FINAL REPORT

Volume 4 of 4
(Appendices C - I)

STUDY TITLE
A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY

STUDY NUMBER
WIL-189205

DATA REQUIREMENT
OECD Guideline 407

STUDY DIRECTOR
Matthew C. Haas, BA, LAT

STUDY INITIATION DATE
16 November 2007

STUDY COMPLETION DATE
22 August 2008

PERFORMING LABORATORY
WIL Research Laboratories, LLC
1407 George Road
Ashland, Ohio 44805-8946
U.S.A.

SPONSOR WORK REQUEST, SERVICE CODE
WR 17568, SC 1023

SPONSOR STUDY NUMBER
DuPont-24447

SPONSOR
E.I. du Pont de Nemours and Company
DuPont Haskell Global Centers for Health and Environmental Sciences
1090 Elkton Road, P.O. Box 50
Newark, Delaware 19714
U.S.A.

APPENDIX C

Analyses Of Dosing Formulations (WIL Research Laboratories, LLC)

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY OF H-28397 IN RATS WITH A 28-DAY RECOVERY

Analysis Of Dosing Formulations

Analytical Chemistry Department

WIL Research Laboratories, LLC

KEY STUDY PERSONNEL AND REPORT SUBMISSION

Report Submitted by: _____ 22 August 2008
Matthew C. Haas, B.A., L.A.T. — Date
Staff Toxicologist, Toxicology
Study Director

Report Prepared by: _____ 21 Aug 2008
W[m]Clue Nethero, B.A. — Date
Senior Chemist
Analytical Chemistry

Report Reviewed by: _____ 21 Aug 2008
Philip L. Stetson, M.D., Ph.D. — Date
Associate Director, Analytical Chemistry

_____ 21 August 2008
Daniel W. Sved, Ph.D. — Date
Director, Metabolism and Analytical Chemistry

Study Personnel:

Melissa A. Sjostrom, B.S.	Chemist I
Joshua J. Hiller, A.A.	Associate Chemist I
Michelle M. Flora, B.A.	Publishing Specialist, Study Analysis and Reports
Melissa A. Hull, B.S.	Group Supervisor, Regulatory Technical Services

TABLE OF CONTENTS

Page

INDEX OF FIGURES

INDEX OF TABLES

INDEX OF ATTACHMENTS

SUMMARY

A high performance liquid chromatography tandem mass spectrometry method in the negative electrospray ionization mode for the determination of H-28397 concentration in aqueous formulations containing deionized (DI) water and test article ranging in concentration from 0.00100 to 50.0 mg/mL was validated in this study. Formulations prepared at target concentrations of 0.03 and 30 mg H-28397/mL and used for dose administration were evaluated for test article homogeneity and, following room temperature storage for 5 hours or refrigerated storage for 10 days, resuspension homogeneity and stability. Formulations prepared at target concentrations of 0.03, 0.3, 3 and 30 mg H-28397/mL and used for dose administration were analyzed to confirm test article concentration. The results of these assessments were excessively variable due to unstable ionization during the analyses. Consequently, prior to the last concentration analysis, the assay was cross-validated for use with modified diluent and mobile phases. Test article stability in processed quality control (QC) samples stored at room temperature for up to 6 days was assessed and verified.

The assay for the determination of H-28397 concentration in aqueous formulations was validated in this study with 3 validation sessions. Quantitation was performed using calibration standards ranging in test article concentration from 100 to 1000 ng/mL. The mean back-calculated standard concentrations had inter-session variability ranging from 3.2% to 6.7% relative standard deviation (RSD) and percent relative error (%RE) ranging from -2.2% to 2.1%, which met the WIL standard operating procedure (SOP) acceptance criteria for calibration standards, i.e., RSD ≤ 15% and %RE within ± 15% (except at the lowest level where ± 20% is acceptable). Assay precision and accuracy were verified by the analysis of QC samples prepared at 0.00100, 0.0500, 0.500 and 50.0 mg/mL. The mean calculated QC concentrations had inter-session variability (precision) ranging from 5.9% to 11% RSD and %RE (accuracy) ranging from -1.7% to 7.2%. The results met the WIL SOP acceptance criteria for precision and accuracy, i.e., RSD ≤ 15% and %RE within ± 15%.

Several pre-initiation formulations (26 and 30 November 2007 and 5 December 2007) were attempted but had results that failed to meet the acceptable range prescribed in the WIL SOPs. Because concentration, homogeneity, stability and resuspension homogeneity of the dosing formulations were not established with any of these pre-initiation formulations, the results will remain in the study records and are not included in this report. Formulations prepared on 7 December 2007 at target test article concentrations of 0.03 and 30 mg/mL were analyzed to assess test article homogeneity and, following room temperature storage for 5 hours or refrigerated storage for 10 days, test article resuspension homogeneity and stability. The assessments met the WIL SOP acceptance criteria for test article homogeneity, i.e., the variability for the mean concentration was ≤ 10% RSD at a concentration within the acceptable limits (85% to 115% of target), resuspension homogeneity, i.e., the variability for the mean concentration was ≤ 10% RSD, and stability, i.e., the post-storage concentration was not less than 90% of the pre-storage value, with the following exceptions. The 7 December 2007

formulation prepared at the target test article concentration of 0.03 mg/mL and assessed for test article homogeneity failed to meet the WIL SOP requirement (11% RSD) and the 7 December 2007 formulation prepared at the target test article concentration of 0.03 mg/mL and stored at room temperature for 5 hours failed to meet the WIL SOP requirement for resuspension homogeneity (14% RSD) and test article stability (post-storage concentration was 82.2% of the pre-storage value). The out-of-specification results were believed to be a result of drift in ionization efficiency in the mass spectrometer. Consequently, the assay was cross-validated using modified diluent and mobile phases that increased and stabilized ionization efficiency.

The assay was cross-validated in this study with a single validation session to include the use of the modified diluent and mobile phases. Quantitation was performed using calibration standards ranging in test article concentration from 100 to 1000 ng/mL. The mean back-calculated standard concentrations had intra-session variability ranging from 3.6% to 6.4% RSD and %RE ranging from -0.47 % to 0.60%, which met the WIL SOP acceptance criteria for calibration standards, i.e., RSD $\leq$ 15% and %RE within $\pm$ 15% (except at the lowest level where $\pm$ 20% is acceptable). Assay precision and accuracy were verified by the analysis of QC samples prepared at 0.00100, 0.0500, 0.500 and 50.0 mg/mL. The mean calculated QC concentrations had intra-session variability (precision) ranging from 2.0% to 5.5% RSD and %RE (accuracy) ranging from -13% to -11%. The results met the WIL SOP acceptance criteria for precision and accuracy, i.e., RSD $\leq$ 15% and %RE within $\pm$ 15%.

Processed QC samples were analyzed, stored at room temperature for 6 days and reanalyzed to assess test article stability. The post-storage test article concentrations met WIL SOP requirement for stability, i.e., the mean post-storage concentration was not less than 90% of the pre-storage value.

Dosing formulations prepared at target test article concentrations of 0, 0.03, 0.3, 3 and 30 mg/mL were analyzed to confirm test article concentration acceptability. The results met the WIL SOP acceptance criteria for concentration acceptability in suspension formulations, i.e., the mean concentration was 85% to 115% of the target concentration with the following exception. The 7 December 2007 Group 3F formulation prepared at the target test article concentration of 3 mg/mL was 83.0% of target (other formulations were in the lower range of acceptable limits). Consequently, the study director changed the dosage volume for all groups from 10 to 12 mL/kg until 3 January 2008 when the dosing formulations prepared for week 3 were all within the specified range for concentration. Also, a formulation stock prepared as a 15% (v/v) solution was analyzed to assess test article concentration.

INTRODUCTION

This report provides a detailed description of a high performance liquid chromatography tandem mass spectrometry (HPLC/MS/MS) method in the negative electrospray ionization (ESI-) mode for the determination of H-28397 concentration in aqueous formulations containing deionized (DI) water and test article ranging in concentration from 0.00100 to 50.0 mg/mL. Method specificity/selectivity, ruggedness, calibration reproducibility, precision and accuracy were assessed. Formulations prepared at target test article concentrations of 0.03 and 30 mg/mL were analyzed to assess homogeneity and, following storage at room temperature for 5 hours or refrigerated for 10 days, test article stability. Dosing formulations prepared at target test article concentrations of 0, 0.03, 0.3, 3 and 30 mg/mL were analyzed to confirm test article concentration acceptability. Also, a formulation stock prepared as a 15% (v/v) solution was assessed for concentration. The assay was cross-validated for the determination of H-28397 concentration in aqueous formulations containing DI water and test article ranging in concentration from 0.00100 to 50.0 mg/mL using modified diluent and mobile phases. Test article stability in processed quality control (QC) samples stored at room temperature for up to 6 days was also assessed.

EXPERIMENTAL (INITIAL METHOD)

A. Instruments

The HPLC/MS/MS system used was a Waters 2695 liquid chromatograph equipped with an autosampler and a Micromass Quattro Micro™ triple quadrupole mass spectrometer equipped with an ESI- interface. Data acquisition and analysis were performed using MassLynx™ software version 4.1 or equivalent. The retention time, run time and mass spectrometer settings may have varied depending on column and mass spectrometer performance.

1. High Performance Liquid Chromatography

Instrument:	Waters 2695 liquid chromatograph equipped with an autosampler, Micromass tandem quadrupole Quattro Micro™ Mass Spectrometer and MassLynx™ software or equivalent system
Column:	Phenomenex Synergi Polar-RP 4 µm 75 × 2.0 mm
Column Temperature:	40ºC
Mobile Phase:	A: 0.15% (v/v) glacial acetic acid in deionized water B: 0.15% (v/v) glacial acetic acid in acetonitrile
Composition:	35% A, 65% B (v/v)
Flow Rate:	0.4 mL/minute
Detector:	Mass spectrometer with conditions as described in Experimental (Initial Method) Section A2. (Mass Spectrometry)
Injection Volume:	10.0 µL
Retention Time:	Approximately 0.6 minutes for H-28397
Run Time:	1.5 minutes
Injector Wash:	90:10 acetonitrile:DI water

2. Mass Spectrometry

Ion Mode:	ESI-
Capillary Voltage:	1.5 kV
Cone:	9.00 V
Extractor:	3.00 V
RF Lens:	0.4 V
Source Temperature:	100°C
Desolvation Temperature:	400°C
Cone Gas Flow:	Approximately 100 L nitrogen/hour
Desolvation Gas Flow:	Approximately 700 L nitrogen/hour

Acquisition Parameters

Function Type:	Multiple reaction monitoring
Precursor/Product Ion:	m/z 328.85/284.85 for H-28397
Collision Gas:	Argon
Collision Cell Pressure:	Approximately 3.28×10^{-3} mbar
Collision Energy:	5.0 V

B. Mobile Phase A Preparation

Mobile phase A was prepared by thoroughly mixing glacial acetic acid (GAA; 1.5 mL) in a final volume of 1000 mL of vacuum-degassed DI water. The preparation was scaled as needed.

C. Mobile Phase B Preparation

Mobile phase B was prepared by thoroughly mixing GAA (1.5 mL) in a final volume of 1000 mL of acetonitrile (ACN). The preparation was scaled as needed.

D. Preparation Of Calibration Stock Solution

A calibration stock solution was prepared at a concentration of 1 mg H-28397/mL as follows. Approximately 114 mg H-28397 (WIL log no. 7741, purity 88.0%) was accurately weighed in a 100-mL volumetric flask. DI water was added, and the preparation was stirred to achieve

complete dissolution. Additional DI water was added to achieve the desired concentration, and the solution was stirred to mix.

E. Preparation Of Secondary Calibration Stock Solution

A secondary calibration stock solution was prepared at a concentration of 0.00100 mg H-28397/mL as follows. An aliquot of the calibration stock solution was diluted 1000-fold with DI water, and the solution was stirred to mix. The secondary stock solution was prepared fresh as needed.

F. Preparation Of Calibration Samples

Calibration samples at test article concentrations of 100, 250, 500, 750 and 1000 ng H-28397/mL were prepared in triplicate for analysis by diluting aliquots of the secondary calibration stock solution with DI water in amber autosampler vials.

G. Preparation Of Quality Control Stock Solution

The QC stock solution was prepared at 50 mg H-28397/mL by accurately weighing approximately 0.57 g of H-28397 (WIL log no.7741, purity 88.0%) in a tared 10-mL volumetric flask. DI water was added, and the preparation was stirred to achieve complete dissolution. Additional DI water was added to achieve the desired concentration, and the solution was stirred to mix.

H. Preparation Of Secondary QC Stock Solution

A secondary QC stock solution was prepared at a concentration of 0.100 mg H-28397/mL as follows. An aliquot of the QC stock solution was diluted 500-fold with DI water, and the solution was stirred to mix. The secondary stock solution was prepared fresh as needed.

I. Preparation Of Quality Control Samples

As detailed in the following table, the lowest (LLQC), low (LQC), middle (MQC) and high concentration (HQC) samples were prepared at 0.00100, 0.0500, 0.500 and 50.0 mg H-28397/mL, respectively, by thoroughly mixing aliquots of the QC stock or secondary QC stock solutions and DI water in polypropylene tubes.

	QC Stock Concentration (mg/mL)	QC Stock Volume (mL)	DI Water Volume (mL)	Total Volume (mL)	QC Concentration (mg/mL)
Blank	0	0	10.0	10.0	0
LLQC	0.100	0.100	9.90	10.0	0.00100
LQC	0.100	5.00	5.00	10.0	0.0500
MQC	50.0	0.100	9.90	10.0	0.500
HQC	50.0	1.00	0	1.00	50.0

J. QC Sample Processing

As detailed in the following table, aliquots of the LLQC, LQC, MQC and HQC samples and DI water were thoroughly mixed in amber autosampler vials (blank and LLQC samples) or polypropylene tubes (LQC, MQC and HQC samples). Aliquots of the MQC and HQC diluted samples were further diluted with DI water, and the samples were mixed by vortex action. The processed LQC, MQC and HQC samples were transferred to amber autosampler vials for analysis.

	Initial Concentration (mg/mL)	QC Sample Aliquot (mL)	DI Water Volume (mL)	Secondary Dilution	Diluted Concentration (µg/mL)
Blank	0	1.00	0	NA	0
LLQC	0.00100	0.500	0.500	NA	0.500
LQC	0.0500	0.100	9.900	NA	0.500
MQC	0.500	0.100	9.900	10-fold	0.500
HQC	50.0	0.100	9.900	1000-fold	0.500

NA = Not applicable

K. Sample Processing

As detailed in the following tables, formulation samples (1.0 mL each) were diluted with DI water in polypropylene tubes. The samples were thoroughly mixed, and portions of the diluted Group 1M and 1F samples were transferred to amber autosampler vials for analysis. Secondary dilutions were performed on the remaining samples with DI water as necessary to achieve a final test article concentration of 0.500 µg/mL. Portions of the processed samples were transferred to amber autosampler vials for analysis.

Group	Nominal Concentration (µg/mL)	Sample Volume (mL)	DI Water Volume (mL)	Total Volume (mL)	Diluted Concentration (µg/mL)
1M and 1F	0	1.0	9.00	10	0
2M	30.0	1.0	9.00	10	3.00
2F and 3M	300	1.0	39.00	40	7.50
4M	3000	1.0	39.00	40	75.0
3F	3000	1.0	39.00	40	75.0
4F	30,000	1.0	39.00	40	750

Secondary Dilutions:

Group	Initial Diluted Concentration (µg/mL)	Aliquot Volume (mL)	DI Water Volume (mL)	Final Volume (mL)	Theoretical Final Concentration (µg/mL)
2M	3.00	0.250	1.250	1.50	0.500
2F + 3M	7.50	0.100	1.40	1.50	0.500
4M	75.0	0.100	14.90	15.0	0.500
3F	75.0	0.100	14.90	15.0	0.500
4F	750	0.100	9.90	10.0	0.500*

* = 15-fold tertiary dilution

L. Preparation Of Backup Samples

Formulation samples (1.0 mL each) were collected and stored in polypropylene tubes. The samples were stored frozen (approximately -20°C) in the Analytical Chemistry Department.

M. Concentration Quantitation

Single injections were made of each calibration standard and processed QC and formulation sample. A calibration curve was constructed for each set of analysis. Using MassLynx™, the H-28397 peak areas (y) and the theoretical concentrations of the calibration standards (x) were fit to a quadratic function using least-squares regression analysis.

$$\ln (y) = a \times [\ln (x)]^2 + b \times \ln (x) + c$$

Concentration and percent relative error (%RE) were calculated using MassLynx™. The concentration data were transferred to an Excel spreadsheet, where appropriate summary statistics, i.e., mean, standard deviation (SD), relative standard deviation (RSD) and %RE, were calculated and presented in tabular form. The concentrations of the dosing formulations and QC samples were calculated by applying any necessary multiplication factors.

EXPERIMENTAL (MODIFIED METHOD)

A. Instruments

The HPLC/MS/MS system used was a Waters 2695 liquid chromatograph equipped with an autosampler and a Micromass Quattro Micro™ triple quadrupole mass spectrometer equipped with an ESI- interface. Data acquisition and analysis were performed using MassLynx™ software version 4.1 or equivalent. The retention time, run time and mass spectrometer settings may have varied depending on column and mass spectrometer performance.

1. High Performance Liquid Chromatography

Instrument:	Waters 2695 liquid chromatograph equipped with an autosampler, Micromass tandem quadrupole Quattro Micro™ Mass Spectrometer and MassLynx™ software or equivalent system
Column:	Phenomenex Synergi Polar-RP 4 µm 75 × 2.0 mm
Column Temperature:	40ºC
Mobile Phase:	A: 90:10 (v/v) 5 mM ammonium acetate in deionized water, pH 2.5:acetonitrile B: 10:90 (v/v) 5 mM ammonium acetate in deionized water, pH 2.5:acetonitrile
Composition:	50% A, 50% B (v/v)
Flow Rate:	0.4 mL/minute
Detector:	Mass spectrometer with conditions as described in Experimental (Modified Method) Section A2. (Mass Spectrometry)
Injection Volume:	10 µL
Retention Time:	Approximately 0.6 minutes for H-28397
Run Time:	1.0 minutes
Injector Wash:	90:10 (v/v) acetonitrile:deionized water

2. Mass Spectrometry

Ion Mode:	ESI-
Capillary Voltage:	1.50 kV
Cone:	9.00 V
Extractor:	3.00 V
RF Lens:	0 .4 V
Source Temperature:	100°C
Desolvation Temperature:	400°C
Cone Gas Flow:	Approximately 100 L nitrogen/hour
Desolvation Gas Flow:	Approximately 700 L nitrogen/hour

Acquisition Parameters

Function Type:	Multiple reaction monitoring
Precursor/Product Ion:	m/z 328.85/284.85 for H-28397
Collision Gas:	Argon
Collision Cell Pressure:	Approximately 3.28×10^{-3} mbar
Collision Energy:	5.0 V

B. Preparation Of Diluent And Buffer (5 mM Ammonium Acetate In DI Water, pH 2.5)

Ammonium acetate (0.77 g) was dissolved in a final volume of 2 liters of DI water and vacuum-filtered through a 0.45-µm nylon membrane. The solution was transferred into a storage bottle, and the pH was adjusted to approximately 2.5 with GAA. The solution was also used as a buffer for preparation of mobile phase A and mobile phase B. In addition, the solution was used as the diluent in the preparation of the calibration and QC stock solutions and the processing of the QC stock solutions and formulation samples. The preparation was scaled as needed.

C. Mobile Phase A Preparation (90:10 [v/v] 5 mM Ammonium Acetate In DI Water, pH 2.5: Acetonitrile)

Ammonium acetate buffer (900 mL) and 100 mL of ACN were thoroughly mixed and degassed by sonication. The solution was stored at room temperature for up to 1 month. The preparation was scaled as needed.

D. Mobile Phase B Preparation (10:90 5 mM Ammonium Acetate In DI Water, pH 2.5: Acetonitrile)

Ammonium acetate buffer (100 mL) and 900 mL of ACN were thoroughly mixed and degassed by sonication. The solution was stored at room temperature for up to 1 month. The preparation was scaled as needed.

E. Preparation Of Calibration Stock Solution

A calibration stock solution was prepared at a concentration of 1 mg H-28397/mL as follows. Approximately 114 mg H-28397 (WIL log no. 7741, purity 88.0%) was accurately weighed in a 100-mL volumetric flask. Diluent was added, and the preparation was stirred to achieve complete dissolution. Additional diluent was added to achieve the desired concentration, and the solution was stirred to mix.

F. Preparation Of Secondary Calibration Stock Solution

A secondary calibration stock solution was prepared at a concentration of 0.00100 mg H-28397/mL as follows. An aliquot of the calibration stock solution was diluted 1000-fold with diluent. The secondary calibration stock solution was prepared fresh as needed.

G. Preparation Of Calibration Samples

Calibration samples at test article concentrations of 100, 250, 500, 750 and 1000 ng H-28397/mL were prepared in triplicate for analysis by diluting aliquots of the secondary calibration stock solution with diluent in amber autosampler vials.

H. Preparation Of Quality Control Stock Solution

The QC stock solution was prepared at 50 mg H-28397/mL by accurately weighing approximately 0.57 g of H-28397 (WIL log no.7741, purity 88.0%) in a tared 10-mL volumetric flask. Diluent was added, and the preparation was stirred to achieve complete dissolution. Additional diluent was added to achieve the desired concentration, and the solution was stirred to mix.

I. Preparation Of Secondary QC Stock Solution

A secondary QC stock solution was prepared at a concentration of 0.100 mg H-28397/mL as follows. An aliquot of the QC stock solution was diluted 500-fold with diluent, and the solution was stirred to mix. The secondary QC stock solution was prepared fresh as needed.

J. Preparation Of Quality Control Samples

As detailed in the following table, the LLQC, LQC, MQC and HQC samples were prepared at 0.00100, 0.0500, 0.500 and 50.0 mg H-28397/mL, respectively, by thoroughly mixing aliquots of the QC stock or secondary QC stock solutions, DI water and diluent in polypropylene tubes.

	QC Stock Concentration (mg/mL)	DI Water Volume (mL)	Diluent Volume (mL)	QC Stock Volume (mL)	Total Volume (mL)	QC Concentration (mg/mL)
Blank	0	1.00	9.00	0	10.0	0
LLQC	0.100	1.00	8.90	0.100	10.0	0.00100
LQC	0.100	1.00	4.00	5.00	10.0	0.0500
MQC	50.0	1.00	8.90	0.100	10.0	0.500
HQC	50.0	0	0	1.00	1.00	50.0

K. QC Sample Processing

As detailed in the following table, aliquots of the LLQC, LQC, MQC and HQC samples and diluent were thoroughly mixed in amber autosampler vials (blank and LLQC samples) or polypropylene tubes (LQC, MQC and HQC samples). Aliquots of the MQC and HQC diluted samples were further diluted with diluent, and the samples were mixed by vortex action. Portions of the processed LQC, MQC and HQC samples were transferred to amber autosampler vials for analysis.

	Initial Concentration (mg/mL)	QC Sample Aliquot (mL)	Diluent Volume (mL)	Secondary Dilution	Diluted Concentration (µg/mL)
Blank	0	0.500	0.500	NA	0
LLQC	0.00100	0.500	0.500	NA	0.500
LQC	0.0500	0.100	9.900	NA	0.500
MQC	0.500	0.100	9.900	10-fold	0.500
HQC	50.0	0.100	9.900	1000-fold	0.500

NA = Not applicable

L. Sample Processing

As detailed in the following tables, formulation samples (1.0 mL each) were diluted with diluent in polypropylene tubes. The samples were thoroughly mixed, and portions of the diluted Group 1M + 1F samples were transferred to amber autosampler vials for analysis. Secondary dilutions were performed on the remaining samples with diluent as necessary to achieve a final test article concentration of 0.500 µg/mL. Portions of the processed samples were transferred to amber autosampler vials for analysis.

Group	Target Concentration (µg/mL)	Sample Volume (mL)	Diluent Volume (mL)	Total Volume (mL)	Diluted Concentration (µg/mL)
1M and 1F	0	1.0	9.00	10	0
2M	30.0	1.0	39.00	40	0.750
2F and 3M	300	1.0	39.00	40	7.50
4M	3000	1.0	39.00	40	75.0
3F	3000	1.0	39.00	40	75.0
4F	30000	1.0	39.00	40	750

Secondary Dilutions:

Group	Initial Diluted Concentration (µg/mL)	Aliquot Volume (mL)	Diluent Volume (mL)	Final Volume (mL)	Theoretical Final Concentration (µg/mL)
2M	0.750	0.800	0.400	1.20	0.500
2F and 3M	7.50	0.800	11.200	12.0	0.500
4M	75.0	0.080	11.920	12.0	0.500
3F	75.0	0.080	11.920	12.0	0.500
4F	750	0.080	119.920	120	0.500

M. Preparation Of Backup Samples

Formulation samples (1.0 mL each) were collected and stored in polypropylene tubes. The samples were stored frozen (approximately -20°C) in the Analytical Chemistry Department.

N. Concentration Quantitation

Single injections were made of each calibration standard and processed QC and formulation sample. A calibration curve was constructed for each set of analysis. Using MassLynx™, the H-28397 peak areas (y) and the theoretical concentrations of the calibration standards (x) were fit to a quadratic function using least-squares regression analysis.

$$\ln (y) = a \times [\ln (x)]^2 + b \times \ln (x) + c$$

Concentration and %RE were calculated using MassLynx™. The concentration data were transferred to an Excel spreadsheet, where appropriate summary statistics, i.e., SD, RSD and %RE, were calculated and presented in tabular form. The concentrations of the dosing formulations and QC samples were calculated by applying any necessary multiplication factors.

RESULTS AND DISCUSSION

Under the described chromatographic conditions, the retention time of the test article was approximately 0.6 minutes. Figures 1, 2, 3 and 4 are typical chromatograms of a processed calibration standard, a processed QC sample, a processed formulation sample, and a processed blank QC sample, respectively, using the initial assay. Figures 5, 6, 7 and 8 are typical chromatograms of a calibration standard, a processed QC sample, a processed formulation sample, and a processed vehicle sample, respectively, using the modified assay. The total analysis time required for each run was approximately 1.5 minutes for the initial assay and 1.0 minute for the modified assay.

