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June 7, 2006

TSCA Confidential Business Information Center (7407M)
EPA EAST - Room 6428 Attn: Section 8(e)
United States Environmental Protection Agency
1201 Constitution Avenue, NW
Washington DC 20460-0001

CONTAIN NO CBI

Attention: TSCA 8(e) Coordinator

RE: Diisobutylene (CASRN 25167-70-8) – Ready Biodegradation by the Carbon Dioxide Evolution Test Method

Dear Sir or Madam:

Lyondell Chemical Company (Lyondell) hereby submits this letter pursuant to Section 8(e) of the Toxic Substances Control Act (TSCA) and EPA's 1991 Section 8(e) Reporting Guide because it includes findings that EPA may consider reportable. The biodegradability of Lyondell's diisobutylene product (CASRN 25167-70-8) was determined by the Carbon Dioxide Evolution Test Method (OECD Guideline 301B). The average cumulative percent biodegradation for diisobutylene was < 1.0%.

This study was conducted by Wildlife International, Ltd. for Lyondell Chemical Company in Easton Maryland in accordance with our product stewardship program for new products.

A copy of the study final report is enclosed for your review.

Should you have any questions or require additional details, please do not hesitate to call me at 713-309-7884. I may also be reached by facsimile at 713-951-1574 or by e-mail at timothy.yagley@lyondell.com.

Sincerely,

Timothy Yagley
Business Consultant – Chemical Control
Corporate TSCA Coordinator
Lyondell Chemical Company



Enclosure



DIISOBUTYLENE: READY BIODEGRADABILITY BY THE CARBON DIOXIDE EVOLUTION
TEST METHOD

WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 620E-101

Organisation for Economic Cooperation and Development
OECD Guideline 301B
and
Council of the European Communities Directive 67/548/EEC
Annex V, Guideline C.4-C

AUTHORS:
Edward C. Schaefer
Molly E. Matthews

STUDY INITIATION DATE: January 27, 2006

STUDY COMPLETION DATE: May 17, 2006

Submitted to:

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Houston, TX 77010

Wildlife International, Ltd.

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Easton, Maryland 21601
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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: Lyondell

TITLE: Diisobutylene: Ready Biodegradability by the Carbon Dioxide Evolution Test Method

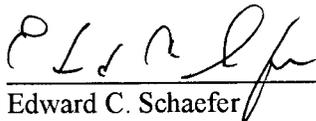
WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 620E-101

STUDY COMPLETION: May 17, 2006

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, 40 CFR Part 792, 17 August 1989, and OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17), with the following exceptions:

The test and reference substances were not characterized in accordance with Good Laboratory Practice Standards, nor were the stability under storage conditions at the test site determined in accordance with Good Laboratory Practice Standards.

STUDY DIRECTOR:



Edward C. Schaefer
Director of Biodegradation

17 May 2004
DATE

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QUALITY ASSURANCE STATEMENT

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, 40 CFR Part 792, 17 August 1989, and OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17). The dates of all inspections and audits and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

ACTIVITY:	DATE CONDUCTED:	DATE REPORTED TO:	
		STUDY DIRECTOR:	MANAGEMENT:
Protocol	February 23, 2006	February 23, 2006	April 26, 2006
Test Initiation	February 22, 2006	February 22, 2006	February 23, 2006
Sample Collection	March 14, 2006	March 14, 2006	March 17, 2006
Data and Draft Report	April 18-19, 2006	April 19, 2006	April 26, 2006
Final Report	May 17, 2006	May 17, 2006	May 17, 2006

All inspections were study-based unless otherwise noted.



Gryphon Perkins, BA
Quality Assurance Representative

17 May 06

DATE

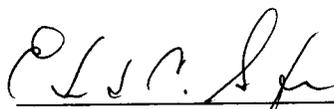
REPORT APPROVAL

SPONSOR: Lyondell

TITLE: Diisobutylene: Ready Biodegradability by the Carbon Dioxide Evolution Test Method

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 620E-101

STUDY DIRECTOR:



Edward C. Schaefer
Director of Biodegradation

17 May 2006

DATE

MANAGEMENT:



Henry O. Krueger, Ph.D.
Director of Aquatic Toxicology/Terrestrial
Plant and Insects

17 May 06

DATE

- 5 -

STUDY INFORMATION

Study Initiation Date: January 27, 2006
Experimental Start Date (OECD): February 21, 2006
Experimental Start Date (EPA): February 22, 2006
Experimental Termination Date: March 23, 2006
Study Completion Date: May 17, 2006

Study Director: Edward C. Schaefer

Sponsor: Lyondell
One Houston Center, Suite 1600
1221 McKinney Street
Houston, TX 77010

Sponsor's Representative: Jim Bootman

Study Personnel: Edward C. Schaefer, B.S., Director of Biodegradation
Henry O. Krueger, Ph.D., Director of Aquatic
Toxicology/Terrestrial Plants and Insects
Molly E. Matthews, B.A., Biologist, Biodegradation
Timothy J. Tefteau, B.S., Biologist, Biodegradation

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SUMMARY

The ready biodegradability of Diisobutylene was determined by the Carbon Dioxide Evolution Test Method (OECD Guideline 301B). Tests of ready biodegradability are stringent tests that provide limited opportunity for acclimation and biodegradation to occur. In the CO₂ test, inoculated mineral medium was dosed with a known amount of test substance as the nominal sole source of organic carbon and aerated with CO₂-free air. The CO₂ produced from the mineralization of organic carbon within the test chambers was displaced by the flow of CO₂-free air and trapped as K₂CO₃ in KOH trapping solution. The amount of CO₂ produced by the test substance (corrected for that evolved by the blank inoculum) is expressed as a percentage of the theoretical amount of CO₂ (TCO₂) that could have been produced if complete biodegradation of the test substance occurred. The test contained a blank control group, a reference group and a treatment group. Each group contained three replicate test chambers. The blank control was used to measure the background CO₂ production of the inoculum and was not dosed with a carbon source. The reference chambers were dosed with sodium benzoate, a substance known to be biodegradable, at a nominal concentration of 10 mg C/L. The treatment group test chambers were used to evaluate Diisobutylene at a nominal concentration of approximately 10 mg C/L. The results indicated that the activated sludge inoculum was active, degrading the reference substance 92.9%. The average cumulative percent biodegradation for Diisobutylene was <1.0%.

