## TRADE SECRET

***Study Title***

# DETERMINATION OF THE DISSOCIATION CONSTANT AND UV-VIS ABSORPTION SPECTRA OF H-28307

***Test Guidelines***

U.S. EPA Product Properties Test Guidelines
OPPTS 830.7370, Dissociation Constant in Water (1996)

OECD Guideline for Testing of Chemicals 112,
Dissociation Constant in Water (1981)

OECD Guidelines for Testing of Chemicals, 101
UV-VIS Absorption Spectra

***Authors***

John R. Murrell, B.S.
Willard B. Nixon, Ph.D.

***Date Study Initiated***

June 25, 2008

***Date Study Completed***

September 17, 2008

***Performing Laboratory***

Wildlife International, Ltd.
8598 Commerce Drive
Easton, Maryland 21601
U.S.A.

***Sponsor***

E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

***DuPont Project Identification Numbers***

DuPont Report No.: Dupont-26349

Work Request No.: 17473

Service Code: 1649

***Wildlife International, Ltd. Study Number***

112C-147

## PAGE RESERVED

## STATEMENT OF CONFIDENTIALITY

## GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with the following GLP principles:

- United States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792 (effective August 17, 1989)

Which are consistent with:

- The OECD Principles of Good Laboratory Practice (ENV/MC/CHEM(98)17) (Paris, 1998)

with the following exceptions:

The reference substance was not characterized in accordance with Good Laboratory Practice Standards.

The stability of the reference substance under the storage conditions at the test site was not determined in compliance with Good Laboratory Practice Standards.

***Study Director***

John R. Murrell, B.S. 9/17/2008
Senior Chemist Date

***Applicant/Sponsor***

E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

DuPont Representative Date

## QUALITY ASSURANCE STATEMENT

***Study Number***
112C-147 (Wildlife International, Ltd.)

***Study Title***
Determination of the Dissociation Constant and UV-VIS Absorption Spectra of H-28307

| *Activity Audited* | *Audit Dates* | *Dates Findings Reported to Study Director* | *Dates Findings Reported to Management* |
|---|---|---|---|
| Protocol | July 9, 2008 | July 9, 2008 | July 11, 2008 |
| Sample Preparation | June 27, 2008 | June 27, 2008 | July 3, 2008 |
| Data and Draft Report | September 2-5, 2008 | September 8, 2008 | September 10, 2008 |
| Final Report | September 17, 2008 | September 17, 2008 | September 17, 2008 |

All inspections were study-based unless otherwise noted.

Linda R. Mitchell
Director of Regulatory and Ecotox Operations
Wildlife International, Ltd.

17 SEPTEMBER 2008
Date

## CERTIFICATION

# DETERMINATION OF THE DISSOCIATION CONSTANT AND UV-VIS ABSORPTION SPECTRA OF H-28307

We, the undersigned, declare that the work described in this report was performed under our supervision, and that this report provides an accurate record of the procedures and results.

***Report by***

John R. Murrell, B.S.
Senior Chemist

9/17/2008
Date

***Approved by***

Willard B. Nixon, Ph.D.
Director of Chemistry

9/17/08
Date

***Date Study Initiated***

June 25, 2008

***Date Study Completed***

September 17, 2008

***Sponsor***

E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

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## BASIC STUDY INFORMATION

***Study Title***

Determination of the Dissociation Constant and UV-VIS Absorption Spectra of H-28307

***Study Objectives***

The objectives of this study are to determine the dissociation constant(s) and absorption spectra of the test substance.

***Study Director***

John R. Murrell, B.S.
Wildlife International, Ltd.
8598 Commerce Drive
Easton, Maryland 21601
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Charles R. Powley, Ph.D.
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***Technical Staff, Wildlife International, Ltd.***

John R. Murrell, B.S.
Frank J. Lezotte, B.S.
Helen Chemere, B.S.

***Test Item***

H-28307

***Testing Facilities***

Wildlife International, Ltd.
8598 Commerce Drive
Easton, Maryland 21601
U.S.A.

# 1.0 SUMMARY

STUDY: Determination of the Dissociation Constant and UV-VIS Absorption Spectra of H-28307

SPONSOR : E.I. du Pont de Nemours and Company

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 112C-147

TEST DATES: Experimental Start (OECD) – June 26, 2008
Experimental Start (EPA) – June 27, 2008
Experimental Termination – August 5, 2008

RESULTS: The pKa value for H-28307 was determined to be 2.84 (SD = 0.0210, CV = 0.738 %) at 20°C.

Absorption spectra of H-28307 test solutions prepared at pH 1.78 showed one absorbance maximum at 203.5 nm. The mean bandwidth at this wavelength was 14.5 nm, and the mean molar absorption coefficient (ε) was 115 L/mol-cm. at 25° C.

Absorption spectra of H-28307 test solutions prepared at pH 7.00 showed one absorbance maximum at 203.5 nm. The mean bandwidth at this wavelength was 17.0 nm, and the mean molar absorption coefficient (ε) was 100 L/mol-cm. at 25° C.

Absorption spectra of H-28307 test solutions prepared at pH 10.0 showed one absorbance maximum at 203.5 and 204.0 nm. The mean bandwidth at this wavelength was 15.0 nm, and the mean molar absorption coefficient (ε) was 106 L/mol-cm. at 25° C.

## 2.0 INTRODUCTION

This study was conducted by Wildlife International, Ltd. for E. I. Du Pont de Nemours and Company and identified as Project Number 112C-147. Dissociation constant determinations were performed between June 26 and June 27, 2008. The UV-VIS absorption spectral analyses were performed on August 5, 2008. The study was performed following procedures in the OECD Guideline for Testing of Chemicals, 112, Dissociation Constants in Water (1), Product Properties Test Guidelines, OPPTS 830.7370, Dissociation Constants in Water (2) and OECD Guidelines for Testing of Chemicals, 101: UV-VIS Absorption Spectra (3). Raw data generated by Wildlife International, Ltd. and a copy of the final report are filed under Project Number 112C-147 in archives located on the Wildlife International, Ltd. Site.

## 3.0 OBJECTIVES

The objectives of this study were to determine the dissociation constant(s) and absorption spectra of the test substance.

## 4.0 EXPERIMENTAL DESIGN

The titration method was used to determine the dissociation constant K (expressed as its log value, pk) of the test substance in HPLC-grade bottled reagent water. A sufficiently strong titrant of appropriate concentration is added incrementally to determine a potentiometric response. The absorption spectra were determined from 190 nm – 800 nm based upon procedures in OECD Guideline 101. The dissociation constant determination was conducted at 20°C and the UV-VIS absorption spectra were analyzed at 25°C.

## 5.0 MATERIALS AND METHODS

The test methods employed in this study were based upon procedures specified in the study protocol entitled "Determination of the Dissociation Constant of H-28307" (Appendix I).

### *5.1 Test Substance*

The test substance was received from E. I. Du Pont de Nemours and Company on December 11, 2007 and was assigned Wildlife International, Ltd. Identification number 8319 upon receipt. The test substance was a liquid, identified as: HFPO Dimer Acid (FRD 903). The test substance had a reported purity of 98% and reanalysis date of October 4, 2008. The certificate of analysis is presented in Appendix II. The test substance was stored at ambient temperature in locked storage at the Wildlife International, Ltd. Facilities in Easton, Maryland.

### *5.2 Reference Substance*

The benzoic acid reference substance was received from Sigma-Aldrich Chemical Company on June 10, 1998, and was assigned Wildlife International, Ltd. Identification number 4505 upon receipt. The reference substance, a solid, was identified as: Benzoic acid; Lot Number 15422JR; CAS Number 65-85-0. The reference substance had a reported purity of 99.6% (Appendix II) and was stored under ambient conditions.

