Biodegradability of ENVIROKLEAN PLUS® in Seawater (Closed-bottle Method)

Guideline: OECD 306

Conducted for:

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STATEMENT OF COMPLIANCE

Test data presented in this report was obtained in accordance with standard laboratory practices and the test guidelines established by the Organization for Economic Cooperation and Development (OECD). This report is an accurate representation of the original data. A copy of this report and the original laboratory records on which this report is based are archived at the PBS&J Environmental Toxicology Laboratory.

Original Signed by JDH — 10/20/00
James D. Horne — Date
Director, Special Projects

Original Signed by FRP — 10/20/00
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1.0 INTRODUCTION

Biodegradation is a major route by which organic chemicals are removed from the environment. In aerobic biodegradation, microbial enzymes break the carbon bonds of a compound in the presence of oxygen, resulting in the consumption of oxygen and the release of carbon dioxide. Using a closed system, it is possible to measure oxygen consumption or carbon dioxide production, and thus to estimate the degree of biodegradation of a compound relative to an empirically derived Theoretical Oxygen Demand (ThOD) or experimentally derived Chemical Oxygen Demand (COD). Aerobic biodegradability is evaluated in aquatic systems using a variety of inocula; natural waters, such as seawater, provide their own mixed inoculum of indigenous microbes, and so are useful for predicting the degree of biodegradability of compounds under relatively natural conditions.

The biodegradability of ENVIROKLEAN PLUS® in natural seawater was evaluated using the closed-bottle method. The test was conducted according to OECD Guideline 306 (1992). A single concentration of the test substance was incubated with nutrient-fortified natural seawater for 28 days. Dissolved oxygen (DO) was measured periodically to determine the degree of oxygen consumption, which was used to calculate the degree of biodegradation relative to the chemical oxygen demand of the test substance. Testing was conducted at the PBS&J Environmental Toxicology Laboratory, Houston, Texas. Original data from this study are archived at PBS&J.

2.0 MATERIALS AND METHODS

2.1 Protocol

The study was conducted according to "Biodegradability in Seawater," OECD Guideline 306.

2.2 Test Substance

The test substance, ENVIROKLEAN PLUS®, was received from The Astonishing Products Company, Houston, TX, on 4 August 2000. Approximately 1 gallon of the test substance (a clear, pale yellow liquid; pH <1) was contained in a linear polyethylene jug. The test substance was stored in a laboratory cabinet at room temperature. Prior to testing, and again after the test was terminated, the test substance was examined to ensure that no overt physical change had occurred. In both cases, the substance was observed to be a clear, pale yellow liquid.

An aliquot of the test substance was provided to North Water District Laboratory Services, The Woodlands, TX, for determination of chemical oxygen demand. The reported COD for the product was 13,600 mg O_2/liter (Appendix A); using the specific gravity (1.12) recorded in the MSDS provided by the study sponsor, this corresponds to 0.0136 mg O_2/mg test substance.

2.3 Reference Substance

Sodium benzoate (benzoic acid sodium salt, $C_7H_6O_2$Na, CAS No. 532-32-1), a readily biodegradable substance, was used as a reference substance. Sodium benzoate, Lot No. 994652, was supplied by Fisher Scientific. The purity of the reference substance was assumed to be 100%.

2.4 Inoculum and Dilution Water

Natural seawater from Galveston Bay (TX) was used as the test medium and inoculum source for the closed-bottle test. The seawater was obtained on 3 August 2000 from a location near the western end of the seawall in Galveston, Texas. The water was conditioned at room temperature in a clean, 10-gallon polyethylene container. During conditioning, the water was filtered continuously through a coarse polyester-fiber filter using a recirculating pump system, and gently aerated with a standard aquarium air stone supplied from a oil-free, low-pressure regenerative blower. The purpose of this pre-test conditioning was to reduce the potential for interference from organic carbon already present in the water. It was assumed that any degradable organic carbon present in the water was largely metabolized during the pre-incubation period.

Just prior to testing, the seawater was fortified with mineral salts (Table 1) as prescribed in the OECD Guideline.