Test Substance	Average Cumulative Percent Biodegradation	Average Final pH	Readily Biodegradable ²
Diisobutylene	<1.0 ¹	7.2 ¹	No

¹ Average of three replicates.
² Meets or exceeds the OECD criteria for ready biodegradability (60% of TCO₂ within a 10-day window of reaching 10% TCO₂).

INTRODUCTION

Tests of ready biodegradability, by definition, provide limited opportunity for acclimation and biodegradation to occur. A positive result in a test of ready biodegradability is an indication that the test substance will undergo rapid and ultimate biodegradation in the environment. A negative result in a test of ready biodegradability does not necessarily mean that the test substance will not be biodegraded under relevant environmental conditions but that additional testing may be needed.

This study was conducted by Wildlife International, Ltd. for Lyondell at the Wildlife International, Ltd. biodegradation facility in Easton, Maryland. Original raw data generated by Wildlife International, Ltd. and the final report are filed under Project number 620E-101 in the archives located on the Wildlife International, Ltd. site.

OBJECTIVE

The objective of the study was to measure the amount of carbon dioxide (CO₂) produced from the biodegradation of a nonvolatile organic test substance. This value is expressed as a percentage of the theoretical amount of CO₂ (TCO₂) that could have been produced if complete biodegradation of the test substance occurred.

EXPERIMENTAL DESIGN

The test contained a blank control group, a reference group and a treatment group. Each group contained three replicate test chambers. The control group was used to measure the background CO₂ production of the inoculum and was not dosed with a carbon source. The reference group chambers were used to check the viability of the inoculum and were dosed with sodium benzoate, a substance known to be biodegradable, at a nominal concentration of 10 mg C/L. The treatment group chambers were used to evaluate Diisobutylene at a nominal concentration of approximately 10 mg C/L.

MATERIALS AND METHODS

This study was conducted according to the procedures outlined in the protocol, "Diisobutylene: Ready Biodegradability by the Carbon Dioxide Evolution Test Method". The protocol was based on the procedures specified in the OECD Guideline for Testing of Chemicals, Method 301B (1), and Commission Directive. Annex V. 67/548/EEC.1992, Method C.4-C, *Carbon Dioxide (CO₂) Evolution* (2).

Test Substance

The test substance was received from Lyondell on September 20, 2005 and was assigned Wildlife International, Ltd. identification number 7355. The following is a description of the test substance used in this study:

Name:	Diisobutylene
Lot Number:	GCRD10401R
CAS Number:	25167-70-8
Physical Description:	Liquid
Purity:	Not Given
Expiration Date:	June 30, 2006
Water Solubility:	Insoluble
Storage Conditions:	Ambient
Carbon Content:	84.5%
(as determined by elemental analysis)	

The test substance was administered to the treatment group test chambers by direct weight addition. Direct weight addition of a test substance that is relatively insoluble in water is the most appropriate route of administration. The amount of test substance used to dose the treatment group test chambers was calculated based on the measured carbon content. The carbon content of the test substance was determined by a non-GLP elemental analysis performed by Quantitative Technologies, Inc.

Reference Substance

The reference substance was received from Mallinckrodt on January 08, 2002 and was assigned Wildlife International, Ltd. Identification number 5861. A stock solution of the reference substance was prepared in NANO[®] pure water at a nominal carbon concentration of 406 mg C/L. The stock solution was analyzed for total organic carbon (TOC). The reference substance was administered to the reference group test chambers by volumetric addition. Dosing amounts were based on the measured carbon content of the reference substance solution. Following is a description of the reference substance used in this study:

Name:	Sodium benzoate
Manufacturer:	Mallinckrodt Baker, Inc. Paris, Kentucky
Lot Number:	0168 V38617
Physical Description:	White powder
Chemical Abstract Number:	532-32-1
Expiration Date:	January 8, 2007
Purity:	99.4%
Storage Conditions:	Ambient

Test Medium

The test medium was a modified biochemical oxygen demand (BOD) test dilution water and was prepared using high quality water as described in the Protocol (Appendix I). All chemicals and reagents used in the preparation of the test medium were reagent grade or better.

Test Apparatus and Conditions

The test chambers were amber 4-liter bottles. The air entering the chambers was passed through Drierite® to remove ambient moisture and then through Ascarite® to produce CO₂ free air. The air exiting the test chambers was passed through a series of three gas washing bottles, each containing approximately 100 mL of 0.5 N KOH to trap the CO₂ that had evolved within the chamber. An additional set of gas washing bottles that were not connected to a chamber were maintained concurrently with the traps connected to the chambers. These bottles contained approximately 100 mL of 0.5 N KOH and the amount of CO₂ detected in the KOH solution was subtracted from the CO₂ in the blank control traps to determine the amount of CO₂ produced by the inoculum in the blank control. The test was conducted at 20±3°C. Test chambers were identified by project number, test substance ID, test concentration and vessel number. Magnetic stir bars and stir plates were used to mix the contents of the test chambers. Stir plate motors were cycled on and off approximately every 15 minutes to prevent the heating of the stirrer motors.