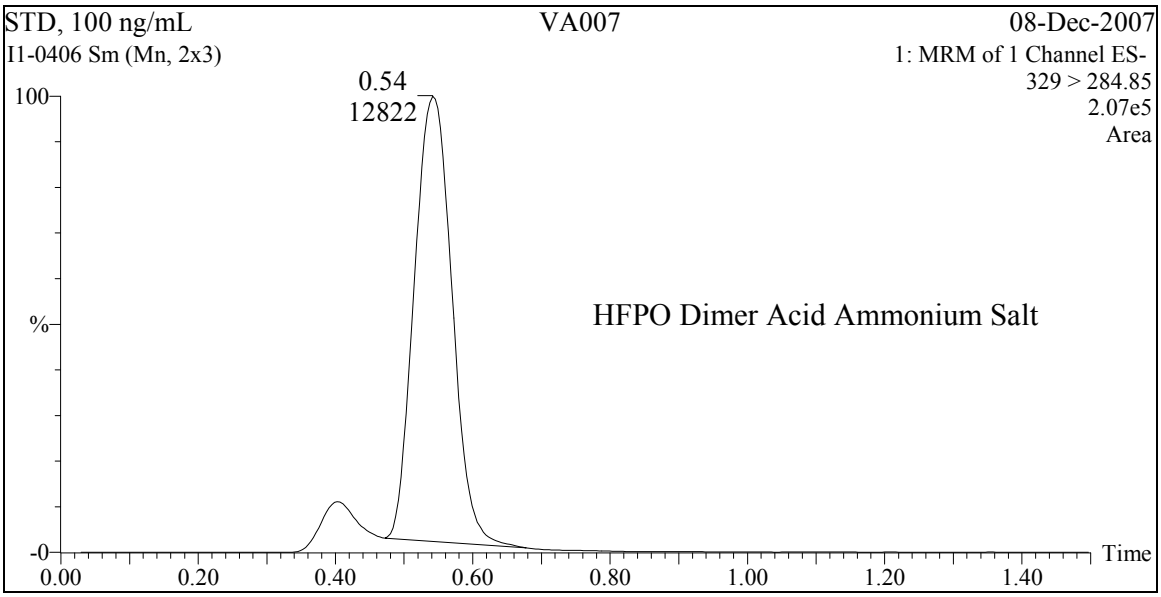

Figure 1: Representative Chromatogram Of A 100 ng H-28397/mL Calibration Standard

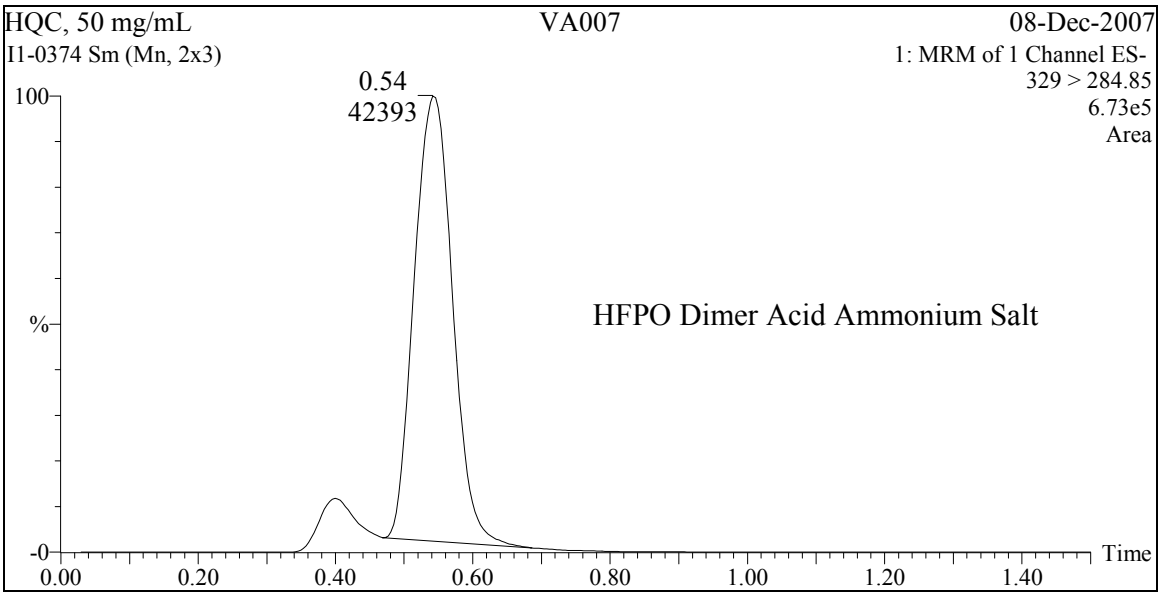

Figure 2: Representative Chromatogram Of A Processed 50 mg H-28397/mL Quality Control Sample

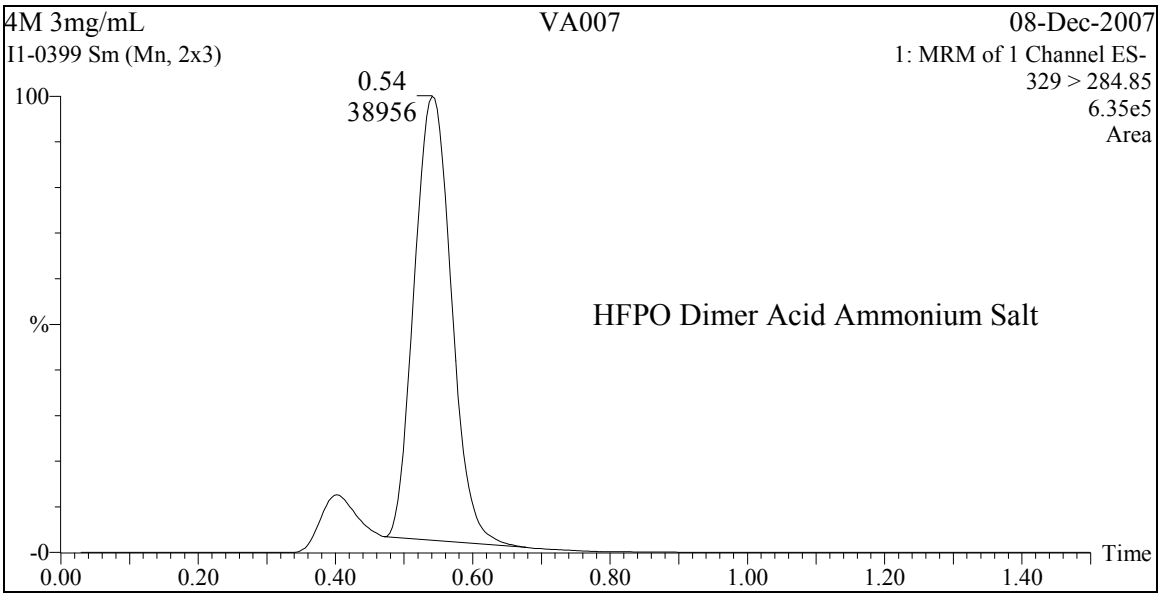

Figure 3: Representative Chromatogram Of A Processed 3 mg H-28397/mL Formulation Sample

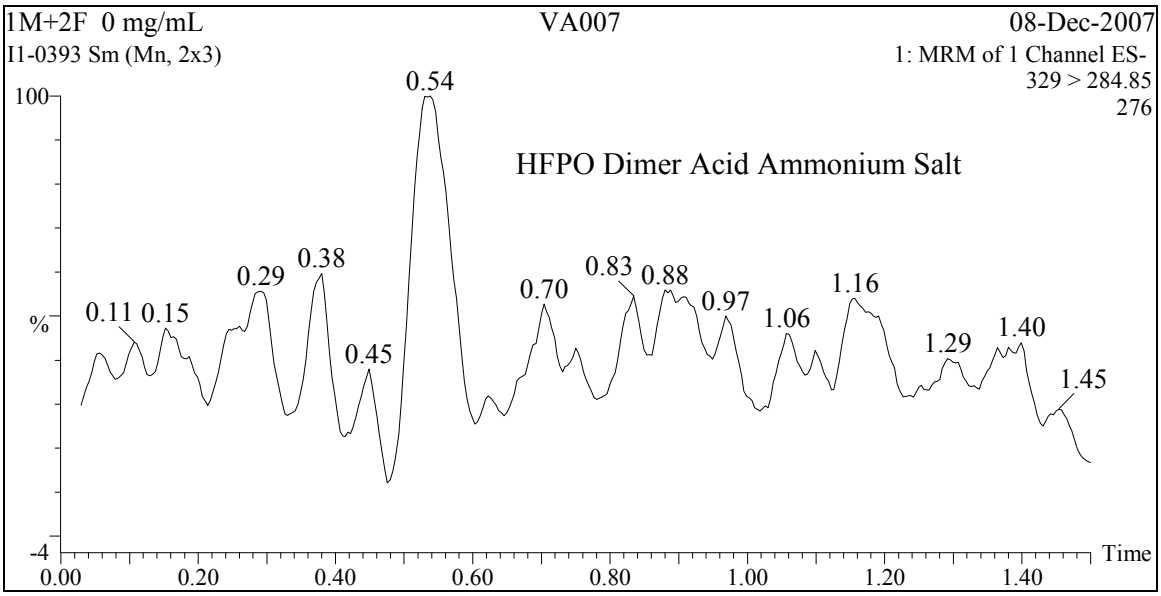

Figure 4: Representative Chromatogram Of A Processed Control Formulation Sample

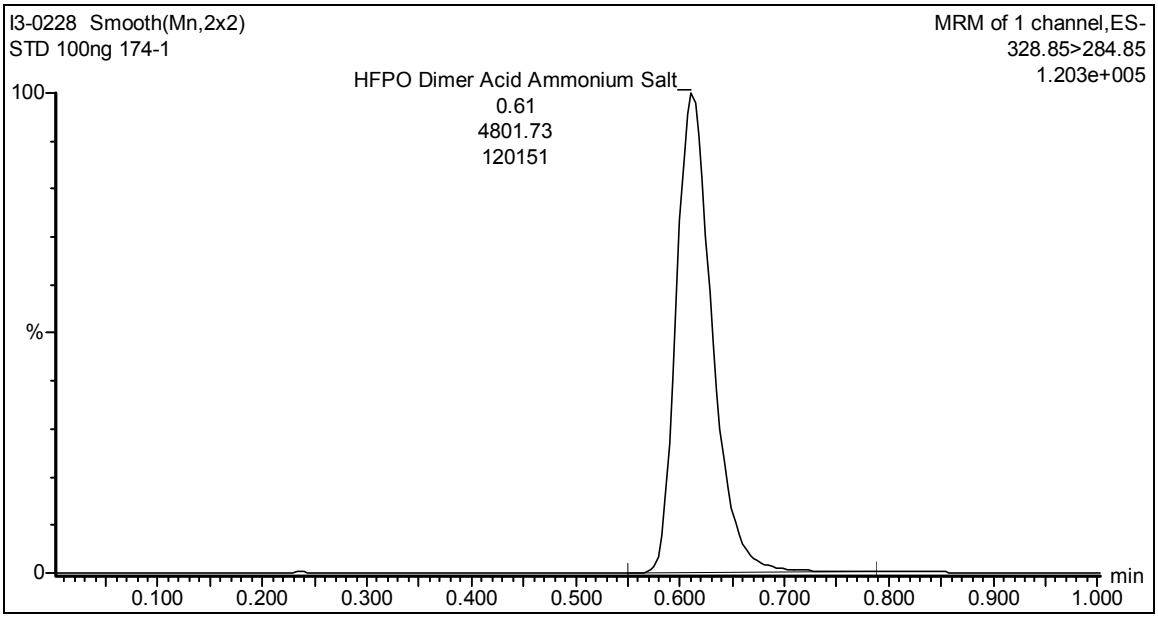

Figure 5: Representative Chromatogram Of A 100 ng H-28397/mL Calibration Standard

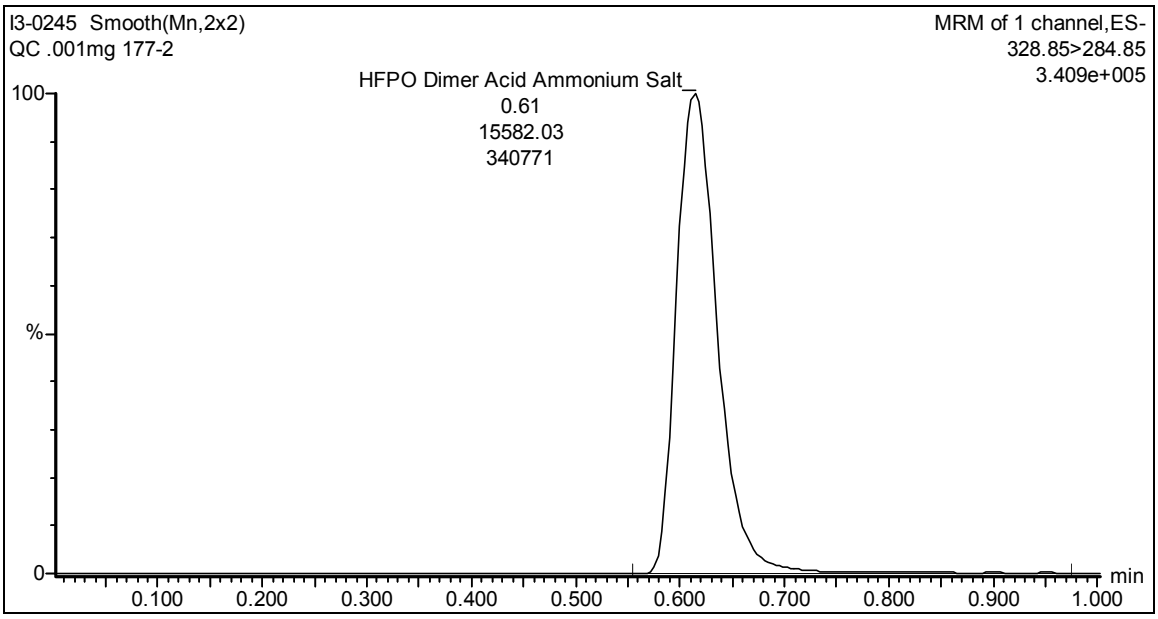

Figure 6: Representative Chromatogram Of A Processed 0.00100 mg H-28397/mL Quality Control Sample

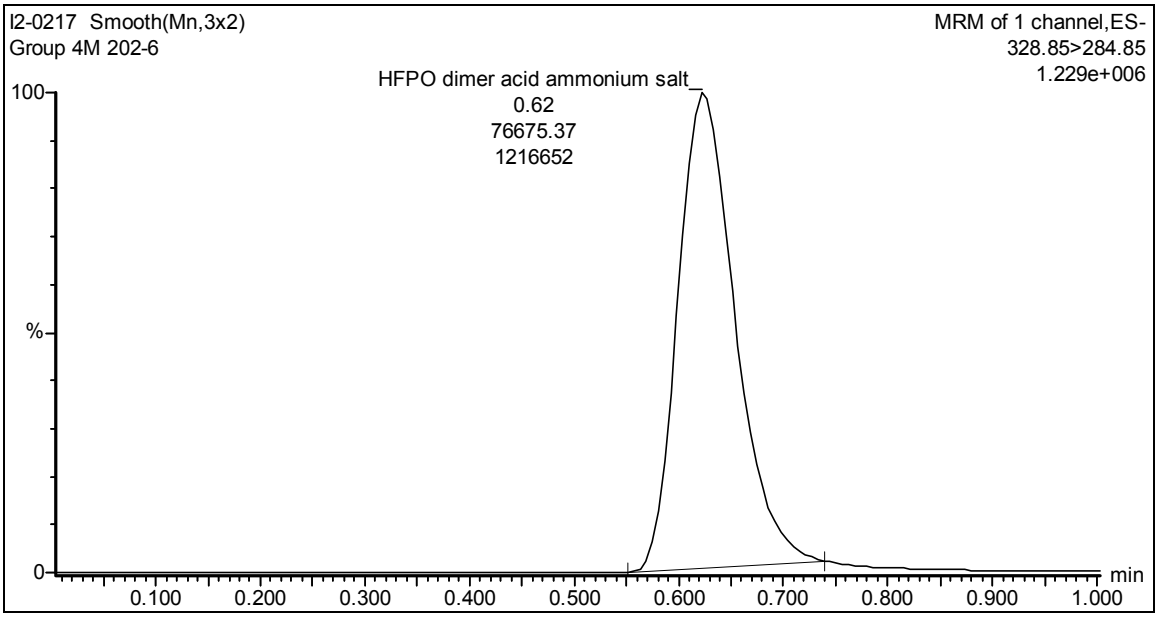

Figure 7: Representative Chromatogram Of A Processed 3 mg H-28397/mL Formulation Sample

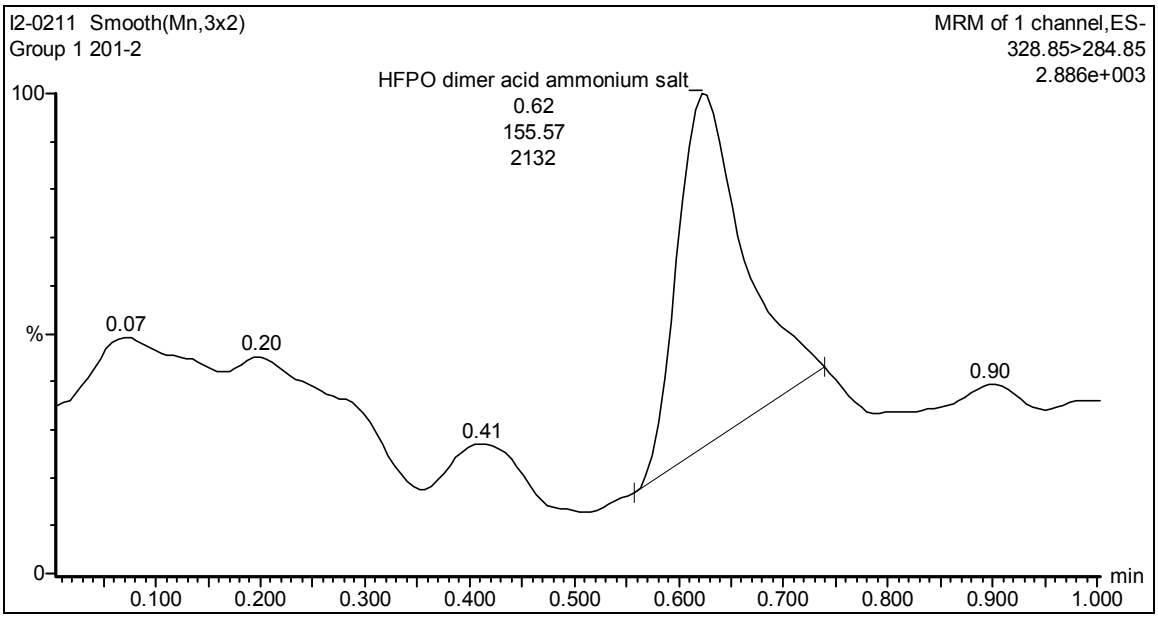

Figure 8: Representative Chromatogram Of A Processed Control Formulation Sample

A. Specificity/Selectivity

As shown in Figures 4 and 8 (and in contrast to the chromatograms shown in Figures 1 through 3 and 5 through 7, respectively), assay specificity/selectivity was confirmed when HPLC/MS/MS analysis of the control formulation samples revealed that there were no significant peaks at or near the retention time for the test article (approximately 0.6 minutes).

B. Assay Validation: Calibration Reproducibility (Initial Assay)

During each of 3 validation sessions, triplicate calibration standards at 5 concentrations were prepared and analyzed as described previously. Single injections were made of each calibration standard. The resulting test article peak area versus theoretical test article concentration data were fit to the ln-quadratic function using least-squares regression analysis. The results of the regression analyses were used to back-calculate the corresponding concentrations from the peak area data. As stated in the WIL standard operating procedures (SOP), the reproducibility of the calibration curve data was considered valid when 1) the inter-session variability of the back-calculated concentrations at each calibration level was ≤ 15% RSD, except at the lowest calibration level where ≤ 20% was acceptable and; 2) the mean back-calculated concentrations at each calibration level were within ± 15% of the theoretical values (%RE within ± 15%), except at the lowest calibration level where %RE within ± 20% was acceptable.

The back-calculated concentrations and the associated inter-session statistics for the H-28397 assay calibration standards are summarized in Table 1. The inter-session variability of the back-calculated concentrations ranged from 3.2 % to 6.7% RSD. The inter-session mean concentrations had %RE values ranging from -2.2% to 2.1%. Based on the stated criteria, the reproducibility of the calibration data was acceptable.

C. Assay Validation: Precision And Accuracy (Initial Assay)

During each of 3 validation sessions, triplicate QC samples at test article concentrations of 0.00100, 0.0500. 0.500 and 50.0 mg/mL were prepared and analyzed as described previously. Single injections were made of each processed QC sample. The results of the regression analyses were used to calculate the corresponding concentrations from the QC peak area data. The variability of the calculated QC concentration data was used as a measure of assay precision. As stated in the WIL SOP, the precision of the method was considered acceptable when the intra- and inter-session RSD of the calculated concentrations at each QC level was ≤ 15%. The difference between the theoretical and calculated mean QC concentrations (%RE) was used as a measure of assay accuracy. As stated in the WIL SOP, the accuracy of the method was considered acceptable when the inter-session calculated mean concentration at each QC level had a %RE value within ± 15%.

The calculated concentrations and the associated intra- and inter-session statistics for the QC samples are summarized in Table 2. The inter-session variability of the calculated concentrations for the QC samples (precision) ranged from 5.9% to 11% RSD. The inter-session mean concentrations had %RE values (accuracy) ranging from -1.7% to 7.2%. Based on the stated criteria, the precision and accuracy of the H-28397 assay were acceptable.

D. Assay Ruggedness

Assay ruggedness, as required by WIL SOP, was successfully demonstrated for this method because more than 1 analyst successfully performed at least 1 of the validation sessions.

E. Assay Cross-Validation (Modified Assay)

The H-28397 assay validation was cross-validated in this study with a single validation session to include the use of modified mobile phases and diluent. The calculated concentrations and the associated intra-session statistics for the calibration standards and QC samples are summarized in Tables 3 and 4, respectively. Quantitation was performed using calibration standards ranging in test article concentration from 100 to 1000 ng/mL. The mean back-calculated standard concentrations had intra-session variability ranging from 3.6% to 6.4% RSD and %RE ranging from -0.47 % to 0.60%, which met the WIL SOP acceptance criteria for calibration standards, i.e., RSD ≤ 15% and %RE within ± 15% (except at the lowest level where 20% is acceptable). Assay precision and accuracy were verified by the analysis of QC samples prepared at

0.00100, 0.0500, 0.500 and 50.0 mg H-28397/mL. The mean calculated QC concentrations had intra-session variability (precision) ranging from 2.0% to 5.5% RSD and %RE (accuracy) ranging from -13% to -11%. The results met the WIL SOP acceptance criteria for precision and accuracy, i.e., RSD ≤ 15% and %RE within ± 15%.

F. Assay Acceptability

In addition to the experimental samples, each analytical session consisted of (but was not limited to) calibration standards at 5 concentrations and triplicate QC samples at each of 3 concentrations. In this study, the formulations were prepared at theoretical test article concentrations of 0.03, 0.3, 3 and 30 mg/mL and the QC samples were prepared at target test article concentrations of 0.00100, 0.0500, 0.500 and 50.0 mg/mL. For an analytical session to be considered valid, at least two-thirds of the QC samples with at least 1 at each concentration level had to be 85% to 115% of the QC target concentration. All reported results were from analytical sessions that met the acceptance criteria.

G. Test Article Stability In Processed QC Samples (Modified Assay)

QC samples, prepared, processed and analyzed on 21 December 2007, were stored at room temperature for 6 days and reanalyzed to assess test article stability. The mean post-storage test article concentrations ranged from 115% to 124% of the pre-storage values (Table 5), which met the WIL SOP requirement for stability, i.e. the post-storage concentration was not less than 90% of the pre-storage value.

H. Assessment Of Test Article Homogeneity And Resuspension Homogeneity

Duplicate samples from the top, middle and bottom strata of formulations prepared at target test article concentrations of 0.03 and 30 mg H-28397/mL on 7 December 2007 were analyzed to assess test article homogeneity. The formulations that remained after sampling were divided into aliquots representative of the volumes used for daily dispensation. Representative aliquots were stored at room temperature for 5 hours and refrigerated for 10 days, at which times, the test article was resuspended by stirring. Samples were collected from the top and bottom strata of the aliquots and analyzed to assess resuspension homogeneity. The results of the homogeneity and the 5-hour and 10-day resuspension homogeneity analyses are presented in Tables 6 through 8 with the overall statistics summarized in the following tables.

Homogeneity Assessment Of The 7 December 2007 Formulations

	Group 2M (0.03 mg/mL)	Group 4F (30 mg/mL)
Mean Concentration (mg/mL)	0.0331	26.0
SD	0.0037	1.3
RSD (%)	11	5.0
Mean % of Target	110	86.6

The formulation prepared at the target test article concentration of 30 mg/mL met the WIL SOP acceptance criteria for test article homogeneity, i.e., the RSD for the mean concentration was 10% or less at a concentration within the acceptable limits (85% to 115% of target concentration). The formulation prepared at the target test article concentration of 0.03 mg/mL failed to meet the stated WIL SOP acceptance criteria (11% RSD).

5-Hour Resuspension Homogeneity Assessment 7 December 2007 Formulations

	Group 2M (0.03 mg/mL)	Group 4F (30 mg/mL)
Mean Concentration (mg/mL)	0.0272	25.0
SD	0.0037	1.1
RSD (%)	14	4.3
Mean % of Target	90.6	83.2

10-Day Resuspension Homogeneity Assessment 7 December 2007 Formulations

	Group 2M (0.03 mg/mL)	Group 4F (30 mg/mL)
Mean Concentration (mg/mL)	0.0338	29.5
SD	0.0014	2.6
RSD (%)	4.1	8.8
Mean % of Target	113	98.5

The formulations stored at room-temperature for 5 hours or refrigerated for 10 days met the WIL SOP acceptance criteria for resuspension homogeneity, i.e., the RSD for the mean concentration was 10% or less, with the following exception. Following 5 hours of room temperature storage, the RSD for the mean concentration of the formulation prepared at 0.03 mg/mL was 14%. The out-of-specification results were believed to be a result of drift in ionization efficiency in the mass spectrometer. Consequently, the assay was cross-validated with modified diluent and mobile phases.