2.5 Incubation Vessels

Incubation vessels were glass Wheaton BOD bottles with a nominal volume of 300 mL. The bottles were fitted with ground-glass stoppers to prevent gas exchange with the atmosphere. Prior to use, the bottles were washed with detergent, rinsed several times with deionized water, then filled with acidulated deionized water (2 drops 12 N hydrochloric acid per bottle) and allowed to stand at least overnight. Each incubation vessel was labeled with test treatment ID and replicate letter.

2.6 Test Initiation

The test treatment group consisted of nine BOD bottles, each containing 130 FL of test substance and filled with nutrient-fortified seawater. Nine additional bottles were filled with nutrient-fortified seawater to serve as treatment blanks. A third set of nine bottles were filled with nutrient-fortified seawater containing 2 mg/L sodium benzoate (the reference control). Finally, nine bottles containing 80 FL test substance were filled with nutrient-fortified seawater which also contained 2 mg/L sodium benzoate. In each group, the bottles were filled to the bottom of the ground-glass neck, then tapered glass stoppers were carefully placed in the bottles to avoid trapping air bubbles below the stopper. Each bottle was filled and stoppered before the next bottle was filled. The bottles were sealed with plastic push-on caps to maintain a water seal on the bottles.

Two bottles from each treatment group were analyzed immediately for dissolved oxygen (DO) concentration using a YSI Model 5000 DO meter with automatic stirrer. The remaining bottles were placed in a darkened BOD incubator programmed to hold a constant temperature of 20 ºC.

2.7 Test Monitoring

The DO concentration was measured in one bottle of each treatment group after two days and, then, in two bottles of each treatment group after 5, 15 and 28 days of incubation. During DO measurements, each bottle was opened and the probe was carefully placed in the test medium to avoid introducing air. The bottles were discarded after measurements were completed.

2.8 Calculation of Percent Biodegradation

The percent biodegradation of the test and reference substance was calculated after correction for the oxygen consumption in the blank group. In this way, the endogenous respiration of the inoculum, and the degradation of any organic carbon present in the test medium was subtracted. All remaining dissolved oxygen consumption was attributed to degradation of the test or reference substance.

Percent biodegradation of the reference substance was calculated as follows (OECD, 1992),

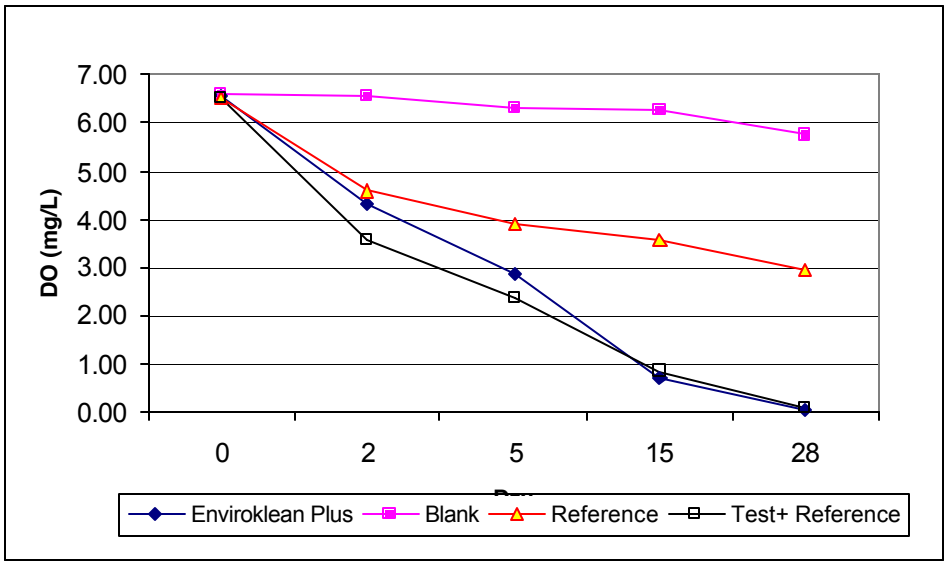
$$\% \text{ biodegradation} = \frac{(M_{t(0)} - M_{t(x)}) - (M_{b(0)} - M_{b(x)})}{\text{Test substance (mg/L)} \times \text{ThOD}} \times 100$$

where $M_{b(0)}$ and $M_{b(x)}$ equal the DO measured in the blank at time(0) and time(x), respectively; $M_{t(0)}$ and $M_{t(x)}$ equal the DO measured in the test or reference treatment at time(0) and time(x), respectively; and ThOD (or COD) equals the theoretical amount of oxygen which would be consumed if all of the test material were mineralized. ThOD was calculated for the reference substance based on the molecular formula; the measured COD was used for the test material.