Test Inoculum

Activated sludge was collected from Denton Wastewater Treatment Plant, Denton, Maryland on February 21, 2006. The Denton facility treats predominantly residential wastes. The sludge was sieved using a 2-mm screen and then aerated for approximately four hours. After the aeration period, an aliquot of the sludge was homogenized in a blender at medium speed for approximately two minutes and then allowed to settle for approximately 30 minutes. The supernatant was used as the inoculum the same day that it was prepared. A total suspended solids measurement and standard plate count were performed on the inoculum. Plates were incubated at 20±3°C for approximately 48 hours.

Preparation of Test Chambers

The following were added to each chamber:

- 1) 2470 mL of NANO[®] pure water
- 2) 3 mL calcium chloride solution (2.75%)
3 mL of ferric chloride solution (0.025%)
3 mL of magnesium sulfate solution (2.25%)
30 mL of phosphate buffer (pH 7.4)
- 3) 30 mL of the activated sludge inoculum

All chambers were aerated with CO₂-free air for approximately 24 hours at a rate of 50 to 100 mL per minute to purge the systems of CO₂. After the aeration period, the flow of CO₂-free air was stopped, and three CO₂ traps, each containing approximately 100 mL of 0.5 N KOH, were connected to the exit air lines of each chamber. Sufficient volumes of reference substance stock solution to achieve a nominal concentration of 10 mg C/L were added to the appropriate chambers. Sufficient amounts of test substance to achieve a nominal concentration of approximately 10 mg C/L were added to the appropriate chambers. The final volume within all chambers was brought up to 3000 mL by the addition of NANO[®] pure water and the airflow was restarted on the system.

Biodegradation Test Initiation

The biodegradation test was started by bubbling CO₂-free air through each of the test chambers at a rate of 50 to 100 mL per minute. The CO₂ produced from the degradation of organic carbon sources within the test chamber was trapped as K₂CO₃ in the KOH solution and the amount of inorganic carbon in the trapping solution was measured at various intervals during the study, using a Shimadzu Model TOC-5000 or Model TOC-VcSH carbon analyzer.

Sample Collection and Analysis

On days 1, 6, 8, 13, 15, 20, 22, 27 and 29 the CO₂ trap nearest the test chamber was removed and analyzed for inorganic carbon. The two remaining traps were placed one position closer to the test chamber and a new trap was placed on the end of the series.

Test Termination

On the 28th day of the test, an aliquot of the contents of each test chamber was removed and the pH determined. The contents of all chambers were then acidified by the addition of 3 mL of concentrated hydrochloric acid to drive off inorganic carbonate. All chambers were aerated overnight

and then a sample from each test chamber was removed for dissolved organic carbon (DOC) analysis and the trapping solutions closest to the test chambers were analyzed for inorganic carbon.

Calculations

The results of the inorganic carbon analyses of the CO₂ traps were converted to mg CO₂ produced using the following equation:

$$\text{mg CO}_2 = \text{result (mg C/L)} \times \text{vol. of KOH (L)} \times 3.67 \text{ mg CO}_2/\text{mg C}$$

The cumulative mg of CO₂ for the test and reference substances were corrected for the amount of CO₂ evolved by the control, using the following equation:

$$\text{Cumulative mg CO}_2 \text{ produced} = \Sigma \text{mg CO}_2 \text{ test} - \text{mean } \Sigma \text{mg CO}_2 \text{ control}$$

The percentage of theoretical carbon dioxide (%TCO₂) evolved was calculated as follows:

$$\% \text{TCO}_2 = \frac{\text{mg CO}_2 \text{ produced}}{(\text{mg of carbon in test}) \times (3.67 \text{ mg CO}_2/\text{mg Carbon})} \times 100$$

RESULTS AND DISCUSSION

Carbon Analysis

The measured total organic carbon (TOC) concentration of the reference substance stock solution was 406.5 mg C/L. The volume of stock solution used to dose the reference chambers was adjusted based on the measured TOC value so that approximately 10 mg C/L was delivered.

Observations and Measurements

The temperature range recorded during the test was 18.0 to 21.8°C and was within the protocol specified range throughout the test. The results of the standard plate count and TSS measurement performed on the inoculum were 1.7 x 10⁶ CFU/mL and 287 mg/L, respectively.

The measured total organic carbon (TOC) value of the dosing stock solution is presented in Table 1. The measured dissolved organic carbon (DOC) on Day 29 and pH values of the test chamber

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contents on Day 28 are presented in Table 2. The measured concentrations of inorganic carbon in the carbon dioxide trapping solutions are presented in Table 3. The cumulative amounts of CO₂ produced over the test period are presented in Tables 4. The cumulative percent of theoretical carbon dioxide (% TCO₂) evolved is presented in tabular and graphical forms in Table 5 and Figure 1, respectively.

The control chambers evolved an average of 23.7 mg CO₂ over the test period. This value has been corrected for the amount of CO₂ in the trapping solution since potassium hydroxide solution, even when freshly prepared, contains carbonates. The amount of CO₂ evolved by the control chambers did not exceed the 40 mg/L (120 mg total) value considered the acceptable limit for CO₂ evolution tests (2).

The viability of the inoculum and validity of the test were supported by the results of the reference substance, sodium benzoate, from which an average of 92.9% of theoretical CO₂ was evolved. An average percent biodegradation of greater than 60% was achieved by Day 8, thereby fulfilling the criteria for a valid test by reaching the pass level by Day 14 (1). The final mean percent biodegradation for Diisobutylene was <1.0%. Diisobutylene may not be considered readily biodegradable, since the pass level of 60% TCO₂ was not achieved.

