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Quality Control
Biodegradation
Antimicrobials

Customer Report

Friday, April 6, 2018

CONTACT

Kafko International
3555 West Howard Street
Skokie IL 60076

George Kafkis
847-763-0333
Gkafkis@kafkointl.com

Project Title

Biodegradation Testing

ID **0218-AML-01 -- 1** **rev. 1** Entry Date 2/8/2018

Project Summary

The OECD 301B method is designed to provide the screening of chemicals for ready biodegradability in an aerobic aqueous medium. Samples are required to achieve a threshold of 60% degradation based on the maximum available carbon from a given sample formulation. Total carbon is determined analytically for each sample and used as the reference for the determination of the percentage of carbon dioxide (% ThCO2) produced by microbiological degradation.

For the purpose of determining biological degradation, two criteria can be achieved. Ready Biodegradability can be achieved by obtaining the 60% threshold within a 10 day-window within the 28 days of testing. The second criteria, Ultimate Biodegradation, can be achieved if the amount of biodegradation meets or exceeds the 60% threshold at a time point determined in the test (e.g. when the rate of degradation reaches a plateau). For each of these criteria, when achieved, the sample also achieves the requirements needed for classification as 'Inherent Biodegradability'.

One test samples were submitted for OECD 301B biodegradation testing. The result data and graphs were analyzed by curve fit to establish a plateau for the rate of biodegradation (see figures).

Sample 1 - CH12142 Oil Eater Original Formula Cleaner & Degreaser – achieved the requirements for Ready Biodegradability by the OECD 301B standard for degradation of 60% ThCO2 within a 10 day window of the test timeframe. The test sample achieved Ready Biodegradability by day 20 of the testing with a degradation plateau of 94% ThCO2, which does meet the requirements for an Ultimate Degradation of 94% by the OECD 301 test standard.

rev.1 revised blank data

Recommended Reading
Online Resource for Product Development, Testing, and Inquiry

http://www.situbiosciences.com/biodegradation/oecd-301b-biodegradation-test-co2-evolution/

http://www.situbiosciences.com/biodegradation-doc-co2-o2-measurements-and-method/

http://www.situbiosciences.com/biodegradation-testing-overview/

Sample List

Method Name

Sample #	*Sample Name*	*Sample Notes*
OECD 301 B - Solution Biodegradation by CO2 Evolution		
1	CH12142 Oil Eater Original Formula Cleaner & Degreaser	
2	Positive Control - Sodium Acetate	
Project - Images		
1	CH12142 Oil Eater Original Formula Cleaner & Degreaser	

Result Table

Contact	Kafko International	George Kafkis	847-763-0333
Title	Biodegradation Testing		
Project ID	0218-AML-01 -- 1	Entry Date 2/8/2018	Test Start Date 2/8/2018

Result Table *

Test Method OECD 301 B - Solution Biodegradation by CO2 Evolution

Sample # 1 **CH12142 Oil Eater Original Formula Cleaner & Degreaser**

	Interval	Result
Inoculum *Environmental Culture (surface water) (1)*		
Notes Section		
Ready Biodegradability Achieved; 10 day-window = day 11 to day 21, achieved on day 20	**20** day	**60** % degradation

Image: **Sample**

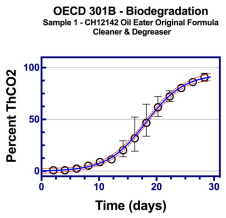


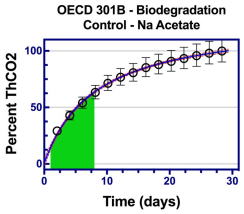
Figure *– The sample graph shows the test chamber carbon dioxide (CO2) measurement as the percent of theoretical maximum (% ThCO2). Average values are plotted with the standard deviation (+/- SD) for the time course of the test. Curve fit is applied to calculate the predicted fit (blue line). If present,* ***(green)*** *shading below the curve fit applies to the biodegradation requirement (10 to 60% ThCO2) for the determination of Biodegradability and shows that the required degradation amount has been met. Ready Biodegradability requires that this degradation occur within a 10-day window during the test duration of 28 days.*

final ultimate degradation	**28** day	**98** %ThCO2
final sample pH	**28** day	**7.1** pH
Sample TOC composition = 3.4%	**0** TOC TEST	**30** mg/L TOC
bacterial concentration	**28** day	**170000** CFU/ml