### *5.3 Reagents and Solvents*

HPLC bottled water was used. The water met ASTM Type II standards (ASTM D 1193-91) (4). All other reagents were ACS grade or better. There were no known levels of contaminants in the reagents or test substance that interfered with the purpose or conduct of the test.

### *5.4 Determination of Dissociation Constant*

For the definitive study, three replicate samples, each at approximately 3 mg/mL in HPLC-grade water, were prepared. For each preparation, approximately 0.3 g of the test substance was brought to a final volume of 100 milliliters in a volumetric flask. The water was degassed with nitrogen to remove carbon dioxide prior to use. Each sample was equilibrated to 20 ± 1°C in a water bath then transferred into a beaker. The samples were then titrated against standardized 0.1N sodium hydroxide solution. The titration parameters used are outlined in Table 1. At least ten incremental additions were made before the equivalence point; the titration was carried out past the equivalence point. The pK value was calculated for a minimum of ten points prior to the equivalence point on the titration curve. The acid dissociation constant (Ka) and pKa were determined as follows:

$$Ka = ([B]/[HB])$$
$$pKa = pH - \log([B]/[HB])$$
$$\text{Antilog } (-pk) = K$$

Where pH = pH of test solution at any point
[B] = concentration of ionized species
[HB] = concentration of unionized species

### *5.5 Determination of Molar Absorptivity*

The absorption spectrum analyses were performed at 25°C. The absorption spectra were obtained at a pH of less than 2 (1.78), pH 7 and pH 10. Triplicate analyses were performed at each pH. A control/blank solution was prepared which contained only the buffer of interest. Acceptable absorbance readings of the blank did not vary more than 0.05 absorbance units from the nominal zero value. An accurately weighed amount of test substance was dissolved its appropriate buffer solution, quantitatively transferred to a volumetric flask and diluted to the mark (with buffer solution). The concentration of the test substance solution was determined to achieve an absorbance maximum in the range 0.5 to 1.5 absorbance units (Table 2). For each test solution, the wavelength and absorbance for the single peak were recorded. The bandwidth at half height was determined. The molar absorption coefficient was calculated using the following equation:

$$\varepsilon = A/bc$$

where $\varepsilon$ = molar absorption coefficient (L/mol-cm)
A = absorbance
b = path length of cuvette (1.00 cm)

c = molar concentration of test substance (moles/L)

The mean and standard deviation of the bandwidths, molar absorption coefficients and log ε values were calculated at each pH level.

## 6.0 RESULTS AND DISCUSSION

The calculated pKa value for the reference substance, benzoic acid, is summarized in Table 3. The averaged calculated pKa value was 4.28 at 20.0°C. Although slightly elevated, the value is consistent with the value cited in OPPTS 830.7370 of 4.19.

The corresponding calculated pKa value for the test substance is summarized in Table 4. The mean pKa value was 2.84 (SD = 0.0210, CV = 0.738%) at 20° C. A representative titration data curve for the analyte is presented in Figure 1.

Absorption spectra of H-28307 test solutions prepared at pH 1.78 showed one absorbance maximum at 203.5 nm. The mean bandwidth at this wavelength was 14.5 nm, and the mean molar absorption coefficient (ε) was 115 L/mol-cm. The blank solution displayed no absorbance (0.0 ± 0.05AU). Table 5 illustrates a summary of the absorption spectra results at pH 1.78. Representative spectra of the aqueous buffer blank and the test substance are presented in Figures 2 and 3, respectively.

Absorption spectra of H-28307 test solutions prepared at pH 7.00 showed one absorbance maximum at 203.5 nm. The mean bandwidth at this wavelength was 17.0 nm, and the mean molar absorption coefficient (ε) was 100 L/mol-cm. The blank solution displayed no absorbance (0.0 ± 0.05AU). Table 6 illustrates a summary of the absorption spectra results at pH 7.00. Representative spectra of the aqueous buffer blank and the test substance are presented in Figures 4 and 5, respectively.

Absorption spectra of H-28307 test solutions prepared at pH 10.0 showed one absorbance maximum at 203.5 and 204.0 nm. The mean bandwidth at this wavelength was 15.0 nm, and the mean molar absorption coefficient (ε) was 106 L/mol-cm. The blank solution displayed no absorbance (0.0 ± 0.05AU). Table 7 illustrates a summary of the absorption spectra results at pH 10.0. Representative spectra of the aqueous buffer blank and the test substance are presented in Figures 6 and 7, respectively.

## 7.0 CONCLUSION

The pKa value for H-28307 was 2.84 (SD = 0.0210, CV = 0.738%) at 20°C.

The UV/Visible absorption characteristics of H-28307 were determined at 25°C in aqueous buffered solutions of pH 1.78, pH 7.00 and pH 10.0. The mean molar absorption coefficient and mean bandwidth for pH 1.78 (at 203.5 nm) were determined to be 115 L/mol-cm and 14.5 nm. The mean molar absorption coefficient and mean bandwidth at pH 7.00 (at 203.5 nm) were determined to be 100 L/mol-cm and 17.0 nm. The mean

molar absorption coefficient and mean bandwidth at pH 10.0 (at 203.5 and 204.0 nm) were determined to be 106 L/mol-cm and 15.0 nm.

## 8.0 REFERENCES

1 **Organization for Economic Cooperation and Development.** 1981. *Dissociation Constants in Water*. OECD Guideline for Testing of Chemicals. Guideline 112.

2 **U.S. Environmental Protection Agency.** 1996. Series 830-Product Properties Test Guidelines, OPPTS Number 830.7370: *Dissociation Constants in Water*.

3 **Organisation for Economic Cooperation and Development.** 1981. Guideline for Testing of Chemicals, 101: *UV-VIS Absorption Spectra.*

4 **American Society for Testing and Materials.** 1999. Standard Specification for Reagent Water, ASTM D1193-99.

Table 1

Operating Parameters for Automatic Titrator

| Titrator: | Metrohm Model 716 DMS Titrino |
|---|---|
| Titration parameters | Definitive Trials |
| Mode | MET |
| Volume Step | 0.10 mL |
| Titration Rate | Max. mL/min |
| Signal Drift | 50 mV/min |
| Equilibration Time | 26 seconds |
| Start Volume | OFF |
| Pause | 0 seconds |
| Measurement Input | 1 |
| Temperature | 20 ± 1°C |
| Stop Volume | 19.9 mL |
| Stop pH | 12.0 |

Table 2

Operating Parameters for UV-VIS Spectrometer

| | |
|---|---|
| INSTRUMENT: | Jasco Model V-550 UV/VIS Spectrophotometer |
| DETECTOR: | Dual-beam Photodiode Array |
| CELL TYPE: | Quartz glass |
| PATH LENGTH: | 1.00 cm |
| TEMPERATURE: | 25 ± 2 °C |
| BAND WIDTH: | 0.5 nm |
| DATA PITCH: | 0.5 nm |
| SCAN RANGE: | 800 to 190 nm |
| SCAN SPEED: | 100 nm/min. |

Table 3

pKa Value of Benzoic Acid in Reagent Water at 20°C

| Sample Number (112C-147-REF-) | Nominal Concentration (mg/mL) | Temperature (°C) | Equivalence Point (pH) | Titrant Volume (mL) | pKa [1] | Mean pKa | Standard Deviation |
|---|---|---|---|---|---|---|---|
| 1 | 1.221 | 20.1 | 8.174 | 9.800 | 4.2953 | | |
| 2 | 1.223 | 20.1 | 8.226 | 9.900 | 4.2669 | 4.28 | 0.0201 (CV = 0.469 %) |
| | | | | | | | Antilog (-pKa) = $5.23 \times 10^{-5}$ |

[1] Results were generated using Excel 2000 in the full precision mode. Manual calculations may differ slightly.

