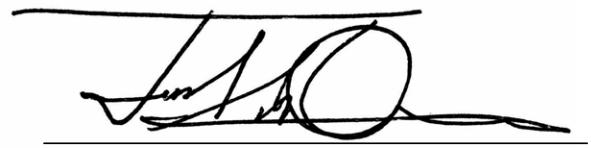
Biodegradation Study Final Report
Method OECD 306: Biodegradability in Seawater

Joel A. Olson, Ph.D.
Associate Professor
Department of Chemistry
Florida Institute of Technology
150 West University Blvd.
Melbourne, FL 32901

Summary Statement

A cosmetic unscreen product (Absolutely Natural Suncare, SPF 4) was tested for biodegradability. The method used was OECD 306: Biodegradability in Seawater (closed bottle method). The test was conducted in the laboratory of Dr. Joel A. Olson, Associate Professor of Chemistry at Florida Institute of Technology. The details of the test are contained in the report that follows this summary statement. The test result was positive (>70% DOC removal) therefore it may be concluded that the test substance was measured to have the potential for biodegradation by this test method (that is, it tested to be biodegradable within the purview of OECD 306). Specifically, 79.1% biodegradation was observed within 15 days of the start of the test. The lag phase (t_L) was measured to be 3.05 days and the time to 50% oxygen uptake (t_{50}) was measured to be 2.28 days. It is important to note, however, that true environmental biodegradation is a complex process that may involve factors beyond the control of this test. Therefore, this report makes no claim of biodegradability in any particular environment. Also, as stated in OECD 306, the method performed in this test is not a test for ready biodegradability, since no inoculum was added in addition to the microorganisms already present in the seawater. Nor did the test simulate the marine environment since nutrients were added, and the concentration of the test substance was significantly higher than would be present in the sea. In any case, OECD 306 is considered by many in the field to be the standard by which biodegradability in seawater may be assessed.



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Test Substance

Absolutely Natural Suncare, SPF 4, viscous suspension of TiO_2 in natural oils and fragrances. Ingredients as listed on the container include: titanium dioxide, aloe extract, walnut oil, vegetable emulsifiers, rose hips oil, squalane, tocopheryl acetate (vitamin E), shea butter, potassium sorbate, natural fragrance.