I. Test Article Stability In Aqueous Formulations

The formulations prepared on 7 December 2007 at target test article concentrations of 0.03 and 30 mg/mL were analyzed, stored at room-temperature for 5 hours or at refrigerated temperature for 10 days and reanalyzed to assess test article stability. The stability results are presented in Tables 6 through 8 and are summarized in the following table.

	Mean Concentration, mg/mL (% of Pre-Storage)	
Time Point	Group 2M (0.03 mg/mL)	Group 4F (30 mg/mL)
5 Hours	0.0272 (82.2)	25.0 (96.0)
10 Days	0.0338 (102)	29.5 (114)

The mean post-storage concentrations ranged from 82.2% to 114% of the pre-storage values, which met the previously stated WIL SOP requirement for stability, with the following exception. The post-storage test article concentration in the formulation prepared at a target concentration of 0.03 mg/mL and stored at room-temperature for 5 hours was 82.2% of the pre-storage value. Again, the out-of-specification results were believed to be a result of drift in ionization efficiency in the mass spectrometer. Consequently, the assay was cross-validated with modified diluent and mobile phases.

J. Test Article Concentration In Aqueous Formulations

The results of the determination of test article concentration in formulations used for dose administration are presented in Tables 9 and 10. The mean concentrations and percent of target values are summarized in the following table.

Date of Preparation	Group 1M and 1F (0 mg/mL)	Group 2M (Low) (0.03 mg/mL)	Group 2F and 3M (0.3 mg/mL)	Group 3F (3 mg/mL)	Group 4M (3 mg/mL)	Group 4F (High) (30 mg/mL)
7 December 2007	ND	0.0336 (112)	0.272 (90.7)	2.49 (83.0)	2.59 (86.5)	27.2 (90.8)
28 December 2007	< LLOQ	0.0282(93.9)	0.296 (98.5)	2.94 (98.1)	3.02 (101)	29.9 (99.6)

ND = No test article chromatographic peak detected
< LLOQ = Less than lower limit of quantitation

The analyzed formulations used for dose administration met the WIL SOP requirement for concentration acceptability for suspension formulations, i.e., the analyzed concentrations were 85% to 115% of the target concentrations with 1 exception. The group 3F formulation prepared on 7 December 2007 at a target test article concentration of 3 mg/mL was 83.0% of target. Consequently, the study director changed the dosage volume from 10 to 12 mL/kg for all of the groups until 3 January 2008, when results of the 28 December 2007 concentration analysis were within all specifications.

K. Test Article Concentration In A 15% (v/v) Formulations Stock Solution

The results of the determination of test article concentration in a 15% (v/v) formulation stock solution used to prepare dose formulations are presented in Table 11. The mean concentrations were between 211 and 241 mg/mL.

CONCLUSION

An HPLC/MS/MS method in the ESI- mode for the determination of H-28397 concentration in aqueous formulations was validated in this study. Aqueous formulations prepared at target test article concentrations of 0.03 and 30 mg/mL met the previously stated WIL SOP acceptance criteria for test article homogeneity, resuspension homogeneity and stability, with the following exceptions. The 7 December 2007 formulation prepared at the target test article concentration of 0.03 mg/mL and assessed for test article homogeneity failed to meet the WIL SOP requirement (11% RSD), and the 7 December 2007 formulation prepared at the target test article concentration of 0.03 mg/mL and stored at room temperature for 5 hours failed to meet the WIL SOP requirement for resuspension homogeneity (14% RSD) and test article stability (post-storage concentration was 82.2% of the pre-storage value). The out-of-specification results were believed to be a result of drift in ionization efficiency in the mass spectrometer. Consequently, prior to the last concentration analysis, the assay was cross-validated for the determination of H-28397 concentration in aqueous formulations containing DI water and test article ranging in concentration from 0.00100 to 50.0 mg/mL using modified diluent and mobile phases. Test article stability in processed QC samples stored at room temperature for up to 6 days was assessed and verified.

The analyzed formulations used for dose administration met the WIL SOP requirement for concentration acceptability for suspension formulations with the following exception. The 7 December 2007 Group 3F formulation prepared at the target test article concentration of 3 mg/mL was 83.0% of target. Other formulations were in the lower range of acceptable limits. Consequently, the study director changed the dosage volume from 10 to 12 mL/kg until 3 January 2008 when the dosing formulations prepared for week 3 were all within the specified range for dose concentration. Also, a formulation stock prepared as a 15% (v/v) solution was analyzed.

TABLES 1 - 11

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY

Table 1: Back-Calculated Concentrations Of The Validation Calibration Standards

Theoretical Concentration (ng/mL)	**100**	**250**	**500**	**750**	**1000**
Set 1	97.8	265	491	795	970
(21 November 2007)	99.4	242	473	753	990
Ruggedness	100	271	475	698	1105
Mean	99.2	259	480	748	1021
SD	1.3	15	10	49	73
%RSD	1.3	5.9	2.1	6.5	7.1
%RE	-0.79	3.7	-4.1	-0.23	2.1
Set 2	95.7	242	491	678	957
(26 November 2007)	106	271	487	775	1093
	97.1	256	481	795	997
Mean	99.6	256	486	749	1016
SD	5.5	15	4.9	63	70
%RSD	5.5	5.7	1.0	8.4	6.9
%RE	-0.43	2.5	-2.8	-0.075	1.6
Set 3	96.8	256	476	771	935
(26 November 2007)	104	248	519	681	1043
	99.0	246	508	799	1029
Mean	100	250	501	750	1003
SD	3.9	5.3	23	62	59
%RSD	3.9	2.1	4.5	8.2	5.9
%RE	0.043	0.038	0.18	0.0010	0.28
Interset Statistics					
n	9	9	9	9	9
Mean	99.6	255	489	749	1013
SD	3.5	12	16	50	59
%RSD	3.5	4.5	3.2	6.7	5.8
%RE	-0.39	2.1	-2.2	-0.10	1.3

189205 results.xls I
Printed: 22May2008 1:24 PM

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY

Table 2: Calculated Concentrations Of The Validation Quality Control Samples

Theoretical Concentration (mg/mL)	**0.00100**	**0.0500**	**0.500**	**50.0**
Set 1	0.00115	0.0522	0.563	44.6
(21November2007)	0.00114	0.0438	0.554	47.0
Ruggedness	0.00123	0.0447	0.545	45.9
Mean	0.00117	0.0469	0.554	45.9
SD	0.000052	0.0046	0.0092	1.2
%RSD	4.5	9.9	1.7	2.6
%RE	17	-6.2	11	-8.3
Set 2	0.000895	0.0461	0.508	50.3
(26November2007)	0.00104	0.0489	0.555	56.7
	0.000928	0.0464	0.524	59.8
Mean	0.000954	0.0472	0.529	55.6
SD	0.000076	0.0016	0.024	4.9
%RSD	7.9	3.3	4.5	8.7
%RE	-4.6	-5.7	5.8	11
Set 3	0.00103	0.0542	0.474	56.2
(26November2007)	0.00114	0.0545	0.540	57.2
	0.00110	0.0514	0.490	52.5
Mean	0.00109	0.0534	0.501	55.3
SD	0.000058	0.0017	0.034	2.5
%RSD	5.3	3.1	6.9	4.5
%RE	9.1	6.7	0.26	11
Interset Statistics				
n	9	9	9	9
Mean	0.00107	0.0491	0.528	52.3
SD	0.00011	0.0041	0.031	5.6
%RSD	10	8.3	5.9	11
%RE	7.2	-1.7	5.6	4.5

189205 results.xls II
Printed: 22May2008 1:24 PM

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY

Table 3: Calculated Concentrations Of The Cross-Validation Calibration Standards
Ammonium Acetate Mobile Phase and Diluent Cross-Validation

Theoretical Concentration (ng/mL)	**100**	**250**	**500**	**750**	**1000**
Cross-Validation (21 Dec 2007)	98.8	259	501	732	971
	97.0	240	522	728	967
	104	255	470	786	1080
Intraset Statistics					
n	3	3	3	3	3
Mean	99.9	251	498	749	1006
SD	3.6	10	26	32	64
%RSD	3.6	4.0	5.3	4.3	6.4
%RE	-0.067	0.53	-0.47	-0.18	0.60

189205 results.xls XVal stds
Printed: 05/22/08 1:24 PM

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY

Table 4: Calculated Concentrations Of The Cross-Validation Quality Control Samples
Ammonium Acetate Mobile Phase and Diluent Cross-Validation

Theoretical Concentration (mg/mL)	**0.00100**	**0.0500**	**0.500**	**50.0**
Cross-Validation (21 Dec 2007)	0.000879	0.0425	0.460	45.1
	0.000885	0.0442	0.415	42.6
	0.000916	0.0436	0.425	42.8
Intraset Statistics				
n	3	3	3	3
Mean	0.000893	0.0434	0.433	43.5
SD	0.000020	0.00086	0.024	1.4
%RSD	2.2	2.0	5.5	3.2
%RE	-11	-13	-13	-13

189205 results.xls XVal QCs
Printed: 05/22/08 1:24 PM

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY

Table 5: 6 Day Room Temperature Process Sample Stability Of The 21 December 2007 QC Samples

(Analyzed 27 December 2007)
Comparison of Concentration

Time Point	Date Analyzed	Theo. Conc (mg/mL)	Ref # (189205 -)	Run #	Calculated Conc. (mg/mL)	Percent of Time Zero (%)	Mean Conc. (mg/mL)	Mean % of Time Zero
Quality Control Samples				(189205 -)				
T = 0	21Dec2007	0.00100	177 - 2	I3-0245	0.000879	NA	0.000893	N/A
			177 - 3	I3-0246	0.000885	NA		
			177 - 4	I3-0247	0.000916	NA		
				(189207 -)				
T = 6-Day	27Dec2007		177 - 2	I3-0397	0.00100	114	0.00103	115
			177 - 3	I3-0398	0.00107	120		
			177 - 4	I3-0399	0.00101	110		
				(189205 -)				
T = 0	21Dec2007	0.0500	177 - 5	I3-0248	0.0425	NA	0.0434	N/A
			177 - 6	I3-0249	0.0442	NA		
			177 - 7	I3-0250	0.0436	NA		
				(189207 -)				
T = 6-Day	27Dec2007		177 - 5	I3-0400	0.0508	119	0.0501	115
			177 - 6	I3-0401	0.0510	115		
			177 - 7	I3-0402	0.0485	111		
				(189205 -)				
T = 0	21Dec2007	0.500	178 - 1	I3-0251	0.460	NA	0.433	N/A
			178 - 2	I3-0252	0.415	NA		
			178 - 3	I3-0253	0.425	NA		
				(189207 -)				
T = 6-Day	27Dec2007		178 - 1	I3-0403	0.549	119	0.535	124
			178 - 2	I3-0404	0.513	124		
			178 - 3	I3-0405	0.545	128		
				(189205 -)				
T = 0	21Dec2007	50.0	178 -4	I3-0254	45.1	NA	43.5	N/A
			178 -5	I3-0255	42.6	NA		
			178 -6	I3-0256	42.8	NA		
				(189207 -)				
T = 6-Day	27Dec2007		178 -4	I3-0406	53.7	119	52.3	120
			178 -5	I3-0407	52.2	122		
			178 -6	I3-0408	50.9	119		

189205 results.xls 6pssQC(12-28-07)
Printed: 05/22/08 1:24 PM

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY

Table 6: Homogeneity Of The 7 December 2007 Formulations

Dose Conc (mg/mL)	Group/ Strata		Ref # (189205 -)	Run #	Analyzed Conc (mg/mL)	Percent of Target (%)	Mean Conc (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)
0.03	2M/Top	*	140 - 1	I1-0376	0.0294	98.0	0.0331	0.0037	11	110
		*	140 - 2	I1-0377	0.0289	96.3				
		**	159 - 1	I3-0169	0.0385	128				
		**	159 - 2	I3-0170	0.0328	109				
	2M/Mid	*	140 - 3	I1-0378	0.0279	93.0				
		*	140 - 4	I1-0379	0.0330	110				
		**	159 - 3	I3-0171	0.0364	121				
		**	159 - 4	I3-0172	0.0369	123				
	2M/Btm	*	140 - 5	I1-0380	0.0300	99.8				
		*	140 - 6	I1-0381	0.0361	120				
		**	159 - 5	I3-0173	0.0309	103				
		**	159 - 6	I3-0174	0.0364	121				
Collected, Processed and Analyzed 8 Dec 07										
30	4F/Top	*	141 - 1	I1-0383	26.0	86.5	26.0	1.3	5.0	86.6
		*	141 - 2	I1-0384	26.1	86.9				
	4F/Mid	*	141 - 3	I1-0385	26.4	87.9				
		*	141 - 4	I1-0386	28.1	93.7				
	4F/Btm	*	141 - 5	I1-0387	24.3	80.9				
		*	141 - 6	I1-0388	25.1	83.7				

**Collected, Processed and Analyzed 8 Dec 07*
***Collected on 8 Dec 07, stored frozen as backups. Processed and Analyzed 11 Dec 07*

189205 results.xls 4H+5H(12-17-07)
Printed: 22May2008 1:24 PM

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY

Table 7: 5-Hour Resuspension Homogeneity And Stability Samples Of The 7 December 2007 Formulations
(Analyzed 8 - 17 December 2007)

Dose Conc (mg/mL)	Group/ Strata		Ref # (189205 -)	Run #	Analyzed Conc (mg/mL)	Percent of Target (%)	Mean Conc (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)	Mean Conc % of Time Zero (%)
0.03	2M/Top	*	147 - 1	I1-0412	0.0273	91.1	0.0272	0.0037	14	90.6	82.2
		**	163 - 1	I3-0058	0.0292	97.3					
		*	147 - 2	I1-0413	0.0264	88.1					
		**	163 - 2	I3-0059	0.0211	70.5					
	2M/Btm	*	147 - 3	I1-0414	0.0274	91.4					
		**	163 - 3	I3-0060	0.0338	113					
		*	147 - 4	I1-0415	0.0282	93.9					
		**	163 - 4	I3-0061	0.0241	80.3					
Collected, Processed and Analyzed 8 Dec 07											
30	4F/Top	*	148 - 1	I1-0417	25.8	86.2	25.0	1.1	4.3	83.2	96.0
		*	148 - 2	I1-0418	24.0	80.1					
	4F/Btm	*	148 - 3	I1-0419	25.9	86.3					
		*	148 - 4	I1-0420	24.0	80.1					

**Collected, Processed and Analyzed 8 Dec 07*
***Collected on 8 Dec 07, stored frozen as backups. Processed 14 Dec 07 and Analyzed 17 Dec 07*

Time Zero Concentration

Group	Conc. (mg/mL)
2M	0.0331
4F	26.0

189205 results.xls Stb (12-17-07) (3)
Printed: 22May2008 1:24 PM

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY

Table 8: 10 Day Resuspension Homogeneity And Refrigerated Storage Stability Analysis Of The 7 December 2007 Formulations
(Analyzed 18 December 2007)

Dose Conc (mg/mL)	Group/ Strata	Ref # (189205 -)	Run #	Analyzed Conc (mg/mL)	Percent of Target (%)	Mean Conc (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)	Mean Conc % of Time Zero (%)
Collected, Processed and Analyzed 18 Dec 07										
0.03	2M/Top	167 - 1	I3-0187	0.0320	107	0.0338	0.0014	4.1	113	102
		167 - 2	I3-0188	0.0351	117					
	2M/Btm	167 - 3	I3-0189	0.0336	112					
		167 - 4	I3-0190	0.0345	115					
Collected, Processed and Analyzed 18 Dec 07										
30	4F/Top	168 - 1	I3-0171	30.3	101	29.5	2.6	8.8	98.5	114
		168 - 2	I3-0172	25.7	85.7					
	4F/Btm	168 - 3	I3-0173	30.7	102					
		168 - 4	I3-0174	31.4	105					

Time Zero Concentration

Group	Conc. (mg/mL)
2M	0.0331
4F	26.0

189205 results.xls 10Dstb(12-18-07)
Printed: 22May2008 1:24 PM

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY

Table 9: Concentration Analysis Of The 7 December 2007 Formulations
(Analyzed 8 December 2007)

Dose Conc (mg/mL)	Group/ Strata	Ref # (189205 -)	Run #	Analyzed Conc (mg/mL)	Percent of Target (%)	Mean Conc (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)
0	1M+1F/Mid	143 - 1	I1-0392		…not detected…				
		143 - 2	I1-0393		…not detected…				
0.03	Low (2M)/Mid	140 - 3	I1-0378	0.0279	93.0	0.0336	0.0042	12	112
		140 - 4	I1-0379	0.0330	110				
	*	159 - 3	I3-0171	0.0364	121				
	*	159 - 4	I3-0172	0.0369	123				
0.3	2F+3M / Mid	144 - 1	I1-0394	0.287	95.6	0.272	0.021	7.6	90.7
		144 - 2	I1-0395	0.257	85.8				
3	3F / Mid	144 - 3	I1-0396	2.54	84.7	2.49	0.069	2.8	83.0
		144 - 4	I1-0397	2.44	81.4				
3	4M / Mid	144 - 5	I1-0398	2.45	81.8	2.59	0.20	7.7	86.5
		144 - 6	I1-0399	2.74	91.2				
30	High (4F)/Mid	141 - 3	I1-0385	26.4	87.9	27.2	1.2	4.5	90.8
		141 - 4	I1-0386	28.1	93.7				

**Collected on 8 Dec 07, stored frozen as backups. Processed and Analyzed 11 Dec 07*

189205 results.xls 4C (2)
Printed: 22May2008 1:24 PM

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY

Table 10: Concentration Analysis Of The 28 December 2007 Formulations
(Analyzed 28 December 2007 - 2 January 2008)

Dose Conc (mg/mL)	Group/ Strata	Ref # (189205 -)	Run #	Analyzed Conc (mg/mL)	Percent of Target (%)	Mean Conc (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)
0	1M+1F/Mid	201 - 1	I2-0210		<LLOQ				
		201 - 2	I2-0211		<LLOQ				
0.03	2M / Mid	202 - 1	I2-0212	0.0282	93.9	0.0282	0.0000020	0.0072	93.9
		202 - 2	I2-0213	0.0282	93.9				
0.3	2F + 3M / Mid	202 - 3	I2-0214	0.297	98.9	0.296	0.0018	0.60	98.5
		202 - 4	I2-0215	0.294	98.1				
3	4M / Mid	202 - 5	I2-0216	2.85	94.8	2.94	0.14	4.7	98.1
		202 - 6	I2-0217	3.04	101				
3	3F / Mid	202 - 7	I2-0218	3.02	101	3.02	0.0088	0.29	101
		202 - 8	I2-0219	3.03	101				
30	4F / Mid	202 - 9	I2-0220	29.9	99.8	29.9	0.068	0.23	99.6
		202 - 10	I2-0221	29.8	99.4				

<LLOQ = Less than lower limit of quantitation

189205 results.xls C(12-29-07)
Printed: 22May2008 1:24 PM

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY

Table 11: Back-Calculated Concentrations Of Formulation Stocks

	Ref #	Run #	Area	Calculated Conc. (mg/mL)	Mean Conc (mg/mL)
Low Group	131-1	I1-0349	58018	203	211
Stock	131-2	I1-0350	60331	211	
	131-3	I1-0351	59778	209	
	131-4	I1-0352	63167	221	
High Group	131-5	I1-0353	43309	243	241
Stock	131-6	I1-0354	43101	242	
	131-7	I1-0355	44500	249	
	131-8	I1-0356	41322	232	

Note: Stock solutions processed under the assumption that the low group was prepared at a concentration of 150 mg/mL and the high group was prepared at 240 mg/mL

189205 results.xls 1Stk
Printed: 05/22/08 3:29 PM

ATTACHMENT I

Supporting Data

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY
Table 1a: Supporting Data
Quantify Compound Summary Report
(Analyzed 21 November 2007)

Dataset : \\Lcms02\lcms02\189205.PRO\189205d.qld
Time : Wednesday, April 23, 2008 16:37:38

Printed Wed Apr 23 16:37:53 2008

Compound 1: HFPO dimer acid ammonium salt

	Run #	Ref. #	Sample Text	Std. Conc. (ng/mL)	RT	Sample Area	Ratio	Flags	Mult.	mg/mL	% RE
1	I2-0127	41-10	System Suit		0.58	97020.438	97020.4	bb	1	0.00205807	
2	I2-0128	41-10	System Suit		0.58	102344.31	102344	bb	1	0.002260346	
3	I2-0129	41-10	System Suit		0.59	100942.38	100942	bb	1	0.002205912	
4	I2-0130	41-10	System Suit		0.58	102292.16	102292	bb	1	0.002258305	
5	I2-0131		DI Water		0.52	148.236	148.236	bb	1	0.000002461	
6	I2-0132	41-1	STD 100ng	0.0001	0.52	9844.186	9844.19	bb	1	0.00009782	-2.18
7	I2-0133	41-2	STD 100ng	0.0001	0.52	9988.151	9988.15	bb	1	0.000099366	-0.634
8	I2-0134	41-3	STD 100ng	0.0001	0.52	10087.536	10087.5	bb	1	0.000100436	0.436
9	I2-0135	41-4	STD 250ng	0.00025	0.52	23408.586	23408.6	bb	1	0.000264937	5.9748
10	I2-0136	41-5	STD 250ng	0.00025	0.52	21735.754	21735.8	bb	1	0.000241956	-3.2176
11	I2-0137	41-6	STD 250ng	0.00025	0.52	23811.818	23811.8	bb	1	0.000270578	8.2312
12	I2-0138	41-7	STD 500ng	0.0005	0.52	37801.223	37801.2	bb	1	0.00049121	-1.758
13	I2-0139	41-8	STD 500ng	0.0005	0.52	36746.918	36746.9	bb	1	0.000472856	-5.4288
14	I2-0140	41-9	STD 500ng	0.0005	0.52	36878.277	36878.3	bb	1	0.000475127	-4.9746
15	I2-0141	41-10	STD 750ng	0.00075	0.52	53258.086	53258.1	bb	1	0.000794541	5.9388
16	I2-0142	41-11	STD 750ng	0.00075	0.52	51306	51306	bb	1	0.00075258	0.344
17	I2-0143	41-12	STD 750ng	0.00075	0.52	48672.73	48672.7	bb	1	0.000697691	-6.9745
18	I2-0144	41-13	STD 1000ng	0.001	0.52	60905.762	60905.8	bb	1	0.000969673	-3.0327
19	I2-0145	41-14	STD 1000ng	0.001	0.52	61750.063	61750.1	bb	1	0.000990083	-0.9917
20	I2-0146	41-15	STD 1000ng	0.001	0.52	66323.742	66323.7	bb	1	0.001104497	10.45
21	I2-0147		DI Water						1		
22	I2-0148		DI Water		0.52	198.983	198.983	bb	1	0.000003067	
23	I2-0149		DI Water		0.52	140.989	140.989	bb	1	0.000002372	
24	I2-0150	44-1	blank						2		
25	I2-0151	44-2	QC .001mg	0.001	0.52	42427.313	42427.3	bb	2	0.001150379	15.038
26	I2-0152	44-3	QC .001mg	0.001	0.52	42036.047	42036	bb	2	0.001135734	13.573
27	I2-0153	44-4	QC .001mg	0.001	0.52	44586.855	44586.9	bb	2	0.001232689	23.269
28	I2-0154	44-5	QC .05 mg	0.05	0.52	39542.316	39542.3	bb	100	0.0522155	4.431
29	I2-0155	44-6	QC .05 mg	0.05	0.52	34668.539	34668.5	bb	100	0.04375146	-12.497
30	I2-0156	44-7	QC .05 mg	0.05	0.52	35217.531	35217.5	bb	100	0.04467421	-10.652
31	I2-0157	45-1	QC .5 mg	0.5	0.52	41777.32	41777.3	bb	1000	0.5630476	12.61
32	I2-0158	45-2	QC .5 mg	0.5	0.52	41288.996	41289	bb	1000	0.5539999	10.8
33	I2-0159	45-3	QC .5 mg	0.5	0.52	40783.723	40783.7	bb	1000	0.5447049	8.941
34	I2-0160	45-4	QC 50 mg	50	0.52	35201.805	35201.8	bb	1E+05	44.64767	-10.705
35	I2-0161	45-5	QC 50 mg	50	0.52	36579.598	36579.6	bb	1E+05	46.99699	-6.006
36	I2-0162	45-6	QC 50 mg	50	0.52	35965.719	35965.7	bb	1E+05	45.94421	-8.1116

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY
Table 2a: Supporting Data
Quantify Compound Summary Report
(Analyzed 26 November 2007)