Table 4

pKa Value of H-28307 in Reagent Water at 20°C

| Sample Number | Nominal Concentration (mg/mL) | Temperature (°C) | Equivalence Point (pH) | Titrant Volume (mL) | pKa [1] | Mean pKa | Standard Deviation |
|---|---|---|---|---|---|---|---|
| 112C-147-1 | 2.871 | 20.0 | 7.777 | 8.500 | 2.8280 | | |
| 112C-147-2 | 3.172 | 20.0 | 8.131 | 9.400 | 2.8664 | 2.84 | 0.0210 (CV = 0.738 %) |
| 112C-147-3 | 3.200 | 20.1 | 8.473 | 9.500 | 2.8326 | | |

Antilog (-pKa) = $1.45 \times 10^{-3}$

[1] Results were generated using Excel 2000 in the full precision mode. Manual calculations may differ slightly.

Table 5

Summary of Absorption Spectra Results at pH 1.78 at 25° C

| Sample I.D. (112C-147-1.78-) | Concentration (Moles/L) | Wavelength (nm) | Absorbance (AU) | [1]Bandwidth (nm) | Molar Absorptivity Coefficient – ε (L/mol-cm) | Log (ε) |
|---|---|---|---|---|---|---|
| 1 | 7.87759E-03 | 203.5 | 0.9160 | 14.5 | 116 | 2.07 |
| 2 | 7.51401E-03 | 203.5 | 0.8627 | 14.5 | 115 | 2.06 |
| 3 | 7.84730E-03 | 203.5 | 0.8919 | 14.5 | 114 | 2.06 |
| | | | Mean: | 14.5 | 115 | 2.06 |
| | | | Std.Dev: | 0.00 | 1.00 | 0.00 |

[1]Extrapolated value, assuming perfect symmetry in peak shape

Table 6

Summary of Absorption Spectra Results at pH 7.00 at 25° C

| Sample I.D. (112C-147-7.00-) | Concentration (Moles/L) | Wavelength (nm) | Absorbance (AU) | [1]Bandwidth (nm) | Molar Absorptivity Coefficient – ε (L/mol-cm) | Log (ε) |
|---|---|---|---|---|---|---|
| 1 | 7.72610E-03 | 203.5 | 0.7874 | 17.0 | 102 | 2.01 |
| 2 | 7.69580E-03 | 203.5 | 0.7773 | 17.0 | 101 | 2.00 |
| 3 | 7.84730E-03 | 203.5 | 0.7638 | 17.0 | 97.3 | 1.99 |
| | | | Mean: | 17.0 | 100 | 2.00 |
| | | | Std.Dev: | 0.00 | 2.65 | 0.00 |

[1]Extrapolated value, assuming perfect symmetry in peak shape

Table 7

Summary of Absorption Spectra Results at pH 10.0 at 25° C

| Sample I.D. (112C-147-10.0-) | Concentration (Moles/L) | Wavelength (nm) | Absorbance (AU) | [1]Bandwidth (nm) | Molar Absorptivity Coefficient – ε (L/mol-cm) | Log (ε) |
|---|---|---|---|---|---|---|
| 1 | 7.69580E-03 | 204.0 | 0.8177 | 15.0 | 106 | 2.03 |
| 2 | 7.63521E-03 | 203.5 | 0.8119 | 15.0 | 106 | 2.03 |
| 3 | 7.57461E-03 | 204.0 | 0.8064 | 15.0 | 106 | 2.03 |
| | | | Mean: | 15.0 | 106 | 2.03 |
| | | | Std.Dev: | 0.00 | 0.00 | 0.00 |

[1]Extrapolated value, assuming perfect symmetry in peak shape

Figure 1

Representative plot of titration curve for H-28307 (Sample ID: 112C-147-1)

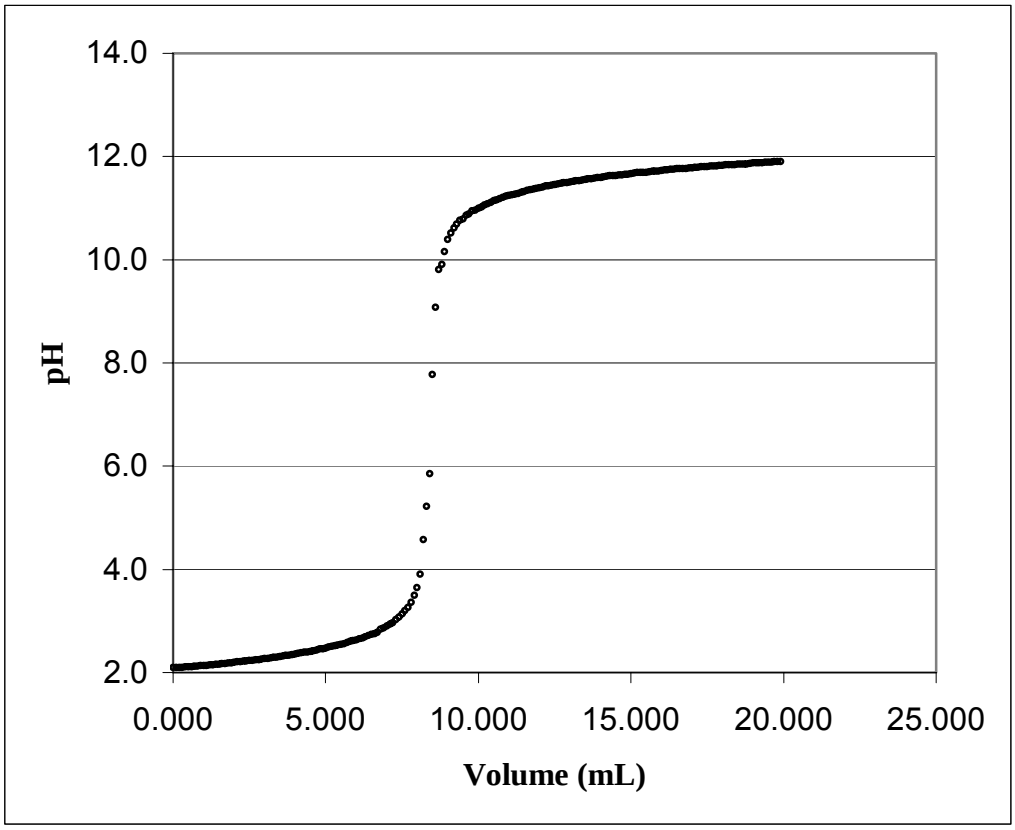


Figure 2

Representative spectra of pH 1.78 aqueous buffer.
(Sample I.D.: 112C-147-1.78-0)

