



RespirTek™
CONSULTING LABORATORY

OECD 301B Ready/Ultimate Biodegradability Assessment

Date of Final Report: November 19, 2014

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Report Prepared For:
Bio-Neat, Inc.
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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



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Client: BIO-2413

Test Product(s): BioNeat NTS™

Test Method: OECD 301B - CO₂ Evolution Test

Report Date: November 19, 2014

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 contained 1 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 H₂O) and allowed to dry.

The mineral salt stock solutions for the project was prepared in media bottles using the appropriate chemicals and DI H₂O. The chemicals were weighed out using an analytical balance, and the DI H₂O 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 H₂O:

8.50 g of KH₂PO₄
21.75 g of K₂HPO₄
33.40 g of Na₂HPO₄ • 2 H₂O
0.50 g of NH₄Cl

The pH of the solution was then adjusted to 7.4.

Solution 2: 36.40 g of CaCl₂ • 2 H₂O was added to 1000 mL of DI H₂O.

Solution 3: 22.50 g of MgSO₄ • 7 H₂O was added to 1000 mL of DI H₂O.

Solution 4: The following compounds were added to 1000 mL of DI H₂O:

0.25 g of FeCl₃ • 6 H₂O
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 CO₂-free aeration system, which uses a CO₂ 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
<u>Solution 4:</u>	<u>60 mL</u>

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 CO₂ 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 CO₂ 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 H₂O 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 CO₂ 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™ BIO-TC1	253.1 mg/L
Sodium Benzoate (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 CO₂ Free Mineral Stock Solution + 41 mL biomass + 441 mL DI water.
- **Sodium Benzoate (PC):** 91 mL Sodium Benzoate PC Stock Solution + 2400 mL CO₂ Free Mineral Stock Solution + 41 mL biomass + 468 DI water.
- **Blank (B):** 2400 mL CO₂ Free Mineral Stock Solution + 41 mL biomass + 559 mL DI water only.

All reactors were delivered CO₂-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 CO₂ produced by each reactor. Scrubber solutions were periodically refreshed to ensure adequate absorption of CO₂ 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, confidentiality 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 CO₂ Evolution Test

Test Chemical
BioNeat NTS™

Biodegradation (%)
61.7

Classification
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.

This report shall not be reproduced, except in full, without written approval of the laboratory.



BIO-2413 Data and Calculations

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

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

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

	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

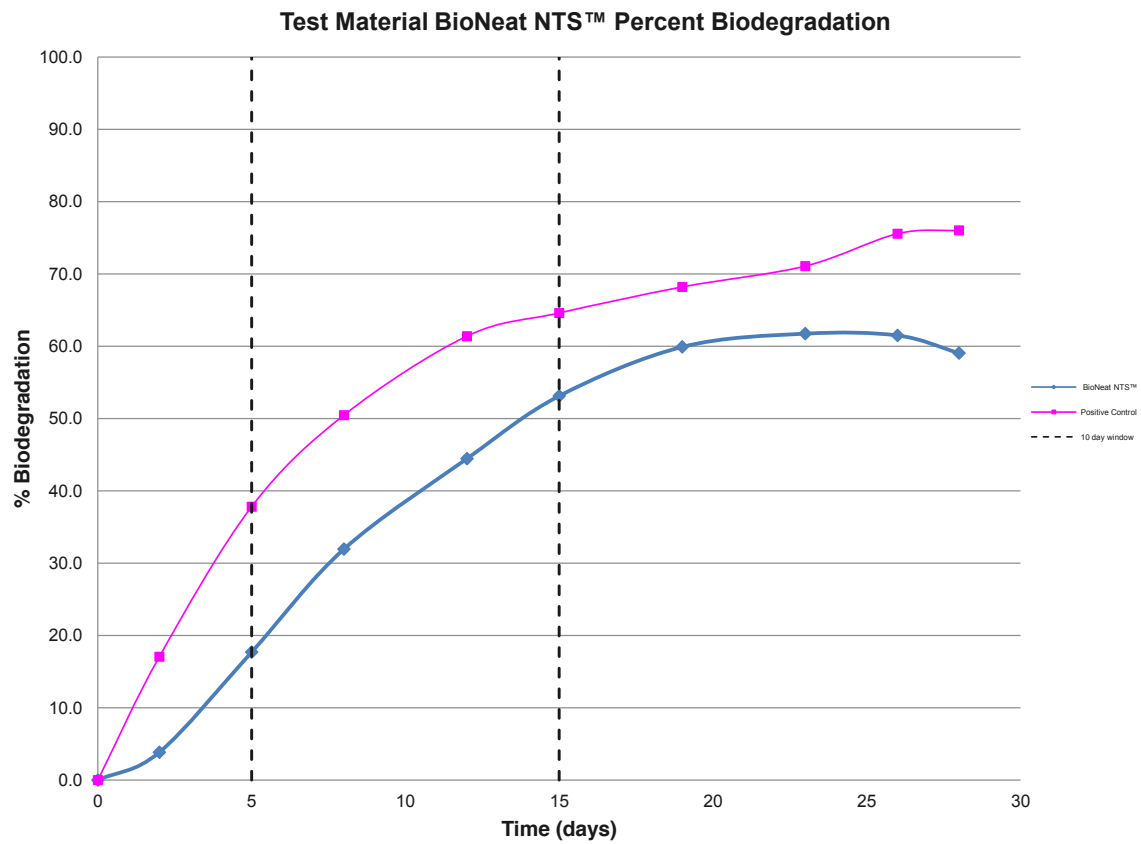
	Total CO ₂ (mg)									
	Day 0	Day 2	Day 5	Day 8	Day 12	Day 15	Day 19	Day 23	Day 26	Day 28
Blank-1	N/A	9.1	12.5	12.1	16.0	11.3	15.1	14.9	13.6	20.6
Blank-2	N/A	10.0	11.3	13.2	15.7	11.1	15.2	16.6	14.6	16.0
PC-1	N/A	26.8	34.9	26.1	26.9	15.5	18.1	19.1	19.5	18.5
PC-2	N/A	29.9	34.6	27.1	28.9	13.9	20.1	18.7	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 CO ₂ (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
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**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:*

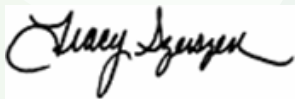
ISO/IEC 17025:2005

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:

September 16, 2011

Issue Date:

March 4, 2014

Expiration Date:

March 4, 2016

Accreditation No.:

69085

Certificate No.:

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	
			ASTM D6400	
	Chemical	Aquatic Aerobic Biodegradation	ISO 15985	
			OECD 301A	
			OECD 301B	
			OECD 301C	
			OECD 301D	
Biological	Chemical Compounds	Aquatic Aerobic Biodegradation	OECD 301E	CO ₂ Gas DL 1% CH ₄ Gas DL 0.10 %
			Internally developed protocols-microcosm studies	
			SM 9215B	
			OECD 301F	
			ASTM D5210	
			OECD 311	
			OECD 302B	
			ASTM D5511	
			ASTM D5864	
			ASTM D5271	
	Aqueous Sample	TOC	ASTM E1720	
			ASTM D5988	
			SM5310B	
			ISO 14593	
			ISO 9439	
Chemical	Water Samples	Biological Oxygen Demand	ISO 15985	
			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 %