



## Final Biodegradability Report

**Project: NAT-2018**

**Report Date: August 7, 2007**

On June 06, 2007 biodegradability testing of Formula 080 (Natural Soap Formulas, Inc.) was initiated and conducted by RespirTek, Inc. according to ASTM E1720-01 (OECD 301B Modified). In this study, Formula 080 was incubated with a dilute inoculum of microbes from a domestic wastewater treatment facility in sealed containers for 20 days. Biodegradability was assessed by measuring carbon dioxide production as dissolved inorganic carbon in the liquid phase after the addition of 10 M sodium hydroxide. It is assumed in the methodology that carbon dioxide gas is the sole product of biodegradation.

The inoculum was collected from the Escatawpa, MS, USA Municipal Wastewater Treatment Plant on May 31, 2007 and conditioned as described in the protocol.

Testing was conducted with no deviations from the protocol. The test results are provided through day 20 when the extent of biodegradation achieved was 66.85%. The testing was halted in accordance with the method given the multiple test days with biodegradation results greater than 60%.

Formula 080 achieved ultimate biodegradability (60% of the theoretical carbon dioxide production) on day 20 (66.85%). It did not achieve ready biodegradability because it did not pass the ten-day window rule (60% production within ten days of exceeding 10%).

All raw data are maintained at RespirTek, Inc. 14373 Jim Byrd Road, Biloxi, MS 39532; (228)-392-7977.



**Report Date: August 7, 2007**

**Project ID: NAT-2018**

**Project Initiation Date: June 6, 2007**

**Test Method: ASTM E1720-01: OECD 301B Modified**

**Test Classification: Ready/Ultimate Biodegradability**

**Test Chemical**

TC-1      Formula 080

**Biodegradation Achieved**

66.85% on Day 20



**Test Chemical 1 - Formula 080**

|         | Day 0 | Day 2 | Day 6 | Day 9 | Day 13 | Day 16 | Day 20 | Day 23 | Day 28 |
|---------|-------|-------|-------|-------|--------|--------|--------|--------|--------|
| Blank 1 | 4.208 | 4.561 | 6.166 | 4.301 | 4.854  | 5.771  | 7.811  |        |        |
| Blank 2 | 4.66  | 4.137 | 7.291 | 5.636 | 5.248  | 5.929  | 6.567  |        |        |
| Blank 3 | 4.978 | 3.672 | 6.158 | 4.742 | 4.444  | 5.229  | 7.112  |        |        |
| TC1-1   | 4.371 | 5.536 | 6.285 | 8.702 | 9.609  | 11.69  | 13.69  |        |        |
| TC1-2   | 4.388 | 5.586 | 7.859 | 9.107 | 9.607  | 12.57  | 13.6   |        |        |
| TC1-3   | 4.566 | 5.356 | 7.016 | 8.992 | 9.963  | 11.67  | 14.72  |        |        |

|            | Day 0 | Day 2 | Day 6 | Day 9 | Day 13 | Day 16 | Day 20 | Day 23  | Day 28  |
|------------|-------|-------|-------|-------|--------|--------|--------|---------|---------|
| Blank Mean | 4.62  | 4.12  | 6.54  | 4.89  | 4.85   | 5.64   | 7.16   | #DIV/0! | #DIV/0! |

| Day 0 Product Mean |         |
|--------------------|---------|
| TC-1               | 4.44167 |

**Test Chemical 1 - Micrograms of Inorganic Carbon as CO2**

| Replicate | Day 0 | Day 2  | Day 6  | Day 9  | Day 13 | Day 16 | Day 20 | Day 23  | Day 28  |
|-----------|-------|--------|--------|--------|--------|--------|--------|---------|---------|
| 1         | 0     | 158.63 | -7.97  | 398.27 | 493.40 | 622.07 | 670.03 | #DIV/0! | #DIV/0! |
| 2         | 0     | 163.63 | 149.43 | 438.77 | 493.20 | 710.07 | 661.03 | #DIV/0! | #DIV/0! |
| 3         | 0     | 140.63 | 65.13  | 427.27 | 528.80 | 620.07 | 773.03 | #DIV/0! | #DIV/0! |

**Organic Carbon Content Per Reactor**

| Sample ID | TOC    | Unit  |
|-----------|--------|-------|
| TC1       | 10.492 | ug/mL |