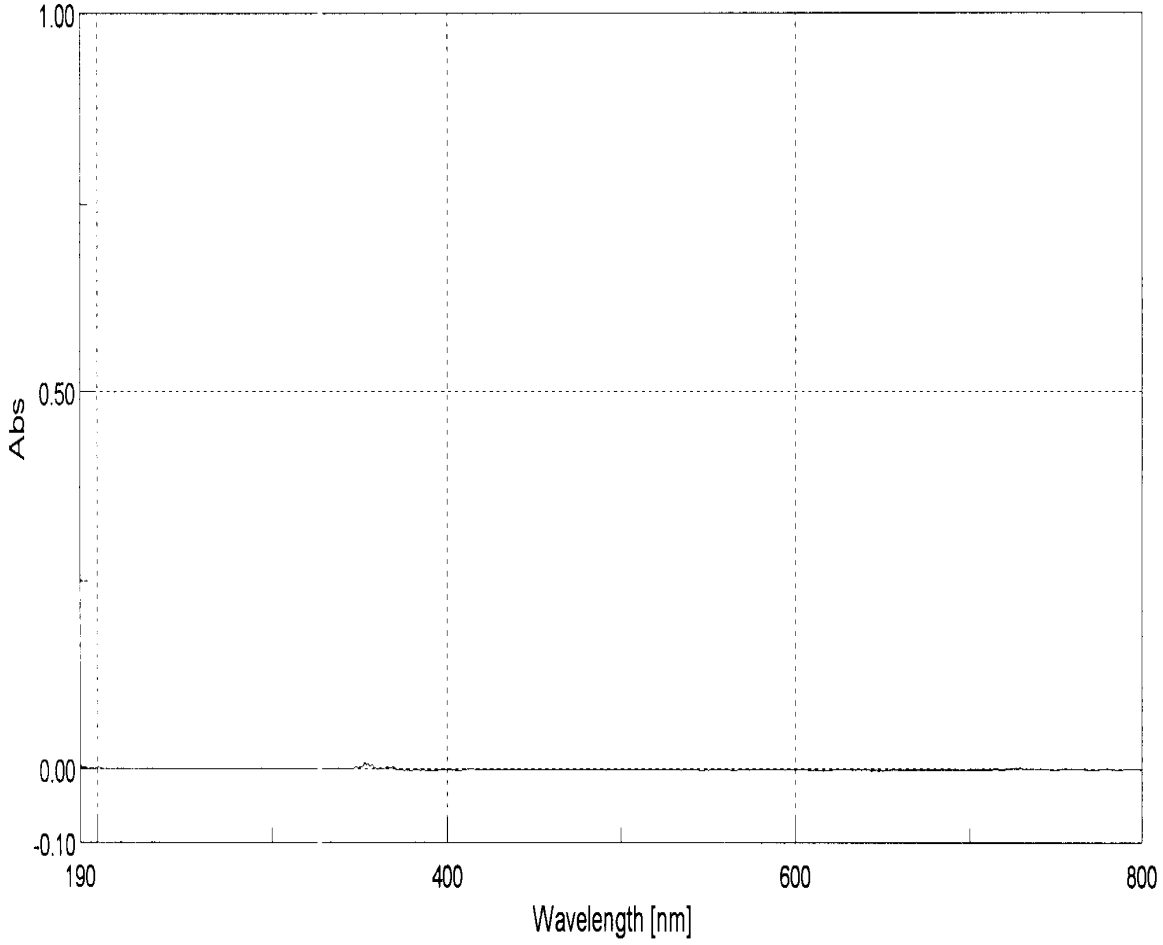


Figure 3

Representative spectra of H-28307 in pH 1.78 aqueous buffer.
(Sample I.D.: 112C-147-1.78-1)

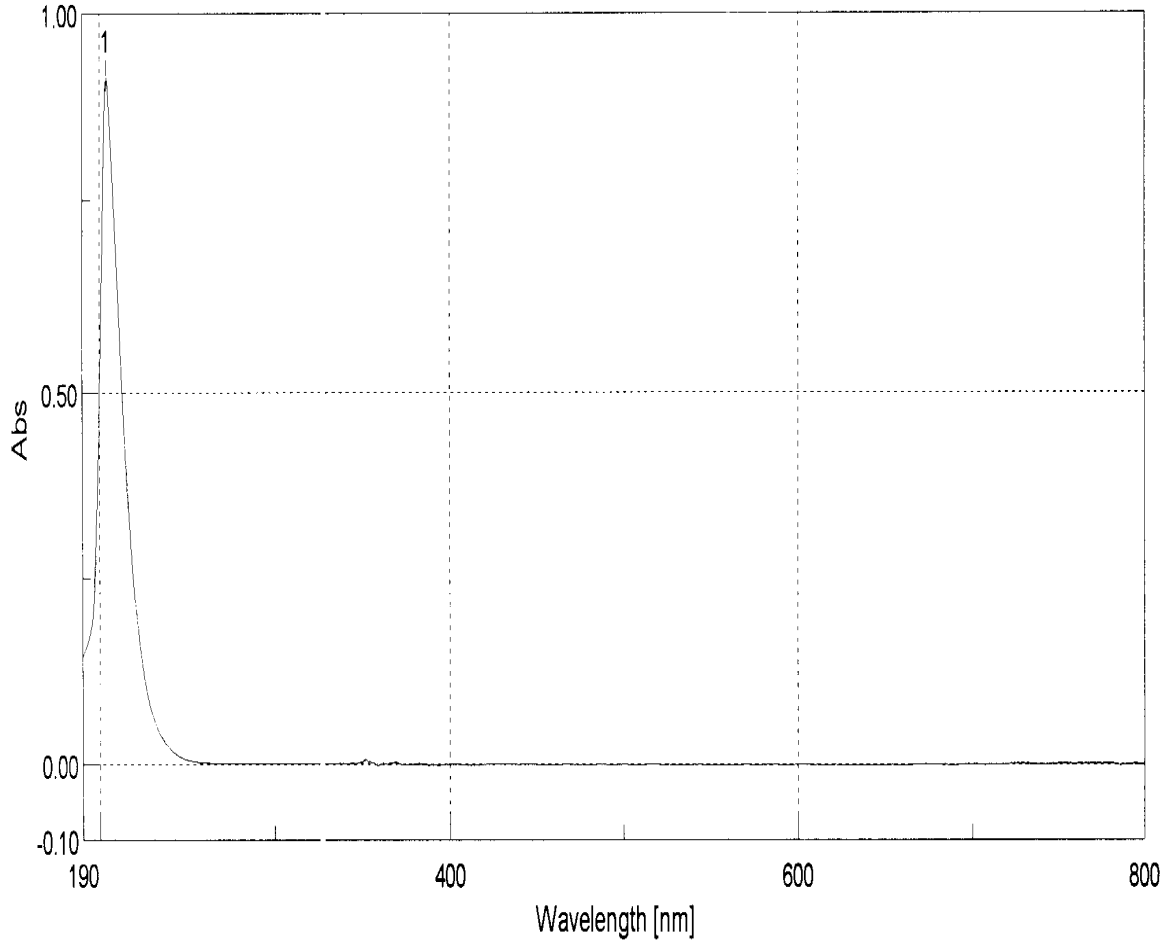


Figure 4

Representative spectra of pH 7.00 aqueous buffer.
(Sample I.D.: 112C-147-7.00-0)

