# SUMMARY REPORT

## Biodegradability of Easy Decon Using the $CO_2$ Evolution Method

A biodegradation test by the $CO_2$ evolution method was conducted at ABC Laboratories, Inc. to determine the aerobic biodegradability of Easy Decon. The test was conducted for 35 days beginning on July 11, 2002.

Stock solutions of the test and reference substances were prepared and used for dosing the test system. A 1.00 mg/mL stock solution of the test substance was prepared by diluting 500.1 mg of Easy Decon Penetrator III RTM and 60.0 mg of the Fortifier in 500 mL of reagent water. A 1.00 mg/mL stock solution of the reference substance was prepared by diluting 1000.1 mg of sodium benzoate in 1000 mL of reagent water.

Activated sludge served as the microbial inoculum. Sludge was collected from aeration basin #1 at the Columbia Wastewater Treatment Plant in Columbia, Missouri. The activated sludge sample was homogenized in a blender for two minutes, solids in the sample were allowed to settle for 30 minutes, and the supernatant was filtered through glass wool.

Each test system consisted of a 5-liter glass carboy filled with a mixture of mineral salts media, activated sludge, reagent water, and the appropriate volume of test or reference substance solution. The final volume in each carboy was 2 liters. Each carboy was placed in a waterbath over a magnetic stir plate and solutions were stirred with a Teflon-coated magnetic stir bar. The samples were connected to a Columbus Instruments Micro-Oxymax respirometer, which measured the $CO_2$ in the sealed headspace above each sample. The Micro-Oxymax respirometer measured the mass of accumulated $CO_2$ (mg) at each 8-hour sampling interval. After odd numbered sampling intervals, the respirometer refreshed the headspace of each carboy with atmospheric air.

The test systems were prepared as summarized in Table 1. The control system contained activated sludge, mineral salts media, and reagent water but no test or reference substance. Duplicate test substance carboys were prepared and contained activated sludge, mineral salts media, reagent water, and Easy Decon at a nominal concentration of 9.90 mg C/L. The reference substance carboy contained activated sludge, mineral salts media, reagent water, and benzoic acid dosed at a nominal concentration of 19.9 mg C/L.

Percent theoretical $CO_2$ (% $ThCO_2$) production from each test and reference system was calculated and determined as follows:

$$\frac{\text{Net Accumulated } CO_2 \text{ (mg)}}{\text{Theoretical } CO_2} \times 100 = \%ThCO_2$$

The net accumulated $CO_2$ was calculated as the difference in the accumulated $CO_2$ from the control systems (mean of the two replicates) and the accumulated $CO_2$ from the test or reference

substance systems. The theoretical $CO_2$ ($ThCO_2$) for the test substance, 71.1 mg $CO_2$, was calculated from the nominal test substance concentration (9.90 mg C/L) the volume of test solution after initiation (1960 mL), and the carbon to carbon dioxide conversion factor. The carbon to carbon dioxide conversion factor used was 3.664 [(44.01 g $CO_2$/mole)/(12.01 g C/mole)]. Similarly, the theoretical $CO_2$ for the reference substance was calculated to be 143 mg $CO_2$.

The test substance, Easy Decon, exhibited % $ThCO_2$ values of 23.5% and 25.7% for replicates 1 and 2, respectively, by day 35 of the study. Easy Decon may be classified as inherently biodegradable (>20% $ThCO_2$).

The reference substance, sodium benzoate, exhibited a % $ThCO_2$ value of 76.1% by day 35 of the study. The results from the reference substance treatment indicate that the inoculum was viable.

TABLE 1. Preparation of the Test Systems

| Carboy ID | Reference Substance Solution (mL) | Test Substance Solution (mL) | Mineral Salts Medium (mL) | Activated Sludge Inoculum (mL) | Reagent Water (mL) |
|---|---|---|---|---|---|
| Control, Replicate 1 | — | — | 1600 | 20 | 400 |
| Control, Replicate 2 | — | — | 1600 | 20 | 400 |
| Reference Substance | 69 [1] | — | 1600 | 20 | 331 |
| Test Substance, Replicate 1 | — | 229 [2] | 1600 | 20 | 171 |
| Test Substance, Replicate 2 | — | 229 [2] | 1600 | 20 | 171 |

Note: The activated sludge inoculum and mineral salts medium were combined in the test carboys on day (-1) and were aerated until test initiation when all other solutions were added.