CONCLUSIONS

Evidence of ready biodegradability in a Carbon Dioxide Evolution Test is 60% TCO₂ within the 28-day test period (3,4). In addition, the pass level must be reached within 10 days of achieving 10% TCO₂ (3,4). The final mean percent biodegradation for Diisobutylene was <1.0%. Diisobutylene may not be considered readily biodegradable since the pass level of 60% TCO₂ was not achieved.

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REFERENCES

1. **Organisation for Economic Cooperation and Development.** 1992. Guideline for the Testing of Chemicals. Guideline 301B: *Ready Biodegradability: CO₂ Evolution (Modified Sturm Test)*. Adopted by the Council on 17 July 1992.
2. **Annex to Commission Directive 67/548/EEC.** 1992. *Carbon Dioxide (CO₂) Evolution*. Official Journal of the European Communities No. L383. Method C.4-C.
3. **Organisation for Economic Cooperation and Development.** 1992. Guideline for the Testing of Chemicals. Guideline 301: *Ready Biodegradability*: Adopted by the Council on 17 July 1992.
4. **Council of the European Communities.** Directive 67/548/EEC. Annex V. Guideline C.4, *Ready Biodegradability*.

Table 1

Total Organic Carbon (TOC) of Reference Substance Dosing Solution

Dosing Stock Solution	TOC (mg C/L)
Sodium Benzoate 5861-022206-1	406.5

Table 2

Dissolved Organic Carbon (DOC) and pH of Test Solutions at Test Termination

Test Chamber/ Nominal Concentration	DOC ¹ (mg C/L)	pH
Control Rep. 1	<1.0	7.2
Control Rep. 2	<1.0	7.3
Control Rep. 3	<1.0	7.2
Sodium Benzoate Rep. 1 (10 mg C/L)	<1.0	7.3
Sodium Benzoate Rep. 2 (10 mg C/L)	<1.0	7.3
Sodium Benzoate Rep. 3 (10 mg C/L)	<1.0	7.2
Diisobutylene Rep. 1 (10 mg C/L)	<1.0	7.3
Diisobutylene Rep. 2 (11 mg C/L)	<1.0	7.2
Diisobutylene Rep. 3 (10 mg C/L)	<1.0	7.2

¹ Samples were filtered (0.45 µm) and purged with CO₂-free air and acidified with 2 drops 2N HCl prior to analysis.

NA – not applicable

Table 3

Measured Inorganic Carbon Concentration of Trapping Solutions (mg C/L)

Date	Day	Control			Sodium Benzoate			Diisobutylene WIL-7355			Blank
		Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	
23-Feb-06	1	11.6	10.3	9.9	9.3	11.3	12.4	11.5	12.5	13.2	7.7
28-Feb-06	6	20.2	16.6	13.0	182.4	162.6	183.4	14.3	33.1	22.8	12.7
02-Mar-06	8	10.6	12.4	10.0	33.1	49.4	24.5	16.6	17.6	13.8	5.2
07-Mar-06	13	26.1	24.2	18.9	43.1	63.9	32.1	23.9	15.1	21.5	6.5
09-Mar-06	15	17.8	11.8	14.3	22.7	24.9	16.8	13.0	11.7	11.1	7.1
14-Mar-06	20	11.9	12.1	16.8	31.0	38.4	22.5	19.0	10.1	13.1	6.5
16-Mar-06	22	9.0	12.2	8.3	16.0	16.4	42.9	7.6	10.9	8.8	5.8
21-Mar-06	27	18.3	22.6	7.9	24.4	23.6	38.4	12.2	9.4	8.5	12.6
23-Mar-06	29	17.1	18.9	20.9	36.7	29.5	48.4	13.4	23.5	13.8	5.8

Wildlife International, Ltd.

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Table 4**Cumulative Milligrams of Carbon Dioxide Evolved^{1,2}**

Date	Day	Control			Sodium ³ Benzoate			Diisobutylene ³ WIL-7355			Diisobutylene ³ WIL-7355			Control ⁴			Blank
		Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	
23-Feb-06	1	4.3	3.8	3.6	-0.5	0.3	0.7	0.3	0.7	1.0	1.4	1.0	1.4	1.0	0.8	2.8	
28-Feb-06	6	11.7	9.9	8.4	60.4	53.8	61.9	-0.5	6.8	3.2	4.2	6.8	2.4	2.4	0.9	7.5	
02-Mar-06	8	15.6	14.4	12.1	68.5	67.9	66.8	1.5	9.2	4.3	6.2	9.2	5.0	5.0	2.7	9.4	
07-Mar-06	13	25.1	23.3	19.0	75.8	82.9	70.1	1.8	6.3	3.7	13.4	6.3	11.5	11.5	7.2	11.8	
09-Mar-06	15	31.7	27.6	24.3	78.8	86.7	70.9	1.2	5.2	2.4	17.3	5.2	13.2	13.2	9.9	14.4	
14-Mar-06	20	36.0	32.1	30.4	85.2	95.8	74.2	3.2	3.9	2.2	19.3	3.9	15.3	15.3	13.7	16.8	
16-Mar-06	22	39.3	36.6	33.5	87.4	98.2	86.3	2.4	4.3	1.8	20.4	4.3	17.7	17.7	14.6	18.9	
21-Mar-06	27	46.1	44.8	36.4	90.4	100.9	94.5	0.9	1.8	-1.0	22.5	1.8	21.3	21.3	12.8	23.5	
23-Mar-06	29	52.3	51.8	44.0	96.9	104.8	105.3	-1.1	3.4	-2.9	26.7	3.4	26.1	26.1	18.4	25.7	

¹The results of the inorganic carbon analyses of the CO₂ traps were converted to mg CO₂ produced using the following equation:

mg CO₂ = cumulative result (mg C/L) X vol. of KOH (L) X 3.67 mg CO₂/mg C

²Calculations performed in Excel 2000 full precision mode. Manual calculations may differ.