Dataset : Z:\189205.PRO\I3-189205a.qld
Time : Wednesday, April 23, 2008 16:35:47

Printed Wed Apr 23 16:37:01 2008

Compound 1: HFPO Dimer Acid Ammonium Salt

	Run #	Ref. #	Sample Text	Std. Conc. (ng/mL)	RT	Sample Area	Ratio	Flags	Mult.	mg/mL	% RE
1	I3-0001	48-9	System Suitability		0.51	7005.059	7005.06	bb	1	0.000557503	
2	I3-0002	48-9	System Suitability		0.51	6549.008	6549.01	bb	1	0.000506892	
3	I3-0003	48-9	System Suitability		0.51	6050.19	6050.19	bb	1	0.00045406	
4	I3-0004	48-9	System Suitability		0.51	6474.38	6474.38	bb	1	0.000498822	
5	I3-0005	48-9	System Suitability		0.51	6486.478	6486.48	bb	1	0.000500126	
6	I3-0006		DI Water						1		
7	I3-0007	48-9	System Suitability		0.51	6695.501	6695.5	bb	1	0.000522906	
8	I3-0008	48-9	System Suitability		0.51	6496.475	6496.48	bb	1	0.000501205	
9	I3-0009	48-9	System Suitability		0.52	6451.076	6451.08	bb	1	0.000496314	
10	I3-0010	48-9	System Suitability		0.52	6202.685	6202.69	bb	1	0.000469936	
11	I3-0011	48-9	System Suitability		0.51	6185.402	6185.4	bb	1	0.000468124	
12	I3-0012		DI Water		0.5	19.642	19.642	bb	1	0.000001984	
13	I3-0013	48-1	STD 100ng	0.0001	0.51	1686.9	1686.9	bb	1	0.000095733	-4.267
14	I3-0014	48-2	STD 100ng	0.0001	0.51	1848.714	1848.71	bb	1	0.000105889	5.889
15	I3-0015	48-3	STD 100ng	0.0001	0.51	1708.633	1708.63	bb	1	0.000097084	-2.916
16	I3-0016	48-4	STD 250ng	0.00025	0.51	3739.877	3739.88	bb	1	0.000242006	-3.198
17	I3-0017	48-5	STD 250ng	0.00025	0.51	4093.266	4093.27	bb	1	0.000271089	8.4356
18	I3-0018	48-6	STD 250ng	0.00025	0.51	3909.349	3909.35	bb	1	0.000255806	2.3224
19	I3-0019	48-7	STD 500ng	0.0005	0.51	6399.498	6399.5	bb	1	0.000490783	-1.843
20	I3-0020	48-8	STD 500ng	0.0005	0.51	6359.747	6359.75	bb	1	0.00048654	-2.692
21	I3-0021	48-9	STD 500ng	0.0005	0.51	6307.487	6307.49	bb	1	0.000480986	-3.803
22	I3-0022	48-10	STD 750ng	0.00075	0.51	8017.355	8017.35	bb	1	0.000677997	-9.6
23	I3-0023	48-11	STD 750ng	0.00075	0.51	8770.451	8770.45	bb	1	0.000775235	3.3647
24	I3-0024	48-12	STD 750ng	0.00075	0.51	8918.124	8918.12	bb	1	0.00079509	6.012
25	I3-0025	48-13	STD 1000ng	0.001	0.52	10056.008	10056	bb	1	0.000957102	-4.29
26	I3-0026	48-14	STD 1000ng	0.001	0.51	10933.354	10933.4	bb	1	0.001093403	9.3403
27	I3-0027	48-15	STD 1000ng	0.001	0.51	10319.729	10319.7	bb	1	0.000997	-0.3
28	I3-0028		DI Water		0.52	11.597	11.597	bb	1	0.000001364	
29	I3-0029	51-1	blank						2		
30	I3-0030	51-2	QC .001mg	0.001	0.51	5984.912	5984.91	bb	2	0.000894677	-10.53
31	I3-0031	51-3	QC .001mg	0.001	0.51	6663.977	6663.98	bb	2	0.001038882	3.8882
32	I3-0032	51-4	QC .001mg	0.001	0.5	6144.96	6144.96	bb	2	0.000927796	-7.22
33	I3-0033	51-5	QC .05 mg	0.05	0.51	6116.215	6116.22	bb	100	0.04609043	-7.819
34	I3-0034	51-6	QC .05 mg	0.05	0.5	6387.426	6387.43	bb	100	0.04894929	-2.101
35	I3-0035	51-7	QC .05 mg	0.05	0.5	6148.24	6148.24	bb	100	0.04642401	-7.152
36	I3-0036	52-1	QC .5 mg	0.5	0.52	6563.132	6563.13	bb	1000	0.5084265	1.6853
37	I3-0037	52-2	QC .5 mg	0.5	0.5	6982.628	6982.63	bb	1000	0.5549609	10.992
38	I3-0038	52-3	QC .5 mg	0.5	0.51	6700.947	6700.95	bb	1000	0.5235062	4.7012
39	I3-0039	52-4	QC 50 mg	50	0.5	36948.742	36948.7	bb	100000	1539.399	2978.8
40	I3-0040	52-5	QC 50 mg	50	0.5	33724.391	33724.4	bb	100000	1155.176	2210.4
41	I3-0041	52-6	QC 50 mg	50	0.5	32338.803	32338.8	bb	100000	1022.088	1944.2
42	I3-0042		DI Water		0.52	65.444	65.444	bb	1	0.00000491	
43	I3-0043		QC 50 mg	50	0.51	6510.865	6510.86	bb	100000	50.27602	0.5521
44	I3-0044	52-5a	QC 50 mg	50	0.51	7091.845	7091.85	bb	100000	56.73878	13.478
45	I3-0045	52-6a	QC 50 mg	50	0.51	7356.255	7356.26	bb	100000	59.80133	19.603

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY
Table 3a: Supporting Data
Quantify Compound Summary Report
(Analyzed 26 November 2007)

Dataset : C:\MassLynx\189205.PRO\I3-189205b.qld
Time : Thursday, November 29, 2007 08:55:15

Quantify Compound Summary Report

Printed Fri Jan 04 09:57:01 2008

Compound 1: HFPO Dimer Acid Ammonium Salt

	Run #	Ref. #	Sample Text	Std. Conc.	RT	Sample Area	Mult.	mg/mL	% RE
1	I3-0046		DI Water		0.51	19.791	1	0.000001735	
2	I3-0047	54-1	STD 100ng	0.0001	0.52	1607.813	1	0.000096764	-3.236
3	I3-0048	54-2	STD 100ng	0.0001	0.51	1725.028	1	0.000104392	4.392
4	I3-0049	54-3	STD 100ng	0.0001	0.51	1641.912	1	0.000098974	-1.026
5	I3-0050	54-4	STD 250ng	0.00025	0.5	3826.748	1	0.000256068	2.4272
6	I3-0051	54-5	STD 250ng	0.00025	0.51	3724.035	1	0.000248028	-0.7888
7	I3-0052	54-6	STD 250ng	0.00025	0.51	3700.394	1	0.000246187	-1.5252
8	I3-0053	54-7	STD 500ng	0.0005	0.52	6383.42	1	0.000475591	-4.8818
9	I3-0054	54-8	STD 500ng	0.0005	0.51	6844.545	1	0.00051904	3.808
10	I3-0055	54-9	STD 500ng	0.0005	0.52	6728.726	1	0.000508019	1.6038
11	I3-0056	54-10	STD 750ng	0.00075	0.51	9311.683	1	0.000770861	2.7815
12	I3-0057	54-11	STD 750ng	0.00075	0.51	8463.558	1	0.000680643	-9.2476
13	I3-0058	54-12	STD 750ng	0.00075	0.51	9564.781	1	0.000798519	6.4692
14	I3-0059	54-13	STD 1000ng	0.001	0.51	10774.351	1	0.000935344	-6.4656
15	I3-0060	54-14	STD 1000ng	0.001	0.5	11685.326	1	0.001043452	4.3452
16	I3-0061	54-15	STD 1000ng	0.001	0.51	11569.495	1	0.001029465	2.9465
17	I3-0062		DI Water		0.51	25.146	1	0.000002102	
18	I3-0063	57-1	blank		0.51	14.677	2	0.000002741	
19	I3-0064	57-2	QC .001mg	0.001	0.52	6786.277	2	0.001026973	2.6973
20	I3-0065	57-3	QC .001mg	0.001	0.51	7376.532	2	0.001141186	14.119
21	I3-0066	57-4	QC .001mg	0.001	0.52	7183.979	2	0.001103515	10.351
22	I3-0067	57-5	QC .05 mg	0.05	0.51	7080.11	100	0.05416799	8.336
23	I3-0068	57-6	QC .05 mg	0.05	0.5	7115.358	100	0.05450933	9.0187
24	I3-0069	57-7	QC .05 mg	0.05	0.53	6796.541	100	0.05144632	2.8926
25	I3-0070	58-1	QC .5 mg	0.5	0.51	6369.21	1000	0.4742709	-5.1458
26	I3-0071	58-2	QC .5 mg	0.5	0.51	7063.357	1000	0.5400599	8.012
27	I3-0072	58-3	QC .5 mg	0.5	0.51	6532.918	1000	0.4895511	-2.0898
28	I3-0073	58-4	QC 50 mg	50	0.51	7284.302	100000	56.15464	12.309
29	I3-0074	58-5	QC 50 mg	50	0.5	7395.835	100000	57.24924	14.498
30	I3-0075	58-6	QC 50 mg	50	0.51	6908.133	100000	52.51219	5.0244
31	I3-0076		DI Water		0.51	20.743	1	0.000001801	
32	I3-0077	60-1	H 0.0100		0.51	15566.598	20	0.03105456	210.55
33	I3-0078	60-2	H 0.0100		0.51	15474.593	20	0.03079481	207.95
34	I3-0079	60-3	H 0.0100		0.5	16556.121	20	0.03390471	239.05
35	I3-0080	60-4	H 0.0100		0.5	17744.42	20	0.03746462	274.65
36	I3-0081	60-5	H 0.0100		0.51	14356.436	20	0.02770926	177.09
37	I3-0082	60-6	H 0.0100		0.51	15768.579	20	0.03162792	216.28
38	I3-0083		DI Water		0.52	24.771	1	0.000002077	

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY
Table 3b: Supporting Data
Quantify Compound Summary Report
(Analyzed 26 November 2007)

	Run #	Ref. #	Sample Text	Std. Conc.	RT	Sample Area	Mult.	mg/mL	% RE
39	I3-0084	61-1	H 30.0		0.5	11914.91	40000	42.85527	42.851
40	I3-0085	61-2	H 30.0		0.52	12280.24	40000	44.65576	48.853
41	I3-0086	61-3	H 30.0		0.51	12177.003	40000	44.14414	47.147
42	I3-0087	61-4	H 30.0		0.51	12468.788	40000	45.59592	51.986
43	I3-0088	61-5	H 30.0		0.5	11952.343	40000	43.03847	43.462
44	I3-0089	61-6	H 30.0		0.52	13187.244	40000	49.24653	64.155
45	I3-0090		DI Water		0.52	34.885	1	0.000002743	
46	I3-0091	64-1	Stb 0.0100		0.5	16331.627	20	0.03324901	232.49
47	I3-0092	64-2	Stb 0.0100		0.53	15050.106	20	0.02960795	196.08
48	I3-0093	64-3	Stb 0.0100		0.52	2967.098	20	0.003814396	-61.856
49	I3-0094	64-4	Stb 0.0100		0.51	16182.209	20	0.03281555	228.16
50	I3-0095		DI Water		0.51	27.999	1	0.000002293	
51	I3-0096	65-1	Stb 30.0		0.5	11740.04	40000	42.00332	40.011
52	I3-0097	65-2	Stb 30.0		0.53	11534.518	40000	41.01021	36.701
53	I3-0098	65-3	Stb 30.0		0.5	11029.738	40000	38.60855	28.695
54	I3-0099	65-4	Stb 30.0		0.53	11571.169	40000	41.18667	37.289

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY
Table 4a: Supporting Data
Quantify Compound Summary Report
(Analyzed 8 December 2007)

Quantify Compound Summary Report

Sample Lis C:\MASSLYNX\189205.PRO\SampleDB\I1-189205h
Last modifi Sat Dec 08 14:18:13 2007
Method: C:\MASSLYNX\189205.PRO\MethDB\I1-189205a
Last modifi Wed Nov 28 09:07:30 2007
Job Code:

Printed: Mon Dec 10 09:02:54 2007

Compound 1: HFPO Dimer Acid Ammonium Salt

Name	ID	Sample Text	RT	Area	Mult.	Flags	mg/mL	%Dev	Comment
I1-0357	134-8	sys suit	0.54	39970	1	bb	0.0004739		
I1-0358	134-8	sys suit	0.54	43980	1	bb	0.0005478		
I1-0359	134-8	sys suit	0.54	43504	1	bb	0.0005388		
I1-0360	134-8	sys suit	0.54	46848	1	bb	0.0006035		
I1-0361	134-8	sys suit	0.54	48738	1	bb	0.0006416		
I1-0362	134-8	sys suit	0.54	45174	1	bb	0.0005707		
I1-0363					1				
I1-0364	134-1	STD, 100 ng/mL	0.55	14784	1	bb	0.0001165	16.47	
I1-0365	134-4	STD, 250 ng/mL	0.54	28859	1	bb	0.0002934	17.35	
I1-0366	134-7	STD, 500 ng/mL	0.55	46275	1	bb	0.0005922	18.44	
I1-0367	134-10	STD, 750 ng/mL	0.55	56353	1	bb	0.0008057	7.42	
I1-0368	134-13	STD, 1000 ng/mL	0.54	70472	1	bb	0.0011564	15.64	
I1-0369					1				
I1-0370	137-1	Blank QC	0.54	4753	2	bb	0.0000563		
I1-0371	137-2	LLQC, 0.001 mg/mL	0.55	40208	2	bb	0.0009562	-4.38	
I1-0372	137-5	LQC, 0.05 mg/mL	0.54	47344	100	bb	0.0613433	22.69	
I1-0373	138-1	MQC, 0.5 mg/mL	0.55	44216	1000	bb	0.5522889	10.46	
I1-0374	138-4	HQC, 50 mg/mL	0.54	42393	100000	bb	51.795837	3.59	
I1-0375					1				
I1-0376	140-1	Low, 0.03 mg/mL	0.55	40860	60	bb	0.0293917	-2.03	
I1-0377	140-2	Low, 0.03 mg/mL	0.54	40389	60	bb	0.0288822	-3.73	
I1-0378	140-3	Low, 0.03 mg/mL	0.54	39483	60	bb	0.0279118	-6.96	
I1-0379	140-4	Low, 0.03 mg/mL	0.54	44087	60	bb	0.0329896	9.97	
I1-0380	140-5	Low, 0.03 mg/mL	0.54	41374	60	bb	0.0299527	-0.16	
I1-0381	140-6	Low, 0.03 mg/mL	0.54	46747	60	bb	0.0360926	20.31	
I1-0382					1				
I1-0383	141-1	High, 30 mg/mL	0.54	37615	60000	bb	25.957127	-13.48	
I1-0384	141-2	High, 30 mg/mL	0.55	37723	60000	bb	26.0684297	-13.11	
I1-0385	141-3	High, 30 mg/mL	0.54	38014	60000	bb	26.3693486	-12.1	
I1-0386	141-4	High, 30 mg/mL	0.54	39680	60000	bb	28.1219106	-6.26	
I1-0387	141-5	High, 30 mg/mL	0.55	35951	60000	bb	24.2666059	-19.11	
I1-0388	141-6	High, 30 mg/mL	0.54	36797	60000	bb	25.1197225	-16.27	
I1-0389		DI water	0.55	687	1	bb	0.0000034		
I1-0390		DI water	0.54	711	1	bb	0.0000035		
I1-0391		DI water	0.55	748	1	bb	0.0000037		

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY

Table 4b: Supporting Data

Quantify Compound Summary Report
(Analyzed 8 December 2007)

Name	ID	Sample Text	RT	Area	Mult.	Flags	mg/mL	%Dev	Comment
I1-0392	143-1				1				
I1-0393	143-2				1				
I1-0394	144-1	2F+3M 0.300 mg/mL	0.54	40192	600	bb	0.286697	-4.43	
I1-0395	144-2	2F+3M 0.300 mg/mL	0.54	37413	600	bb	0.2574901	-14.17	
I1-0396	144-3	3F 3 mg/mL	0.54	37075	6000	bb	2.5403646	-15.32	
I1-0397	144-4	3F 3 mg/mL	0.54	36105	6000	bb	2.442171	-18.59	
I1-0398	144-5	4M 3mg/mL	0.54	36217	6000	bb	2.4533953	-18.22	
I1-0399	144-6	4M 3mg/mL	0.54	38956	6000	bb	2.7354199	-8.82	
I1-0400		DI water	0.55	656	1	bb	0.0000032		
I1-0401	137-3	LLQC, 0.001 mg/mL	0.55	42729	2	bb	0.0010485	4.85	
I1-0402	137-6	LQC, 0.05 mg/mL	0.55	41398	100	bb	0.0499642	-0.07	
I1-0403	138-2	MQC, 0.5 mg/mL	0.55	39928	1000	bb	0.4731102	-5.38	
I1-0404	138-5	HQC, 50 mg/mL	0.54	34975	100000	bb	38.8302879	-22.34	
I1-0405					1				
I1-0406	134-2	STD, 100 ng/mL	0.54	12822	1	bb	0.0000966	-3.44	
I1-0407	134-5	STD, 250 ng/mL	0.54	25368	1	bb	0.0002441	-2.38	
I1-0408	134-8	STD, 500 ng/mL	0.54	41198	1	bb	0.000496	-0.8	
I1-0409	134-11	STD, 750 ng/mL	0.54	54864	1	bb	0.0007722	2.97	
I1-0410	134-14	STD, 1000 ng/mL	0.54	66940	1	bb	0.0010629	6.29	
I1-0411		DI water	0.54	676	1	bb	0.0000033		
I1-0412	147-1	Low, 0.03 mg/mL	0.55	38920	60	bb	0.0273167	-8.94	
I1-0413	147-2	Low, 0.03 mg/mL	0.54	38063	60	bb	0.0264206	-11.93	
I1-0414	147-3	Low, 0.03 mg/mL	0.54	39016	60	bb	0.0274171	-8.61	
I1-0415	147-4	Low, 0.03 mg/mL	0.55	39731	60	bb	0.0281754	-6.08	
I1-0416		DI water	0.55	714	1	bb	0.0000035		
I1-0417	148-1	High, 30 mg/mL	0.54	37508	60000	bb	25.8473925	-13.84	
I1-0418	148-2	High, 30 mg/mL	0.54	35716	60000	bb	24.0323217	-19.89	
I1-0419	148-3	High, 30 mg/mL	0.55	37564	60000	bb	25.9046955	-13.65	
I1-0420	148-4	High, 30 mg/mL	0.54	35706	60000	bb	24.0222182	-19.93	
I1-0421					1				
I1-0422	137-4	LLQC, 0.001 mg/mL	0.54	40131	2	bb	0.0009535	-4.65	
I1-0423	137-7	LQC, 0.05 mg/mL	0.54	35363	100	bb	0.0394687	-21.06	
I1-0424	138-3	MQC, 0.5 mg/mL	0.55	38900	1000	bb	0.4549127	-9.02	
I1-0425	138-6	HQC, 50 mg/mL	0.54	33861	100000	bb	37.0200319	-25.96	
I1-0426					1				
I1-0427	134-3	STD, 100 ng/mL	0.55	12098	1	bb	0.0000895	-10.48	
I1-0428	134-6	STD, 250 ng/mL	0.55	23174	1	bb	0.0002149	-14.05	
I1-0429	134-9	STD, 500 ng/mL	0.55	37297	1	bb	0.0004272	-14.56	
I1-0430	134-12	STD, 750 ng/mL	0.54	51846	1	bb	0.0007065	-5.8	
I1-0431	134-15	STD, 1000 ng/mL	0.54	55800	1	bb	0.0007932	-20.68	

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY
Table 5a: Supporting Data
Quantify Compound Summary Report
(Analyzed 11 December 2007)

Quantify Compound Summary Report

Printed Wed Dec 12 09:05:39 2007

Compound 1: HFPO Dimer Acid Ammonium Salt

	Run #	Ref. #	Sample Text	Std. Conc. (ng/mL)	RT	Sample Area	Ratio	Flags	Mult.	mg/mL	% RE
1	I3-0142	153-7	STD 500ng		0.5	1451.564	1451.56	bb	1	0.000432678	
2	I3-0143	153-7	STD 500ng		0.5	1521.35	1521.35	bb	1	0.000456172	
3	I3-0144	153-7	STD 500ng		0.5	1296.37	1296.37	bb	1	0.000380939	
4	I3-0145	153-7	STD 500ng		0.5	1399.929	1399.93	bb	1	0.000415384	
5	I3-0146	153-7	STD 500ng		0.5	1335.058	1335.06	bb	1	0.000393768	
6	I3-0147		DI Water		0.5	16.04	16.04	bb	1	2.63539E-06	
7	I3-0148	153-1	STD 100ng	0.0001	0.51	416.847	416.847	MM	1	0.000105937	5.9366
8	I3-0149	153-2	STD 100ng	0.0001	0.5	340.921	340.921	MM	1	8.44097E-05	-15.59
9	I3-0150	153-4	STD 250ng	0.00025	0.5	835.613	835.613	MM	1	0.000232221	-7.1115
10	I3-0151	153-5	STD 250ng	0.00025	0.5	991.523	991.523	bb	1	0.000281621	12.648
11	I3-0152	153-7	STD 500ng	0.0005	0.5	1610.431	1610.43	bb	1	0.000486356	-2.7288
12	I3-0153	153-8	STD 500ng	0.0005	0.49	1524.202	1524.2	MM	1	0.000457135	-8.5729
13	I3-0154	153-10	STD 750ng	0.00075	0.5	2237.957	2237.96	bb	1	0.000704353	-6.0862
14	I3-0155	153-11	STD 750ng	0.00075	0.5	2858.379	2858.38	MM	1	0.000927487	23.665
15	I3-0156	153-13	STD 1000ng	0.001	0.5	2880.762	2880.76	MM	1	0.000935657	-6.4343
16	I3-0157	153-14	STD 1000ng	0.001	0.5	3203.234	3203.23	MM	1	0.001054195	5.4195
17	I3-0158		DI Water		0.5	12.02	12.02	bb	1	1.89741E-06	
18	I3-0159	156-1	blank					MM-	2		
19	I3-0160	156-2	QC .001mg	0.001	0.51	1966.164	1966.16	bb	2	0.001217726	21.773
20	I3-0161	156-3	QC .001mg	0.001	0.51	1522.118	1522.12	bb	2	0.000912863	-8.7137
21	I3-0162	156-5	QC .05 mg	0.05	0.49	1565.628	1565.63	bb	100	0.04711487	-5.7703
22	I3-0163	156-6	QC .05 mg	0.05	0.5	1776.131	1776.13	bb	100	0.05430448	8.609
23	I3-0164	157-1	QC .5 mg	0.5	0.5	1721.781	1721.78	bb	1000	0.5243757	4.8751
24	I3-0165	157-2	QC .5 mg	0.5	0.5	1485.9	1485.9	bb	1000	0.4442204	-11.156
25	I3-0166	157-4	QC 50 mg	50	0.51	1994.678	1994.68	bb	100000	61.88087	23.762
26	I3-0167	157-5	QC 50 mg	50	0.5	1668.466	1668.47	MM	100000	50.61326	1.2265
27	I3-0168		DI Water		0.5	14.142	14.142	bb	1	2.28337E-06	
28	I3-0169	159-1	0.0300 mg/mL		0.49	2059.647	2059.65	bb	60	0.03849208	28.307
29	I3-0170	159-2	0.0300 mg/mL		0.5	1788.082	1788.08	MM	60	0.03282957	9.4319
30	I3-0171	159-3	0.0300 mg/mL		0.5	1962.212	1962.21	MM	60	0.03644917	21.497
31	I3-0172	159-4	0.0300 mg/mL		0.5	1983.818	1983.82	bb	60	0.03690112	23.004
32	I3-0173	159-5	0.0300 mg/mL		0.5	1693.138	1693.14	MM	60	0.03087396	2.9132
33	I3-0174	159-6	0.0300 mg/mL		0.5	1961.875	1961.88	MM	60	0.03644212	21.474
34	I3-0175		DI Water		0.51	1736.945	1736.94	MM	1	0.000529577	
35	I3-0176	153-3	STD 100ng	0.0001	0.51	431.754	431.754	MM	1	0.000110225	10.225
36	I3-0177	153-6	STD 250ng	0.00025	0.49	910.798	910.798	MM	1	0.000255912	2.3646
37	I3-0178	153-9	STD 500ng	0.0005	0.5	1625.195	1625.19	MM	1	0.000491379	-1.7242
38	I3-0179	153-12	STD 750ng	0.00075	0.5	2281.856	2281.86	bb	1	0.000719915	-4.0113
39	I3-0180	153-15	STD 1000ng	0.001	0.51	3016.799	3016.8	MM	1	0.000985476	-1.4524
40	I3-0181		DI Water		0.5	1657.737	1657.74	bb	1	0.00050247	
41	I3-0182	156-4	QC .001mg	0.001	0.5	2132.205	2132.2	bb	2	0.001334039	33.404
42	I3-0183	156-7	QC .05 mg	0.05	0.5	1690.537	1690.54	MM	100	0.05136762	2.7352
43	I3-0184	157-3	QC .5 mg	0.5	0.51	1835.578	1835.58	MM	1000	0.5635454	12.709
44	I3-0185	157-6	QC 50 mg	50	0.51	1425.527	1425.53	MM	100000	42.39474	-15.211
45	I3-0186		DI Water						1		

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY
Table 6a: Supporting Data
Quantify Compound Summary Report
(Analyzed 17 December 2007)

Dataset : Z:\CLIENT\189\189207\Dosing\Instrument Data\LC03\I3-189207b.qld
Time : Wednesday, December 19, 2007 08:19:35