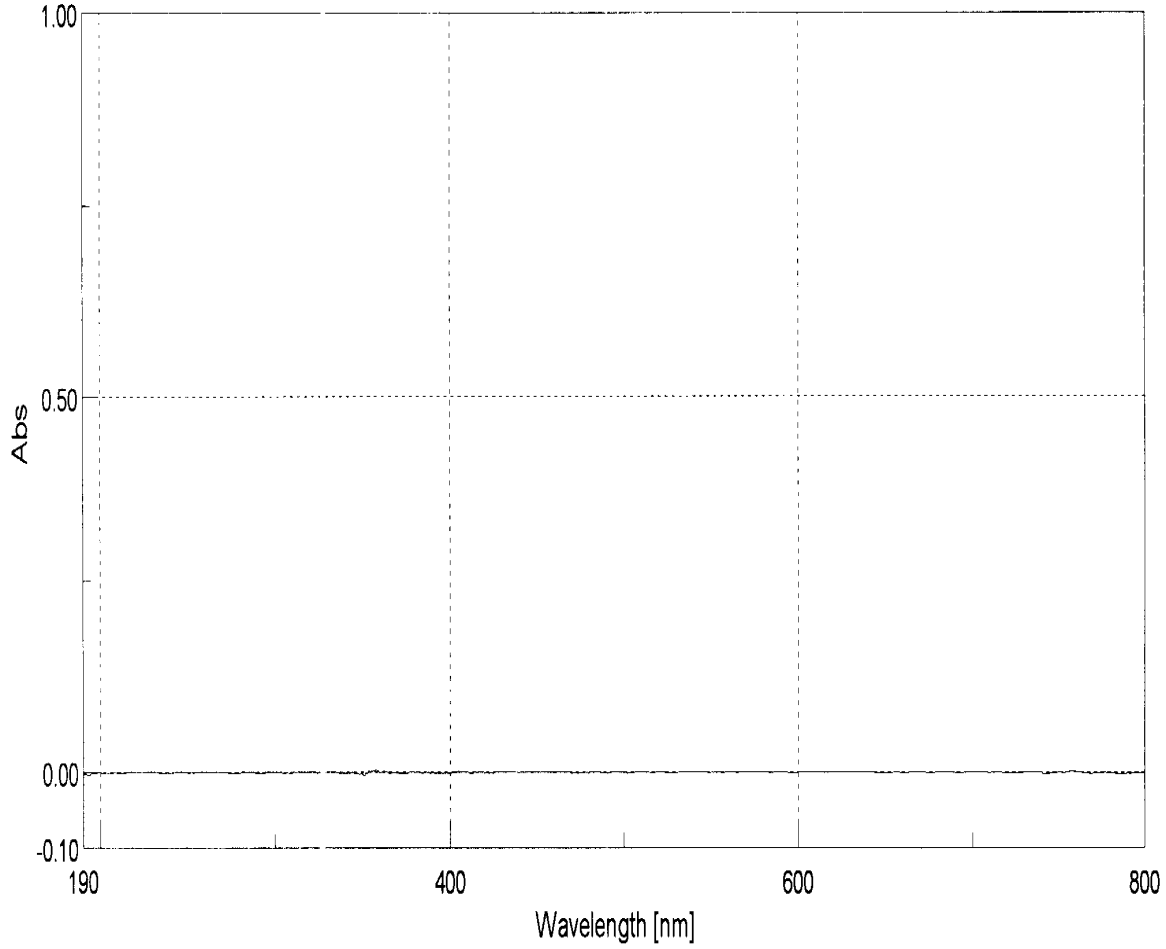


Figure 5

Representative spectra of H-28307 in pH 7.00 aqueous buffer.
(Sample I.D.: 112C-147-7.00-1)

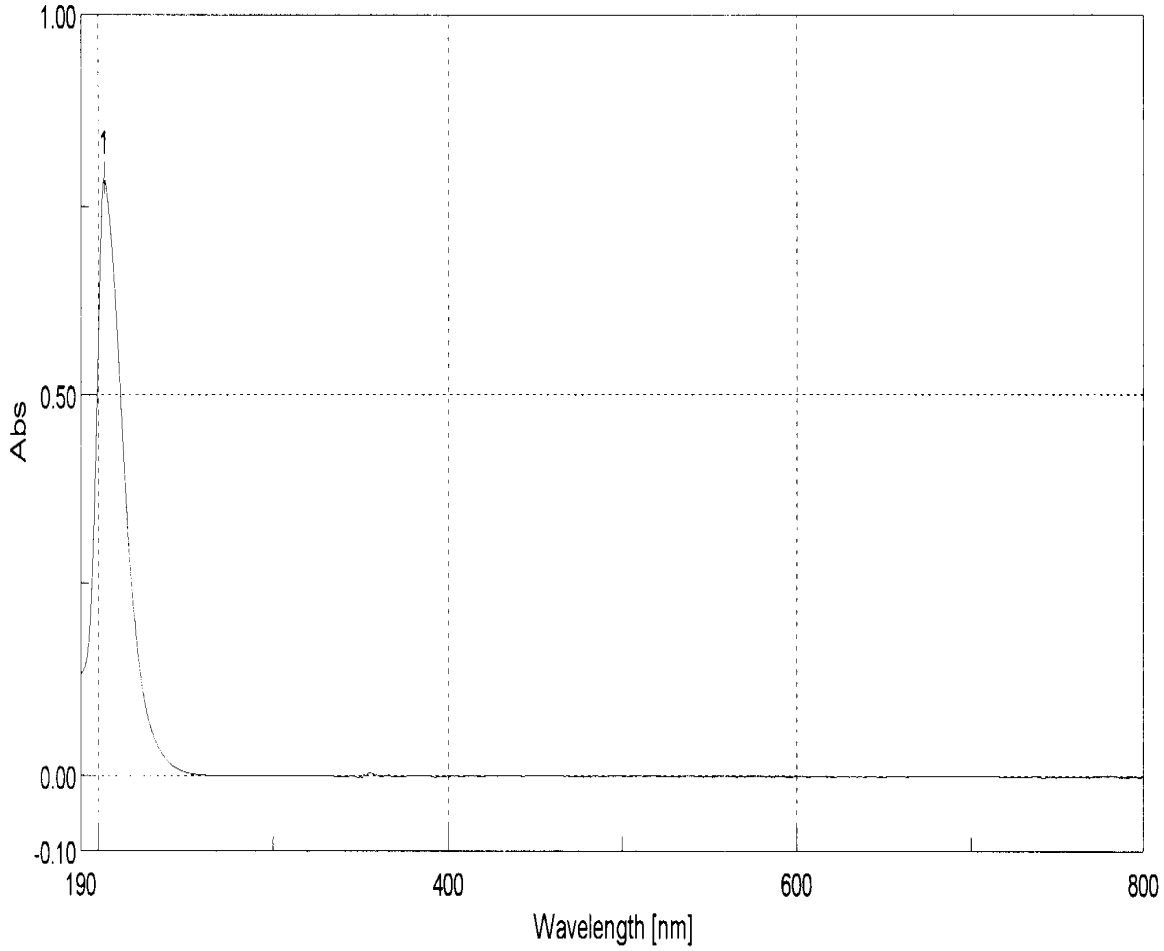


Figure 6

Representative spectra of pH 10.0 aqueous buffer.
(Sample I.D.: 112C-147-10.0-0)