3.0 RESULTS AND DISCUSSION

As shown in Figure 1, the test treatments exhibited varying degrees of dissolved oxygen depletion during the course of the biodegradation experiment. All treatments except the blank exhibited strong dissolved oxygen depletion throughout the test. End-of-test oxygen concentration in the reference substance treatment was $2 mg/L; therefore, oxygen concentration was not a limiting factor. Oxygen consumption in the test substance and test substance plus reference substance treatment group, however, resulted in oxygen concentrations which were < 1 mg/L at the end of the test; consequently, the oxygen concentration in these treatments may have been a limiting factor

Figure 1. Residual dissolved oxygen during a 28-day closed bottle biodegradation test of ENVIROKLEAN PLUS®



3.1 Control Performance

The blank control exhibited a decrease in dissolved oxygen concentration from 6.61 mg/L at test initiation to 5.76 mg/L after 28 days of incubation (Table 2). This decrease represents a 12.8% consumption of the available oxygen by the endogenous respiration of the inoculum. This is within the OECD guideline (<30%) for oxygen depletion in control media and shows that there was not a large amount of dissolved organic carbon in the natural seawater.

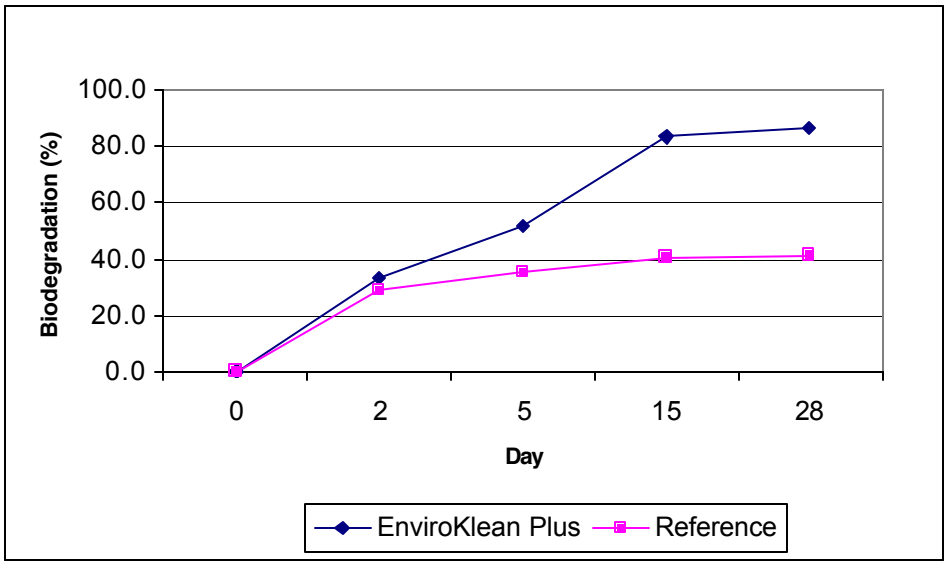
3.2 Reference Substance Performance

By day 5 of the experiment, sodium benzoate was biodegraded by ~35%, and by day 28 the reference substance was degraded by approximately 42% (Table 2). The acceptance criterion for sodium benzoate biodegradation by natural seawater microbes is >50% within 5 days. This criterion was not met, indicating that the viability of the seawater inoculum was less than optimal; accordingly, the biodegradability of the test material may also be underestimated.

3.3 Biodegradation of ENVIROKLEAN PLUS®

The mixture of ENVIROKLEAN PLUS® and the reference substance consumed oxygen a rate approximately equal to that of the test substance alone (Figure 1); therefore, there was no indication that the material might be toxic or inhibitory to seawater microbes. Dissolved oxygen in the test substance treatment decreased from 6.56 mg/L at the beginning to 0.06 mg/L at the end of the test (Table 2). Based on these data, the biodegradability of ENVIROKLEAN PLUS® is estimated to be 86% (Figure 2).