³Corrected for the CO₂ attributed to the inoculum and the KOH by subtracting the average amount of CO₂ evolved by the controls.

⁴Corrected for the amount of CO₂ in the blank to determine the amount of CO₂ evolved by the inoculum.

Wildlife International, Ltd.

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Table 5

Cumulative Percent of Theoretical Carbon Dioxide Evolved^{1,2}

Date	Day	Control			Sodium Benzoate			Diisobutylene WIL-7355		
		Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
23-Feb-06	1	NA	NA	NA	-0.4	0.2	0.6	0.3	0.6	0.9
28-Feb-06	6	NA	NA	NA	54.8	48.9	56.2	-0.5	5.6	2.9
02-Mar-06	8	NA	NA	NA	62.2	61.7	60.7	1.4	7.6	3.9
07-Mar-06	13	NA	NA	NA	68.9	75.3	63.7	1.7	5.2	3.3
09-Mar-06	15	NA	NA	NA	71.6	78.7	64.4	1.1	4.3	2.2
14-Mar-06	20	NA	NA	NA	77.4	87.0	67.4	2.9	3.2	2.0
16-Mar-06	22	NA	NA	NA	79.4	89.2	78.4	2.2	3.5	1.7
21-Mar-06	27	NA	NA	NA	82.1	91.6	85.8	0.8	1.5	-0.9
23-Mar-06	29	NA	NA	NA	88.0	95.1	95.6	-1.0	2.8	-2.7
Cumulative Average (N=3)					92.9				-0.3	
Std. Dev.					4.2				2.8	

¹The percentage of TCO₂ was calculated using the following equation:

$$\% TCO_2 = \frac{\text{mg } CO_2 \text{ produced}}{(\text{mg of carbon in test}) \times (3.67 \text{ mg } CO_2 / \text{mg Carbon})} \times 100$$

²Calculations performed in Excel 2000 full precision mode. Manual calculations may differ.
NA - Not Applicable.

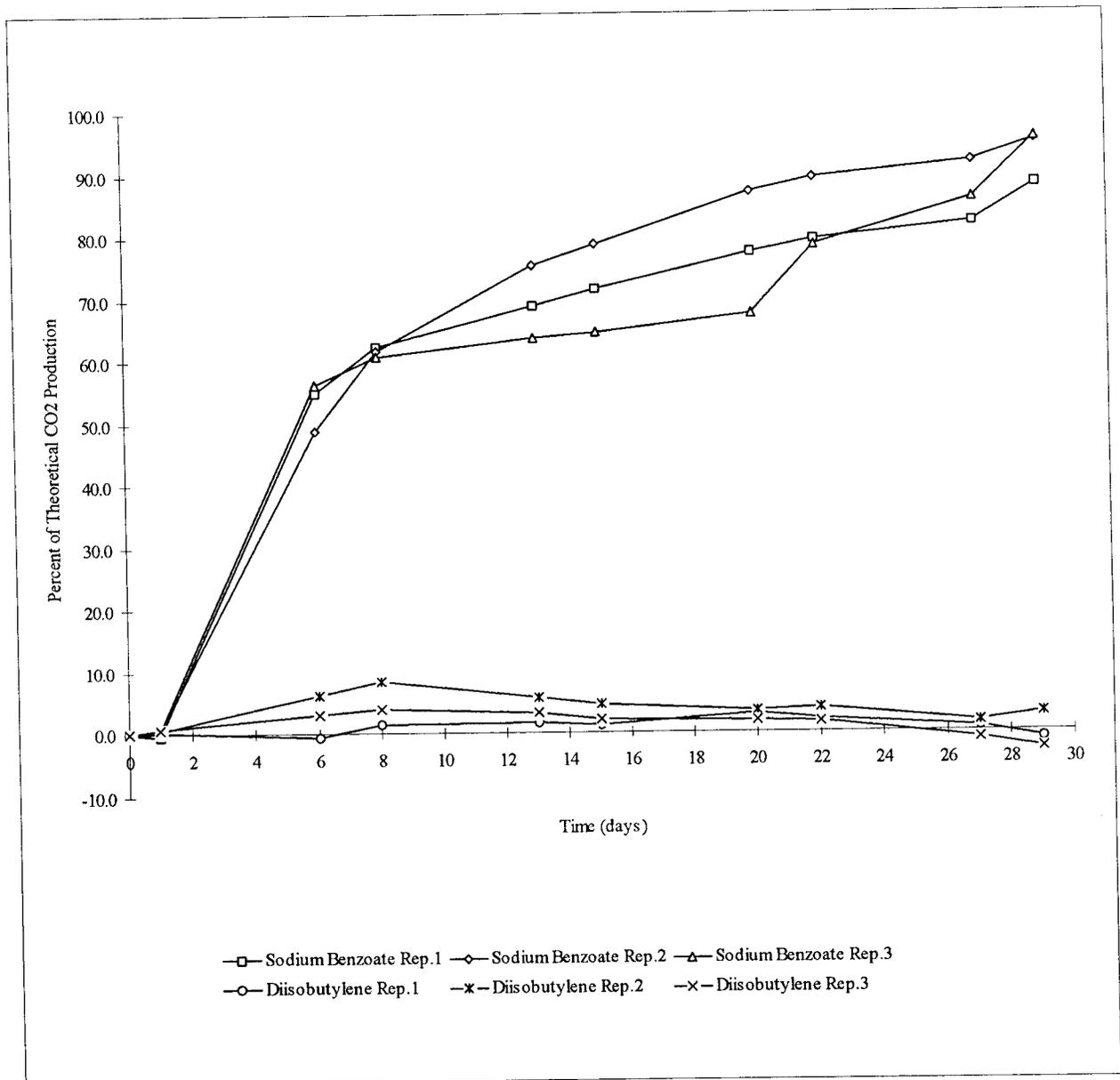


Figure 1. Cumulative Percentage of Theoretical Carbon Dioxide Evolved

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APPENDIX I

Protocol, Amendment and Deviation

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PROTOCOL

DIISOBUTYLENE: READY BIODEGRADABILITY BY THE
CARBON DIOXIDE EVOLUTION TEST METHOD

Organisation for Economic Cooperation and Development
OECD Guideline 301B

and

Council of the European Communities Directive 67/548/EEC
Annex V, Guideline C.4-C