Compound 1: HFPO Dimer Acid Ammonium Salt

	Run #	Ref. #	Sample Text	Std. Conc. (ng/mL)	RT	Sample Area	Ratio	Flags	Mult.	mg/mL	% RE
1	I3-0143	17-7	STD 500ng		0.51	7831.5	7831.5	bb	1	0.000540729	
2	I3-0144	17-7	STD 500ng		0.51	7396.205	7396.2	bb	1	0.000500802	
3	I3-0145	17-7	STD 500ng		0.5	7705.55	7705.55	bb	1	0.000529085	
4	I3-0146	17-7	STD 500ng		0.5	7364.435	7364.44	bb	1	0.000497923	
5	I3-0147	17-7	STD 500ng		0.51	7850.402	7850.4	bb	1	0.000542483	
6	I3-0148	17-7	STD 500ng		0.5	7481.581	7481.58	bb	1	0.000508563	
7	I3-0149	17-7	STD 500ng		0.5	7525.181	7525.18	bb	1	0.000512539	
8	I3-0150	17-7	STD 500ng		0.51	7601.481	7601.48	bb	1	0.00051952	
9	I3-0151	17-7	STD 500ng		0.5	7114.625	7114.63	bb	1	0.000475452	
10	I3-0152		DI Water						1		
11	I3-0153	17-1	STD 100ng	0.0001	0.51	2115.85	2115.85	bb	1	9.82575E-05	-1.7425
12	I3-0154	17-4	STD 250ng	0.00025	0.5	4954.264	4954.26	bb	1	0.000294281	17.713
13	I3-0155	17-7	STD 500ng	0.0005	0.5	7027.122	7027.12	bb	1	0.000467652	-6.4695
14	I3-0156	17-10	STD 750ng	0.00075	0.5	11464.348	11464.3	bb	1	0.000906536	20.871
15	I3-0157	17-13	STD 1000ng	0.001	0.5	13111.881	13111.9	bb	1	0.00109013	9.013
16	I3-0158		DI Water		0.51	14.444	14.444	bb	1	3.13522E-07	
17	I3-0159	20-1	blank		0.5	5548.58	5548.58	bb	2	0.000683333	
18	I3-0160	20-2	QC .001mg	0.001	0.51	7483.615	7483.61	bb	2	0.001017496	1.7496
19	I3-0161	20-5	QC .05 mg	0.05	0.5	7604.846	7604.85	bb	100	0.05198283	3.9657
20	I3-0162	21-1	QC .5 mg	0.5	0.51	8329.763	8329.76	bb	1000	0.5875108	17.502
21	I3-0163	21-4	QC 50 mg	50	0.51	6466.751	6466.75	bb	1E+05	41.85942	-16.281
22	I3-0164		DI Water		0.51	19.378	19.378	bb	1	4.28471E-07	
23	I3-0165	26-1	High, bckups, 3.00mg/mL		0.5	7234.393	7234.39	bb	6000	2.917127	-2.7624
24	I3-0166	26-2	High, bckups, 3.00mg/mL		0.5	7852.074	7852.07	bb	6000	3.255831	8.5277
25	I3-0167	26-3	High, bckups, 3.00mg/mL		0.51	7190.354	7190.35	bb	6000	2.893394	-3.5535
26	I3-0168	26-4	High, bckups, 3.00mg/mL		0.5	7089.119	7089.12	db	6000	2.839049	-5.365
27	I3-0169		DI Water		0.51	15.289	15.289	bb	1	3.32981E-07	
28	I3-0170		DI Water		0.5	11.853	11.853	bb	1	2.54495E-07	
29	I3-0171	189205-168-1	High, 30 mg/mL		0.5	7443.589	7443.59	bb	60000	30.30628	1.0209
30	I3-0172	189205-168-2	High, 30 mg/mL		0.51	6582.499	6582.5	bb	60000	25.71598	-14.28
31	I3-0173	189205-168-3	High, 30 mg/mL		0.5	7518.217	7518.22	bb	60000	30.7142	2.3807
32	I3-0174	189205-168-4	High, 30 mg/mL		0.5	7645.213	7645.21	bb	60000	31.41199	4.7066
33	I3-0175		DI Water		0.51	22.688	22.688	bb	1	5.07252E-07	
34	I3-0176	20-3	QC .001mg	0.001	0.5	6539.204	6539.2	bb	2	0.000849699	-15.03
35	I3-0177	20-6	QC .05 mg	0.05	0.5	8204.299	8204.3	bb	100	0.05756243	15.125
36	I3-0178	21-2	QC .5 mg	0.5	0.5	7789.451	7789.45	bb	1000	0.5368337	7.3667
37	I3-0179	21-5	QC 50 mg	50	0.5	6760.524	6760.52	bb	1E+05	44.4119	-11.176
38	I3-0180		DI Water		0.5	10.767	10.767	bb	1	2.30059E-07	
39	I3-0181	17-2	STD 100ng	0.0001	0.51	2139.205	2139.2	bb	1	9.9632E-05	-0.368
40	I3-0182	17-5	STD 250ng	0.00025	0.51	4072.667	4072.67	bb	1	0.000227729	-8.9085
41	I3-0183	17-8	STD 500ng	0.0005	0.5	6747.403	6747.4	bb	1	0.00044297	-11.406
42	I3-0184	17-11	STD 750ng	0.00075	0.51	10582.507	10582.5	bb	1	0.000812644	8.3526
43	I3-0185	17-14	STD 1000ng	0.001	0.51	10510.114	10510.1	bb	1	0.000805076	-19.492
44	I3-0186		DI Water		0.51	24.399	24.399	bb	1	5.48454E-07	
45	I3-0187	189205-167-1	Low, 0.03 mg/mL		0.5	7746.476	7746.48	bb	60	0.03197164	6.5721

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY
Table 6b: Supporting Data
Quantify Compound Summary Report
(Analyzed 17 December 2007)

	Run #	Ref. #	Sample Text	Std. Conc. (ng/mL)	RT	Sample Area	Ratio	Flags	Mult.	mg/mL	% RE
46	I3-0188	189205-167-2	Low, 0.03 mg/mL		0.51	8311.007	8311.01	bb	60	0.03514376	17.146
47	I3-0189	189205-167-3	Low, 0.03 mg/mL		0.51	8039.894	8039.89	bb	60	0.03360941	12.031
48	I3-0190	189205-167-4	Low, 0.03 mg/mL		0.5	8191.164	8191.16	bb	60	0.03446304	14.877
49	I3-0191		DI Water		0.5	23.579	23.579	bb	1	5.28669E-07	
50	I3-0192	23-1	Grp 2, .1mg/mL		0.5	1258.98	1258.98	bb	200	0.01026229	2.6229
51	I3-0193	23-2	Grp 2, .1mg/mL		0.5	1274.351	1274.35	bb	200	0.01041765	4.1765
52	I3-0194	23-3	Grp 3, 0.3 mg/mL		0.5	6178.526	6178.53	bb	600	0.2363847	-21.205
53	I3-0195	23-4	Grp 3, 0.3 mg/mL		0.5	6048.675	6048.68	bb	600	0.2298132	-23.396
54	I3-0196	23-5	Grp 4, 3.0 mg/mL		0.5	7987.051	7987.05	bb	6000	3.33127	11.042
55	I3-0197	23-6	Grp 4, 3.0 mg/mL		0.5	7730.253	7730.25	bb	6000	3.188179	6.2726
56	I3-0198		DI Water		0.51	22.721	22.721	bb	1	5.08044E-07	
57	I3-0199	20-4	QC .001mg	0.001	0.51	6650.086	6650.09	bb	2	0.000868946	-13.105
58	I3-0200	20-7	QC .05 mg	0.05	0.5	7431.879	7431.88	bb	100	0.05040403	0.80805
59	I3-0201	21-3	QC .5 mg	0.5	0.5	7298.219	7298.22	bb	1000	0.4919373	-1.6125
60	I3-0202	21-6	QC 50 mg	50	0.5	8316.497	8316.5	bb	1E+05	58.62506	17.25
61	I3-0203		DI Water		0.5	19.909	19.909	bb	1	4.41024E-07	
62	I3-0204	17-3	STD 100ng	0.0001	0.5	2251.254	2251.25	bb	1	0.000106291	6.291
63	I3-0205	17-6	STD 250ng	0.00025	0.5	3818.057	3818.06	bb	1	0.000209389	-16.244
64	I3-0206	17-9	STD 500ng	0.0005	0.51	7953.942	7953.94	bb	1	0.00055212	10.424
65	I3-0207	17-12	STD 750ng	0.00075	0.5	11167.385	11167.4	bb	1	0.000874569	16.609
66	I3-0208	17-15	STD 1000ng	0.001	0.51	11183.623	11183.6	bb	1	0.000876308	-12.369
67	I3-0209		DI Water		0.51	20.351	20.351	bb	1	4.51499E-07	
68	I3-0210		DI Water		0.51	17.224	17.224	bb	1	3.77903E-07	
69	I3-0211										
70	I3-0212	22-3	Grp 2, .01mg/mL		0.5	3997.349	3997.35	bb	40	0.008890436	-11.096
71	I3-0213	22-4	Grp 2, .01mg/mL		0.5	3825.244	3825.24	bb	40	0.008396033	-16.04
72	I3-0214		DI Water		0.5	19.437	19.437	bb	1	4.29865E-07	
73	I3-0215		DI Water		0.5	17.462	17.462	bb	1	3.83462E-07	
74	I3-0216		DI Water		0.5	18.286	18.286	bb	1	4.02763E-07	
75	I3-0217		DI Water		0.5	20.74	20.74	bb	1	4.60736E-07	
76	I3-0218		DI Water		0.5	20.518	20.518	bb	1	4.55462E-07	
77	I3-0219		DI Water		0.5	20.965	20.965	bb	1	4.66087E-07	
78	I3-0220		DI Water		0.5	23.152	23.152	bb	1	5.18394E-07	
79	I3-0221		DI Water		0.5	26.274	26.274	bb	1	5.93959E-07	
80	I3-0222		DI Water		0.5	46.341	46.341	bb	1	1.10096E-06	
81	I3-0223	22-1	Grp 1, blank						1		
82	I3-0224	22-2	Grp 1, blank		0.5	12.653	12.653	bb	1	2.72614E-07	
83	I3-0225	23-7	Grp 3, 0.3 mg/mL		0.51	6257.873	6257.87	bb	600	0.2404259	-19.858
84	I3-0226	23-8	Grp 3, 0.3 mg/mL		0.5	5722.473	5722.47	bb	600	0.2135377	-28.821
85	I3-0227	23-9	Grp 3, 0.3 mg/mL		0.5	5827.888	5827.89	bb	600	0.2187605	-27.08
86	I3-0228	23-10	Grp 3, 0.3 mg/mL		0.5	6668.684	6668.68	bb	600	0.2616559	-12.781

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY
Table 7a: Supporting Data
Quantify Compound Summary Report
(Analyzed 18 December 2007)

Printed Tue Dec 18 09:08:13 2007

Compound 1: HFPO Dimer Acid Ammonium Salt

	Run #	Ref. #	Sample Text	Std. Conc. (ng/mL)	RT		Sample Are	Ratio	Flags	Mult.	mg/mL	% RE
1	I3-0001	3-9	sys suit			0.5	8454.431	8454.43	bb	1	0.000544788	
2	I3-0002	3-9	sys suit			0.5	8032.443	8032.44	bb	1	0.000506219	
3	I3-0003	3-9	sys suit			0.51	8356.329	8356.33	bb	1	0.000535743	
4	I3-0004	3-9	sys suit			0.51	7776.669	7776.67	bb	1	0.000483274	
5	I3-0005	3-9	sys suit			0.51	7851.756	7851.76	bb	1	0.000489976	
6	I3-0006	3-9	sys suit			0.51	8992.606	8992.61	bb	1	0.000595236	
7	I3-0007	3-9	sys suit			0.51	8492.016	8492.02	bb	1	0.000548266	
8	I3-0008	3-9	sys suit			0.5	7706.339	7706.34	bb	1	0.000477024	
9	I3-0009	3-9	sys suit			0.5	8042.122	8042.12	bb	1	0.000507093	
10	I3-0010		DI Water			0.51	233.435	233.435	bb	1	3.52154E-06	
11	I3-0011	3-1	STD 100ng	0.0001		0.5	2648.605	2648.61	bb	1	0.000104271	4.2711
12	I3-0012	3-4	STD 250ng	0.00025		0.51	5496.446	5496.45	bb	1	0.000294216	17.686
13	I3-0013	3-7	STD 500ng	0.0005		0.5	8746.965	8746.97	bb	1	0.000572037	14.407
14	I3-0014	3-10	STD 750ng	0.00075		0.51	10419.72	10419.7	bb	1	0.000735543	-1.9276
15	I3-0015	3-13	STD 1000ng	0.001		0.51	13325.26	13325.3	bb	1	0.001048256	4.8256
16	I3-0016		DI Water			0.51	248.596	248.596	bb	1	3.84008E-06	
17	I3-0017	6-1	blank			0.5	227.324	227.324	bb	2	6.79072E-06	
18	I3-0018	6-2	QC .001mg	0.001		0.51	8014.214	8014.21	bb	2	0.001009145	0.9145
19	I3-0019	6-5	QC .05 mg	0.05		0.5	7773.297	7773.3	bb	100	0.0482974	-3.4052
20	I3-0020	7-1	QC .5 mg	0.5		0.5	8851.867	8851.87	bb	1000	0.581909	16.382
21	I3-0021	7-4	QC 50 mg	50		0.5	8333.473	8333.47	bb	1E+05	53.36426	6.7285
22	I3-0022		DI Water			0.5	268.214	268.214	bb	1	4.26356E-06	
23	I3-0023	9-1	Low, 0.01 mg/mL			0.51	9077.026	9077.03	bb	20	0.01206549	20.655
24	I3-0024	9-2	Low, 0.01 mg/mL			0.51	7674.343	7674.34	bb	20	0.009483764	-5.1624
25	I3-0025	9-3	Low, 0.01 mg/mL			0.51	8490.25	8490.25	bb	20	0.01096204	9.6204
26	I3-0026	9-4	Low, 0.01 mg/mL			0.5	8731.226	8731.23	bb	20	0.01141121	14.112
27	I3-0027	9-5	Low, 0.01 mg/mL			0.51	8960.951	8960.95	bb	20	0.0118446	18.446
28	I3-0028	9-6	Low, 0.01 mg/mL			0.5	8256.438	8256.44	bb	20	0.01053164	5.3164
29	I3-0029		DI Water			0.5	219.395	219.395	bb	1	3.23359E-06	
30	I3-0030	9-7	High, 30 mg/mL			0.5	8460.103	8460.1	bb	60000	32.71874	9.0625
31	I3-0031	9-8	High, 30 mg/mL			0.51	8266.514	8266.51	bb	60000	31.65022	5.5007
32	I3-0032	9-9	High, 30 mg/mL			0.51	7784.156	7784.16	bb	60000	29.03647	-3.2118
33	I3-0033	9-10	High, 30 mg/mL			0.5	7848.092	7848.09	bb	60000	29.37888	-2.0704
34	I3-0034	9-11	High, 30 mg/mL			0.5	7186.349	7186.35	bb	60000	25.89615	-13.68
35	I3-0035	9-12	High, 30 mg/mL			0.5	8287.562	8287.56	bb	60000	31.76586	5.8862
36	I3-0036		DI Water			0.51	254.891	254.891	bb	1	3.9746E-06	
37	I3-0037	6-3	QC .001mg	0.001		0.51	7162.087	7162.09	bb	2	0.000859036	-14.096
38	I3-0038	6-6	QC .05 mg	0.05		0.5	7538.359	7538.36	bb	100	0.04621967	-7.5607
39	I3-0039	7-2	QC .5 mg	0.5		0.51	8536.022	8536.02	bb	1000	0.5523463	10.469
40	I3-0040	7-5	QC 50 mg	50		0.5	8637.119	8637.12	bb	1E+05	56.17565	12.351
41	I3-0041		DI Water			0.5	281.719	281.719	bb	1	4.56222E-06	
42	I3-0042	3-2	STD 100ng	0.0001		0.51	2641.927	2641.93	bb	1	0.000103899	3.8993
43	I3-0043	3-5	STD 250ng	0.00025		0.51	5217.003	5217	bb	1	0.00027311	9.244
44	I3-0044	3-8	STD 500ng	0.0005		0.5	8369.72	8369.72	bb	1	0.000536975	7.395
45	I3-0045	3-11	STD 750ng	0.00075		0.51	10995.61	10995.6	bb	1	0.000794731	5.9641
46	I3-0046	3-14	STD 1000ng	0.001		0.51	13977	13977	bb	1	0.001123031	12.303

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY
Table 7a: Supporting Data
Quantify Compound Summary Report
(Analyzed 18 December 2007)

	Run #	Ref. #	Sample Text	Std. Conc. (ng/mL)	RT		Sample Are	Ratio	Flags	Mult.	mg/mL	% RE
47	I3-0047		DI Water			0.5	253.303	253.303	bb	1	3.94055E-06	
48	I3-0048	13-1	Low, 0.01 mg/mL			0.51	7846.273	7846.27	bb	20	0.009789708	-2.1029
49	I3-0049	13-2	Low, 0.01 mg/mL			0.5	8284.399	8284.4	bb	20	0.01058283	5.8283
50	I3-0050	13-3	Low, 0.01 mg/mL			0.51	8254.316	8254.32	bb	20	0.01052776	5.2776
51	I3-0051	13-4	Low, 0.01 mg/mL			0.5	8006.453	8006.45	bb	20	0.01007744	0.7744
52	I3-0052		DI Water			0.5	271.854	271.854	bb	1	4.3435E-06	
53	I3-0053	13-5	High, 30 mg/mL			0.51	8735.132	8735.13	bb	60000	34.25561	14.185
54	I3-0054	13-6	High, 30 mg/mL			0.51	7320.736	7320.74	bb	60000	26.59234	-11.359
55	I3-0055	13-7	High, 30 mg/mL			0.5	6908.144	6908.14	bb	60000	24.47325	-18.422
56	I3-0056	13-8	High, 30 mg/mL			0.5	7883.856	7883.86	bb	60000	29.57096	-1.4301
57	I3-0057		DI Water			0.5	257.637	257.637	bb	1	4.03369E-06	
58	I3-0058	189205-163-1	Low, 0.03 mg/mL			0.51	7811.712	7811.71	bb	60	0.0291839	-2.7203
59	I3-0059	189205-163-2	Low, 0.03 mg/mL			0.5	6237.398	6237.4	bb	60	0.02114726	-29.509
60	I3-0060	189205-163-3	Low, 0.03 mg/mL			0.5	8657.328	8657.33	bb	60	0.03381861	12.729
61	I3-0061	189205-163-4	Low, 0.03 mg/mL			0.51	6829.907	6829.91	bb	60	0.02407762	-19.741
62	I3-0062		DI Water			0.5	261.032	261.032	bb	1	4.10708E-06	
63	I3-0063	6-4	QC .001mg	0.001		0.5	6948.858	6948.86	bb	2	0.000822664	-17.734
64	I3-0064	6-7	QC .05 mg	0.05		0.51	6075.193	6075.19	bb	100	0.03394298	-32.114
65	I3-0065	7-3	QC .5 mg	0.5		0.5	8146.491	8146.49	bb	1000	0.5165555	3.3111
66	I3-0066	7-6	QC 50 mg	50		0.51	8275.933	8275.93	bb	1E+05	52.83659	5.6732
67	I3-0067		DI Water			0.5	235.656	235.656	bb	1	3.56772E-06	
68	I3-0068	3-3	STD 100ng	0.0001		0.51	2380.734	2380.73	bb	1	8.96801E-05	-10.32
69	I3-0069	3-6	STD 250ng	0.00025		0.51	4441	4441	bb	1	0.000217114	-13.155
70	I3-0070	3-9	STD 500ng	0.0005		0.51	6362.703	6362.7	bb	1	0.000362619	-27.476
71	I3-0071	3-12	STD 750ng	0.00075		0.5	10144.18	10144.2	bb	1	0.000707737	-5.6351
72	I3-0072	3-15	STD 1000ng	0.001		0.5	11959.88	11959.9	bb	1	0.000896988	-10.301
73	I3-0073		DI Water			0.5	254.027	254.027	bb	1	3.95606E-06	

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN MICE WITH A 28-DAY RECOVERY
Quantify Compound Summary Report
Table 8a: Supporting Data
(Analyzed 19 December 2007)

Dataset : Z:\CLIENT\189\189207\Dosing\Instrument Data\LC03\I3-189207b.qld
Time : Wednesday, December 19, 2007 08:19:35

Compound 1: HFPO Dimer Acid Ammonium Salt

	Run #	Ref. #	Sample Text	Std. Conc. (ng/mL)	RT	Sample Area	Ratio	Flags	Mult.	mg/mL	% RE
1	I3-0143	17-7	STD 500ng		0.51	7831.5	7831.5	bb	1	0.000540729	
2	I3-0144	17-7	STD 500ng		0.51	7396.205	7396.2	bb	1	0.000500802	
3	I3-0145	17-7	STD 500ng		0.5	7705.55	7705.55	bb	1	0.000529085	
4	I3-0146	17-7	STD 500ng		0.5	7364.435	7364.44	bb	1	0.000497923	
5	I3-0147	17-7	STD 500ng		0.51	7850.402	7850.4	bb	1	0.000542483	
6	I3-0148	17-7	STD 500ng		0.5	7481.581	7481.58	bb	1	0.000508563	
7	I3-0149	17-7	STD 500ng		0.5	7525.181	7525.18	bb	1	0.000512539	
8	I3-0150	17-7	STD 500ng		0.51	7601.481	7601.48	bb	1	0.00051952	
9	I3-0151	17-7	STD 500ng		0.5	7114.625	7114.63	bb	1	0.000475452	
10	I3-0152		DI Water						1		
11	I3-0153	17-1	STD 100ng	0.0001	0.51	2115.85	2115.85	bb	1	9.82575E-05	-1.7425
12	I3-0154	17-4	STD 250ng	0.00025	0.5	4954.264	4954.26	bb	1	0.000294281	17.713
13	I3-0155	17-7	STD 500ng	0.0005	0.5	7027.122	7027.12	bb	1	0.000467652	-6.4695
14	I3-0156	17-10	STD 750ng	0.00075	0.5	11464.348	11464.3	bb	1	0.000906536	20.871
15	I3-0157	17-13	STD 1000ng	0.001	0.5	13111.881	13111.9	bb	1	0.00109013	9.013
16	I3-0158		DI Water		0.51	14.444	14.444	bb	1	3.13522E-07	
17	I3-0159	20-1	blank		0.5	5548.58	5548.58	bb	2	0.000683333	
18	I3-0160	20-2	QC .001mg	0.001	0.51	7483.615	7483.61	bb	2	0.001017496	1.7496
19	I3-0161	20-5	QC .05 mg	0.05	0.5	7604.846	7604.85	bb	100	0.05198283	3.9657
20	I3-0162	21-1	QC .5 mg	0.5	0.51	8329.763	8329.76	bb	1000	0.5875108	17.502
21	I3-0163	21-4	QC 50 mg	50	0.51	6466.751	6466.75	bb	100000	41.85942	-16.281
22	I3-0164		DI Water		0.51	19.378	19.378	bb	1	4.28471E-07	
23	I3-0165	26-1	High, bckups, 3.00mg/mL		0.5	7234.393	7234.39	bb	6000	2.917127	-2.7624
24	I3-0166	26-2	High, bckups, 3.00mg/mL		0.5	7852.074	7852.07	bb	6000	3.255831	8.5277
25	I3-0167	26-3	High, bckups, 3.00mg/mL		0.51	7190.354	7190.35	bb	6000	2.893394	-3.5535
26	I3-0168	26-4	High, bckups, 3.00mg/mL		0.5	7089.119	7089.12	db	6000	2.839049	-5.365
27	I3-0169		DI Water		0.51	15.289	15.289	bb	1	3.32981E-07	
28	I3-0170		DI Water		0.5	11.853	11.853	bb	1	2.54495E-07	
29	I3-0171	189205-168-1	High, 30 mg/mL		0.5	7443.589	7443.59	bb	60000	30.30628	1.0209
30	I3-0172	189205-168-2	High, 30 mg/mL		0.51	6582.499	6582.5	bb	60000	25.71598	-14.28
31	I3-0173	189205-168-3	High, 30 mg/mL		0.5	7518.217	7518.22	bb	60000	30.7142	2.3807
32	I3-0174	189205-168-4	High, 30 mg/mL		0.5	7645.213	7645.21	bb	60000	31.41199	4.7066
33	I3-0175		DI Water		0.51	22.688	22.688	bb	1	5.07252E-07	
34	I3-0176	20-3	QC .001mg	0.001	0.5	6539.204	6539.2	bb	2	0.000849699	-15.03
35	I3-0177	20-6	QC .05 mg	0.05	0.5	8204.299	8204.3	bb	100	0.05756243	15.125
36	I3-0178	21-2	QC .5 mg	0.5	0.5	7789.451	7789.45	bb	1000	0.5368337	7.3667
37	I3-0179	21-5	QC 50 mg	50	0.5	6760.524	6760.52	bb	100000	44.4119	-11.176
38	I3-0180		DI Water		0.5	10.767	10.767	bb	1	2.30059E-07	
39	I3-0181	17-2	STD 100ng	0.0001	0.51	2139.205	2139.2	bb	1	9.9632E-05	-0.368
40	I3-0182	17-5	STD 250ng	0.00025	0.51	4072.667	4072.67	bb	1	0.000227729	-8.9085
41	I3-0183	17-8	STD 500ng	0.0005	0.5	6747.403	6747.4	bb	1	0.00044297	-11.406
42	I3-0184	17-11	STD 750ng	0.00075	0.51	10582.507	10582.5	bb	1	0.000812644	8.3526
43	I3-0185	17-14	STD 1000ng	0.001	0.51	10510.114	10510.1	bb	1	0.000805076	-19.492
44	I3-0186		DI Water		0.51	24.399	24.399	bb	1	5.48454E-07	
45	I3-0187	189205-167-1	Low, 0.03 mg/mL		0.5	7746.476	7746.48	bb	60	0.03197164	6.5721
46	I3-0188	189205-167-2	Low, 0.03 mg/mL		0.51	8311.007	8311.01	bb	60	0.03514376	17.146

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN MICE WITH A 28-DAY RECOVERY
Quantify Compound Summary Report
Table 8b: Supporting Data
(Analyzed 19 December 2007)

Run #	Ref. #	Sample Text	Std. Conc. (ng/mL)	RT	Sample Area	Ratio	Flags	Mult.	mg/mL	% RE
47 I3-0189	189205-167-3	Low, 0.03 mg/mL		0.51	8039.894	8039.89	bb	60	0.03360941	12.031
48 I3-0190	189205-167-4	Low, 0.03 mg/mL		0.5	8191.164	8191.16	bb	60	0.03446304	14.877
49 I3-0191		DI Water		0.5	23.579	23.579	bb	1	5.28669E-07	
50 I3-0192	23-1	Grp 2, .1mg/mL		0.5	1258.98	1258.98	bb	200	0.01026229	2.6229
51 I3-0193	23-2	Grp 2, .1mg/mL		0.5	1274.351	1274.35	bb	200	0.01041765	4.1765
52 I3-0194	23-3	Grp 3, 0.3 mg/mL		0.5	6178.526	6178.53	bb	600	0.2363847	-21.205
53 I3-0195	23-4	Grp 3, 0.3 mg/mL		0.5	6048.675	6048.68	bb	600	0.2298132	-23.396
54 I3-0196	23-5	Grp 4, 3.0 mg/mL		0.5	7987.051	7987.05	bb	6000	3.33127	11.042
55 I3-0197	23-6	Grp 4, 3.0 mg/mL		0.5	7730.253	7730.25	bb	6000	3.188179	6.2726
56 I3-0198		DI Water		0.51	22.721	22.721	bb	1	5.08044E-07	
57 I3-0199	20-4	QC .001mg	0.001	0.51	6650.086	6650.09	bb	2	0.000868946	-13.105
58 I3-0200	20-7	QC .05 mg	0.05	0.5	7431.879	7431.88	bb	100	0.05040403	0.80805
59 I3-0201	21-3	QC .5 mg	0.5	0.5	7298.219	7298.22	bb	1000	0.4919373	-1.6125
60 I3-0202	21-6	QC 50 mg	50	0.5	8316.497	8316.5	bb	100000	58.62506	17.25
61 I3-0203		DI Water		0.5	19.909	19.909	bb	1	4.41024E-07	
62 I3-0204	17-3	STD 100ng	0.0001	0.5	2251.254	2251.25	bb	1	0.000106291	6.291
63 I3-0205	17-6	STD 250ng	0.00025	0.5	3818.057	3818.06	bb	1	0.000209389	-16.244
64 I3-0206	17-9	STD 500ng	0.0005	0.51	7953.942	7953.94	bb	1	0.00055212	10.424
65 I3-0207	17-12	STD 750ng	0.00075	0.5	11167.385	11167.4	bb	1	0.000874569	16.609
66 I3-0208	17-15	STD 1000ng	0.001	0.51	11183.623	11183.6	bb	1	0.000876308	-12.369
67 I3-0209		DI Water		0.51	20.351	20.351	bb	1	4.51499E-07	
68 I3-0210		DI Water		0.51	17.224	17.224	bb	1	3.77903E-07	
69 I3-0211										
70 I3-0212	22-3	Grp 2, .01mg/mL		0.5	3997.349	3997.35	bb	40	0.008890436	-11.096
71 I3-0213	22-4	Grp 2, .01mg/mL		0.5	3825.244	3825.24	bb	40	0.008396033	-16.04
72 I3-0214		DI Water		0.5	19.437	19.437	bb	1	4.29865E-07	
73 I3-0215		DI Water		0.5	17.462	17.462	bb	1	3.83462E-07	
74 I3-0216		DI Water		0.5	18.286	18.286	bb	1	4.02763E-07	
75 I3-0217		DI Water		0.5	20.74	20.74	bb	1	4.60736E-07	
76 I3-0218		DI Water		0.5	20.518	20.518	bb	1	4.55462E-07	
77 I3-0219		DI Water		0.5	20.965	20.965	bb	1	4.66087E-07	
78 I3-0220		DI Water		0.5	23.152	23.152	bb	1	5.18394E-07	
79 I3-0221		DI Water		0.5	26.274	26.274	bb	1	5.93959E-07	
80 I3-0222		DI Water		0.5	46.341	46.341	bb	1	1.10096E-06	
81 I3-0223	22-1	Grp 1, blank						1		
82 I3-0224	22-2	Grp 1, blank		0.5	12.653	12.653	bb	1	2.72614E-07	
83 I3-0225	23-7	Grp 3, 0.3 mg/mL		0.51	6257.873	6257.87	bb	600	0.2404259	-19.858
84 I3-0226	23-8	Grp 3, 0.3 mg/mL		0.5	5722.473	5722.47	bb	600	0.2135377	-28.821
85 I3-0227	23-9	Grp 3, 0.3 mg/mL		0.5	5827.888	5827.89	bb	600	0.2187605	-27.08
86 I3-0228	23-10	Grp 3, 0.3 mg/mL		0.5	6668.684	6668.68	bb	600	0.2616559	-12.781

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY
Table 9a: Supporting Data
Quantify Compound Summary Report
(Analyzed 21 December 2007)

Printed Fri Dec 21 14:25:37 2007

Compound 1: HFPO Dimer Acid Ammonium Salt

	Run #	Ref. #	Sample Text	Std. Conc. (ng/mL)	RT		Sample Are	Ratio	Flags	Mult.	mg/mL	
1	I3-0215	174-9	STD 500ng			0.61	20705.47	20705.5	bb	1	0.0006769	% RE
2	I3-0216	174-9	STD 500ng			0.61	19855.72	19855.7	bb	1	0.0006336	
3	I3-0217	174-9	STD 500ng			0.61	19614.55	19614.5	bb	1	0.0006216	
4	I3-0218	174-9	STD 500ng			0.61	19943.88	19943.9	bb	1	0.000638	
5	I3-0219	174-9	STD 500ng			0.61	19161.57	19161.6	bb	1	0.0005994	
6	I3-0220	174-9	STD 500ng			0.61	18940.61	18940.6	bb	1	0.0005887	
7	I3-0221	174-9	STD 500ng			0.61	18841.31	18841.3	bb	1	0.000584	
8	I3-0222	174-9	STD 500ng			0.61	18842.11	18842.1	bb	1	0.000584	
9	I3-0223	174-8	STD 500ng			0.61	18099.3	18099.3	bb	1	0.0005491	
10	I3-0224	174-8	STD 500ng			0.61	18919.85	18919.8	bb	1	0.0005877	
11	I3-0225	174-8	STD 500ng			0.61	19031.79	19031.8	bb	1	0.0005931	
12	I3-0226	174-8	STD 500ng			0.61	18275.54	18275.5	bb	1	0.0005573	
13	I3-0227	170-1	Diluent			0.61	213.15	213.15	bb	1	5.60E-06	
14	I3-0228	174-1	STD 100ng	0.0001		0.61	4801.734	4801.73	bb	1	9.88E-05	
15	I3-0229	174-2	STD 100ng	0.0001		0.61	4723.288	4723.29	bb	1	9.70E-05	-1.1949
16	I3-0230	174-3	STD 100ng	0.0001		0.61	5023.827	5023.83	bb	1	0.000104	-3.004
17	I3-0231	174-4	STD 250ng	0.00025		0.61	10616.36	10616.4	bb	1	0.0002586	3.9743
18	I3-0232	174-5	STD 250ng	0.00025		0.61	10042.31	10042.3	bb	1	0.0002404	3.425
19	I3-0233	174-6	STD 250ng	0.00025		0.61	10520.48	10520.5	bb	1	0.0002555	-3.8297
20	I3-0234	174-7	STD 500ng	0.0005		0.61	17039.36	17039.4	bb	1	0.0005014	2.1981
21	I3-0235	174-8	STD 500ng	0.0005		0.61	17492.97	17493	bb	1	0.0005215	0.2762
22	I3-0236	174-9	STD 500ng	0.0005		0.61	16314.42	16314.4	bb	1	0.0004701	4.3061
23	I3-0237	174-10	STD 750ng	0.00075		0.61	21733.19	21733.2	bb	1	0.0007316	-5.9893
24	I3-0238	174-11	STD 750ng	0.00075		0.61	21674.68	21674.7	bb	1	0.0007285	-2.4468
25	I3-0239	174-12	STD 750ng	0.00075		0.61	22711.22	22711.2	bb	1	0.0007861	-2.8712
26	I3-0240	174-13	STD 1000ng	0.001		0.61	25743.67	25743.7	bb	1	0.0009707	4.8128
27	I3-0241	174-14	STD 1000ng	0.001		0.61	25678.83	25678.8	bb	1	0.0009665	-2.9265
28	I3-0242	174-15	STD 1000ng	0.001		0.61	27368.09	27368.1	bb	1	0.0010802	-3.3476
29	I3-0243	170-1	Diluent			0.61	205.935	205.935	bb	1	5.45E-06	8.0243
30	I3-0244	177-1	blank			0.61	263.903	263.903	bb	2	1.33E-05	
31	I3-0245	177-2	QC .001mg	0.001		0.61	15582.03	15582	bb	2	0.0008789	
32	I3-0246	177-3	QC .001mg	0.001		0.61	15657.06	15657.1	bb	2	0.0008851	-12.105
33	I3-0247	177-4	QC .001mg	0.001		0.61	16026.82	16026.8	bb	2	0.0009158	-11.488
34	I3-0248	177-5	QC .05 mg	0.05		0.61	15231.48	15231.5	bb	100	0.042521	-8.4163
35	I3-0249	177-6	QC .05 mg	0.05		0.61	15640.34	15640.3	bb	100	0.044187	-14.958
36	I3-0250	177-7	QC .05 mg	0.05		0.61	15504.36	15504.4	bb	100	0.0436293	-11.626
37	I3-0251	178-1	QC .5 mg	0.5		0.61	16087.45	16087.5	bb	1000	0.460463	-12.741
38	I3-0252	178-2	QC .5 mg	0.5		0.61	14977.85	14977.8	bb	1000	0.4150376	-7.9074
39	I3-0253	178-3	QC .5 mg	0.5		0.61	15215.02	15215	bb	1000	0.4245458	-16.992
40	I3-0254	178-4	QC 50 mg	50		0.61	15863.97	15864	bb	100000	45.112	-15.091
41	I3-0255	178-5	QC 50 mg	50		0.61	15262.54	15262.5	bb	100000	42.64641	-9.776
42	I3-0256	178-6	QC 50 mg	50		0.61	15294.68	15294.7	bb	100000	42.77639	-14.707
43	I3-0257	170-1	Diluent			0.61	212.582	212.582	bb	1	5.59E-06	-14.447

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY
Table 10a: Supporting Data
Quantify Compound Summary Report
(Analyzed 27 December 2007)

Dataset : Z:\189207.PRO\I3-189207d_r1.qld
Time : Monday, April 14, 2008 13:40:16

Compound 1: HFPO Dimer Acid Ammonium Salt

	Run #	Ref. #	Sample Text	Std. Conc.	RT	Sample Area	Ratio	Flags	Mult.	mg/mL	% RE
1	I3-0325	47-7	STD 500ng		0.59	12492.672	12492.7	bb	1	0.000491872	
2	I3-0326	47-7	STD 500ng		0.61	11943.259	11943.3	bb	1	0.000461702	
3	I3-0327	47-7	STD 500ng		0.61	12169.127	12169.1	bb	1	0.000474004	
4	I3-0328	47-7	STD 500ng		0.61	12341.121	12341.1	bb	1	0.000483466	
5	I3-0329	47-7	STD 500ng		0.61	12465.429	12465.4	bb	1	0.000490357	
6	I3-0330	47-7	STD 500ng		0.61	12282.093	12282.1	bb	1	0.000480209	
7	I3-0331	47-7	STD 500ng		0.61	12625.358	12625.4	bb	1	0.000499285	
8	I3-0332	47-7	STD 500ng		0.61	12725.863	12725.9	bb	1	0.000504932	
9	I3-0333	47-7	STD 500ng		0.61	12047.822	12047.8	bb	1	0.000467379	
10	I3-0334		Diluent		0.04	19.828	19.828	bb	1	9.8554E-07	
11	I3-0335	47-1	STD 100ng	0.0001	0.61	3577.054	3577.05	bb	1	0.000101247	1.2471
12	I3-0336	47-4	STD 250ng	0.00025	0.61	7805.657	7805.66	bb	1	0.000260722	4.2888
13	I3-0337	47-7	STD 500ng	0.0005	0.61	12587.336	12587.3	bb	1	0.000497156	-0.56886
14	I3-0338	47-10	STD 750ng	0.00075	0.61	16455.465	16455.5	bb	1	0.000735077	-1.9897
15	I3-0339	47-13	STD 1000ng	0.001	0.61	19751.744	19751.7	bb	1	0.000973702	-2.6298
16	I3-0340		Diluent		0.04	44.871	44.871	bb	1	1.81672E-06	
17	I3-0341	36-1	blank		0.61	3119.999	3120	bb	2	0.000173464	
18	I3-0342	36-2	QC .001mg	0.001	0.61	15665.404	15665.4	bb	2	0.001365809	36.581
19	I3-0343	36-5	QC .05 mg	0.05	0.61	15149.693	15149.7	bb	100	0.06498583	29.972
20	I3-0344	37-1	QC .5 mg	0.5	0.61	14583.725	14583.7	bb	1000	0.6144967	22.899
21	I3-0345	37-4	QC 50 mg	50	0.61	12769.289	12769.3	bb	100000	50.73812	1.4762
22	I3-0346		Diluent		0.61	17.243	17.243	bb	1	8.90222E-07	
23	I3-0347	39-1	Low, Stab, 0.01mg/mL		0.56	11.287	11.287	bb	20	1.31398E-05	-99.869
24	I3-0348	39-2a	Low, Stab, 0.01mg/mL		0.61	12442.148	12442.1	bb	20	0.009781257	-2.1874
25	I3-0349	39-3a	Low, Stab, 0.01mg/mL		0.61	13468.224	13468.2	bb	20	0.01095057	9.5057
26	I3-0350	39-4	Low, Stab, 0.01mg/mL		0.61	18336.24	18336.2	bb	20	0.01734001	73.4
27	I3-0351		Diluent		0.61	16.895	16.895	bb	1	8.77162E-07	
28	I3-0352		Diluent		0.61	17.18	17.18	bb	1	8.87862E-07	
29	I3-0353	39-5	High, Stab, 3 mg/mL		0.61	12263.065	12263.1	bb	6000	2.87497	-4.1677
30	I3-0354	39-6	High, Stab, 3 mg/mL		0.61	12360.235	12360.2	bb	6000	2.907138	-3.0954
31	I3-0355	39-7	High, Stab, 3 mg/mL		0.61	12456.538	12456.5	bb	6000	2.939174	-2.0275
32	I3-0356	39-8	High, Stab, 3 mg/mL		0.61	12341.143	12341.1	bb	6000	2.900805	-3.3065
33	I3-0357		Diluent		0.05	13.254	13.254	bb	1	7.36579E-07	
34	I3-0358	36-3	QC .001mg	0.001	0.61	14732.837	14732.8	bb	2	0.001247444	24.744
35	I3-0359	36-6	QC .05 mg	0.05	0.61	16569.266	16569.3	bb	100	0.07427483	48.55
36	I3-0360	37-2	QC .5 mg	0.5	0.62	14525.371	14525.4	bb	1000	0.6109042	22.181
37	I3-0361	37-5	QC 50 mg	50	0.61	13193.27	13193.3	bb	100000	53.15699	6.314
38	I3-0362		Diluent		0.62	17.909	17.909	bb	1	9.15059E-07	
39	I3-0363	47-2	STD 100ng	0.0001	0.61	3553.047	3553.05	bb	1	0.000100471	0.47143
40	I3-0364	47-5	STD 250ng	0.00025	0.61	7265.604	7265.6	bb	1	0.000237812	-4.8752
41	I3-0365	47-8	STD 500ng	0.0005	0.61	12324.365	12324.4	bb	1	0.000482541	-3.4918
42	I3-0366	47-11	STD 750ng	0.00075	0.61	18234.566	18234.6	bb	1	0.000859585	14.611
43	I3-0367	47-14	STD 1000ng	0.001	0.61	20073.014	20073	bb	1	0.000998834	-0.11663
44	I3-0368		Diluent		0.05	36.525	36.525	bb	1	1.55283E-06	
45	I3-0369	42-3	Grp 2, .01mg/mL		0.61	13033.57	13033.6	bb	20	0.01044798	4.4798

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY
Table 10b: Supporting Data
Quantify Compound Summary Report
(Analyzed 27 December 2007)

	Run #	Ref. #	Sample Text	Std. Conc.	RT	Sample Area	Ratio	Flags	Mult.	mg/mL	% RE
46	I3-0370	42-4	Grp 2, .01mg/mL		0.61	13710.542	13710.5	bb	20	0.01123543	12.354
47	I3-0371	43-1	Grp 3, 0.3 mg/mL		0.61	14154.519	14154.5	bb	600	0.3529826	17.661
48	I3-0372	43-2	Grp 3, 0.3 mg/mL		0.61	13489.919	13489.9	bb	600	0.329278	9.7593
49	I3-0373	43-3	Grp 4, 3.0 mg/mL		0.61	12460.103	12460.1	bb	6000	2.940363	-1.9879
50	I3-0374	43-4	Grp 4, 3.0 mg/mL		0.61	12660.408	12660.4	bb	6000	3.007507	0.25022
51	I3-0375		Diluent		0.61	15.484	15.484	bb	1	8.2358E-07	
52	I3-0376	36-4	QC .001mg	0.001	0.61	15534.162	15534.2	bb	2	0.001348839	34.884
53	I3-0377	36-7	QC .05 mg	0.05	0.61	15937.784	15937.8	bb	100	0.07006787	40.136
54	I3-0378	37-3	QC .5 mg	0.5	0.61	13900.369	13900.4	bb	1000	0.5730463	14.609
55	I3-0379	37-6	QC 50 mg	50	0.61	13180.649	13180.6	bb	100000	53.08425	6.1685
56	I3-0380		Diluent		0.61	17.252	17.252	bb	1	8.90559E-07	
57	I3-0381	47-3	STD 100ng	0.0001	0.61	3493.359	3493.36	bb	1	9.85494E-05	-1.4507
58	I3-0382	47-6	STD 250ng	0.00025	0.61	7585.784	7585.78	bb	1	0.000251304	0.52156
59	I3-0383	47-9	STD 500ng	0.0005	0.61	12721.201	12721.2	bb	1	0.00050467	0.93395
60	I3-0384	47-12	STD 750ng	0.00075	0.61	16228.391	16228.4	bb	1	0.000719889	-4.0148
61	I3-0385	47-15	STD 1000ng	0.001	0.62	19921.584	19921.6	bb	1	0.000986945	-1.3055
62	I3-0386		Diluent		0.05	36.437	36.437	bb	1	1.54999E-06	
63	I3-0387		Diluent		0.61	18.854	18.854	bb	1	9.49959E-07	
64	I3-0388		Diluent		0.61	15.391	15.391	bb	1	8.20011E-07	
65	I3-0389		Diluent		0.61	17.074	17.074	bb	1	8.83887E-07	
66	I3-0390		Diluent		0.61	15.578	15.578	bb	1	8.27182E-07	
67	I3-0391		Diluent		0.61	16.251	16.251	bb	1	8.52834E-07	
68	I3-0392	42-1	Grp 1, blank		0.61	21.468	21.468	bb	1	1.04463E-06	
69	I3-0393	42-2	Grp 1, blank		0.61	25.433	25.433	bb	1	1.18381E-06	
70	I3-0394		Diluent		0.62	14.843	14.843	bb	1	7.98882E-07	
71	I3-0395		Diluent		0.62	15.278	15.278	bb	1	8.15668E-07	
72	I3-0396		Diluent		0.61	17.829	17.829	bb	1	9.12086E-07	
73	I3-0397	177-2	QC .001 mg		0.61	12671.216	12671.2	bb	2	0.001003716	0.37163
74	I3-0398	177-3	QC .001 mg		0.61	13219.157	13219.2	bb	2	0.001066126	6.6126
75	I3-0399	177-4	QC .001 mg		0.61	12697.865	12697.9	bb	2	0.001006712	0.67125
76	I3-0400	177-5	QC .05 mg		0.61	12782.253	12782.3	bb	100	0.05081133	1.6227
77	I3-0401	177-6	QC .05 mg		0.61	12817.794	12817.8	bb	100	0.05101228	2.0246
78	I3-0402	177-7	QC .05 mg		0.61	12369.916	12369.9	bb	100	0.04850585	-2.9883
79	I3-0403	178-1	QC .5 mg		0.61	13494.399	13494.4	bb	1000	0.5490588	9.8118
80	I3-0404	178-2	QC .5 mg		0.61	12863.526	12863.5	bb	1000	0.5127138	2.5428
81	I3-0405	178-3	QC .5 mg		0.61	13416.332	13416.3	bb	1000	0.5445001	8.9
82	I3-0406	178-4	QC 50 mg		0.61	13289.744	13289.7	bb	100000	53.71448	7.429
83	I3-0407	178-5	QC 50 mg		0.61	13027.686	13027.7	bb	100000	52.20627	4.4125
84	I3-0408	178-6	QC 50 mg		0.61	12801.501	12801.5	bb	100000	50.92012	1.8402
85	I3-0409		Diluent		0.61	20.342	20.342	bb	1	1.00417E-06	
86	I3-0410		Diluent		0.61	21.491	21.491	bb	1	1.04545E-06	
87	I3-0411		Diluent		0.61	21.295	21.295	bb	1	1.03844E-06	
88	I3-0412	39-1	Low, Stab, 0.01mg/mL		0.61	19169.131	19169.1	bb	20	0.01857992	85.799
89	I3-0413	39-2a	Low, Stab, 0.01mg/mL		0.61	12748.167	12748.2	bb	20	0.01012379	1.2379
90	I3-0414	39-3a	Low, Stab, 0.01mg/mL		0.61	13633.857	13633.9	bb	20	0.01114491	11.449
91	I3-0415	39-4	Low, Stab, 0.01mg/mL		0.61	18986.916	18986.9	bb	20	0.01830483	83.048
92	I3-0416		Diluent		0.61	7288.208	7288.21	bb	1	0.000238756	
93	I3-0417	39-1a	Low, Stab, 0.01mg/mL		0.61	11909.852	11909.9	bb	20	0.009197885	-8.0212
94	I3-0418	39-4a	Low, Stab, 0.01mg/mL		0.62	11816.611	11816.6	bb	20	0.009097312	-9.0269
95	I3-0419		Diluent		0.61	23.11	23.11	bb	1	1.10286E-06	
96	I3-0420	50-1	blank		0.62	23.508	23.508	bb	2	2.23368E-06	

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY
Table 10c: Supporting Data
Quantify Compound Summary Report
(Analyzed 27 December 2007)

	Run #	Ref. #	Sample Text	Std. Conc.	RT	Sample Area	Ratio	Flags	Mult.	mg/mL	% RE
97	I3-0421	50-2	QC .001mg	0.001	0.61	12437.376	12437.4	bb	2	0.000977596	-2.2404
98	I3-0422	50-3	QC .001mg		0.61	13193.417	13193.4	bb	2	0.001063157	6.3157
99	I3-0423	50-4	QC .001mg		0.61	12665.197	12665.2	bb	2	0.00100304	0.30401
100	I3-0424	50-5	QC .05 mg	0.05	0.62	12793.309	12793.3	bb	100	0.05087381	1.7476
101	I3-0425	50-6	QC .05 mg	0.05	0.62	12788.629	12788.6	bb	100	0.05084736	1.6947
102	I3-0426	50-7	QC .05 mg	0.05	0.61	12702.056	12702.1	bb	100	0.0503592	0.7184
103	I3-0427	51-1	QC .5 mg	0.5	0.61	13737.457	13737.5	bb	1000	0.5633636	12.673
104	I3-0428	51-2	QC .5 mg	0.5	0.62	12721.494	12721.5	bb	1000	0.5046862	0.93725
105	I3-0429	51-3	QC .5 mg	0.5	0.61	12389.452	12389.5	bb	1000	0.4861401	-2.772
106	I3-0430	51-4	QC 50 mg	50	0.61	12284.569	12284.6	bb	100000	48.03459	-3.9308
107	I3-0431	51-5	QC 50 mg	50	0.61	12392.128	12392.1	bb	100000	48.62883	-2.7423
108	I3-0432	51-6	QC 50 mg	50	0.61	12956.257	12956.3	bb	100000	51.79855	3.5971
109	I3-0433		Diluent		0.61	20.95	20.95	bb	1	1.02607E-06	
110	I3-0434	39-1a	Low, Stab, 0.01mg/mL		0.61	12520.454	12520.5	bb	20	0.009868407	-1.3159
111	I3-0435	39-2a	Low, Stab, 0.01mg/mL		0.61	12403.895	12403.9	bb	20	0.009738808	-2.6119
112	I3-0436	39-3a	Low, Stab, 0.01mg/mL		0.61	12724.229	12724.2	bb	20	0.01009681	0.96805
113	I3-0437	39-4a	Low, Stab, 0.01mg/mL		0.61	13261.877	13261.9	bb	20	0.01071064	7.1064

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY
Table 11a: Supporting Data
Quantify Compound Summary Report
(Analyzed 2 January 2008)

Dataset : C:\MassLynx\189205.PRO\189205f.qld
Time : Wednesday, January 02, 2008 15:47:14

Compound 1: HFPO dimer acid ammonium salt

	Run #	Ref. #	Sample Text	Std. Conc. (ng/mL)	RT	Sample Area	Ratio	Flags	Mult.	mg/mL	% RE
1	I2-0194	200-10	System Suit		0.57	623.596	623.596	bb	1	0.000004316	
2	I2-0195	200-10	System Suit						1		
3	I2-0196	200-10	System Suit		0.57	531.542	531.542	bb	1	0.000003819	
4	I2-0197	200-10	System Suit						1		
5	I2-0198		Diluent		0.62	568.735	568.735	bb	1	0.000004022	
6	I2-0199	200-1	STD 100ng		0.62	20260.709	20260.7	bb	1	0.000099886	-0.114
7	I2-0200	200-4	STD 250ng		0.62	44481.938	44481.9	bb	1	0.000248509	-0.5964
8	I2-0201	200-7	STD 500ng		0.62	77326.719	77326.7	bb	1	0.000512539	2.5078
9	I2-0202	200-10	STD 750ng						1		
10	I2-0203	200-13	STD 1000ng		0.62	121625.99	121626	bb	1	0.000997909	-0.2091
11	I2-0204		Diluent		0.62	122652.43	122652	bb	1	0.001011128	
12	I2-0205	189207-50-1	QC .001mg	0.001	0.62	326.225	326.225	bb	2	0.000005299	-99.47
13	I2-0206	189207-50-4	QC .05 mg	0.05	0.62	75281.727	75281.7	bb	100	0.04938691	-1.2262
14	I2-0207	189207-51-1	QC .5 mg	0.5	0.63	76184.969	76185	bb	1000	0.5020772	0.41544
15	I2-0208	189207-51-4	QC 50 mg	50	0.62	75845.164	75845.2	bb	100000	49.89822	-0.2036
16	I2-0209		Diluent		0.62	75973	75973	bb	1	0.000500146	
17	I2-0210	201-1	Group 1		0.63	175.854	175.854	bb	1	0.000001695	
18	I2-0211	201-2	Group 1		0.62	155.569	155.569	bb	1	0.000001555	
19	I2-0212	202-1	Group 2M		0.62	72585.016	72585	bb	60	0.02818311	-6.0563
20	I2-0213	202-2	Group 2M		0.62	72579.594	72579.6	bb	60	0.02818023	-6.0659
21	I2-0214	202-3	Group 2F/3M		0.62	75378.32	75378.3	bb	600	0.2968464	-1.0512
22	I2-0215	202-4	Group 2F/3M		0.62	74914.102	74914.1	bb	600	0.2943273	-1.8909
23	I2-0216	202-5	Group 4M		0.62	73092.516	73092.5	bb	6000	2.845338	-5.1554
24	I2-0217	202-6	Group 4M		0.62	76675.367	76675.4	bb	6000	3.039353	1.3118
25	I2-0218	202-7	Group 3F		0.62	76237.57	76237.6	bb	6000	3.015342	0.51142
26	I2-0219	202-8	Group 3F		0.62	76464.508	76464.5	bb	6000	3.027778	0.92594
27	I2-0220	202-9	Group 4F		0.62	75822.594	75822.6	bb	60000	29.92662	-0.2446
28	I2-0221	202-10	Group 4F		0.62	75645.688	75645.7	bb	60000	29.83016	-0.5661
29	I2-0222		Diluent		0.62	610.512	610.512	bb	60000	0.2547854	-99.151
30	I2-0223	189207-50-2	QC .001mg	0.001	0.62	76268.094	76268.1	bb	2	0.001005671	0.5671
31	I2-0224	189207-50-5	QC .05 mg	0.05	0.62	76525.641	76525.6	bb	100	0.05051887	1.0377
32	I2-0225	189207-51-2	QC .5 mg	0.5	0.62	76773.867	76773.9	bb	1000	0.5074612	1.4922
33	I2-0226	189207-51-5	QC 50 mg	50	0.62	73982.352	73982.4	bb	100000	48.21661	-3.5668
34	I2-0227		Diluent		0.62	620.477	620.477	bb	1	0.0000043	
35	I2-0228	200-2	STD 100ng	0.0001	0.62	20504.863	20504.9	bb	1	0.000101199	1.199
36	I2-0229	200-5	STD 250ng	0.00025	0.62	45036.625	45036.6	bb	1	0.000252356	0.9424
37	I2-0230	200-8	STD 500ng	0.0005	0.62	75933.195	75933.2	bb	1	0.000499783	-0.0434
38	I2-0231	200-11	STD 750ng	0.00075	0.62	101355.23	101355	bb	1	0.000755851	0.78013
39	I2-0232	200-14	STD 1000ng	0.001	0.62	122491.04	122491	bb	1	0.001009043	0.9043
40	I2-0233		Diluent		0.62	647.514	647.514	bb	60000	0.266608	
41	I2-0234	189207-50-3	QC .001mg	0.001	0.62	74531.141	74531.1	bb	2	0.000974188	-2.5812
42	I2-0235	189207-50-6	QC .05 mg	0.05	0.62	74686.461	74686.5	bb	100	0.04884924	-2.3015
43	I2-0236	189207-51-3	QC .5 mg	0.5	0.62	74867.727	74867.7	bb	1000	0.4901269	-1.9746
44	I2-0237	189207-51-6	QC 50 mg	50	0.62	73882.539	73882.5	bb	100000	48.12723	-3.7455
45	I2-0238		Diluent		0.62	608.554	608.554	bb	1	0.000004236	

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY
Table 11b: Supporting Data
Quantify Compound Summary Report
(Analyzed 2 January 2008)

	Run #	Ref. #	Sample Text	Std. Conc. (ng/mL)	RT	Sample Area	Ratio	Flags	Mult.		mg/mL	% RE
46	I2-0239	200-3	STD 100ng	0.0001	0.62	20093.426	20093.4	bb		1	0.000098988	-1.012
47	I2-0240	200-6	STD 250ng	0.00025	0.62	44189.785	44189.8	bb		1	0.000246491	-1.4036
48	I2-0241	200-9	STD 500ng	0.0005	0.62	75580.086	75580.1	bb		1	0.000496574	-0.6852
49	I2-0242	200-12	STD 750ng	0.00075	0.62	102185.48	102185	bb		1	0.000765078	2.0104
50	I2-0243	200-15	STD 1000ng	0.001	0.62	119757.77	119758	bb		1	0.000974096	-2.5904
51	I2-0244		Diluent		0.62	649.23	649.23	bb		1	0.000004453	

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN RATS WITH A 28-DAY RECOVERY
Table 12a: Supporting Data
Quantify Compound Summary Report
(Analyzed 7 December 2007)

Sample Lis C:\MASSLYNX\189205.PRO\SampleDB\I1-189205g
Last modifi Fri Dec 07 13:45:46 2007
Method: C:\MASSLYNX\189205.PRO\MethDB\I1-189205a
Last modifi Wed Nov 28 09:07:30 2007
Job Code:

Printed: Thu Dec 20 14:54:01 2007

Compound 1: HFPO Dimer Acid Ammonium Salt

Name	ID	Sample Text	RT	Area	Mult.	Flags	Conc
I1-0343	114-7	sys suit	0.54	37584	1	bb	
I1-0344	114-7	sys suit	0.54	43239	1	bb	
I1-0345	114-7	sys suit	0.54	41818	1	bb	
I1-0346	114-7	sys suit	0.54	44049	1	bb	
I1-0347	114-7	sys suit	0.54	44804	1	bb	
I1-0348	114-7	sys suit	0.54	45388	1	bb	
I1-0349	131-1	Low stock, 150 mg/mL	0.54	58018	300000	bb	203.2692
I1-0350	131-2	Low stock, 150 mg/mL	0.55	60331	300000	bb	211.3729
I1-0351	131-3	Low stock, 150 mg/mL	0.54	59778	300000	bb	209.4355
I1-0352	131-4	Low stock, 150 mg/mL	0.54	63167	300000	bb	221.309
I1-0353	131-5	High stock, 240 mg/mL	0.54	43309	480000	bb	242.7767
I1-0354	131-6	High stock, 240 mg/mL	0.54	43101	480000	bb	241.6107
I1-0355	131-7	High stock, 240 mg/mL	0.54	44500	480000	bb	249.4531
I1-0356	131-8	High stock, 240 mg/mL	0.54	41322	480000	bb	231.6382

Concentration calculated by comparing average area of system suitability injections (500 ng/mL) to area obtained from stock solutions and applying the multiplication factor

APPENDIX D

Pretest Clinical Observations

PRETEST CLINICAL OBSERVATIONS (MALES)

PROJECT NO.:WIL-189205P A 28-DAY ORAL STUDY OF H-28397 IN RATS WITH 28-DAY RECOVERY PAGE 1
SPONSOR:E.I. DUPONT SUMMARY OF CLINICAL FINDINGS: TOTAL OCCURRENCE/NO. OF ANIMALS
SPONSOR NO.:DUPONT-24447

	----- M A L E -----
TABLE RANGE:	11-30-07 TO 12-09-07
GROUP:	1
NORMAL	
-NO SIGNIFICANT CLINICAL OBSERVATIONS	136/69
EYES/EARS/NOSE	
-DRIED RED MATERIAL AROUND RIGHT EYE	2/ 1
-DRIED RED MATERIAL AROUND NOSE	1/ 1
BODY/INTEG II	
-SCABBING DORSAL HEAD	1/ 1

1- PRETEST

PCSUv4.07
04/22/2008

PRETEST CLINICAL OBSERVATIONS (FEMALES)

PROJECT NO.:WIL-189205Q A 28-DAY ORAL STUDY OF H-28397 IN RATS WITH 28-DAY RECOVERY PAGE 1
SPONSOR:E.I. DUPONT SUMMARY OF CLINICAL FINDINGS: TOTAL OCCURRENCE/NO. OF ANIMALS
SPONSOR NO.:DUPONT-24447

----- F E M A L E -----

TABLE RANGE:	11-30-07 TO 12-10-07
GROUP:	1
NORMAL	
-NO SIGNIFICANT CLINICAL OBSERVATIONS	140/70

1- PRETEST

PCSUv4.07
04/22/2008

APPENDIX E

Animal Room Environmental Conditions

A 28-DAY ORAL STUDY OF H-28397 IN RATS WITH 28-DAY RECOVERY PAGE 1
PROJECT NO.:WIL- 189205 TEMPERATURE/HUMIDITY - DAILY SUMMARY REPORT BY STUDY
SPONSOR: E.I. DUPONT

STUDY SPECIFICATIONS: 189205 DATE IN: 11/27/07 TIME IN: 7:00
DATE OUT: 02/05/08 TIME OUT: 16:00

ROOM SPECIFICATIONS: B ROOM 106 LOW TEMPERATURE ºF: 66.0 HIGH TEMPERATURE ºF: 76.0 LOW HUMIDITY: 30.0
SPECIES: RAT LOW TEMPERATURE ºC: 18.9 HIGH TEMPERATURE ºC: 24.4 HIGH HUMIDITY: 70.0

	TEMPERATURE		HUMIDITY
DATE	MEAN (ºF)	MEAN (ºC)	MEAN (%RH)
27-Nov-07	70.4	21.3	40.1
28-Nov-07	70.4	21.4	40.0
29-Nov-07	70.5	21.4	38.8
30-Nov-07	70.3	21.3	38.5
01-Dec-07	70.5	21.4	36.6
02-Dec-07	70.6	21.4	43.8
03-Dec-07	70.7	21.5	41.5
04-Dec-07	70.6	21.4	38.2
05-Dec-07	70.4	21.3	41.2
06-Dec-07	70.8	21.6	34.7
07-Dec-07	70.4	21.4	45.5
08-Dec-07	70.4	21.3	45.9
09-Dec-07	70.9	21.6	48.1
10-Dec-07	70.5	21.4	51.0
11-Dec-07	70.6	21.5	55.6
12-Dec-07	70.7	21.5	48.2
13-Dec-07	70.7	21.5	49.4
14-Dec-07	70.2	21.2	44.7
15-Dec-07	70.8	21.5	44.0
16-Dec-07	70.7	21.5	45.9
17-Dec-07	70.7	21.5	38.9
18-Dec-07	71.0	21.7	40.7
19-Dec-07	70.2	21.2	47.5

NOTE: + = VALUE WAS GREATER THAN HIGH RANGE
\- = VALUE WAS LESS THAN LOW RANGE
NOTE: MEANS REPRESENT THE MEAN OF THE DAILY VALUES

REPORT 4
VERSION 1.09
4/15/2008 10:43

A 28-DAY ORAL STUDY OF H-28397 IN RATS WITH 28-DAY RECOVERY PAGE 2

PROJECT NO.:WIL- 189205 TEMPERATURE/HUMIDITY - DAILY SUMMARY REPORT BY STUDY

SPONSOR: E.I. DUPONT

STUDY SPECIFICATIONS: 189205 DATE IN: 11/27/07 TIME IN: 7:00
DATE OUT: 02/05/08 TIME OUT: 16:00

ROOM SPECIFICATIONS: B ROOM 106 LOW TEMPERATURE ºF: 66.0 HIGH TEMPERATURE ºF: 76.0 LOW HUMIDITY: 30.0
SPECIES: RAT LOW TEMPERATURE ºC: 18.9 HIGH TEMPERATURE ºC: 24.4 HIGH HUMIDITY: 70.0

	TEMPERATURE		HUMIDITY
DATE	MEAN (ºF)	MEAN (ºC)	MEAN (%RH)
20-Dec-07	70.5	21.4	44.4
21-Dec-07	70.7	21.5	47.4
22-Dec-07	70.6	21.4	56.6
23-Dec-07	70.5	21.4	48.0
24-Dec-07	70.6	21.4	41.6
25-Dec-07	70.7	21.5	44.7
26-Dec-07	70.1	21.2	46.6
27-Dec-07	70.7	21.5	51.3
28-Dec-07	70.5	21.4	52.5
29-Dec-07	70.2	21.2	46.7
30-Dec-07	70.2	21.2	44.6
31-Dec-07	70.7	21.5	48.0
01-Jan-08	70.8	21.6	44.4
02-Jan-08	70.4	21.4	40.4
03-Jan-08	70.2	21.2	36.5
04-Jan-08	70.6	21.5	38.5
05-Jan-08	70.2	21.2	49.1
06-Jan-08	70.9	21.6	54.0
07-Jan-08	70.6	21.5	60.3
08-Jan-08	70.8	21.5	55.1
09-Jan-08	70.6	21.5	50.2
10-Jan-08	70.6	21.5	49.9
11-Jan-08	70.5	21.4	50.8

NOTE: + = VALUE WAS GREATER THAN HIGH RANGE
\- = VALUE WAS LESS THAN LOW RANGE
NOTE: MEANS REPRESENT THE MEAN OF THE DAILY VALUES

REPORT 4
VERSION 1.09
4/15/2008 10:43

A 28-DAY ORAL STUDY OF H-28397 IN RATS WITH 28-DAY RECOVERY PAGE 3

PROJECT NO.:WIL- 189205 TEMPERATURE/HUMIDITY - DAILY SUMMARY REPORT BY STUDY

SPONSOR: E.I. DUPONT

STUDY SPECIFICATIONS: 189205 DATE IN: 11/27/07 TIME IN: 7:00
DATE OUT: 02/05/08 TIME OUT: 16:00

ROOM SPECIFICATIONS: B ROOM 106 LOW TEMPERATURE ºF: 66.0 HIGH TEMPERATURE ºF: 76.0 LOW HUMIDITY: 30.0
SPECIES: RAT LOW TEMPERATURE ºC: 18.9 HIGH TEMPERATURE ºC: 24.4 HIGH HUMIDITY: 70.0

	TEMPERATURE		HUMIDITY	
DATE	MEAN (ºF)	MEAN (ºC)	MEAN (%RH)	
12-Jan-08	70.6	21.4	45.1	
13-Jan-08	70.6	21.4	48.1	
14-Jan-08	70.5	21.4	45.0	
15-Jan-08	70.6	21.5	40.6	
16-Jan-08	70.5	21.4	39.8	
17-Jan-08	70.5	21.4	45.9	
18-Jan-08	70.5	21.4	38.0	
19-Jan-08	70.5	21.4	35.1	
20-Jan-08	70.5	21.4	29.8	-
21-Jan-08	70.5	21.4	30.2	
22-Jan-08	70.5	21.4	39.4	
23-Jan-08	70.6	21.4	33.9	
24-Jan-08	70.5	21.4	34.9	
25-Jan-08	70.5	21.4	31.6	
26-Jan-08	70.6	21.4	38.4	
27-Jan-08	70.6	21.4	42.8	
28-Jan-08	70.3	21.3	42.2	
29-Jan-08	70.5	21.4	49.2	
30-Jan-08	70.7	21.5	36.5	
31-Jan-08	70.3	21.3	40.5	
01-Feb-08	70.4	21.3	53.5	
02-Feb-08	70.5	21.4	51.6	
03-Feb-08	70.7	21.5	49.0	

NOTE: + = VALUE WAS GREATER THAN HIGH RANGE
- = VALUE WAS LESS THAN LOW RANGE
NOTE: MEANS REPRESENT THE MEAN OF THE DAILY VALUES

REPORT 4
VERSION 1.09
4/15/2008 10:43

A 28-DAY ORAL STUDY OF H-28397 IN RATS WITH 28-DAY RECOVERY PAGE 4

PROJECT NO.:WIL- 189205 TEMPERATURE/HUMIDITY - DAILY SUMMARY REPORT BY STUDY

SPONSOR: E.I. DUPONT

STUDY SPECIFICATIONS: 189205 DATE IN: 11/27/07 TIME IN: 7:00
DATE OUT: 02/05/08 TIME OUT: 16:00

ROOM SPECIFICATIONS: B ROOM 106 LOW TEMPERATURE ºF: 66.0 HIGH TEMPERATURE ºF: 76.0 LOW HUMIDITY: 30.0
SPECIES: RAT LOW TEMPERATURE ºC: 18.9 HIGH TEMPERATURE ºC: 24.4 HIGH HUMIDITY: 70.0

	TEMPERATURE		HUMIDITY
DATE	MEAN (ºF)	MEAN (ºC)	MEAN (%RH)
04-Feb-08	70.3	21.3	50.9
05-Feb-08	70.6	21.4	60.9

GRAND STATS	MEAN	MIN	MAX
TEMPERATURE ºF	70.5	70.1	71.0
TEMPERATURE ºC	21.4	21.2	21.7
HUMIDITY (%RH)	44.4	29.8 -	60.9
N DAYS	71		

NOTE: + = VALUE WAS GREATER THAN HIGH RANGE
\- = VALUE WAS LESS THAN LOW RANGE
NOTE: MEANS REPRESENT THE MEAN OF THE DAILY VALUES

REPORT 4
VERSION 1.09
4/15/2008 10:43

ROOM SPECIFICATIONS:	B ROOM 106		
SPECIES:	RAT		
LOW TEMPERATURE:	66.0	DATE IN:	11/27/07
HIGH TEMPERATURE:	76.0	TIME IN:	7:00
LOW HUMIDITY:	30.0	DATE OUT:	02/05/08
HIGH HUMIDITY:	70.0	TIME OUT:	16:00

ROOM B ROOM 106 SUMMARY

	TEMPERATURE	HUMIDITY
MEAN	70.5	44.4
MIN	68.2	28.0
MAX	72.9	76.3
SD	0.93	7.54
N SAMPLES	1686	1686
FIRST DAY	11/27/07	
LAST DAY	02/05/08	
N DAYS	71	

NOTE: TEMPERATURE UNITS = DEGREES FAHRENHEIT
HUMIDITY UNITS = % RELATIVE HUMIDITY
NOTE: MEANS REPRESENT THE MEAN OF ALL VALUES

STUDY 189205 SUMMARY

MEAN	70.5	44.4
MIN	68.2	28.0
MAX	72.9	76.3
SD	0.93	7.54
N SAMPLES	1686	1686
FIRST DAY	11/27/07	
LAST DAY	02/05/08	
N DAYS	71	

NOTE: TEMPERATURE UNITS = DEGREES FAHRENHEIT
HUMIDITY UNITS = % RELATIVE HUMIDITY
NOTE: MEANS REPRESENT THE MEAN OF ALL VALUES

APPENDIX F

Clinical Pathology Methods, Procedures And References

CLINICAL PATHOLOGY METHODS, PROCEDURES AND REFERENCES

Serum Chemistry - Hitachi 912

Albumin - Bromcresol Green (BCG) Method, Modification of the Doumas reaction. Default unit: g/dL. Hitachi 912 Application Code 413. Roche Reagent, catalog number 11970569.

A/G Ratio - Calculated from Albumin and Globulin Results. Default unit: %.

Alkaline Phosphatase - based on the International Federation of Clinical Chemistry (IFCC) Method (Tietz *et al.* J Clin Chem Clin Biochem 1983; 21:731-748). Default unit: U/L. Hitachi 912 Application Code 158. Roche Reagent, catalog number 2172933.

Alanine Aminotransferase - Modification of the International Federation of Clinical Chemistry (IFCC) recommended method. Default unit: U/L. Hitachi 912 Application Code 706. Roche Reagent, catalog number 450065.

Aspartate Aminotransferase - Kinetic method based on the International Federation of Clinical Chemistry (IFCC) recommendations. Default unit: U/L. Hitachi 912 Application Code 713. Roche Reagent, catalog number 450064.

Bilirubin (Total) - Diazo method developed by Wahlefeld *et al*. Scand J Clin Lab Invest 1972; 29: Supplement 126. Default unit: mg/dL. Hitachi 912 Application Code 018. Roche Reagent, catalog number 1822730.

Blood Urea Nitrogen (BUN) - A urease-triggered methodology based upon the method of Talke and Schubert Klin Wschr, 1965; 43:174. Default unit: mg/dL. Hitachi 912 Application Code 427. Roche Reagent, catalog number 1489321.

Calcium - Modified method using a gamma-amino-butyric acid (GABA) buffer. Default unit: mg/dL. Hitachi 912 Application Code 180. Roche Reagent, catalog number 1125621.

Chloride - An ion-selective electrode that measures the electrical potential of the ions present in solution. Default unit: mEq/L. Hitachi 912 Application. Roche Reagent, catalog numbers 450043,450042, and 450041.

Cholesterol - Enzymatic reaction as described by Trinder. Ann Clin Biochem 1974; 12:266. Default unit: mg/dL. Hitachi 912 Application Code 722. Roche Reagent, catalog number 450061.

Creatinine - Modified Jaffe reaction based on the work of Poper *et al.* Biochem Z 1937; 291:354, and Seelig and Wuest. Aerztl Labor 1969; 15:34. Default unit: mg/dL. Hitachi 912 Application Code 727. Roche Reagent, catalog number 450019.

Gamma-Glutamyltransferase (GGT) - Method based on the studies of Persijn and Van der Slik, J Clin Chem Clin Biochem, 1976; 14:421-427. Default unit: U/L. Hitachi 912 Application Code 479. Roche Reagent, catalog number 2016885.

Globulin - Calculation obtained by subtracting Albumin from Total Protein. Default unit: g/dL.

Glucose - Glucose hexokinase method based on the work of Schmidt, Peterson and Young. Klin Wschr 1961; 39:1244. Methods of Enzymatic Analysis, 2nd Eng ed. New York, Academic Press, 1974; 1196. Anal Biochemistry 1958; 23:301. Default unit: mg/dL. Hitachi 912 Application Code 767. Roche Reagent, catalog number 450058.

Phosphorus - Method involves the formation of ammonium phosphomolybdate. Default unit: mg/dL. Hitachi 912 Application Code 714. Roche Reagent, catalog number 1040898.

Potassium - An ion-selective electrode that measures the electrical potential of the ions present in solution. Default unit: mEq/L. Hitachi 912 Application. Roche Reagent, catalog numbers 450043,450042, and 450041.

Sodium - An ion-selective electrode that measures the electrical potential of the ions present in solution. Default unit: mEq/L. Hitachi 912 Application. Roche Reagent, catalog number 450043,450042, and 450041.

Sorbitol Dehydrogenase (SDH) - An ultraviolet, kinetic method utilizing the following principal: Fructose + NADH $\xleftarrow{\text{SDH}}\rightarrow$ Sorbitol + NAD. Default unit: U/L. Diagnostic Chemicals Limited, catalog number 740-10B-2, Hitachi 912 Application Code 342.

Total Protein - Endpoint biuret method that utilizes a sample blank. Default unit: g/dL. Hitachi 912 Application Code 756. Roche Reagent, catalog number 1040901.

Triglycerides - Method that utilizes lipase from a microorganism to promote rapid and complete hydrolysis of triglycerides to glycerol. Default unit: mg/dL. Hitachi 912 Application Code 781. Roche Reagent, catalog number 1488899.

Hematology - Manual Methods

White Cell Differential - Manual method of counting 100 white cells stained with Wright Giemsa and entered on-line into the data files.

Reticulocyte Count - Manual method of counting the reticulocytes present in 1000 red blood cells stained with New Methylene Blue and entered on-line into the data files.

Red Blood Cell Morphology - Manual method of evaluating red blood cells on a Wright Giemsa-stained slide and entered on-line into the data files.

Platelet Estimate- Manual method of evaluating platelets on a Wright Giemsa-stained slide. Platelet estimation is evaluated and entered on-line into the data files as decreased, adequate or increased. Platelet clumps present on the slide will be reported as part of the RBC morphology.

AMAX Destiny Amelung Coagulation

Mechanical (Ball Method) Measurement – Mechanical measurement methods depend on the physical formation of fibrin strands that attach to a moving mechanical device that either completes or opens an electrical circuit. The elapsed time from the addition of the starting reagent, up to the beginning of fibrin formation is measured.

Prothrombin Time (Protime-PT)/(PTM)- mechanical clot detection is used to measure and record the time required for plasma specimens to clot by adding an excess of thromboplastin reagent and an optional amount of calcium to citrated plasma. The PT measures from factor VII through fibrin formation (extrinsic and common pathways of coagulation). Default unit: sec.

Activated Partial Thromboplastin Time (APTT)/(PTTM)- mechanical clot detection is used to measure and record clotting time in a one-stage procedure which consists of recalcifying plasma in the presence of an excess of platelet-like reagent (cephalin) containing a plasma activator. The APTT measures the clotting time from factor XII through fibrin formation (intrinsic and common pathways of coagulation) Default unit: sec.

Urine Chemistry-Bayer CLINITEK® 500-Siemens Healthcare Diagnostics/(Formerly: Bayer CLINITEK®500)

Bilirubin - This test is based on the coupling of bilirubin with diazotized dichloroaniline in a strongly acid medium. The color ranges through various shades of tan.

Blood - This test is based on the peroxidase-like activity of hemoglobin, which catalyzes the reaction of diisopropylbenzene dihydroperoxide and $3,3^{1},5,5^{1}$-tetramethylbenzidine. The resulting color ranges from orange through green; very high levels of blood may cause the color development to continue to blue.

Glucose - This test is based on a double sequential enzyme reaction. One enzyme, glucose oxidase, catalyzes the formation of gluconic acid and hydrogen peroxide from the oxidation of glucose. A second enzyme, peroxidase, catalyzes the reaction of hydrogen peroxide with potassium iodide chromogen to oxidize the chromogen to colors ranging from green to brown.

Ketone - This test is based on the development of colors ranging from buff-pink to purple when acetoacetic acid reacts with nitroprusside.

Leukocytes - Granulocytic leukocytes contain esterases that catalyze the hydrolysis of the derivatized pyrrole amino acid ester to liberate 3-hydroxy-5-phenyl pyrrole. This pyrrole then reacts with a diazonium salt to produce a purple product.

Nitrite - This test depends upon the conversion of nitrate to nitrite by the action of Gram-negative bacteria in the urine. At the acid pH of the reagent area, nitrite in the urine reacts with p-arsanilic acid to form a diazonium compound. This diazonium compound in turn couples with 1,2,3,4-tetrahydrobenzo(h)quinolin-3-ol to produce a pink color.

pH - This test is based on a double indicator principle that gives a broad range of colors covering the entire urinary pH range. Colors range from orange to yellow and green to blue.

Protein - This test is based on the protein-error-of-indicators principle. The development of any green color is due to the presence of protein. Colors range from yellow to green-blue.

Urobilinogen - This test is based on a modified Ehrlich reaction, in which p-diethylaminobenzaldehyde in conjunction with a color enhancer reacts with urobilinogen in a strongly acid medium to produce a pink-red color.

Urine Chemistry

Macroscopic

Color and Clarity - Characteristics that are visually inspected and entered on-line into the data files.

Specific Gravity - Measurement obtained by use of hand refractometer which allows for direct determination of specific gravity of urine; entered on-line into the data files. Reichert VET 360 (A/B) - Urine Specific Gravity Refractometer. Reichert Analytical Instruments, Inc., Depew, N.Y.

Microscopic

Examination of urine sediment under a microscope and entered on-line into the data files.

Urine Chemistry Advanced Osmometer Model 3900

Osmolality - Measurement of osmotic concentration by means of the freezing point method.

Hematology-Bayer Advia® 120-Siemens Healthcare Diagnostics/(Formerly: Bayer Advia®120)

WBC Count - The whole blood sample is mixed with ADVIA® 120 BASO reagent that contains acid and surfactant. The red cells are hemolyzed, and the white blood cells are then analyzed using two angle laser light scatter signals. Default unit: x 10^3 cells/μL

RBC / Platelet Count - Both red blood cells and platelets are analyzed by a single optical cytometer after appropriate dilution of the blood sample with ADVIA® 120 RBC/PLT reagent. The red blood cells are isovolumetrically sphered and lightly fixed with glutaraldehyde to preserve the spherical shape. Red cells and platelets are counted from the signals from a common detector with 2 different gain settings. Default unit RBC: x 10^6cells/μL. Default unit PLT: x 10^3 cells/μL

Hgb - Hemoglobin: The hemoglobin method is a modification of the manual cyanmethemoglobin method developed by the International Committee for standardization in Hematology (ICSH). Default unit: g/dL.