Sample # 2 **Positive Control - Sodium Acetate**

	Interval	Result
Inoculum *Environmental Culture (surface water) (1)*		
Notes Section		
Ready Biodegradability Achieved; 10 day-window = day 1 to day11, achieved on day 8	**8** day	**60** % degradation

Image: **Control**



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final ultimate degradation	**28** day	**94** %ThCO2

Result Table *

Test Method OECD 301 B - Solution Biodegradation by CO2 Evolution

Sample # 2 **Positive Control - Sodium Acetate**

Inoculum *Environmental Culture (surface water) (1)*

Notes Section

	Interval	Result
final sample pH	28 day	7.4 pH
Sample TOC composition = 24%	0 TOC TEST	30 mg/L TOC
bacterial concentration	28 day	450000 CFU/ml

Test Method Project - Images

Sample # 1 **CH12142 Oil Eater Original Formula Cleaner & Degreaser**

Inoculum *None ()*

Notes Section

	Interval	Result
	time - 0	sample image
Image: Sample		

Test Method - Additional Information

OECD 301 B - Solution Biodegradation

Test conditions:
- inoculum: Surface water from Skokie, IL water district.
- proportion and nature of industrial waste water in sewage: unknown, discharge from waste treatment facility within 1 mile.
- test duration and temperature: 28 days or as indicated, 22C +/- 2C
- bacterial inoculum ~1E5 CFU/ml

Legend
Sample Analysis
TC - Total Carbon determined by catalytic oxidation of the test sample.
IC - Inorganic Carbon
TOC - Total Organic Carbon - determined by the subtraction of TC from IC.
%S - Percent Solids- is the dry (non-volatile) percent of the test sample.

For the sample analysis, percent solids is determined when estimating the weight of material to test. The total carbon (TC) provides an indication of the material composition, but does not provide information on chemical structure or function. Inorganic carbon is typically low in most biodegradable materials, and increases over the course of the test due to the action of the microorganisms in creating waste, or biological compounds that are generated from the consumption of the carbon based test sample. Sample results are provided as a graph showing the raw data and curve fit analysis. Determinations of the percent degradation is based on the curve fit analysis performed with guidance from *OECD Guidelines for the Testing of Chemicals / Section 3: Degradation and Accumulation Test No. 314: Simulation Tests to Assess the Biodegradability of Chemicals Discharged in Wastewater.*

Current Laboratory Standard:
Test measurement uncertainty is based on the established standard provided in SOP 050410 Measurement Uncertainty Estimates.
The current measurement uncertainty for this test method can be found in FORM 050430 measurement uncertainty - Sodium Acetate ThCO2.

Expanded Uncertainty for the test method of k=2 is for a 95% confidence of (0.08) [+/- 8%] for the 14 day determination.
Expanded Uncertainty for the test method of k=2 is for a 95% confidence of (0.13) [+/- 14%] for the 28 day determination.

Uncertainty Values in theoretical CO2 production are obtained by the plateau value of a series of sodium acetate test measurements (ThCO2)) and multiply this result by the Expanded Uncertainty (ThCO2 * EU), then add and subtract from the value (ThCO2 +/- (ThCO2 * EU)); which provides the upper and lower limits of 95% confidence in percent theoretical carbon dioxide production.