Submitted to

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November 23, 2005

Wildlife International, Ltd.

DIISOBUTYLENE: READY BIODEGRADABILITY BY THE CARBON DIOXIDE EVOLUTION TEST METHOD

SPONSOR: Lyondell
One Houston Center, Suite 1600
1221 McKinney Street
Houston, TX 77010

SPONSOR'S REPRESENTATIVE: Dr. George Cruzan
ToxWorks
1153 Roadstown Road
Bridgeton, NJ 08302

TESTING FACILITY: Wildlife International, Ltd.
8598 Commerce Drive
Easton, Maryland 21601

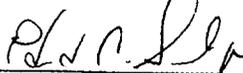
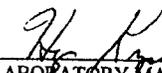
STUDY DIRECTOR: Edward C. Schaefer
Wildlife International, Ltd.

LABORATORY MANAGEMENT: Henry O. Krueger, Ph.D.
Director of Aquatic Toxicology and Non-Target Plants

FOR LABORATORY USE ONLY

Proposed Dates:	
Experimental	Experimental
Start Date: <u>01 Feb 2006</u>	Termination Date: <u>01 MARCH 2006</u>
Project No.: <u>620E-101</u>	
Test Concentrations: <u>10 mg C/L</u>	
Test Substance No.: <u>7355</u>	Reference Substance No. (if applicable): <u>5861</u>

PROTOCOL APPROVAL

<u></u>	<u>27 JAN 06</u>
STUDY DIRECTOR	DATE
<u></u>	<u>27 Jan 06</u>
LABORATORY MANAGEMENT	DATE
<u></u>	<u>Nov. 23, 2005</u>
SPONSOR'S REPRESENTATIVE	DATE

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INTRODUCTION

Tests of ready biodegradability, by definition, provide limited opportunity for acclimation and biodegradation to occur. A positive result in a test of ready biodegradability is an indication that the test substance will undergo rapid and ultimate biodegradation in the environment. A negative result in a test of ready biodegradability does not necessarily mean that the test substance will not be biodegraded under relevant environmental conditions but that additional testing may be needed.

OBJECTIVE

The objective of the study will be to measure the amount of carbon dioxide (CO₂) produced from the biodegradation of a nonvolatile organic test substance and express it as a percentage of the theoretical amount of CO₂ (TCO₂) that could have been produced assuming complete biodegradation of the test substance to CO₂ and other inorganic constituents.

EXPERIMENTAL DESIGN

The test will contain a blank control group, a reference group, and a treatment group. Each group will contain three replicate test chambers. The blank control is used to measure the background CO₂ production of the inoculum and will not be dosed with a carbon source. The reference chambers will be dosed with sodium benzoate, a substance known to be biodegradable, at a concentration of 10 mg C/L. The treatment group will be used to evaluate the test substance at 10 mg C/L.

MATERIALS AND METHODS

Test methods are based on the procedures specified in the OECD Guideline for Testing of Chemicals, Guideline 301B (1) and Council of the European Communities, Guideline C.4-C, *Carbon Dioxide (CO₂) Evolution* (2).

Test Substance

Information on the characterization of test, control or reference substances is required by Good Laboratory Practice (GLP) Standards and Principles. The Sponsor is responsible for providing Wildlife International, Ltd. verification that the test substance has been characterized according to GLPs prior to its use in the study. If verification of GLP test substance characterization is not provided to Wildlife International, Ltd., it will be noted in the compliance statement of the final report.

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The Sponsor is responsible for all information related to the test substance and agrees to accept any unused test substance and/or test substance containers remaining at the end of the study.

If the test substance is relatively water soluble, a stock solution of the test substance will be prepared at a nominal concentration of 400 mg C/L in high quality water. Volumetric addition of a test substance stock solution is the most appropriate route of administration for water-soluble materials. The dosing volume of the test substance stock solution will be calculated based on the measured TOC. The stock solution may be stored in a refrigerator for a maximum of three days.

For poorly soluble materials, the test substance will be administered by direct weight addition. Direct weight addition of a test substance that is relatively insoluble in water is the most appropriate route of administration. Dosing amounts will be based on the carbon content of the test substance. If the carbon content of the test substance is not provided by the Sponsor, the carbon content will be determined by elemental analysis and will be performed by Quantitative Technologies Inc. (Whitehouse, NJ). The carbon analysis will not be conducted in accordance with Good Laboratory Practice Standards.

Reference Substance

A stock solution of sodium benzoate will be prepared at a nominal concentration of 400 mg C/L in high quality water. The TOC of the stock solution must within 15% of the nominal concentration to be acceptable. If the TOC is not acceptable, a new stock solution will be prepared. The dosing volume of the reference substance stock solution will be calculated based on the measured TOC. The stock solution may be stored in a refrigerator for a maximum of three days.

Test Medium

The test medium is a modified biochemical oxygen demand (BOD) test dilution water and will be prepared using high quality water as described in Appendix I.