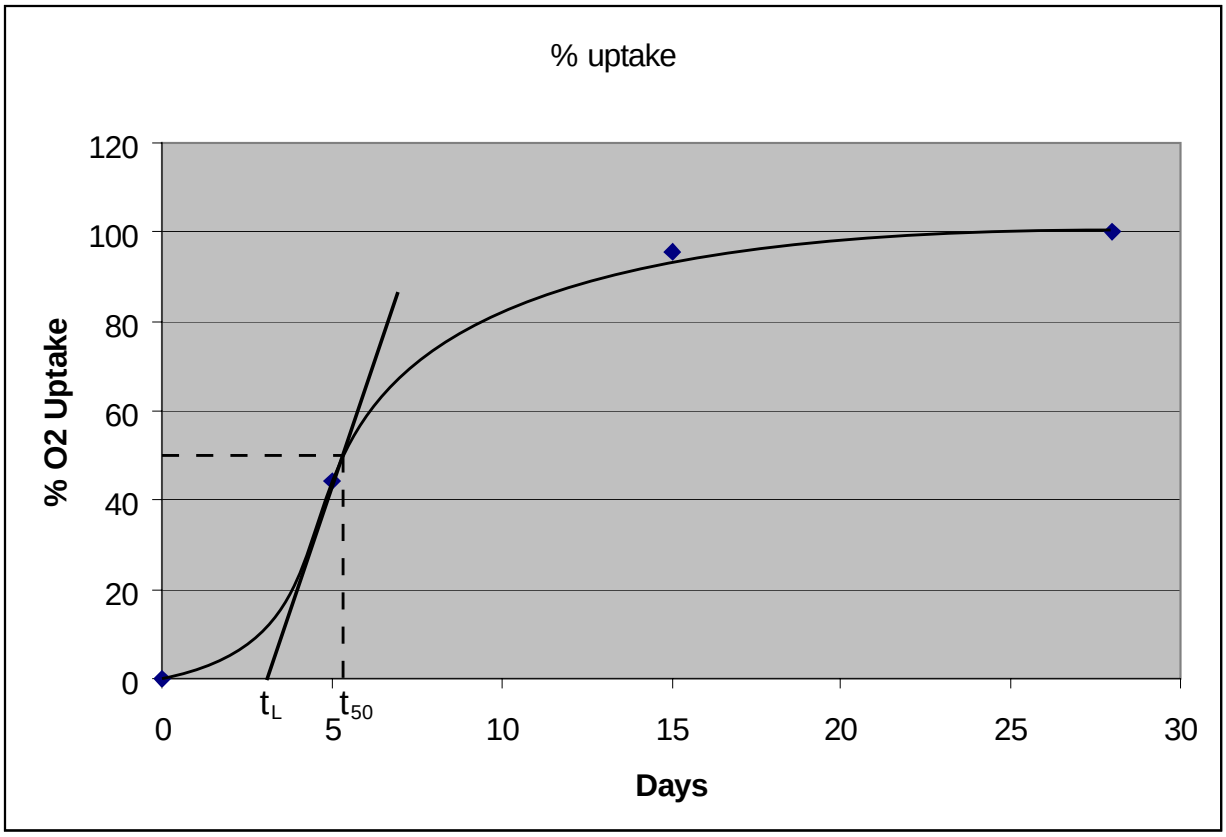
Test Conditions

Seawater was collected from the sea water intake site of the Vero Beach Laboratory (805 East 46th Place Vero Beach, FL 32963). Specifics of the seawater sampling and treatment follow:

Seawater sampling date: April 28th, 2010
Seawater temperature: 72ºF
Seawater appearance: clear
Seawater salinity: 36 ppt
Seawater delay between collection and test: 24 h (stored at 4ºC in the absence of light)
Seawater aging: none
Seawater pretreatment: Rough filtration with a paper filter
Test substance COD method: EPA 410.4 performed by ABC Research Corp, Gainesville, FL
Dissolved oxygen measurement method: ASTM D 888-05 Test Method B
Dispersion procedure: magnetic stirrer
Determination of heterotrophs: not performed
Determination of DOC in seawater: not performed
Specific analysis method: not performed
Other optional methods: none

Results
Analytical data are attached as Appendix 1 (Annex 5 from OECD 306)

Graphical representation of the course of the degradation test:



Lag phase (t_L): 3.05 days
Time to reach 50% of final oxygen uptake (from end of lag phase, t_{50}): 2.28 days
Percent degradation after 15 days (maximum measured): 79.1%

Validity and interpretation of results
The blank respiration was 16.5% over the 28 day test, well below the validation threshold maximum of 30%

Results obtained with a test substance (sodium acetate) were comparable to the results obtained for the test substance. Thus the test need not be repeated with another seawater sample.

The test substance was not found to be inhibitory to bacteria at the concentration used.

In spite of the relatively high concentrations used for this test as compared to most natural systems, the test substance was found to be easily biodegradable. Thus no further study is necessary to confirm biodegradability.

Appendix 1: ANNEX 5 from OECD 306

ANNEX 5

BIODEGRADATION IN SEAWATER
CLOSED BOTTLE METHOD
DATA SHEET

1. **LABORATORY:** Florida Institute of Technology

2. **DATE AT START OF TEST:** April 29th, 2010

3. **TEST SUBSTANCE:**

   Name: Absolutely Natural Suncare, SPF 4
   Stock solution concentration: N/A (used as solid)
   Initial conc. in seawater medium: 3.0 mg/L
   ThOD or COD: 0.632 or 63.2% (COD)

4. **SEAWATER:**

   Source: 805 East 46th Place Vero Beach, FL 32963
   Date of collection: April 28th, 2010
   Depth of collection: Near shore, underwater
   Appearance at time of collection (e.g. turbid, etc.): clear with some plant material

   Salinity at collection: 36 ppt
   Temperature at collection: 72ºF
   DOC "x" hours after collection: not performed

   Pretreatment prior to testing (e.g. filtration, sedimentation, ageing, etc.): rough filtration with paper filter

   Microbial colony count: not performed

   Other characteristics: none

5. **TEST MEDIUM:**

   Temperature after aeration: compensated for automatically by the test apparatus

   O_2 concentration after aeration and
   standing before start of test: within normal limits

6. **DO DETERMINATION:**

Method: electrode

	Flask no.		mg O_2/l after n days			
			0	5	15	28
Test: nutrient-fortified seawater with test substance	1	a_1	5.70	4.57	3.78	3.73
	2	a_2	5.80	4.87	3.38	3.33
	Mean test	$m_t = (a_1 + a_2)/2$	5.75	4.72	3.58	3.53
Blank: nutrient-fortified seawater, but without test substance	1	c_1	5.70	5.47	5.08	4.93
	2	c_2	5.70	5.47	5.08	4.73
	Mean blank	$m_b = (c_1 + c_2)/2$	5.70	5.47	5.08	4.83

7. **DO DEPLETION: % DEGRADATION (%D):**

	DO depletion after n days		
	5	15	28
$(m_b - m_t)$	0.75	1.50	1.30
$\%D = [(m_b - m_t)/(\text{test substance (mg/l)} \times \text{COD})] \times 100$	39.6%	79.1%	68.6%

*Note that the DO depletion value is higher for day 15 than for day 28. This result is due to the test product's rapid degradation earlier in the test. The lower value for day 28 is due to the decrease in m_b during that period of the test.