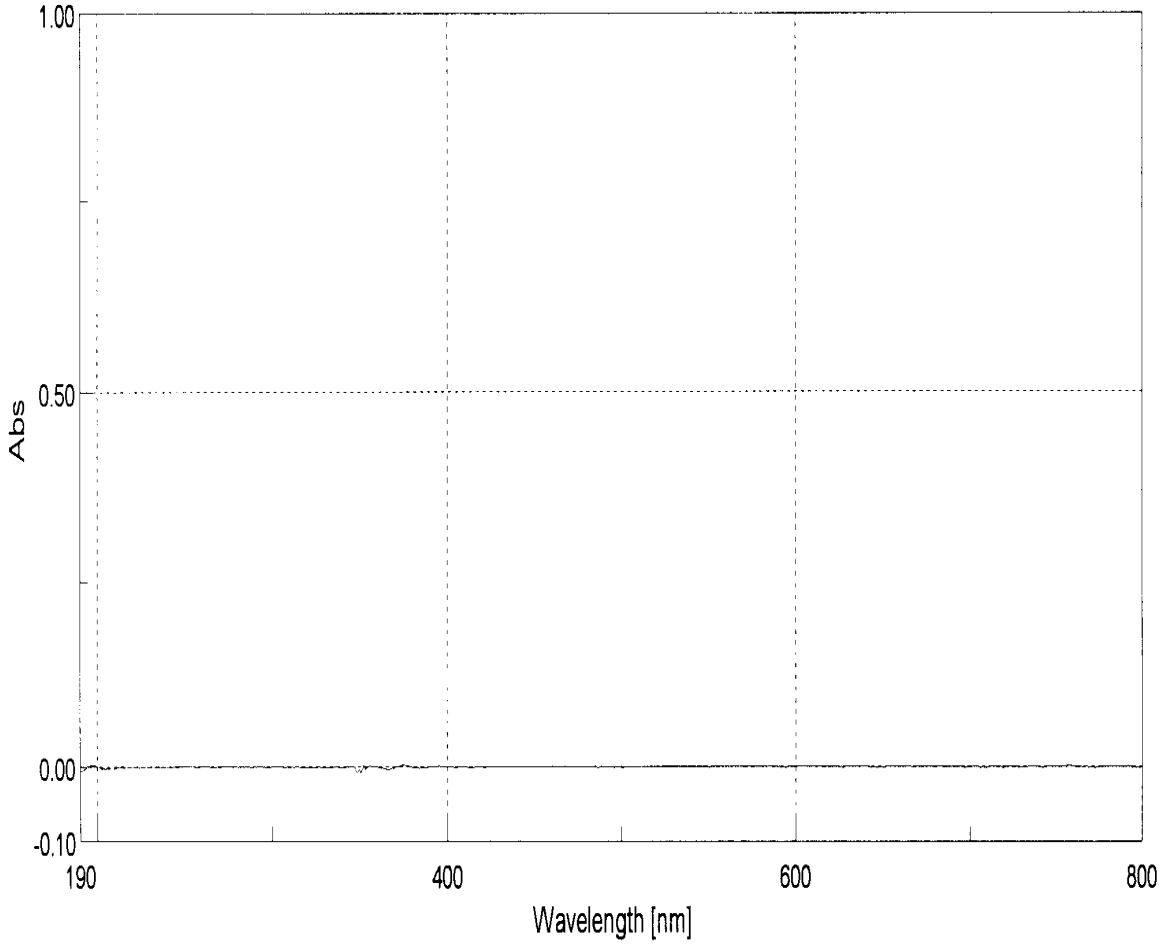
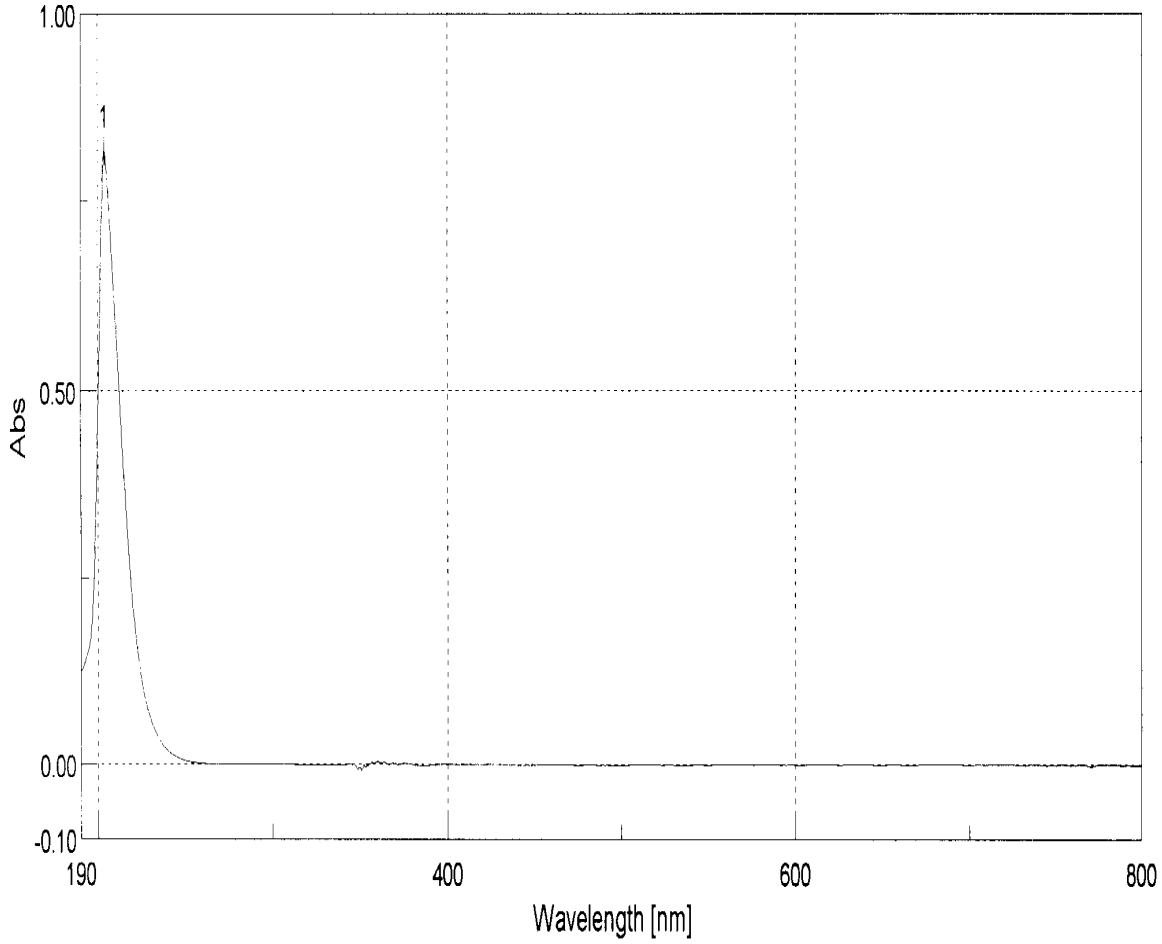


Figure 7

Representative spectra of H-28307 in pH 10.0 aqueous buffer.
(Sample I.D.: 112C-147-10.0-1)



# Appendix I

## Protocol and Amendments

P R O T O C O L

DETERMINATION OF THE DISSOCIATION CONSTANT OF H-28307

OECD Guideline for Testing of Chemicals, 112
Dissociation Constants in Water

Submitted to

E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

DuPont Report: DuPont-
Work Request:
Service Code:

*Wildlife International, Ltd.*

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

June 3, 2008

DETERMINATION OF THE DISSOCIATION CONSTANT OF H-28307

SPONSOR: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

SPONSOR'S REPRESENTATIVE: Charles R. Powley, Ph.D.

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

STUDY DIRECTOR: ~~Frank L. Lezotte~~ John R. Murrell, Senior Chemist
Wildlife International, Ltd. JRM 6/25/08

LABORATORY MANAGEMENT: Willard B. Nixon, Ph.D., Director of Chemistry
Wildlife International, Ltd.