[1] Concentration of the reference substance stock solution was 1.00 mg/mL.

[2] Concentration of the test substance stock solution was 1.00 mg/mL.

The nominal test concentrations for the test substance treatment, 9.90 mg C/L, and reference substance treatment, 19.9 mg C/L, were based on the percent carbon in the test and reference substances. The percent carbon in the reference substance was calculated to be 58.3% based on the molecular formula ($C_7H_5O_2Na$). The percent carbon in the test substance, 8.73%, was supplied by the sponsor.

TABLE 2. Accumulated $CO_2$ from the Control Systems and Net Accumulated $CO_2$ and Percent $ThCO_2$ from the Test and Reference Substance Systems

| | | Control | Test Substance, Rep. 1 | | Test Substance, Rep. 2 | | Reference Substance | |
|---|---|---|---|---|---|---|---|---|
| Sampling Interval | Sampling Time (Days) | Mean Accumulated $CO_2$ (mg) | Net Accumulated $CO_2$ (mg) | Percent $ThCO_2$ | Net Accumulated $CO_2$ (mg) | Percent $ThCO_2$ | Net Accumulated $CO_2$ (mg) | Percent $ThCO_2$ |
| 1 | 0.35 | -0.07 | -0.01 | 0.0% | -0.03 | 0.0% | 0.05 | 0.0% |
| 2 | 0.68 | -0.19 | -0.02 | 0.0% | -0.05 | -0.1% | 0.52 | 0.4% |
| 3 | 1.02 | -0.23 | -0.03 | 0.0% | -0.06 | -0.1% | 2.97 | 2.1% |
| 4 | 1.35 | -0.35 | -0.05 | -0.1% | -0.10 | -0.1% | 6.12 | 4.3% |
| 5 | 1.68 | -0.37 | -0.07 | -0.1% | -0.10 | -0.1% | 7.84 | 5.5% |
| 6 | 2.02 | -0.45 | -0.12 | -0.2% | -0.15 | -0.2% | 11.64 | 8.1% |
| 7 | 2.35 | -0.48 | -0.12 | -0.2% | -0.14 | -0.2% | 13.13 | 9.2% |
| 8 | 2.69 | -0.58 | -0.17 | -0.2% | -0.19 | -0.3% | 16.99 | 11.9% |