Figure 2. Percent biodegradation of ENVIROKLEAN PLUS® and sodium benzoate during a 28-day closed bottle test



4.0 CONCLUSION

Enviroklean Plus degraded to > 60% in fortified natural seawater during a 28-day incubation period; therefore, it may be classified as “readily biodegradable.”

5.0 REFERENCE

Organization for Economic Cooperation and Development (OECD) 1992. OECD Guideline for Testing of Chemicals. 306: Biodegradability in Seawater (Closed Bottle Test). Adopted by the Council on 17 July 1992.

Table 1. Concentration of mineral salts in fortified natural seawater

Solution ID.	Nutrient Supplied	Stock Concentration (g/L)	Final Concentration in Seawater (mg/L)[a]
A	$CaCl_2$	27.5	27.5
B	$FeCl_3 \cdot 6H_2O$	0.25	0.25
C	$MgSO_4 \cdot 7H_2O$	22.5	22.5
D	KH_2PO_4	8.5	8.5
	K_2HPO_4	21.75	21.8
	$Na_2HPO_4 \cdot 2H_2O$	33.3	33.3
	NH_4Cl	0.50	0.50

[a] The test medium is prepared by adding 1 mL of each stock solution per liter of seawater.

Table 2. Mean dissolved oxygen concentration and percent biodegradation of ENVIROKLEAN PLUS® and sodium benzoate in a 28-day closed bottle test.

Test Day	Dissolved Oxygen (mg/L)			
	Blank	ENVIROKLEAN PLUS	Reference	ENVIROKLEAN PLUS + Reference
0	6.61	6.56	6.54	6.52
2	6.55	4.32	4.60	3.58
5	6.30	2.86	3.90	2.35
15	6.27	0.73	3.55	0.85
28	5.76	0.06	2.95	0.09
Percent Biodegradation @ day 28	NA	86	42	NA

APPENDIX: TEST DATA

28-day Closed-Bottle Biodegradability (Seawater)

Astonishing Products Job No. 470122-09

Substance: ENVIROKLEAN® PLUS

Treatment Group	Replicate No.	mg/L O_2 after n days				
		0	2	5	15	28
Test: nutrient-fortified seawater, plus 130 µL ENVIROKLEAN® PLUS	1	96.7 | 10X / 6.52	63.6 | 11Y / 4.32	45.8 | 13X / 2.89	10.1 | 14B / 0.69	0.8 | 87 / 0.05
	2	96.5 | 19C / 6.56		44.9 | 31D / 2.83	11.2 | 23X / 0.76	0.9 | 24A / 0.06
	Mean	6.56	4.32	2.86	0.73	0.06
Carrier Blank: (not required for this product)	1					
	2					
	Mean					
Blank: nutrient-fortified seawater, without test substance	1	97.5 | 4B / 6.62	95.7 | 6Z / 6.55	99.2 | 3X / 6.30	91.1 | 16X / 6.26	83.6 | 20Z / 5.74
	2	97.3 | 4Y / 6.60		99.8 | 8Z / 6.30	91.4 | 3Y / 6.28	84.5 | 21C / 5.78
	Mean	6.61	6.55	6.30	6.27	5.76
Reference: nutrient-fortified seawater, plus 2 mg/L sodium benzoate	1	96.9 | 11Y / 6.56	67.3 | 23Y / 4.60	61.0 | 21 / 3.85	51.4 | 12Z / 3.54	42.8 | 16Z / 2.92
	2	96.3 | 10Y / 6.52		62.5 | 13Y / 3.94	51.6 | 23B / 3.55	43.3 | 2B / 2.97
	Mean	6.54	4.60	3.90	3.55	2.95
Toxicity (Inhibition): nutrient-fortified seawater, plus 2 mg/L sodium benzoate and 80 µL ENVIROKLEAN® PLUS	1	96.1 | 10X / 6.52	52.7 | 22C / 3.58	38.1 | 15X / 2.41	13.2 | 8Y / 0.90	1.4 | 7Y / 0.09
	2	96.0 | 1D / 6.52		35.9 | 23Z / 2.28	11.8 | 16Y / 0.80	1.5 | 1A / 0.09
	Mean	6.52	3.58	2.35	0.85	0.09
	Init.	SB	SB	SB	SB	SB
	Date	8/30/00	9/1/00	9/4/00	9/14/00	9/27/00
	Time	1200	1330	1100	1300	1300

Comments: