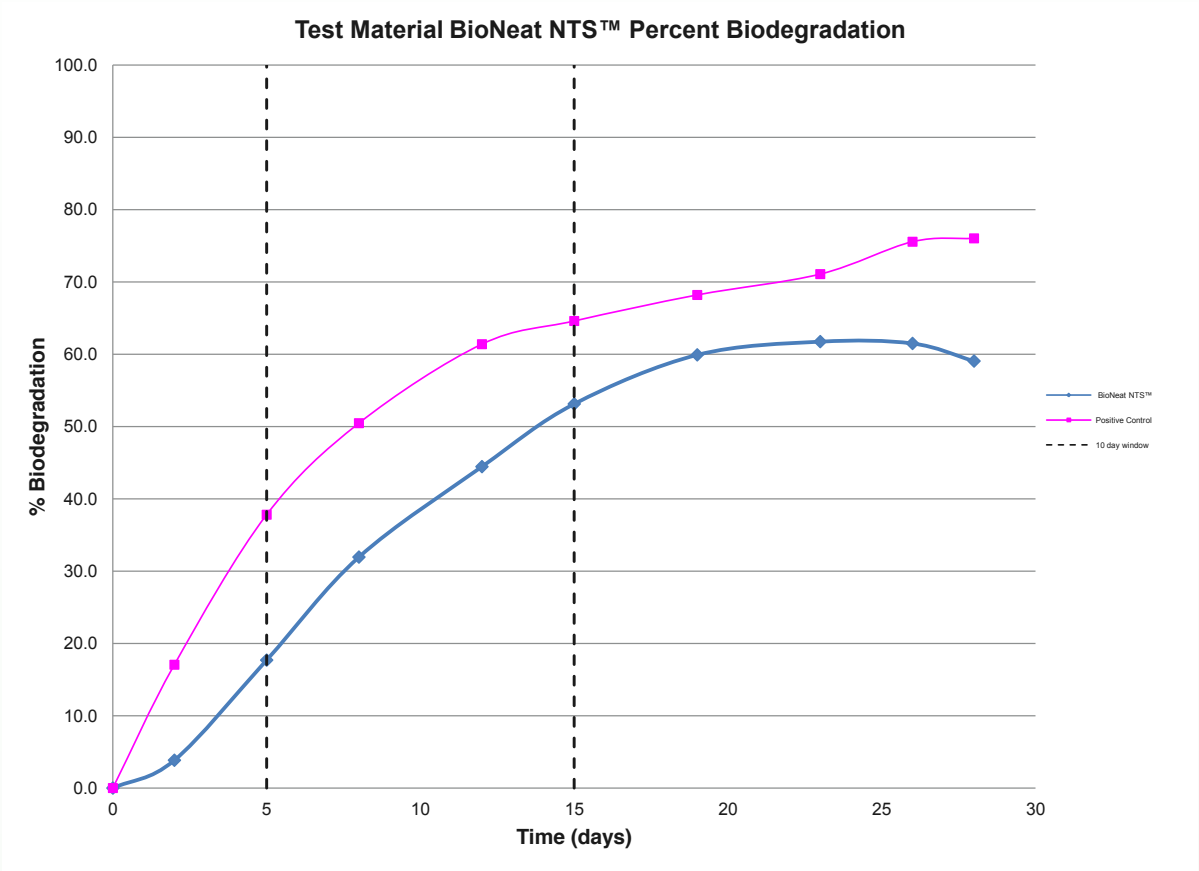
**Percent Biodegradation (%)**

| | Day 0 | Day 2 | Day 5 | Day 8 | Day 12 | Day 15 | Day 19 | Day 23 | Day 26 | Day 28 |
|---|---|---|---|---|---|---|---|---|---|---|
| **PC-1** | N/A | 15.6 | 36.5 | 48.7 | 58.7 | 62.7 | 65.3 | 68.4 | 73.3 | 73.4 |
| **PC-2** | N/A | 18.5 | 39.1 | 52.2 | 64.0 | 66.5 | 71.1 | 73.8 | 77.8 | 78.7 |
| **TC1-1** | N/A | 4.4 | 17.5 | 31.1 | 46.4 | 57.7 | 65.4 | 68.1 | 67.9 | 66.7 |
| **TC1-2** | N/A | 3.3 | 17.9 | 32.8 | 42.5 | 48.6 | 54.5 | 55.4 | 55.0 | 51.4 |
| **PC Average** | **0.00** | **17.1** | **37.8** | **50.5** | **61.4** | **64.6** | **68.2** | **71.1** | **75.6** | **76.0** |
| **TC1 Average** | **0.00** | **3.8** | **17.7** | **31.9** | **44.5** | **53.1** | **59.9** | **61.7** | **61.5** | **59.1** |

**Standard Deviation**

| | Day 0 | Day 2 | Day 5 | Day 8 | Day 12 | Day 15 | Day 19 | Day 23 | Day 26 | Day 28 |
|---|---|---|---|---|---|---|---|---|---|---|
| **PC** | N/A | 3.0 | 0.2 | 0.9 | 1.9 | 1.6 | 2.0 | 0.3 | 0.9 | 0.7 |
| **TC1** | N/A | 1.2 | 1.5 | 1.4 | 5.9 | 5.5 | 1.8 | 1.9 | 0.2 | 2.5 |
| **Blank** | N/A | 0.8 | 1.1 | 1.0 | 0.3 | 0.2 | 0.1 | 1.7 | 1.0 | 4.4 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Sample Days** | **0** | **2** | **5** | **8** | **12** | **15** | **19** | **23** | **26** | **28** |


Test Material BioNeat NTS™ Percent Biodegradation
% Biodegradation
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0
5
10
15
20
25
30
Time (days)
BioNeat NTS™
Positive Control
10 day window

# PERRY JOHNSON LABORATORY ACCREDITATION, INC.

# *Certificate of Accreditation*

*Perry Johnson Laboratory Accreditation, Inc. has assessed the Laboratory of:*

***RespirTek, Inc.***
***12450 Shortcut Road, Building F, Biloxi, MS 39532***

*(Hereinafter called the Organization) and hereby declares that Organization is accredited in accordance with the recognized International Standard:*

**ISO/IEC 17025:2005**

This accreditation demonstrates technical competence for a defined scope and the operation of a laboratory quality management system
(as outlined by the joint ISO-ILAC-IAF Communiqué dated January 2009):

***Biological and Chemical Testing***
***(As detailed in the supplement)***

Accreditation claims for such testing and/or calibration services shall only be made from addresses referenced within this certificate. This Accreditation is granted subject to the system rules governing the Accreditation referred to above, and the Organization hereby covenants with the Accreditation body's duty to observe and comply with the said rules.

For PJLA:

Tracy Szerszen
President/Operations Manager

Perry Johnson Laboratory
Accreditation, Inc. (PJLA)
755 W. Big Beaver, Suite 1325
Troy, Michigan 48084

| *Initial Accreditation Date:* | *Issue Date:* | *Expiration Date:* |
|---|---|---|
| September 16, 2011 | March 4, 2014 | March 4, 2016 |

| *Accreditation No.:* | *Certificate No.:* |
|---|---|
| 69085 | L14-71 |

*The validity of this certificate is maintained through ongoing assessments based on a continuous accreditation cycle. The validity of this certificate should be confirmed through the PJLA website: www.pjlabs.com*

# *Certificate of Accreditation: Supplement*

**Respirtek, Inc.**
12450 Shortcut Road, Building F, Biloxi, MS 39532
Jude Martin Phone: 228-392-7977

*Accreditation is granted to the facility to perform the following testing:*

| FIELD OF TEST | ITEMS, MATERIALS OR PRODUCTS TESTED | SPECIFIC TESTS OR PROPERTIES MEASURED | SPECIFICATION, STANDARD METHOD OR TECHNIQUE USED | RANGE (WHERE APPROPRIATE) AND DETECTION LIMIT |
|---|---|---|---|---|
| Environmental Biological | Plastic Material | Aerobic Biodegradation | ISO 14855 | % Biodegradation |
| | | | ASTM D5338 | |
| | | | ISO 14852 | |
| | | Oxobiodegradation & Biodegradation | ASTM D6954 | |
| | | Compostability | ASTM D6400 | |
| | | Anaerobic Biodegradability | ISO 15985 | |
| | Chemical | Aquatic Aerobic Biodegradation | OECD 301A | |
| | | | OECD 301B | |
| | | | OECD 301C | |
| | | | OECD 301D | |
| | | | OECD 301E | |
| | Water/Soil Samples | Treatability/Toxicity Testing<br><br>HPC | Internally developed protocols-microcosm studies<br>SM 9215B | |
| Biological | Chemical Compounds | Aquatic Aerobic Biodegradation | OECD 301F | |
| | | | ASTM D5210 | CO2 Gas<br>DL 1%<br>CH4 Gas<br>DL 0.10 % |
| | | | OECD 311<br>OECD 302B | |
| | | | ASTM D5511 | |
| | | | ASTM D5864 | |
| | | | ASTM D5271 | |
| | | | ASTM E1720 | |
| | | | ASTM D5988 | |
| | Aqueous Sample | TOC | SM5310B | |
| | | | ISO 14593 | |
| | | | ISO 9439 | |
| | | | ISO 15985 | |
| Chemical | Water Samples | Biological Oxygen Demand | Standard Methods 5210 D | IDL 1 mg/L |
| | Water Samples | Total Organic/Inorganic Carbon | Standard Methods 5310 C | MDL 0.5 mg/L |
| | Gas Samples | Carbon Dioxide Instruments | Gas Chromatography | IDL 1% |
| | | Methane Instruments | Gas Chromatography | IDL 1 % |

# COMPARATIVE ANALYSIS

BioNeat NTS™- All Purpose Cleaner

vs.