| 9 | 3.02 | -0.61 | -0.16 | -0.2% | -0.16 | -0.2% | 18.41 | 12.9% |
| 10 | 3.36 | -0.65 | -0.19 | -0.3% | -0.19 | -0.3% | 22.25 | 15.6% |
| 11 | 3.69 | -0.68 | -0.19 | -0.3% | -0.18 | -0.3% | 23.65 | 16.5% |
| 12 | 4.02 | -0.75 | -0.22 | -0.3% | -0.22 | -0.3% | 27.38 | 19.1% |
| 13 | 4.36 | -0.79 | -0.21 | -0.3% | -0.21 | -0.3% | 28.70 | 20.1% |
| 14 | 4.69 | -0.88 | -0.24 | -0.3% | -0.24 | -0.3% | 32.28 | 22.6% |
| 15 | 5.02 | -0.93 | -0.24 | -0.3% | -0.24 | -0.3% | 33.55 | 23.5% |
| 16 | 5.36 | -0.97 | -0.29 | -0.4% | -0.28 | -0.4% | 36.96 | 25.8% |
| 17 | 5.69 | -1.02 | -0.26 | -0.4% | -0.26 | -0.4% | 38.12 | 26.7% |
| 18 | 6.03 | -1.08 | -0.26 | -0.4% | -0.15 | -0.2% | 41.35 | 28.9% |
| 19 | 6.36 | -1.10 | -0.16 | -0.2% | -0.07 | -0.1% | 42.40 | 29.7% |
| 20 | 6.70 | -1.16 | -0.05 | -0.1% | 0.07 | 0.1% | 45.44 | 31.8% |
| 21 | 7.03 | -1.18 | 0.01 | 0.0% | 0.19 | 0.3% | 46.46 | 32.5% |
| 22 | 7.37 | -1.24 | 0.16 | 0.2% | 0.48 | 0.7% | 49.35 | 34.5% |
| 23 | 7.70 | -1.26 | 0.26 | 0.4% | 0.60 | 0.9% | 50.30 | 35.2% |
| 24 | 8.04 | -1.34 | 0.43 | 0.6% | 0.94 | 1.3% | 52.99 | 37.1% |
| 25 | 8.37 | -1.39 | 0.55 | 0.8% | 1.07 | 1.5% | 53.93 | 37.7% |
| 26 | 8.70 | -1.51 | 0.86 | 1.2% | 1.41 | 2.0% | 56.53 | 39.5% |
| 27 | 9.04 | -1.52 | 1.00 | 1.4% | 1.54 | 2.2% | 57.43 | 40.2% |
| 28 | 9.37 | -1.52 | 1.35 | 1.9% | 1.95 | 2.7% | 59.88 | 41.9% |
| 29 | 9.70 | -1.55 | 1.50 | 2.1% | 2.15 | 3.0% | 60.70 | 42.4% |
| 30 | 10.04 | -1.56 | 1.95 | 2.7% | 2.67 | 3.8% | 63.04 | 44.1% |
| 31 | 10.37 | -1.57 | 2.12 | 3.0% | 2.84 | 4.0% | 63.82 | 44.6% |
| 32 | 10.71 | -1.57 | 2.56 | 3.6% | 3.34 | 4.7% | 66.01 | 46.2% |