Test Apparatus and Conditions

The test chambers will be amber 4-liter bottles. The air entering the chambers will be passed through Drierite to remove ambient moisture and then through Ascarite to produce CO₂ free air. The air

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exiting the test chambers will be passed through a series of three gas washing bottles each containing approximately 100 mL of 0.5 N KOH to trap the CO₂ that has evolved within the chamber. An additional set of gas washing bottles that are not connected to a chamber will be maintained concurrently with the traps connected to the chambers. The amount of CO₂ detected in these traps will be subtracted from the blank control traps to determine the amount of CO₂ produced by the blank control. The test will be incubated at 20±3°C. Test temperatures will be measured each working day using a min/max thermometer. Magnetic stirrers will be employed to mix the contents of the chambers. The stirrers will be cycled on and off approximately every 15 minutes to prevent the transfer of heat from the stirrer motors to the test chambers. Test chambers will be identified by project number, test substance ID, test concentration, and vessels number.

Test Inoculum

Activated sludge will be collected from Denton Wastewater Treatment Facility, Denton, Maryland. The sludge will be sieved using a 2 mm screen and then aerated for approximately four hours. After the aeration period, the sludge will be homogenized in a blender at medium speed for approximately 2 minutes and then allowed to settle for approximately 30 minutes. If in the opinion of the Study Director the supernatant above the settled solids contains high levels of suspended sludge solids after 30 minutes of settling, the sludge may be settled for an additional 30 minutes. The supernatant will be used as the inoculum the same day that it is prepared. A total suspended solids measurement and standard plate count will be performed on the inoculum. Plates will be incubated at 20 ± 3°C for approximately 48 hours.

Preparation of Test Chambers

The following will be added to each chamber:

- 1) 2470 mL of high quality water
- 2) 3 mL calcium chloride solution
3 mL of ferric chloride solution
3 mL of magnesium sulfate solution
30 mL of phosphate buffer
- 3) 30 mL of the activated sludge inoculum

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All chambers will be aerated with CO₂ free air for approximately 24 hours at a rate of 50 -100 mL per minute to purge the systems of CO₂. After the aeration period, the flow of CO₂ free air will be stopped, and three CO₂ traps each containing approximately 100 mL of 0.5 N KOH will be connected to the exit air lines of each chamber. A volume reference substance stock solution necessary to deliver 10 mg C/L will be added to the reference group chambers. Treatment group test chambers will be dosed with sufficient test substance to deliver 10 mg C/L. The volume within all chambers will be brought up to 3000 mL by the addition of high quality water.

Biodegradation Test Initiation

The biodegradation test will be started by bubbling CO₂ free air through the test media at a rate of 50-100 mL per minute. The CO₂ produced from the degradation of organic carbon sources within the test chamber is trapped as K₂CO₃ in the KOH solution and measured using a carbon analyzer.

Sample Collection and Analysis

The CO₂ traps will be removed for analysis twice a week over the 4-week test period. More or less frequent removal and analysis of the traps may be conducted at the discretion of the study director with consultation with the Sponsor. The CO₂ trap nearest the chamber will be removed and analyzed for inorganic carbon. The two remaining traps are placed one position closer to the test chamber and a new trap is placed on the end of the series.

Test Termination

On the 28th day of the test an aliquot of the contents of each test chamber will be removed and the pH determined. The contents of chamber will then be acidified by the addition of 3 mL of concentrated hydrochloric acid to drive off inorganic carbonate. The chambers will be aerated overnight and then a sample from each chamber will be removed for dissolved organic carbon (DOC) analysis. The remaining trapping solutions closest to the test chambers will be analyzed for inorganic carbon.

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Calculations

The results of the carbon analysis will be converted to mg CO₂ produced and used to determine the percentage of theoretical CO₂ (%TCO₂) evolved at each sampling interval. The cumulative %TCO₂ will be plotted against time. The results of the carbon analyses will be converted to mg CO₂ using the following equation:

$$mg\ CO_2 = result\ (mg\ C/L) \times vol.\ of\ KOH\ (L) \times 3.67\ mg\ CO_2 / mg\ C$$

The cumulative mg of CO₂ for the test and reference substances will be corrected for the amount of CO₂ evolved by control, using the following equation:

$$Cumulative\ mg\ CO_2\ evolved = \sum\ mg\ CO_2\ test - mean\ \sum\ CO_2\ control$$

The percent of theoretical CO₂ produced will be calculated using the following equation:

$$\% TCO_2 = \frac{mg\ CO_2\ produced}{(mg\ carbon\ in\ test)(3.67\ mg\ CO_2 / mg\ Carbon)} \times 100$$

Results

No bias is expected in this study and statistical methods will not be used in the analysis of the data. Interpretation of the results will be based on the following:

1. The test is considered valid if the reference substance produces at least 60% of its TCO₂ within 14 days, and the CO₂ production of the blank control does not exceed 40 mg/L.
2. Test substances achieving 60% of TCO₂ within 10 days of reaching 10% TCO₂ are regarded as readily biodegradable. If the 60% criteria is not met, biodegradation studies conducted under more favorable conditions should be performed to evaluate the substances biodegradation potential.

RECORDS TO BE MAINTAINED

Records to be maintained will include, but not limited to, the following:

1. A copy of the signed protocol.
2. Identification and characterization of the test substance as provided by Sponsor.

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3. Study initiation and termination dates.
4. Experimental initiation and termination dates.
5. Stock solution concentration calculations and solution preparation.
6. Inoculum source and pretreatment data.
7. Results of CO₂ analysis.
8. Temperature range recorded during test period.
9. Copy of final report.