BioSolve® Pinkwater®

STERLING LABORATORIES
6800 W. Central Ave, Ste. L1
Toledo, OH 43617
419.885.3183
www.sterlinglabsinc.com

July 27, 2021

# Bioneat, Inc.
# Project 21148
# All Purpose Cleaners
# Hydrocarbon Soil Removal

Bioneat
Biosolve Pink Water

STERLING LABORATORIES
6800 W. Central Ave, Ste. L1
Toledo, OH 43617
419.885.3183
www.sterlinglabsinc.com

July 27, 2021

Vincent Gizzi
Bioneat, Inc.
101 S. East 10th Street
Ft Lauderdale, FL 33316

Dear Vincent,

You sent us a sample of **All Purpose Cleaner** identified as **Bioneat.** We evaluated it for soil removals against **Biosolve-Pink Water**. We used various hydrocarbon-based soils on stainless steel. A summary of the testing is below.

**The Samples are comparable in Overall Soil Removals.**

| Samples | % Soil Removals | % of Best |
|---|---|---|
| Bioneat | 497.4 | Best |
| Pink Water | 496.6 | 99.8% |

Descriptions of the test method, summaries of the results and sample information are attached.

Sincerely,

Tod Losey
Sterling Laboratories

STERLING LABORATORIES
6800 W. Central Ave, Ste. L1
Toledo, OH 43617
419.885.3183
www.sterlinglabsinc.com

# Sample Information

All Purpose Cleaners

1. **Bioneat**, All Purpose Cleaner,
   USDA Certified Biobased Product / Product 100%,
   Made With Natural, Organic And Biobased Ingredients,
   Non-Toxic, 100% Biodegradable, Non Caustic, Hypoallergenic, Non Hazardous,
   Lot #: None Found, UPC #: Non Found,
   Received 1-6-21 from Bioneat

2. **Biosolve, Pinkwater**, Hydrocarbon Mitigation Agent,
   Effective Across A Wide Spectrum Of Crude Oil & Hydrocarbon Products,
   Commonly Used For: Remediation 1 US Gallon (3.8 Liters) Bottle,
   Lot #: None Found, UPC #: None Found,
   Received 7-16-21 from RVR, LLC.

STERLING LABORATORIES
6800 W. Central Ave, Ste. L1
Toledo, OH 43617
419.885.3183
www.sterlinglabsinc.com

# Photographs of Samples Tested

All Purpose Cleaners


Bioneat


Biosolve
Pink Water

STERLING LABORATORIES
6800 W. Central Ave, Ste. L1
Toledo, OH 43617
419.885.3183
www.sterlinglabsinc.com

# TEST METHOD

All Purpose Cleaners

## Substrate and Soils Used

Q-Lab Stainless Steel SS-36 was the substrate used. We soiled six (6) panels of each for the following soils: Cutting Oil, Used Motor Oil, Hydraulic Fluid, Gear Oil, and Lube Oil. They were allowed to set at room temperature for two (2) days. We then measured the reflectance using a Konica Minolta CR410 Colorimeter. (D65/2°, Y - Color Scale)

## Sample Dilutions

- All samples were tested using a 12% by volume dilution with tap water.

## Gardner Runs: (3 replicates / sample)

We applied 2 mL of sample onto soiled area, allowed it to soak for 30 seconds and then scrubbed it 10 cycles with a water-dampened sponge using a Gardner In-Line abrasion apparatus. Samples were rinsed under the tap and allowed to air dry before measuring again.

## Soil Removal Calculations

The percent (%) soil removal (SR) was computed by %SR=100(A-B)/(C-B) where A is the reflectance of the tile after cleaning, B is the reflectance before cleaning, and C is the reflectance of the unsoiled tile. We generated the significant difference at the 95% confidence level for each of the soil groups. Samples within 10% are said to be comparable.

## %Soil Removal Data Summary

| Soil | Substrate | Bioneat | Pink Water | Sig. Dif. |
|---|---|---|---|---|
| Cutting Oil | Stainless Steel | 99.2 | 99.7 | 0.5 |
| Gear Oil | Stainless Steel | 99.3 | 99.2 | 0.7 |
| Hydraulic Fluid | Stainless Steel | 99.6 | 99.2 | 0.7 |
| Lube Oil | Stainless Steel | 99.6 | 99.4 | 0.7 |
| Used Motor Oil | Stainless Steel | 99.7 | 99.1 | 0.8 |
| ***Overall Soil Removals*** | | ***497.4*** | ***496.6*** | |
| % of Best | | Best | 99.8% | |

Values are the average of 3 data points per soil.