TABLE 2 (Cont.). Accumulated $CO_2$ from the Control Systems and Net Accumulated $CO_2$ and Percent $ThCO_2$ from the Test and Reference Substance Systems

| Sampling Interval | Sampling Time (Days) | Control Mean Accumulated $CO_2$ (mg) | Test Substance, Rep. 1 Net Accumulated $CO_2$ (mg) | Test Substance, Rep. 1 Percent $ThCO_2$ | Test Substance, Rep. 2 Net Accumulated $CO_2$ (mg) | Test Substance, Rep. 2 Percent $ThCO_2$ | Reference Substance Net Accumulated $CO_2$ (mg) | Reference Substance Percent $ThCO_2$ |
|---|---|---|---|---|---|---|---|---|
| 33 | 11.04 | -1.59 | 2.72 | 3.8% | 3.51 | 4.9% | 66.73 | 46.7% |
| 34 | 11.38 | -1.60 | 3.17 | 4.5% | 4.00 | 5.6% | 68.81 | 48.1% |
| 35 | 11.71 | -1.63 | 3.33 | 4.7% | 4.18 | 5.9% | 69.52 | 48.6% |
| 36 | 12.05 | -1.73 | 3.75 | 5.3% | 4.70 | 6.6% | 71.35 | 49.9% |
| 37 | 12.38 | -1.75 | 3.90 | 5.5% | 4.86 | 6.8% | 71.96 | 50.3% |
| 38 | 12.71 | -1.75 | 4.32 | 6.1% | 5.33 | 7.5% | 73.66 | 51.5% |
| 39 | 13.05 | -1.76 | 4.45 | 6.3% | 5.48 | 7.7% | 74.22 | 51.9% |
| 40 | 13.38 | -1.77 | 4.86 | 6.8% | 5.94 | 8.4% | 75.84 | 53.0% |
| 41 | 13.72 | -1.78 | 4.99 | 7.0% | 6.09 | 8.6% | 76.38 | 53.4% |
| 42 | 14.05 | -1.85 | 5.40 | 7.6% | 6.53 | 9.2% | 77.90 | 54.5% |
| 43 | 14.38 | -1.86 | 5.53 | 7.8% | 6.67 | 9.4% | 78.42 | 54.8% |
| 44 | 14.72 | -1.85 | 5.92 | 8.3% | 7.11 | 10.0% | 79.86 | 55.8% |
| 45 | 15.05 | -1.86 | 6.06 | 8.5% | 7.25 | 10.2% | 80.36 | 56.2% |
| 46 | 15.39 | -1.86 | 6.46 | 9.1% | 7.69 | 10.8% | 81.74 | 57.2% |
| 47 | 15.72 | -1.86 | 6.60 | 9.3% | 7.84 | 11.0% | 82.20 | 57.5% |
| 48 | 16.06 | -1.86 | 7.00 | 9.8% | 8.26 | 11.6% | 83.51 | 58.4% |
| 49 | 16.39 | -1.87 | 7.14 | 10.0% | 8.42 | 11.8% | 83.94 | 58.7% |
| 50 | 16.73 | -1.88 | 7.53 | 10.6% | 8.83 | 12.4% | 85.19 | 59.6% |
| 51 | 17.06 | -1.88 | 7.66 | 10.8% | 8.97 | 12.6% | 85.58 | 59.8% |
| 52 | 17.39 | -1.85 | 8.05 | 11.3% | 9.38 | 13.2% | 86.74 | 60.7% |
| 53 | 17.73 | -1.85 | 8.16 | 11.5% | 9.50 | 13.4% | 87.13 | 60.9% |
| 54 | 18.06 | -1.85 | 8.54 | 12.0% | 9.90 | 13.9% | 88.27 | 61.7% |
| 55 | 18.39 | -1.85 | 8.65 | 12.2% | 10.03 | 14.1% | 88.65 | 62.0% |
| 56 | 18.73 | -1.86 | 9.00 | 12.7% | 10.41 | 14.6% | 89.72 | 62.7% |
| 57 | 19.97 | -1.89 | 9.12 | 12.8% | 10.55 | 14.8% | 90.09 | 63.0% |
| 58 | 20.31 | -1.95 | 9.49 | 13.3% | 10.94 | 15.4% | 91.14 | 63.7% |
| 59 | 22.02 | -1.96 | 9.62 | 13.5% | 11.08 | 15.6% | 91.49 | 64.0% |
| 60 | 22.35 | -1.93 | 9.97 | 14.0% | 11.46 | 16.1% | 92.49 | 64.7% |
| 61 | 22.69 | -1.92 | 10.10 | 14.2% | 11.57 | 16.3% | 92.83 | 64.9% |
| 62 | 23.02 | -1.88 | 10.45 | 14.7% | 11.93 | 16.8% | 93.78 | 65.6% |
| 63 | 23.35 | -1.88 | 10.56 | 14.9% | 12.06 | 17.0% | 94.09 | 65.8% |
| 64 | 23.69 | -1.83 | 10.88 | 15.3% | 12.40 | 17.4% | 94.99 | 66.4% |

TABLE 2. Accumulated $CO_2$ from the Control Systems and Net Accumulated $CO_2$ and Percent $ThCO_2$ from the Test and Reference Substance Systems