**Test Chemical 1 - % Theoretical CO2 Production Realized**

| Replicate | Day 0 | Day 2 | Day 6 | Day 9 | Day 13 | Day 16 | Day 20 | Day 23  | Day 28  |
|-----------|-------|-------|-------|-------|--------|--------|--------|---------|---------|
| 1         | 0     | 15.12 | -0.76 | 37.96 | 47.03  | 59.29  | 63.86  | #DIV/0! | #DIV/0! |
| 2         | 0     | 15.60 | 14.24 | 41.82 | 47.01  | 67.68  | 63.00  | #DIV/0! | #DIV/0! |
| 3         | 0     | 13.40 | 6.21  | 40.72 | 50.40  | 59.10  | 73.68  | #DIV/0! | #DIV/0! |

**Summary Statistics**

**Average % Theoretical CO2 Production**

| Mean | Day 0 | Day 2 | Day 6 | Day 9 | Day 13 | Day 16 | Day 20 | Day 23  | Day 28  |
|------|-------|-------|-------|-------|--------|--------|--------|---------|---------|
| TC1  | 0.00  | 14.71 | 6.56  | 40.17 | 48.14  | 62.02  | 66.85  | #DIV/0! | #DIV/0! |

**Standard Deviation Between Replicates at Each Sample Point**

| St. Dev. | Day 0 | Day 2 | Day 6 | Day 9 | Day 13 | Day 16 | Day 20 | Day 23  | Day 28  |
|----------|-------|-------|-------|-------|--------|--------|--------|---------|---------|
| TC1      | N/A   | 1.153 | 7.507 | 1.989 | 1.954  | 4.898  | 5.931  | #DIV/0! | #DIV/0! |

**95% Confidence Interval Values (+- Value Calculated) at n-1 Degrees of Freedom**

| 95% CI | Day 0 | Day 2 | Day 6  | Day 9 | Day 13 | Day 16 | Day 20 | Day 23  | Day 28  |
|--------|-------|-------|--------|-------|--------|--------|--------|---------|---------|
| TC1    | N/A   | 2.862 | 18.638 | 4.938 | 4.850  | 12.161 | 14.724 | #DIV/0! | #DIV/0! |



**Positive Control - Dextrose**

|           | Day 0 | Day 2 | Day 6 | Day 9 |
|-----------|-------|-------|-------|-------|
| Blank 1   | 4.208 | 4.561 | 6.166 | 4.301 |
| Blank 2   | 4.66  | 4.137 | 7.291 | 5.636 |
| Blank 3   | 4.978 | 3.672 | 6.158 | 4.742 |
| Control 1 | 4.682 | 10.73 | 13.65 | 12.79 |
| Control 2 | 4.301 | 8.923 | 13.25 | 13.66 |
| Control 3 | 4.076 | 11.37 | 13.41 | 12.64 |

|            | Day 0 | Day 2 | Day 6 | Day 9 |
|------------|-------|-------|-------|-------|
| Blank Mean | 4.62  | 4.12  | 6.54  | 4.89  |

| Day 0 Product Mean |       |
|--------------------|-------|
| Control            | 4.353 |

**Test Chemical 1 - Micrograms of Inorganic Carbon as CO<sub>2</sub>**

| Replicate | Day 0 | Day 2  | Day 6  | Day 9  |
|-----------|-------|--------|--------|--------|
| 1         | 0     | 686.90 | 737.40 | 815.93 |
| 2         | 0     | 506.20 | 697.40 | 902.93 |
| 3         | 0     | 750.90 | 713.40 | 800.93 |

**Organic Carbon Content Per Reactor**

| Sample ID | TOC    | Unit  |
|-----------|--------|-------|
| Control   | 10.352 | ug/mL |

**Test Chemical 1 - % Theoretical CO<sub>2</sub> Production Realized**

| Replicate | Day 0 | Day 2 | Day 6 | Day 9 |
|-----------|-------|-------|-------|-------|
| 1         | 0     | 66.35 | 71.23 | 78.82 |
| 2         | 0     | 48.90 | 67.37 | 87.22 |
| 3         | 0     | 72.54 | 68.91 | 77.37 |

**Summary Statistics**

Average % Theoretical CO<sub>2</sub> Production (>60% indicates passing conditions)

| Mean    | Day 0 | Day 2 | Day 6 | Day 9 |
|---------|-------|-------|-------|-------|
| Control | 0.00  | 62.60 | 69.17 | 81.14 |

**Standard Deviation Between Replicates at Each Sample Point**

| St. Dev. | Day 0 | Day 2  | Day 6 | Day 9 |
|----------|-------|--------|-------|-------|
| Control  | N/A   | 12.259 | 1.945 | 5.320 |

**95% Confidence Interval Values (+- Value Calculated) at n-1 Degrees of Freedom**

| 95% CI  | Day 0 | Day 2  | Day 6 | Day 9  |
|---------|-------|--------|-------|--------|
| Control | N/A   | 30.434 | 4.828 | 13.207 |

### Biodegradation Curves