FINAL REPORT

A final report of the results of the study will be prepared by Wildlife International, Ltd. The report is to include, but is not limited to, the following, when applicable:

1. Name and address of facility performing the study.
2. Dates on which the study was initiated and completed.
3. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.
4. Objectives and procedures stated in the approved protocol, including any changes in the original protocol.
5. Identification and characterization of the test substance as provided by Sponsor including name, CAS number, percent active, percent carbon, theoretical TOC, and other characteristics, if provided by the Sponsor.
6. A description of the transformations and calculations performed on the data.
7. Results of the TOC analysis performed on test and reference substance stock solutions.
8. A description of the test system.
9. A description of the preparation of the test solutions, the testing concentrations, the route of administration, and the duration of the test.
10. A description of all circumstances that may have affected the quality or integrity of the data.
11. The name of the study director, the names of other scientists or professionals, and the names of all supervisory personnel, involved in the study.
12. The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable.
13. The location where the raw data and final report are to be stored.

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14. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made and findings reported to the Study Director and Management.

CHANGES TO THE FINAL REPORT

If it is necessary to make corrections or additions to the final report after it has been accepted, such changes shall be made in the form of an amendment issued by the Study Director. The amendment shall clearly identify the part of the study that is being amended and the reasons for the alteration. Amendments shall be signed and dated by the Study Director and Laboratory QA.

CHANGES TO PROTOCOL

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and approved by the Sponsor's Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. Any other changes will be in the form of written deviations signed by the Study Director and filed with the raw data. All changes to the protocol will be indicated in the final report.

GOOD LABORATORY PRACTICES

This study will be conducted in accordance with Good Laboratory Practice Standards for EPA (40 CFR Part 792) and OECD (ENV/MC/CHEM (98) 17). Each study conducted by Wildlife International, Ltd. is routinely examined by the Wildlife International, Ltd. Quality Assurance Unit for compliance with Good Laboratory Practices, Standard Operating Procedures and the specified protocol. A statement of compliance with Good Laboratory Practices will be prepared for all portions of the study conducted by Wildlife International, Ltd. The Sponsor will be responsible for compliance with Good Laboratory Practices for procedures performed by other laboratories (e.g., residue analyses or pathology). Raw data for all work performed at Wildlife International, Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International, Ltd. site or at an alternative location to be specified in the final report.

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REFERENCES

1. **Organisation for Economic Cooperation and Development.** 1992. *Ready Biodegradability: CO₂ Evolution (Modified Sturm Test)*. OECD Guideline 301B.
2. **Council of the European Communities.** Directive 67/548/EEC. Annex V. Guideline C.4-C, *Carbon Dioxide (CO₂) Evolution*.

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APPENDIX I. Test Medium

The following stock solutions used to prepare the test medium may be purchased or prepared as described below:

Calcium chloride solution (2.75%) - dissolve 27.5 g of anhydrous CaCl_2 in 1 liter of high quality water.

Ferric chloride solution (0.025%) - dissolve 0.25 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 1 liter of high quality water.

Magnesium sulfate solution (2.25%) - dissolve 22.5 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in 1 liter of high quality water.

Phosphate buffer solution (pH 7.4)- dissolve 8.5 g KH_2PO_4 , 21.75 g K_2HPO_4 , 33.4 g $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, and 0.5 g NH_4Cl in 1 liter of high quality water.

The test medium will contain the following standard reagent solutions per liter of high quality water (e.g. Nanopure):

1 mL of calcium chloride solution (2.75%)

1 mL of ferric chloride solution (0.025%)

1 mL of magnesium sulfate solution (2.25%)

10 mL of phosphate buffer solution (pH 7.4)

The constituents of the test medium are not known to contain any contaminants that are reasonably expected to be present and are known to be capable of interfering with the study.

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AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: Diisobutylene: Ready Biodegradability by the Carbon Dioxide Evolution Test Method
PROTOCOL NO: 620/112305/301B/SUB620 **AMENDMENT NO.:** 1
SPONSOR: Lyondell **PROJECT NO.:** 620E-101
EFFECTIVE DATE: April, 03 2006

AMENDMENT: Sponsor's Representative, Page -2 -

REMOVE: Dr. George Cruzan
ToxWorks
1153 Roadstown Road
Bridgeton, NJ 08302

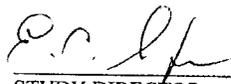
ADD: Jim Bootman
Bootman Chemical Safety Ltd
Diss Business Centre
Diss, Norfolk
IP21 4HD, U.K.

REASON: Sponsor change of Study Monitor

AMENDMENT: Test Inoculum, Page -5 -

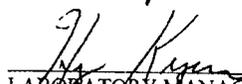
ADD: The test system was selected to be consistent with the referenced study guidelines.

REASON: Statement inadvertently omitted from protocol.



STUDY DIRECTOR

26 Apr 2006
DATE



LABORATORY MANAGEMENT

26 Apr 06
DATE

Wildlife International Ltd.

PROJECT NO.: 620E-101
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DEVIATION TO STUDY PROTOCOL

STUDY TITLE: Diisobutylene: Ready Biodegradability by the Carbon Dioxide Evolution Test Method

PROTOCOL NO: 620/112305/301B/SUB620

DEVIATION NO.: 1

SPONSOR: Lyondell

PROJECT NO.: 620E-101

DEVIATION: Experimental Design, Page -3-

The second replicate vessel for Diisobutylene was dosed at a nominal concentration of 11 mg C/L.

REASON: Oversight by study personnel

IMPACT: In the best judgment of the Study Director, this deviation did not impact the integrity of study.

P. P. L. L. L.
STUDY DIRECTOR

24 Apr 2006
DATE

J. K.
LABORATORY MANAGEMENT

27 Apr 06
DATE