| Sampling Interval | Sampling Time (Days) | Control Mean Accumulated $CO_2$ (mg) | Test Substance, Rep. 1 Net Accumulated $CO_2$ (mg) | Test Substance, Rep. 1 Percent $ThCO_2$ | Test Substance, Rep. 2 Net Accumulated $CO_2$ (mg) | Test Substance, Rep. 2 Percent $ThCO_2$ | Reference Substance Net Accumulated $CO_2$ (mg) | Reference Substance Percent $ThCO_2$ |
|---|---|---|---|---|---|---|---|---|
| 65 | 24.02 | -1.83 | 11.00 | 15.5% | 12.52 | 17.6% | 95.30 | 66.6% |
| 66 | 24.36 | -1.78 | 11.33 | 15.9% | 12.87 | 18.1% | 96.17 | 67.3% |
| 67 | 24.69 | -1.78 | 11.45 | 16.1% | 12.99 | 18.3% | 96.46 | 67.5% |
| 68 | 25.03 | -1.78 | 11.77 | 16.6% | 13.31 | 18.7% | 97.30 | 68.0% |
| 69 | 25.36 | -1.79 | 11.88 | 16.7% | 13.42 | 18.9% | 97.59 | 68.2% |
| 70 | 25.70 | -1.76 | 12.18 | 17.1% | 13.75 | 19.3% | 98.39 | 68.8% |
| 71 | 26.03 | -1.75 | 12.31 | 17.3% | 13.86 | 19.5% | 98.66 | 69.0% |
| 72 | 26.37 | -1.72 | 12.62 | 17.8% | 14.18 | 19.9% | 99.44 | 69.5% |
| 73 | 26.70 | -1.70 | 12.74 | 17.9% | 14.30 | 20.1% | 99.72 | 69.7% |
| 74 | 27.03 | -1.76 | 13.03 | 18.3% | 14.58 | 20.5% | 100.39 | 70.2% |
| 75 | 27.37 | -1.77 | 13.13 | 18.5% | 14.67 | 20.6% | 100.64 | 70.4% |
| 76 | 27.70 | -1.75 | 13.38 | 18.8% | 14.91 | 21.0% | 101.24 | 70.8% |
| 77 | 28.03 | -1.74 | 13.48 | 19.0% | 15.02 | 21.1% | 101.50 | 71.0% |
| 78 | 28.37 | -1.73 | 13.76 | 19.3% | 15.30 | 21.5% | 102.13 | 71.4% |
| 79 | 28.70 | -1.72 | 13.84 | 19.5% | 15.39 | 21.6% | 102.34 | 71.6% |
| 80 | 29.04 | -1.70 | 14.10 | 19.8% | 15.66 | 22.0% | 102.95 | 72.0% |
| 81 | 29.37 | -1.69 | 14.19 | 20.0% | 15.75 | 22.1% | 103.16 | 72.1% |
| 82 | 29.71 | -1.66 | 14.46 | 20.3% | 16.02 | 22.5% | 103.76 | 72.6% |
| 83 | 30.04 | -1.66 | 14.55 | 20.5% | 16.11 | 22.7% | 103.96 | 72.7% |
| 84 | 30.38 | -1.61 | 14.80 | 20.8% | 16.38 | 23.0% | 104.53 | 73.1% |
| 85 | 30.71 | -1.60 | 14.88 | 20.9% | 16.46 | 23.2% | 104.73 | 73.2% |
| 86 | 31.05 | -1.56 | 15.12 | 21.3% | 16.71 | 23.5% | 105.28 | 73.6% |
| 87 | 31.38 | -1.55 | 15.19 | 21.4% | 16.79 | 23.6% | 105.45 | 73.7% |
| 88 | 31.71 | -1.50 | 15.42 | 21.7% | 17.04 | 24.0% | 105.97 | 74.1% |
| 89 | 32.05 | -1.50 | 15.51 | 21.8% | 17.13 | 24.1% | 106.14 | 74.2% |
| 90 | 32.38 | -1.48 | 15.73 | 22.1% | 17.35 | 24.4% | 106.66 | 74.6% |
| 91 | 32.71 | -1.48 | 15.80 | 22.2% | 17.44 | 24.5% | 106.82 | 74.7% |
| 92 | 33.05 | -1.51 | 16.02 | 22.5% | 17.63 | 24.8% | 107.32 | 75.0% |
| 93 | 33.38 | -1.51 | 16.11 | 22.7% | 17.71 | 24.9% | 107.49 | 75.2% |
| 94 | 33.72 | -1.45 | 16.33 | 23.0% | 17.93 | 25.2% | 107.98 | 75.5% |
| 95 | 34.05 | -1.44 | 16.40 | 23.1% | 18.00 | 25.3% | 108.13 | 75.6% |
| 96 | 34.39 | -1.38 | 16.61 | 23.4% | 18.23 | 25.6% | 108.61 | 76.0% |
| 97 | 34.72 | -1.36 | 16.68 | 23.5% | 18.30 | 25.7% | 108.75 | 76.1% |

FIGURE 1. Cumulative Percent Theoretical $CO_2$ versus Time for the Test and Reference Substances