# SAFETY DATA SHEET (SDS)

Material Name: **BioNeat NTS™-1** All Purpose Cleaner
Issue Date: 06/14/2021
MSDS No.: Formula NTS-01

## Section 1- CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

| | |
|---|---|
| Chemical Name: | Colloid |
| Internal Part Number: | NTS-01 |
| Product Use: | All-Purpose Cleaner |
| Mfg. Part #: | Formula NTS-01 |
| Brand Names: | BioNeat NTS™-1 |
| Synonyms: | Cleaner |
| Sup. Part #: | Not applicable |

Manufacturer Information:
BIONEAT INC.
Address: 101 SE 10th Street
Fort Lauderdale, FL 33316
Phone: (800) 749-2466

## Section 2 – COMPOSITON/INFORMATION ON INGREDIENTS

Component Information/Information on Non-Hazardous Components:
All components have been identified and evaluated under the criteria specified in 29 CFR 1910.1200 (Hazard Communication Standard).
**Active ingredients:**
Bioneat is a proprietary, Nano-technology/Colloidal Micelle Formula made from Natural resource Ingredients including:

| Ingredient | Function | CAS # | Concentration |
|---|---|---|---|
| **Aqua(Water)** | **Diluent** | **773-18-5** | **98.3999%** |
| **Fatty Acids** | **Cleaning Agent** | **61790-12-3** | **1.45%** |
| **Coconut Oil** | **Plant based cleanser** | **8001-31-8** | **0.15%** |
| **Sodium Bicarbonate** | **Stabilizer** | **144-55-8** | **0.0001%** |

## Section 3 – HAZARDS IDENTIFICATION

**Overview**: Product is slightly viscous, clear-amber solution.
**Potential Health Effects**:
**Eyes**: This product may cause irritation to the eyes after direct contact.
**Skin**: Exposure to skin is not likely to result in irritation or redness. There is no level of toxicity associated with the material being absorbed through the skin.
**Ingestion**: There is no level of toxicity associated with ingestion of this product. However, ingesting very large amounts may cause minor gastrointestinal distress such as diarrhea due to emulsification of grease and oil in the digestive tract.

## Section 4 – FIRST AID MEASURES

**Eyes**: In case of direct contact with eyes, rinse with water if irritation occurs
**Skin**: If desired wash after handling; but no level of toxicity is associated with the material being absorbed through the skin.
**Ingestion:** There is no known level of toxicity associated with ingestion of this product. (See above, Section #3)
**Inhalation**: There is no level of toxicity associated with the material being inhaled. If desired, remove to fresh air.

## Section 5 – FIRE FIGHTING MEASURES

| | |
|---|---|
| **Flash Point:** | Not established but > 200°F |
| **Method Use**: | Not available |
| **Upper Flammable Limit** (UFL): | Not applicable |
| **Lower Flammable Limit** (LFL): | Not applicable |
| **Auto Ignition**: | Not applicable |
| **Flammability Classification:** | Non-flammable |
| **Rate of Burning:** | Not applicable |

**General Fire Hazards:** This product is 83.999% aqueous mixture that will not burn.
**Hazardous Combustion Products:** Upon decomposition this product emits carbon dioxide.
**Extinguishing Media**: Use extinguishing media appropriate to surrounding fire conditions.
**NFPA Ratings: Health: 0 Fire: 0 Reactivity: 0 Other: 0**
**HMIS Ratings: Health: 0 Fire: 0 Reactivity: 0 Other: 0**
**Personal Protection**: None

## Section 6 - ACCIDENTAL RELEASE MEASURES

**Containment Procedures**: None necessary.
**Clean-Up Procedures**: Rinse area with water. Dispose of material in accordance with local regulations.
**Evacuation Procedures:** Not necessary.
**Special Instructions**: Surfaces may become slippery after spillage.

## Section 7 – HANDLING AND STORAGE

**Procedures for Handling:** No special precautions required.
**Recommended Storage Methods**: N/A

## Section 8 – EXPOSURE CONTROLS/PERSONAL PROTECTION

**Exposure Guidelines:**
**A.** General Product Information:
None required.
**B.** Component Exposure Limits:
No ACGIH, NIOSH OR OSHA exposure guidelines listed for this product's components.
**Engineering Control**: Use general ventilation.
**Personal Protective Gear:**
**Eye/Face:** None required for normal usage.
**Skin:** None required.
**Respiratory:** None required
**General:** None required.

## Section 9 – PHYSICAL & CHEMICAL PROPERTIES

| | |
|---|---|
| Appearance: | Clear Amber |
| Physical State: | Slightly Viscous Liquid |
| Vapor Pressure: | Not available |
| Boiling Point: | 213°F @ 760 mm Hg |
| Melting Point: | Not applicable |
| Specific Gravity: | 1.1119 (Water = 1) |
| Softening Point: | Not applicable |
| Viscosity: | Not available |
| Percent Volatile: | Not applicable |
| Odor: | None |
| pH: | 9.85 to 10.35 |
| Vapor Density: | Not available |
| Freezing Point: | 29°F |
| Solubility ($H_2O$): | Miscible |
| Particle Size: | 1.2 – 4 nanometers |

| | |
|---|---|
| Evaporation Rate: | Not applicable |
| Bulk Density: | Not applicable |
| Molecular Weight: | Mixture |
| Additional Properties: | No additional properties available |



## Section 9 – PHYSICAL & CHEMICAL PROPERTIES CONT'D

VOC's: Testing was performed using Gas Chromatography/Mass Spectrometer instrumentation to determine the presence of any contaminating compounds. Results indicated that NO contaminants of any kind, including VOC's, were present.

## Section 10 – CHEMICAL STABILITY & REACTIVTY INFORMATION

**Chemical Stability**: Stable.
**Conditions to Avoid**: None documented.
**Incompatibility**: None documented.
**Hazardous Decomposition Products**: Upon decomposition this product emits carbon dioxide.
**Hazardous Polymerization**: Will not occur

## Section 11 – TOXICOLOGICAL INFORMATION

**Acute Toxicity/Target Organ Information**
A. General Product/Component Information
**No irritation or toxicity associated with this product.**
B. Component LD50/LC50 – Not applicable – NO such testing conducted on animals. NO toxicity associated with this product.
**Epidemiology**: No information available.
**Carcinogenicity**
A. General Product/Component Information
None Identified.
B. Component Carcinogenicity Listings
**None of this product's components are listed by** ACGIH, IARC, NIOSH, NTP or OSHA.
**Neurotoxicity**: None
**Mutagenicity:** None
**Other Information**: None available.

## Section 12– ECOLOGICAL INFORMATION

**Ecotoxicity**: There is no known ecotoxicity associated with this product.
**Environmental Fate**: Contains Biodegradable Surfactants

## Section 13 – DISPOSAL CONSIDERATIONS

**US EPA Waste Number & Description**
A. General Product Information: Not listed by the EPA as a hazardous waste.
B. Component Waste Number: No EPA Waste Numbers are applicable for this product's components.
**Disposal Instructions**: Clean spill area and dispose of waste in accordance with all applicable federal, state and local regulations.

## Section 14 – TRANSPORTATION INFORMATION

**DOT Information**

| | |
|---|---|
| Shipping Name: | Non-regulated |
| Hazard Class: | N/A |
| UN/NA: | N/A |
| Packing Group: | N/A |
| Label(s) Required: | None |
| Additional Shipping | None |
| Information: | None |
| International Transportation Regulations: | N/A |

## Section 15 – REGULATORY INFORMATION

**US Federal Regulations**
A. General Product Information
No information available.
B. Component Information
None of this product's components are listed under SARA Section 302 (40CFR Appendix A), SARA Section 313 (40CFR 372.65) or CERCLA (40CFR 302.4).
All of this product's components are on the EPA's TSCA Inventory
All of this product's components are on the FDA's GRAS list
**State Regulations**
A. General Product Information
No information available
B. Component Information N/A
Other Regulations
**Local Regulations**
A. General Product Information
No information available.
B. Component Information N/A
Other Regulations

## Section 16 – OTHER INFORMATION

**Other Information:**
Reasonable care has been taken in the preparation of this information, but the manufacturer makes no warranty of merchantability or any other warranty, expressed or implied, with respect to this information. The manufacturer makes no representation and assumes no liability for any direct, incidental or consequential damages resulting from its use. The above sets forth technical data supplied to the manufacturer and has been edited to avoid disclosure of proprietary information and avoid redundancy. All inquiries concerning the content of this document should be directed to the Distributor or Manufacturer.

**Key/Legend**
Y = Yes; N = No; EPA = Environmental Protection Agency; TSCA = Toxic Substance Control Act; ACGIH = American Conference of Governmental Industrial Hygienists; FDA = Food and Drug Administration; GRAS = Generally Regarded As Safe; IARC = International Agency for Research Cancer; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; N/A = Not Available or Applicable; g = grams; ml = milliliter; C = Celsius; F = Fahrenheit; DOT = Department of Transportation.

SDS preparation: 06/14/2021
Contact: Technical Department
Phone: 800-749-Bioneat

End of SDS #cc-01 Print date 06/14/2021

# Safety Data Sheet



## Section 1 – Chemical Products and Company Identification

**Product Names:** BioSolve® Pinkwater®

**Product Uses:** Remediation of hydrocarbon (oil, fuel, petrochemical) contamination, including: impacted soils, suppression of VOCs, surface cleaning of equipment and protective clothing.

**Manufacturer:** The BioSolve Company
329 Massachusetts Avenue
Lexington, MA 02420 USA

**Contact Information:**

| | |
|---|---|
| +1 (800) 225-3909 | US, Canada, Mexico and Puerto Rico |
| +1 (781) 482-7900 | All other locations |

## Section 2 – Hazards Identification

**Health Hazards:**

| | |
|---|---|
| Eye Contact: | Causes transient eye irritation |
| Skin Contact: | May cause mild, transient irritation |
| Ingestion: | May be harmful if swallowed; can cause gastrointestinal irritation, nausea, vomiting and/or diarrhea |

**Hazard Mitigation:** Wear protective gloves and eye/face protection
Avoid prolonged breathing of spray

**Environmental Hazards:** Moderately toxic to aquatic life. Avoid discharge to storm drains and waterways

**GHS Classification:** Toxic to aquatic life, Acute Category 2

## Section 3 – Composition/Information on Ingredients

Proprietary formulation with nonionic surfactants (32% active ingredients in water)

BioSolve products contain no caustic, d-limonene or hydrocarbon solvents.

BioSolve products do not contain any hazardous ingredients as defined by CERCLA, Massachusetts Right to Know Law and California Prop 65. All ingredients are TSCA compliant.

## Section 4 – First Aid Measures

**Eyes:** Immediately flush eyes with water for at least 15 minutes. Hold eyelids apart while flushing to rinse entire surface of eye and lids with water. Seek medical attention for lasting irritation.

**Skin:** Rinse exposed area and wash with mild soap and water for several minutes. Seek medical attention if irritation develops.

**Ingestion:** Seek medical attention in the event of serious or persistent abdominal discomfort, nausea or diarrhea.

**Inhalation:** Inhalation of concentrated vapors resulting from spraying or heating in confined or poorly ventilated areas may cause irritation of nose and throat. Remove person to fresh air and seek medical attention if irritation persists.

## Section 5 – Fire Fighting Measures

**Suitable Extinguishing Media:** None required; BioSolve products are non-flammable

**Special Protective Equipment for Firefighters:** None necessary

**Unusual Fire or Explosive Hazards:** None

## Section 6 – Accidental Release Measures

In case of accidental release, breakage or leakage: Eliminate or contain source with inert material, such as sand, earth, absorbent pads, etc. Transfer liquid to suitable containers for recovery, re-use or disposal. Wipe up or mop up using water. Hard surfaces (e.g., floors, driveways) may be slippery; use care to avoid falling.

Rinse area with water. Avoid flow of run-off to surface waters. Always check with local regulations before discharging effluent to storm drains or sewers.

## Section 7 – Handling and Storage

**Handling:** Minimize periods of exposure to extreme temperatures. Keep from freezing. If frozen, separation may occur; thaw and stir thoroughly prior to use. Freezing will not affect product performance.

**Precautions:** Chemical resistant gloves and eye protection are recommended while mixing and using.

**Incompatibilities:** Avoid contact with strong acids or strong oxidants.

**Storage:** Recommended storage temperature: $35^{o} – 120^{o}$ F ($1^{o} – 48^{o}$ C).

**Shelf Life:** If unopened, more than 10 years.

## Section 8 – Exposure Controls / Personal Protection

**Eyes Protection:** Safety glasses; chemical goggles or face shield recommended when spraying to protect against backsplash and drift.

**Skin Protection:** Rubber or latex gloves recommended.

**Respiratory Protection:** None required, except if application results in significant misting of product. If so, use of an approved air purifying respirator is recommended.

**Engineering Controls:** For indoor use or for use in a confined space, normal ventilation is generally satisfactory.

## Section 9 – Physical and Chemical Properties

**Appearance:** Deep red

**Odor:** Mild, pleasant sassafras fragrance

**Concentration:** ~32% active ingredients as sold

| | | | |
|---|---|---|---|
| **Boiling Point** | 265°F/129°C | **Vapor Pressure mm/Hg** | Not available |
| **Melting/Freezing Point** | 28°F/-2°C | **Vapor Density (Air=1)** | Not available |
| **Flash Point** | Non-flammable | **Surface Tension*** | 29 Dyne/cm @25°C |
| **Flammability Limits** | Not applicable | **Viscosity (concentrate)** | 490 centipoise |
| **Reactivity with Water** | None | **Viscosity (6% solution)** | 1.5 centipoise |
| **Evaporation Rate** | Not determined | **Solubility in Water** | 100% |
| **Specific Gravity** | 1.01 gms/cc | **VOC Content** | Not determined |
| | 8.43 lbs/U.S. gal | **pH** | 9.1 +/- 0.3 |

*6% solution

## Section 10 – Stability and Reactivity

**Chemical Stability:** Stable; will not decompose if used according to manufacturer's directions.

**Conditions to Avoid:** Prolonged exposure to heat may cause product degradation. Freezing should also be avoided as discussed in Section 7.

**Incompatible Materials:** Normally unreactive. Avoid strong alkalis, strong acids, strong oxidizing agents and materials with reactive hydroxyl compounds. These materials could damage the product and reduce its effectiveness during application.

**Hazardous Decomposition Products:** None are known.

**Hazardous Polymerization:** Will not occur.

## Section 11 – Toxicological Information

**Overview:** No adverse acute or chronic health effects expected if product used in accordance with manufacturer's directions.

**Carcinogenicity:** No ingredient has been shown to cause cancer in laboratory animals.

**Specific Organ Toxicity:** None are known.

## Section 12 – Ecological Considerations

**Persistence and Degradability:** The total of the organic components contained in this product is not classified as readily biodegradable (OECD-301 A-F). However, this product is inherently biodegradable with 60% degradation in 28 days (OECD-301B) and estimated >95% degradation in 120 days.

**Bioaccumulation Potential:** The bioaccumulation factor in fish has been estimated to be low, ranging from 87 to 344.

**Mobility:** No data available

**Aquatic Toxicity:**

| | | |
|---|---|---|
| **$LC_{50}$ of Concentrate** (As shipped) | | |
| *Mysidopsis bahia* | 48-hours | 3.6 mg/L |
| *Menidia beryllina* | 96-hours | 6.4 mg/L |
| **LC50 of 3% Dilute Solution** (As Used) | | |
| *Mysidopsis bahia* | 48-hours | 185 mg/L |
| *Menidia beryllina* | 96-hours | 247 mg/L |
| **LC50 of 6% Dilute Solution** (As Used) | | |
| *Daphnia magna* | 48-hours | 287 mg/L |
| *Pimephales promelas* | 96-hours | 124 mg/L |
| *Onchorhynchus mykiss* | 96-hours | 177 mg/L |

## Section 13 - Disposal

DO NOT DUMP INTO STORM DRAINS OR INTO ANY BODY OF WATER. All disposal practices must be in compliance with all Federal, State/Provincial and local laws and regulations. As manufactured, BioSolve products do not meet the definition of a hazardous waste. Small quantities of unused and uncontaminated product may be discharged to a qualified wastewater treatment facility. Always obtain approval from local and Federal regulatory agencies prior to discarding this product into public sewers.

As your supplier, we have no control over your handling and use of this product. However, the intended use of this product as a remediation and/or surface washing agent may produce wastewater containing emulsified or dispersed hydrocarbons that may be classified as a hazardous waste and should be treated and disposed of accordingly.

## Section 14 – Transportation Information

USDOT Freight Class 55 (Liquid Cleaning Compound, Non-Hazardous)
This product is not regulated by USDOT or Canadian TDG when shipped domestically by land.

North American Industry Classification System (NAICS) # 325613

U.S. ITC, Harmonized Tariff Schedule B Classification: 3402.90.30.00

## Section 15 – Regulatory Information

This product is considered non-hazardous as defined by CERCLA, according to OSHA, Massachusetts Right to Know Law and California Prop 65.

| | |
|---|---|
| **Toxic Substances Control Act:** | All components of this product are on the TSCA inventory or are exempt from TSCA Inventory requirements under 40 CFR 720.30. |
| **CEPA – Domestic Substances List:** | All substances contained in this product are listed on the Canadian Domestic Substances List (DSL) or not required to be listed. |
| **Canadian CPR Compliance:** | This product has been classified in accordance with the hazard criteria of the Canadian Controlled Products Regulations (CPR) and the SDS contains all the information required by the CPR |
| **WHMIS Classification:** | D2B Eye or skin irritant |

Regulatory requirements are subject to change and may differ from one location to another; it is the buyer's responsibility to ensure that its activities comply with Federal, state or provincial and local laws.

## Section 16 – Other Information

| | | |
|---|---|---|
| **HMIS Rating** | Health Hazard: | 1 (Eye/Skin Irritant) |
| | Fire Hazard: | 0 |
| | Reactivity: | 0 |
| | Personal Protective Equipment: | Rubber gloves, safety glasses or face shield |
| **NFPA Rating** | Health: | 1 (Eye/Skin Irritant) |
| | Flammability: | 0 |
| | Reactivity: | 0 |
| | Other Hazard: | None |

BioSolve Pinkwater is on the US Environmental Protection Agency's NCP Product Schedule. This listing does NOT mean that EPA approves, recommends, licenses, certifies or authorizes the use of BioSolve Pinkwater on an oil discharge. This listing means only that data have been submitted to EPA as required by Subpart J of the National Contingency Plan, 40 CFR Section 300.915.

**SDS Effective Date:** May 12, 2016

The information contained herein is accurate to the best of our knowledge. The BioSolve Company makes no warranty of any kind, express or implied, concerning the safe use of this material in your process or application or in combination with other substances.

For more information, visit: www.biosolve.com

# OIL MARKET WELL TEST

Low Volume Non-Problematic Well Treatment Test

# Low Volume Non-Problematic Well Treatment Test

Bioneat Degreaser was used to treat two oil wells in Cleveland County, OK to determine if the product could enhance production from the wells whose production has dropped from high volumes when initially completed to very low production volumes over the last 24 years. The wells were drilled in 1987 and both had initial production of approximately 250 barrels of oil per day. Prior to the Bioneat treatment, both well were only producing 1 barrel or less per day. The wells are considered low volume and non-problematic well because neither have inherent barriers to production such as mineral scale formation or paraffin precipitation that would occur over time to impede production. Both wells produce a high-quality sweet crude oil. The well depths range from 1100 feet deep to 1600 feet deep. The thickness of the producing sandstone intervals are approximately 10 feet thick.

The objective for treating these wells was to achieve the following goals:

1. Create documentation of the product being used as a well intervention product.

   This documentation provides prospective customers with a degree of confidence the new product is a viable well intervention product and enhance the ability to persuade the use of the product in their wells. This objective has been achieved with the treatment of these two wells, however more documentation of well treatments is always a big advantage.

2. Establish or show the product has little to no risk of damaging the producing formation.

   Low volume producing wells have high risk of production loss when foreign fluids are introduced into the formation that create any impairment to the permeability of the production interval. If these treated well only return to initial production volumes, then this objective is achieved.

3. Determine if treatment increases the inflow of formation fluids.

   Commonly long term producing wells that have achieved steady state flow for years will have a very consistent ration of formation water to oil. The water portion of this ration is generally multiple time higher than the oil portion. If the fluid volume produced is increased the corresponding portion of oil is expected to increase proportionately.

4. Chemically alter the physical properties or characteristics of the producing formation to stimulate it ability to produce fluids.

   Mother nature dictates the formation properties and characteristic. If we can temporarily alter these properties to be more favorable for the fluids to flow then we have achieved stimulation. Oil producing formation are almost all oil wet, meaning oil is what is chemically bonded to the rock. Oil bonded to the rock will not flow and is left behind. The oil molecules attached to the rock also reduced the effective size of the pore throats all fluids must flow through to get to the well bore, and the oil flowing through the pore throats is attracted to the oil coating the walls creating drag/friction to flow. Temporarily changing the formation rock from oil wet to water wet accomplishes two major advantages. This will strip oil from the rock and making it mobile. A water wet rock will repeal oil from the surface and allow oil to flow without the drag/friction associated with oil wet rock.

## Ten Day Post Treatment Results

Well results will be reported in 10-day increments. Because the wells are low volume producers, a 10-day report cycle is used to provide a better representation of daily averages. Both wells have minimal gas production that is not of sufficient volume to be sold.

Both well were treated with 40 barrels of a 9:1 Produced water:Bioneat Concentrate solution and shut in for 48 hours. The well were put back on production and monitored for fluid production.

Well Name F-6 Results Summary:

- 24 hours after treatment well has gas break through
- 48 hours after treatment well is turned to production
- 72 hours after treatment well is producing oil and water
- 10 days after treatment well is producing 250 percent more fluid on average and the oil production has doubled.

Well Name S-3 Results Summary:

- 24 hours after treatment well has gas break through
- 48 hours after treatment well is turned to production

- 10 days after treatment well has returned to pre-treatment production rates with a possible slight increase in total fluid production.
    - This well has half the pump capacity to move fluid as the F-6 well. The next ten-day report will give a more representative analysis of the treatment results.

## Results Discussion:

The first two objectives of this project have been successfully satisfied. Objectives 3 and 4 have been validated and awaiting additional data to quantify. This short term data is encouraging because it satisfied our objectives. It is however short term data and does not give us ability to evaluate economical feasibility yet. Keeping in mind that these well are not problematic well and we are not treating a physical restriction to production, I believe this product, with these results, has given it strong merit as an oil well intervention product.

## Additional Note:

My preliminary testing in other oil related applications show good promise. This being related to the presentation I sent of my testing. We currently have the product in front of several chemical companies to evaluate. Will update on this when available.