**FOR LABORATORY USE ONLY**

Proposed Dates:

Experimental Start Date: 6/25/08 Experimental Termination Date: 7/25/08

Project No.: 1120-147

Test Substance No.: 8319 Reference Substance No. (if applicable): (WR # 6456) NITROPHENOL

PROTOCOL APPROVAL

STUDY DIRECTOR — DATE 6/25/08

LABORATORY MANAGEMENT — DATE 6/26/08

SPONSOR'S REPRESENTATIVE — DATE 06 Jun 2008

PROTOCOL NO.: 112/060308/112/SUB112

## INTRODUCTION

Wildlife International, Ltd. will determine the dissociation constant of the test substance in water at 20°C. The study will be conducted at the Wildlife International, Ltd. analytical chemistry facility in Easton, Maryland. The study will be performed following procedures in the OECD Guideline for Testing of Chemicals, 112, *Dissociation Constants in Water* (1) and Product Properties Test Guidelines, OPPTS 830.7370, *Dissociation Constants in Water* (2). 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 at Wildlife International, Ltd. or at an alternative location to be specified in the final report.

## OBJECTIVE

The objective of this study is to determine the dissociation constant(s) of the test substance in water at 20°C.

## EXPERIMENTAL DESIGN

The titration method is used to determine the dissociation constant K (expressed as its log value, pK) of a test substance in water. This method is not suitable for low solubility compounds.

## MATERIALS AND METHODS

### 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.

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.

### Reagents

All reagents and solvents will be ACS reagent grade or better.

**Test Procedure**

The water used for preparation of the titrant solutions will be degassed to remove carbon dioxide prior to preparation of the titrants. A reference substance (e.g., citric acid) may be used to verify calibration of the procedure. A test solution will be prepared at a concentration not to exceed the lesser of 0.01M or half the saturation concentration in distilled water. A minimum amount of co-solvent may be used to aid solubility. The type of titrant will be determined from the pH of the test solution (i.e., < pH 7 - NaOH; > pH 7 - HCl). The test substance will be titrated with a 0.1 N solution of titrant. A titration curve will be prepared by plotting the pH of the test solution versus the milliliters (mL) of titrant added. The center point of the inflection in the titration curve will be used as the approximate equivalence point. The concentration of the titrant to be used for the definitive determination will be calculated by determining the milliliters used for titration to the equivalence point as follows:

*Normality of Titrant = (0.1N titrant) X (mL of titrant used/20 mL)*

For the definitive study, the test chemical, in a solution not to exceed the lesser of 0.01M or half the saturation concentration, will be titrated. The solution will be maintained at 20 ± 1°C. A minimum amount of co-solvent may be used to aid solubility. The pH of the solution and milliliters of titrant (to nearest 0.1 mL) added will be recorded after each addition of titrant. At least ten incremental additions will be made before the equivalence point. A plot of pH versus milliliters of titrant will be generated. Values of pK will be calculated for a minimum of ten points on the titration curve. Titration will be carried out past the equivalence point. The equivalence point is defined as that section of the titration curve in which a small addition of titrant results in a large change in pH. The test chemical will be titrated a minimum of three times. The dissociation constant (K) and pK (log(K)) will be determined as follows:

$pK_a = pH - \log([B]/[HB])$ *for acidic test substances*

$pK_b = pH - \log([HB]/[B])$ *for basic test substances*

Antilog (pK) = K

where pH = pH of test solution at any point
[B] = concentration of ionized species
[HB] = concentration of un-ionized species

PROTOCOL NO.: 112/060308/112/SUB112

The mean and standard deviation of values will be reported. When the average $pK_a$ values of the replicates differ by more than ± 0.1 log units, additional determination will be made. A plot of pH versus volume of standard base or acid will be included along with appropriate tabulated values.

**Sample Handling and Safety**

The Sponsor will identify any special handling or safety precautions to be used with the above referenced test substance. All normal precautions with respect to handling and storage will be taken.

**Sample and Test Substance Retention**

Upon completion of testing, portions of the test substance used as part of this study will be disposed of in accordance with federal, state and local regulations. Any unused portion of the test substance will be returned to the Sponsor.

**RECORDS TO BE MAINTAINED**

Records to be maintained for data generated by Wildlife International, Ltd. will include, but not be limited to:

1. A copy of the signed protocol.
2. Identification and characterization of the test substance, if provided by the Sponsor.
3. Dates of initiation and completion of the study.
4. Dates of experimental start and termination.
5. Storage conditions of the test substance.
6. Test substance use log.
7. Concentration calculations and records of solution preparation.
8. Titrator operating conditions.
9. Statistical calculations.
10. Test conditions.
11. A copy of the final report.

**FINAL REPORT**

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

1. Name and address of the facility performing the study.

2. Dates upon 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. Purpose and procedure, as stated in the approved protocol, including all amendments and deviations to the protocol.
5. The test substance identification, including name, chemical abstract number or code number, purity, composition, empirical formula, molecular weight, manufacturer's lot/batch number, dissociation in water, method of analysis, and any other information, if provided by the Sponsor.
6. Description of the test method or reference to the method used along with any modifications made.
7. Titrant volumes, measured values of pKa and plots of pH versus titrant volumes.
8. Description of any problems experienced and how they were resolved.
9. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made and the dates that any findings were reported to the Study Director and Management.

**CHANGING OF 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 according to the Good Laboratory Practices described in OECD (ENV/MC/CHEM (98) 17) and EPA (40 CFR Parts 160 and/or 792). 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. 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.

**REFERENCES**

1. **Organisation for Economic Cooperation and Development.** 1981. Guideline for Testing of Chemicals, 112: *Dissociation Constants in Water.*

2. **Product Properties Test Guidelines.** 1996. OPPTS 830.7370. *Dissociation Constants in Water.*

Project Number 112C-147

*Wildlife International, Ltd.*

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**AMENDMENT TO STUDY PROTOCOL**

**STUDY TITLE:** Determination of the Dissociation Constant of H-28307

**PROTOCOL NO.:** 112/060308/112/SUB112 **AMENDMENT NO.:** 1

**SPONSOR:** E.I. du Pont de Nemours and Company **PROJECT NUMBER:** 112C-147

**SPONSOR STUDY NO.:** 26349 **EFFECTIVE DATE:** June 27, 2008

**AMENDMENT:** Page 2

Change: Nitrophenol (WIL # 6458)

To: Benzoic Acid (WIL # 4505)

**REASON:** Nitrophenol was discarded from our test substance inventory. Benzoic acid was available and used for this study.

STUDY DIRECTOR — DATE 7/11/08

LABORATORY MANAGEMENT — DATE 7/11/08

**AMENDMENT TO STUDY PROTOCOL**

**STUDY TITLE:** Determination of the Dissociation Constant of H-28307

**PROTOCOL NO.:** 112/060308/112/SUB112 **AMENDMENT NO.:** 2

**SPONSOR:** E.I. du Pont de Nemours and Company **PROJECT NUMBER:** 112C-147

**SPONSOR STUDY NO.:** 26349 **EFFECTIVE DATE:** July 15, 2008

**AMENDMENT:** Page 3, Introduction

Change: "Wildlife International, Ltd. will determine the dissociation constant of the test substance in water at 20°C. The study will be conducted at the Wildlife International, Ltd. analytical chemistry facility in Easton, Maryland. The study will be performed following procedures in the OECD Guideline for Testing of Chemicals, 112, Dissociation Constants in Water (1) and Product Properties Test Guidelines, OPPTS 830.7370, Dissociation Constants in Water (2)."

To: "Wildlife International, Ltd. will determine the dissociation constant (at 20°C) and absorption spectra (at 25°C) of the test substance. The study will be conducted at the Wildlife International, Ltd. analytical chemistry facility in Easton, Maryland. The study will be performed following procedures in the OECD Guideline for Testing of Chemicals, 112, Dissociation Constants in Water (1), Product Properties Test Guidelines, OPPTS 830.7370, Dissociation Constants in Water (2) and OECD Guidelines for Testing of Chemicals, 101: UV-VIS Absorption Spectra (3)."