Stated Method Standard:

For OECD Solution Biodegradability Testing , a interlaboratory test demonstrated a results with Standard deviation of ~ +/- 20% ThCO2
Ref. *CONCAWE Report no. 99/59 A Test Method to ASSESS the Inherent Biodegradability of oil Products*

Result Table *

This report sets forth our findings solely with respect to test samples identified herein. The results set forth in this report are not indicative or representative of the quality or characteristics of the lot from which a test sample was taken or any similar identical product unless specifically and expressly noted. Our report includes all tests requested and the results thereof based upon the information provided. Written notification within 60 days from the date of issuance of this report is required to address any material error or omission caused by the handling of the samples. Any such notification shall specifically address the issues related to the test samples supplied and testing conducted as provided in this report. A failure to raise such an issue within the prescribed time shall constitute your unqualified acceptance of the completeness of this report, the testing conducted, and the correctness of the report contents.

d p satchell Ph.D.
Technology Director

Report Addendum

Friday, April 6, 2018

Project ID **0218-AML-01 -- 1** Entry Date 2/8/2018 Test Start Date 2/8/2018

Image Table

Sample # **1** **CH12142 Oil Eater Original Formula Cleaner & Degreaser**

Test Method **OECD 301 B - Solution Biodegradation by CO2 Evolution**

Inoculum ***Environmental Culture (surface water)***

Image: **Sample** ***Timepoint:*** **20 day**

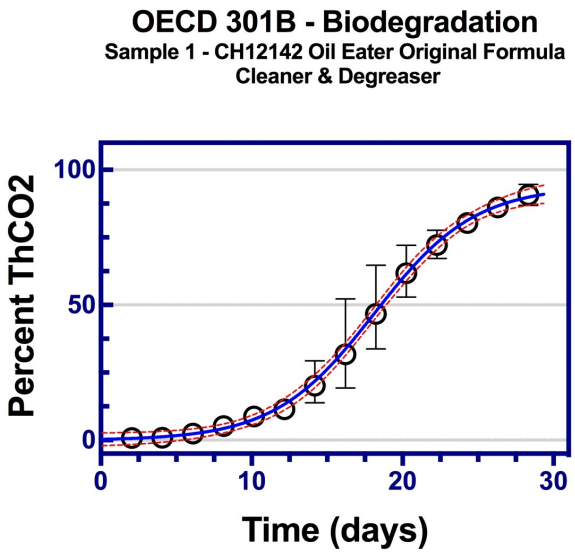

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Image Table

Sample # 1 **CH12142 Oil Eater Original Formula Cleaner & Degreaser**

Test Method Project - Images

Inoculum *None*

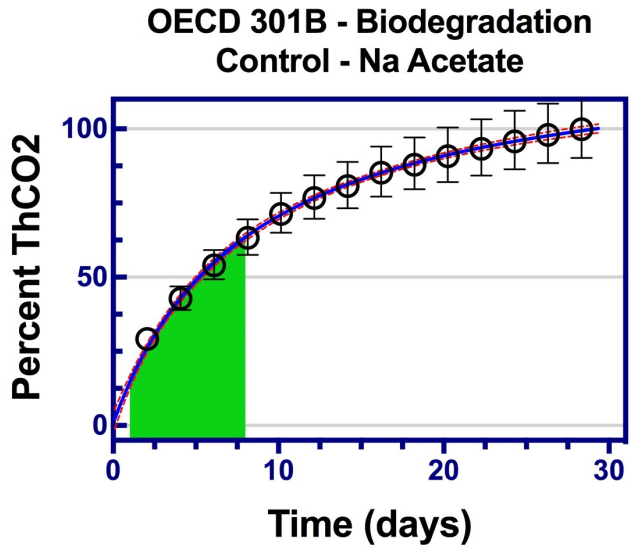
Image: Sample *Timepoint:* time - 0

Sample # 2 **Positive Control - Sodium Acetate**

Test Method OECD 301 B - Solution Biodegradation by CO2 Evolution

Inoculum *Environmental Culture (surface water)*

Image: Control *Timepoint:* 8 day



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