**AMENDMENT:** Page 3, Objective

Change: "The objective of this study is to determine the dissociation constant(s) of the test substance in water at 20°C."

To: "The objectives of this study are to determine the dissociation constant(s) and absorption spectra of the test substance. "

**AMENDMENT:** Page 3, Experimental Design

Add: "The absorption spectra will be determined (from 190 nm – 800 nm) based upon procedures in OECD Guideline 101."

**AMENDMENT:** Page 3, Reagents

Change: "All reagents and solvents will be ACS reagent grade or better."

To: "Water that meets or exceeds ASTM Type II standards (ASTM D 1193-91) will be used as the solvent of choice. If the test substance has low water solubility or is insoluble in water, organic solvents such as methanol or acetonitrile may be used. All solvents used in the procedure will be of HPLC grade or better. All reagents will be of ACS grade or better. There are no known levels of contaminants in the reagents or test substance that are expected to interfere with the purpose or conduct of the test."

**AMENDMENT:** Page 4, Test Procedure

Add: "The absorption spectrum determinations will be performed at 25°C (±2°C) and will be done in triplicate. The water bath temperature will be recorded upon initial placement of samples in the water bath. For test substances that reversibly ionize or protonate, the absorption spectrum will be obtained at a pH of less than 2, at pH 7 and at pH 10. The solvent for the neutral solution, and for preparing the acidic and basic solutions, will be reagent water. Sufficient quantity of the acidic and basic solutions will be prepared at the start of the study. The pH of each solution will be recorded at that time. If methanol or acetonitrile will be used, the acidic and basic solutions will be prepared by adding 10% by volume of 1 M HCl or NaOH in an aqueous solution. A control solution will be prepared which contains the solvent and all chemical species other than the test substance. The absorption spectrum of this solution will be recorded in a manner identical to that of the test substance. Acceptable absorbance readings of the blank will not vary more than 0.05 absorbance units from the nominal zero value. The test substance solution will be prepared in triplicate by adding an accurately known amount of test substance to a volumetric flask and diluting to the mark with the appropriate solvent. The test solution will be scanned using freshly prepared solutions. The concentration of the test substance solution will be such that the resulting concentration will achieve at least one absorbance maximum in the range 0.5 to 1.5 units."

**AMENDMENT:** Page 5, Data Analysis

Add: "The molar absorption (extinction) coefficient, ε, will be calculated for all absorbance maxima of each replicate from all pH levels of the test substance using the equation below. No statistical evaluations other than means and standard deviations will be performed. For each peak which is capable of being resolved, either as recorded or by extrapolated symmetrical peaks, the band width will be recorded."

$$\varepsilon = \frac{A}{bc}$$

ε = Molar absorption coefficient (L/mol-cm)
A = Absorbance
c = Test substance solution concentration (mol/L)
b = Path length of cell (cm)

Project Number 112C-147

*Wildlife International, Ltd.*



**AMENDMENT:** Page 7, References

Add: 3. **Organisation for Economic Cooperation and Development.** 1981. Guideline for Testing of Chemicals, 101: *UV-VIS Absorption Spectra.*

**REASON:** UV-VIS spectral analysis of the test material was added to the study per Sponsor's request.

STUDY DIRECTOR — DATE 8/7/08

LABORATORY MANAGEMENT — DATE 8/7/08

QA reviewed
RSA 29 Jul 2008

*Wildlife International, Ltd.*

Project No.: 112C-147
Page 1 of 1

**DEVIATION TO STUDY PROTOCOL**

**STUDY TITLE:** Determination of the Dissociation Constant of H-28307

**PROTOCOL NO.:** 112/060308/112/SUB112 **DEVIATION NO.:** 1

**SPONSOR:** E.I.DuPont de Nemours and Company **PROJECT NO.:** 112C-147

**DATE OF DEFACTO DEVIATION:** August 5, 2008

**DEVIATION:** The protocol states: "Sufficient quantity of the acidic and basic solutions will be prepared at the start of the study. The pH of each solution will be recorded at that time." and "The test solution will be scanned using freshly prepared solutions." The pH 7.00 and pH 10.0 buffer solutions were prepared approximately two months prior to the experimental start of the UV/VIS analysis. The pH values for each buffer were recorded at the time of preparation. Although the pH values for these buffers were verified less than twenty-four hours prior to the experimental start date, they were not recorded.

**REASON:** Analyst oversight.

**IMPACT:** There is no impact to the study. The age of the buffer is not of consequence if the pH is verified prior to use. The pH of each buffer solution was verified to within acceptable tolerances immediately (< 24 hours) prior to use in the study.

STUDY DIRECTOR
John R. Murrell, B.S.

9/9/08
Date

LABORATORY MANAGEMENT
Willard B. Nixon, Ph.D.

9/10/08
Date

Appendix II

Certificates of Analysis

E. I. du Pont de Nemours and Company
Wilmington, DE 19898
USA

**CERTIFICATE OF ANALYSIS**

This Certificate of Analysis fulfills the requirement for characterization of a test substance prior to a study subject to GLP regulations. It documents the identity and content of the test substance. This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 792).

| | |
|---|---|
| Haskell Code Number | H-28307 |
| Common Name | HFPO Dimer Acid |
| Purity Percent | 98% |
| Other Components | Water – 0.61%<br>Perfluorooctanoic acid – 8.3 ppm |
| Date of Analysis | October 4, 2007 |
| Recommended reanalysis interval | 1 year |
| Instructions for storage | NRT&H |
| Reference | DuPont-24003 |
| Analysis performed at | E. I. DuPont de Nemours and Company<br>DuPont Haskell Laboratories<br>Newark, Delaware<br>USA |

Approver:

Peter A. Bloxham, Ph.D.
Senior Research Chemist

18-OCT-2007
Date

# CERTIFICATE OF ANALYSIS

WILDLIFE INTERNATIONAL LTD
410 822 0632
MIKE SCHUETTPELZ

PO NBR:

PRODUCT NUMBER: 24238-1 LOT NUMBER: 15422JR

PRODUCT NAME: BENZOIC ACID, 99.5%, A.C.S. REAGENT

FORMULA: C7H6O2 FORMULA WEIGHT: 122.12

| | | |
|---|---|---|
| APPEARANCE | WHITE GRANULAR POWDER | |
| INFRARED SPECTRUM | CONFORMS TO STRUCTURE AND STANDARD AS ILLUSTRATED ON PAGE 186A OF EDITION I, VOLUME 2 OF "THE ALDRICH LIBRARY OF FT-IR SPECTRA". | |
| TITRATION | 99.6 % (WITH NAOH) | |
| RESIDUE ON IGNITION | 0.00% | * |
| FREEZING POINT | 122.5 DEGREES C | * |
| INSOLUBLE MATTER | 0.00 % (C=10, MEOH) | * |
| CHLORINE COMPOUNDS | <0.005% | * |
| HEAVY METALS | < 5PPM (AS PB) | * |
| SUBSTANCES REDUCING PERMANGANATE | PASSES TEST | * |
| SULFUR COMPOUNDS | <0.002 % | * |
| QUALITY CONTROL ACCEPTANCE DATE | JULY 1997 | |

* SUPPLIER INFORMATION.

ALDRICH CHEMICAL COMPANY
DAVID SWESSEL
JUNE 10, 1998



** TOTAL PAGE.001 **

# Appendix III

## Personnel Involved in the Study

The following key Wildlife International, Ltd. personnel were involved in the conduct or management of this study:

1. Willard B. Nixon, Ph.D., Director of Chemistry
2. John R. Murrell, B.S., Senior Chemist
3. Frank J. Lezotte, B.S., Senior Chemist