Hematocrit - The percentage of blood volume that is occupied by red blood cells. Also referred to as the packed red cell volume. On the ADVIA® 120 Hematology System this parameter is derived from the measured red cell volume (MCV) and the red cell count (RBC). Default unit: %.

MCH - Mean Corpuscular Hemoglobin: the average weight of hemoglobin in the red blood cells, calculated from the RBC and Hgb measurements. Default unit: pg.

MCHC - Mean Corpuscular Hemoglobin Concentration: the average concentration of hemoglobin in the red blood cells. This parameter is computed from the measured hemoglobin and the computed hematocrit. Default unit: g/dL.

MCV - Mean Corpuscular Volume: the average volume of the red blood cells. Default unit: fL

White blood cell differential - The ADVIA® 120 Hematology System White Blood Cell Differential (WBC DIFF) methods, consists of both the Peroxidase method and the Basophil/Lobularity method. The ADVIA® 120 Hematology System performs a six-part differential that consists of basophils, eosinophils, large unstained cells, lymphocytes, monocytes, and neutrophils. The white blood cell differential count is reported in percent and the actual number of each type of cell per microliter of blood.

Reticulocyte - This method uses a nucleic acid dye (oxazine 750) to stain cellular RNA. The ADVIA® 120 autoRETIC reagent isovolumetrically spheres the erythroid cells and stains cellular RNA. Low-angle laser light scatter, high-angle laser light scatter, and absorption characteristics of all cells are counted and measured. The absorption data are used to classify each cell as a reticulocyte or mature red blood cell based on its RNA content. The reticulocyte is reported in percent and actual number x 10^9cells/Liter = thous/µl.

References:

Bayer Reagent Strips, Multistix® - Siemens Healthcare Diagnostics/ (Formerly: Bayer Corporation Diagnostics Division). Package Insert, 5/95.

ADVIA® 120 Hematology System Operator's Guide: Copyright© 1997, 1998. Siemens Healthcare Diagnostics/ (Formerly: Bayer Corporation Diagnostics Division).

AMAX Destiny Operation Manual SW V 1.1.4, 31/08/05. Trinity Biotech Plc.

The Advanced™ Osmometer Model 3900. Advanced Instruments, Inc.

APPENDIX G

Pathology Report (WIL Research Laboratories, LLC)

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY OF H-28397 IN RATS WITH A 28-DAY RECOVERY

PATHOLOGY REPORT

Pathology Department

WIL Research Laboratories, LLC

TABLE OF CONTENTS

Page

Page

1. INTRODUCTION

The objective of this study was to evaluate the potential toxicity, and recovery therefrom, of H-28397 when administered to rats by oral gavage for 28 consecutive days.

2. STUDY DESIGN

Male and female Crl:CD(SD) rats were administered H-28397 via oral gavage once daily for 28 consecutive days as indicated in the following table. The dosage volume was 10 mL/kg for all groups.

Group Number	Test Substance	Dosage Level (mg/kg/day) Males	Dosage Level (mg/kg/day) Females	Number of Animals [a] Males	Number of Animals [a] Females
1	Vehicle [b]	0	0	20	20
2	H-28397	0.3	3	10	10
3	H-28397	3	30	10	10
4	H-28397	30	300	20	20

[a] = 10 animals/sex/group were euthanized following a minimum of 28 days of dose administration; the remaining ≤ 10 animals/sex in Groups 1 and 4 were euthanized following a 28-day nondosing (recovery) period.

[b] = The vehicle was deionized water.

3. METHODS

3.1. CLINICAL PATHOLOGY

Hematology, coagulation, serum chemistry and urine parameters were evaluated on all animals just prior to the scheduled necropsies (i.e., 10 animals/sex/group at the primary necropsy and ≤10 animals/sex/group for Groups 1 and 4 at the recovery necropsy). Animals were fasted overnight prior to blood collection. Blood was collected for hematology and serum chemistry evaluation via the retro-orbital sinus of animals anesthetized by inhalation of isoflurane. Blood was collected for coagulation parameters at the time of euthanasia via the vena cava of animals euthanized by inhalation of carbon dioxide. Urine was collected overnight prior to blood collection using metabolism cages. Anticoagulants were potassium EDTA for the hematology parameters and sodium citrate

for the coagulation parameters. Anticoagulants were not used for serum chemistry samples.

The following parameters were evaluated.

3.1.1. HEMATOLOGY AND COAGULATION

Total leukocyte count (White Cells)
Erythrocyte count (Red Cells)
Hemoglobin
Hematocrit
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular hemoglobin concentration (MCHC)
Platelet count (Platelet)
Prothrombin time (ProTime)
Activated partial thromboplastin time (APTT)
Reticulocyte count
  Percent (Reticulocyte)
  Absolute (Retic Absolute)
Differential leukocyte count - Percent and absolute
  -Neutrophil
  -Lymphocyte
  -Monocyte
  -Eosinophil
  -Basophil
  -Large unstained cell
Blood smears [a]

( ) - Designates tabular abbreviation
a - Blood smears were made for all animals receiving a hematology evaluation. Blood smears were evaluated when scientifically warranted. Parameters evaluated from these smears included a differential leukocyte count, platelet estimates and RBC morphology.

3.1.2. SERUM CHEMISTRY

Albumin
Total protein
Globulin [by calculation]
Albumin/globulin ratio (A/G Ratio) [by calculation]
Total bilirubin (Total Bili)
Urea nitrogen
Creatinine
Alkaline phosphatase (AlkalinePhos'tse)
Alanine aminotransferase (Alanine Transfer)
Aspartate aminotransferase (AspartatTransfer)
Gamma glutamyltransferase (GlutamylTransfer)
Glucose
Total cholesterol (Cholesterol)
Calcium
Chloride
Phosphorus
Potassium
Sodium
Triglycerides (Triglyceride)
Sorbitol dehydrogenase (Sorbitol'Genase) [a]

( ) - Designates tabular abbreviation
[a] - Presented on special chemistry tables

3.1.3. URINALYSIS

Specific gravity (SG)
pH
Urobilinogen (URO)
Total volume (TVOL)
Osmolality [a]
Color (COL)
Clarity (CLA)
Protein (PRO)
Glucose (GLU)
Ketones (KET)
Bilirubin (BIL)
Occult blood (BLD)
Leukocytes (LEU)
Nitrites (NIT)
Microscopy of sediment [Tabular abbreviations appear on individual tables]

( ) - Designates tabular abbreviation
[a] - Presented on urine chemistry tables

3.2. ANATOMIC PATHOLOGY

3.2.1. MACROSCOPIC EXAMINATION

Complete postmortem examinations were performed on all animals at the scheduled necropsies. Animals were euthanized by carbon dioxide inhalation and exsanguinated.

At the time of necropsy, the following tissues and organs were collected and placed in 10% neutral-buffered formalin fixative unless otherwise noted:

Adrenals (2)
Aorta
Bone with marrow
  Femur
  Sternum
Bone marrow smear [a]
Brain
  Cerebrum (2 levels)
  Cerebellum with pons/medulla
Cervix
Epididymides [b]
Exorbital lacrimal glands (2)
Eyes with optic nerves (2) [c]
Gastrointestinal tract
  Esophagus
  Stomach
  Duodenum
  Jejunum
  Ileum
  Peyer's patches
  Cecum
  Colon
  Rectum
Heart
Kidneys (2)
Larynx
Liver (sections of 2 lobes)
Lungs (including bronchi, fixed by inflation with fixative)
Lymph nodes
  Mandibular
  Mesenteric
Nasal cavity [d]
Ovaries (2) with oviducts [e]
Pancreas
Peripheral nerve (sciatic)
Pharynx
Pituitary
Prostate
Salivary glands [mandibular (2)]
Seminal vesicles (2)
Skeletal muscle (rectus femoris)
Skin with mammary gland [f]
Spinal cord (cervical, thoracic, lumbar)
Spleen
Testes (2) [b]
Thymus
Thyroids (with parathyroids) (2) [e]
Tongue
Trachea
Urinary bladder
Uterus
Vagina
Gross lesions and masses (when possible)

[a] - Bone marrow smears were obtained at the scheduled necropsies, but not placed in formalin; slides were examined only if scientifically warranted.
[b] - Fixed in Bouin's solution
[c] - Fixed in Davidson's solution
[d] - Levels I and III were examined (Young, 1981).
[e] - Oviducts and parathyroids were examined microscopically if in the plane of section and in all cases where a gross lesion of the organ was present.
[f] - For females; a corresponding section of skin was collected from the same anatomic area for males.

3.2.2. ORGAN WEIGHTS

The following organs were weighed from all animals at the scheduled necropsies:

Adrenals	Ovaries (with oviducts)
Brain	Spleen
Epididymides	Testes
Heart	Thymus
Kidneys	Uterus
Liver	

Paired organs were weighed together. Organ-to-final-body-weight and organ-to-brain-weight ratios were calculated.

3.2.3. MICROSCOPIC EXAMINATION

Microscopic examination of routinely prepared hematoxylin-eosin stained paraffin sections was performed on all tissues collected at necropsy from all animals in the control and high-dose groups euthanized at the scheduled primary necropsy. Gross lesions were examined from animals in the low- and mid-dose groups euthanized at the primary necropsy and animals in the control and high-dose groups euthanized at the recovery necropsy. In addition, the liver was evaluated from animals in the low- and mid-dose groups at the primary necropsy as well as from animals in the control and high-dose groups at the recovery necropsy. Stained histologic sections were examined by light microscopy and observations were entered in the WIL Toxicology Data Management System (WTDMS™) by the pathologist. All gross necropsy observations were addressed. Histologic sections were of adequate size and quality for detailed evaluation. The number of tissues examined from each dosage group may not necessarily equal the number of animals included in the group due to sectioning difficulties. The number of missing tissues was negligible and did not interfere with detection of test substance-related histologic alterations in the study. Histopathologic lesions were classified using standard published terminology to the extent possible. The WTDMS™ histopathology tables contain all of the recorded data and serve as the basis for this narrative report.

3.3. ABBREVIATIONS

The following abbreviations may apply to this report:

Interval - point in the study at which event occurred (specimen collection, necropsy, etc.)
PN - study day 28 primary necropsy (end of dosing)
RN - study day 56 recovery necropsy

3.4. DATA INTERPRETATION

In the discussion of clinical pathology parameters, values derived from the control group animals at all time points evaluated were considered as concurrent control values for purposes of constructing a 'normal' range for the present study. In addition, historical control values for this laboratory were consulted to refine data interpretation. Unless otherwise stated in this report, the 'normal' historical control range was represented by values within the WIL Historical control reference range (essentially a 95% confidence interval).

In the discussion of organ weight changes, the indication of higher or lower mean organ weights refers to a statistically significant ($p<0.05$ or $p<0.01$ using Dunnett's test) difference between test substance-treated versus control group animals in the present study. In addition, historical control values for this laboratory were consulted to refine data interpretation.

4. RESULTS

4.1. SURVIVAL

All animals survived until the scheduled euthanasia on study day 28 primary necropsy (PN) and study day 56 recovery necropsy (RN).

4.2. CLINICAL PATHOLOGY

4.2.1. HEMATOLOGY AND COAGULATION

4.2.1.1. CHANGES ASSOCIATED WITH TEST SUBSTANCE ADMINISTRATION

There were no test substance-related alterations in coagulation parameters.

Minimal, statistically significant decreases in red cell mass parameters (RBC, hemoglobin and hematocrit) were present in the 3 and 30 mg/kg/day male groups (Text Table 1). These decreases were associated with minimal increases in absolute reticulocyte counts. The decreases in red cell mass parameters were minimal (≤ 7.9% below the control mean for all parameters), and values for red cell mass parameters and reticulocyte counts in individual animals in the 3 and 30 mg/kg/day male groups were within WIL historical control reference ranges for the respective parameters. Therefore, the hematological changes in the 3 and 30 mg/kg/day male groups were considered to be test substance-related but nonadverse. There were no statistically significant changes in red cell mass parameters or reticulocytes following the 4-week recovery period.

Text Table 1. Selected Hematology Findings - Males

Analysis	Group (mg/kg/day):	0	0.3	3	30
Red Cells (mil/uL)					
Week 4 Mean		8.44	8.27	8.12*	7.97**
% Difference			-2.0	-3.8	-5.6
Week 8 Mean		8.89	NA	NA	8.75
% Difference					-1.6
Hemoglobin (g/dL)					
Week 4 Mean		16.3	16.3	15.8*	15.2**
% Difference			0.0	-3.1	-6.7
Week 8 Mean		16.0	NA	NA	15.8
% Difference					-1.3
Hematocrit (%)					
Week 4 Mean		45.6	44.9	43.4**	42.0**
% Difference			-1.5	-4.8	-7.9
Week 8 Mean		44.6	NA	NA	44.2
% Difference					-0.9
Absolute Reticulocytes (thous/uL)					
Week 4 Mean		188.9	189.9	196.1	224.9
% Difference			0.5	3.8	19.1
Week 8 Mean		219.9	NA	NA	228.3
% Difference					3.8

NA = Not applicable
* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test

4.2.1.2. CHANGES UNRELATED TO TEST SUBSTANCE ADMINISTRATION

There were no other test substance-related effects on hematology (including coagulation) parameters. However, some statistically significant differences were observed when the control and test substance-treated groups were compared. These findings included slightly higher mean hemoglobin corpuscular concentration (MCHC) and lower absolute reticulocyte counts in the 300 mg/kg/day group females at study week 8, which were not considered test substance-related because the change occurred only at the recovery interval.

Statistically significant findings that involved percentage reticulocyte or leukocyte differential counts were not itemized above, and were not considered toxicologically important because absolute cell counts are utilized for interpretative purposes.

4.2.2. SERUM CHEMISTRY

4.2.2.1. CHANGES ASSOCIATED WITH TEST SUBSTANCE ADMINISTRATION

Alterations in serum chemistry parameters that were considered to be related to test substance administration are summarized below in Text Tables 2 and 3. Changes in serum chemistry parameters were mostly minimal and were not considered to be adverse. Administration of the test material was also associated with increases in beta-oxidation (see Liver Metabolic Enzymes section), an indicator of peroxisome proliferator alpha (PPARα) receptor activation, and the clinical chemistry changes observed were consistent with PPARα activation (Staels et al., 1998; Sheikh et al, 2006; Grevois et al., 2004).

Test substance-related and statistically significant decreases in cholesterol were present in all treated male groups. Decreases were minimal, as values for most animals were within or only slightly below the WIL historical control range. Based on the minimal nature of the changes, as well as the direction of change (decreased rather than increased), these changes in cholesterol were not considered to be adverse. Effects on cholesterol were reversible as cholesterol values were actually increased compared to controls following the approximately 4-week recovery period, although cholesterol values for all animals in the 30 mg/kg/day recovery group were within the WIL historical control range.

Higher albumin and lower globulin levels, as well as associated increases in albumin/globulin ratio, were present in the 3 and 30 mg/kg/day male groups. Increased albumin and albumin/globulin ratio were also present in the 300 mg/kg/day female group. Changes in globulin were minimal, as individual values for all animals in the 3 and 30 mg/kg/day male groups were within the WIL historical control range, with the exception of 1 rat in the 30 mg/kg/day group whose value was just below the WIL historical control range. Similarly, increases in albumin in the affected male and female

groups were within the WIL historical control range, or, for some animals in the 30 mg/kg/day male group, were only slightly above the WIL historical control range. The changes in serum proteins were considered to be test substance-related. However, these changes were not considered to be adverse based on their minimal nature at all dose levels. In addition, all serum protein changes were reversible, as mean values were similar to controls following the 4-week recovery period.

Urea nitrogen was minimally increased in the 30 mg/kg/day group males. This increase was not associated with changes in creatinine or with treatment-related microscopic changes in the kidney. Thus, the minimal increase in urea nitrogen is likely of non-renal origin and was considered to be nonadverse. The pattern of changes in urea nitrogen, as well as those noted above for serum proteins, is consistent with those reported for other peroxisome proliferators (Sheikh et al., 2006; Grevois et al., 2004). Changes in urea nitrogen were reversible in males, as there were no statistically significant changes in these parameters following the recovery period.

Glucose levels were minimally increased (15.2% higher than the control group mean) in the 30 mg/kg/day group males at study week 4, but were lower than the control group at study week 8. These increases were within WIL Historical Control ranges and were not considered adverse.

Mean triglyceride values in treated male groups were lower than controls. These decreases did not occur in a dose-related manner and were statistically significant only in the 3 mg/kg/day group. The group means for the treated groups were actually similar to the historical mean, while the concurrent study control group mean of 72 mg/dL was higher than the mean of the historical population, which was 48 mg/dL. In addition, individual triglyceride values in animals from all treated male groups were within the WIL historical control range. Thus, while some peroxisome proliferators have been shown to lower triglycerides in rodents, it is unclear if the triglyceride effects in the current study are test substance-related. Regardless, the effects are not considered to be

adverse, as changes were minimal and individual triglyceride values in treated groups were similar to those seen normally in this species and strain.

There were no significant elevations in group mean liver enzyme values in test substance-treated males and females. Sorbital dehydrogenase levels were lower in all treated male groups at the end of dosing and in the 30 mg/kg/day group following the recovery period. However, these changes in sorbitol dehydrogenase were not dose-related and did not occur in a biologically relevant direction (i.e., values were decreased rather than increased). Therefore, these changes in sorbitol dehydrogenase were not considered to be adverse.

Text Table 2. Selected Serum Findings - Males

Analysis	Group (mg/kg/day):	0	0.3	3	30
Albumin (g/dL)					
Week 4 Mean		4.1	4.1	4.3	4.7**
% Difference			0.0	4.9	14.6
Week 8 Mean		4.3	NA	NA	4.2
% Difference					-2.3
Globulin (g/dL)					
Week 4 Mean		2.3	2.1	2.0*	1.8**
% Difference			-8.7	-13.0	-21.7
Week 8 Mean		2.4	NA	NA	2.4
% Difference					0.0
A/G ratio					
Week 4 Mean		1.84	1.93	2.13**	2.59**
% Difference			4.9	15.8	40.8
Week 8 Mean		1.81	NA	NA	1.73
% Difference					-4.4
Urea Nitrogen (mg/dL)					
Week 4 Mean		14.9	15.0	15.1	18.4**
% Difference			0.7	1.3	23.5
Week 8 Mean		14.4	NA	NA	14.0
% Difference					-2.8

NA = Not applicable
* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test

Text Table 2. Selected Serum Findings - Males (continued)

Analysis	Group (mg/kg/day):	0	0.3	3	30
Glucose (mg/dL)					
Week 4 Mean		105	95	105	121**
% Difference			-9.5	0.0	15.2
Week 8 Mean		114	NA	NA	107
% Difference					-6.1
Cholesterol (mg/dL)					
Week 4 Mean		51	40*	41*	37*
% Difference			-21.6	-19.6	-27.5
Week 8 Mean		45	NA	NA	61**
% Difference					35.6

NA = Not applicable
* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test

Text Table 3. Selected Serum Findings - Females

Analysis	Group (mg/kg/day):	0	3	30	300
Albumin (g/dL)					
Week 4 Mean		4.5	4.6	4.6	4.7
% Difference			2.2	2.2	4.4
Week 8 Mean		4.8	NA	NA	4.6
% Difference					-4.2
Globulin (g/dL)					
Week 4 Mean		2.3	2.4	2.4	2.1*
% Difference			4.3	4.3	-8.7
Week 8 Mean		2.5	NA	NA	2.5
% Difference					0.0
A/G ratio					
Week 4 Mean		1.93	1.97	1.97	2.32**
% Difference			2.1	2.1	20.2
Week 8 Mean		1.96	NA	NA	1.87
% Difference					-4.6

NA = Not applicable
* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test

4.2.2.2. CHANGES UNRELATED TO TEST SUBSTANCE ADMINISTRATION

There were no other test substance-related effects on serum chemistry parameters. However, some statistically significant ($p<0.05$ or $p<0.01$ using Dunnett's test) differences were observed when the control and test substance-treated groups were compared. These findings included slightly higher creatinine and potassium levels in the 30 mg/kg/day group males and lower bilirubin levels in the 300 mg/kg/day group females at study week 8. These group mean differences were not considered to be test substance-related because the values occurred at the recovery interval.

4.2.3. URINALYSIS

4.2.3.1. CHANGES ASSOCIATED WITH TEST SUBSTANCE ADMINISTRATION

There were no test substance-related alterations in urinalysis parameters.

4.3. GROSS OBSERVATIONS

Review of the gross necropsy observations revealed no findings that were considered to be associated with administration of the test substance.

4.4. ORGAN WEIGHTS

There were no test substance-related alterations in final body weight. Organ weight changes presented in Text Table 4 were considered to be associated with administration of the test substance.

Text Table 4. Test Substance-Related Organ Weight Changes

Parameter	Direction and magnitude of change	Dosage level (mg/kg/day)	Sex	Interval
Liver				
Absolute	↑ 24.4%**, ↑ 58.7%**	3, 30	M	PN
Relative to body weight	↑ 18.6%**, ↑ 55.5%**			
Relative to brain weight	↑ 24.3%**, ↑ 58.7%**			
Liver				
Absolute	↑ 8.1%	300	F	PN
Relative to body weight	↑12.1%**			
Relative to brain weight	↑ 8.3%			

** = Significantly different from the control group at 0.01 using Dunnett's test

Significantly higher liver weights occurred in a dose-related manner in males administered 3 or 30 mg/kg/day group and in females in the 300 mg/kg/day group. These findings correlated with histologic evidence of centrilobular hypertrophy. Following the recovery period, the absolute liver weight and organ to body weight ratios of males from the 30 mg/kg/day group and females from the 300 mg/kg/day group did not significantly differ from the control group values.

There were no other test substance-related effects on organ weights. However, some statistically significant differences were observed when the control and test substance-treated groups were compared. The absolute kidney weight was higher for the 3 and 30 mg/kg/day group males relative to the control group and kidney weights relative to body or brain weight were higher for the 30 mg/kg/day group males relative to the control group. These differences were small in magnitude, and lacked a morphologic or clinical pathology correlate. Therefore, the kidney weight effects were not considered to be adverse.

4.5. HISTOLOGIC CHANGES

4.5.1. CHANGES ASSOCIATED WITH TEST SUBSTANCE ADMINISTRATION

Test substance-related changes of multifocal centrilobular hypertrophy were observed in the liver of 3 and 30 mg/kg/day group males and the 300 mg/kg/day group females (Text Table 5). The tissue alteration was characterized by enlargement of hepatocytes surrounding central veins. Changes, graded minimal and mild, were diagnosed as a relative change when compared to periportal hepatocytes.

Text Table 5. Incidence Of Selected Histopathologic Findings, Study Day 28 Primary Necropsy

	Males				Females			
Dosage (mg/kg/day):	**0**	**0.3**	**3**	**30**	**0**	**3**	**30**	**300**
Liver [a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Hypertrophy, centrilobular	0	0	4	7	0	0	0	4
Minimal	0	0	4	6	0	0	0	4
Mild	0	0	0	1	0	0	0	0

[a] = Number of tissues examined from each group.
NA = not applicable

Although females were administered higher doses of H-28397, changes were more subtle than in males. Histologic examination of the liver from recovery animals revealed no evidence of centrilobular hypertrophy.

4.5.2. CHANGES UNRELATED TO TEST SUBSTANCE ADMINISTRATION

There were no other test substance-related histologic changes. Remaining histologic changes were considered to be incidental findings or related to some aspect of experimental manipulation other than administration of the test substance. There was no test substance-related alteration in the prevalence, severity or histologic character of those incidental tissue alterations.

5. DISCUSSION

There were no test substance-related changes in coagulation or urinalysis parameters. Statistically significant alterations in hematologic parameters that were considered to test substance-related changes were limited to males. There were dose-related, nonadverse decreases in red cell mass parameters (RBC, hemoglobin and hematocrit) in the 3 and 30 mg/kg/day group males at study week 4 that was partially regenerative. A few nonadverse changes in serum chemistry parameters at study week 4 were considered related to test substance administration and consistent with PPARα activation: lower cholesterol levels in the 0.3, 3 and 30 mg/kg/day group males, which were reversible by study week 8; higher albumin and lower globulin levels as well as associated increases in albumin/globulin ratio in the 3 and 30 mg/kg/day group males and higher albumin levels and higher albumin/globulin ratios in the 300 mg/kg/day group females, which were reversible by study week 8; minimal increases in urea nitrogen in the 30 mg/kg/day males, which were reversible by study week 8; and minimally increased glucose levels in the 30 mg/kg/day males. Elevations in liver enzyme activity were not evident; in fact, there were lower sorbital dehydrogenase levels in the 0.3, 3 and 30 mg/kg/day group males at study week 4 and remained 33.3% lower in the 30 mg/kg/day group males than the control group at study week 8. These changes in sorbital dehydrogenase levels were not dose-related, however.

Increased liver weight and centrilobular hepatocellular hypertrophy were observed in the liver of male and female rats, albeit at a higher dose in females. Hepatocellular centrilobular hypertrophy was observed in males from the 3 and 30 mg/kg/day groups and in females that received 300 mg/kg/day. The change was minimal to mild and correlated with higher absolute and relative liver weights. Reversibility of centrilobular hepatocellular hypertrophy was observed in male and female rats necropsied after a 28-day nondosing period. This reversibility taken together with increases in liver weights suggested microsomal enzyme induction that was probably adaptive in nature (Amacher et al., 1998; Williams and Iatropoulos, 2002) and nonadverse.

6. CONCLUSIONS

Administration of H-28397 via daily oral gavage to Sprague-Dawley rats at dosage levels of 0, 0.3, 3 and 30 mg/kg/day in males and 0, 3, 30 and 300 mg/kg/day in females for a minimum of 28 consecutive days followed by a 28-day nondosing (recovery) period was well tolerated with no adverse effects, including premature deaths. Effects noted in treated groups were consistent with a peroxisome proliferator (PPARα agonist) and were generally more consistent, and occurred at lower doses, in males compared to females. Changes included increased liver weights, minimal hepatocellular hypertrophy, changes in serum lipids and proteins, and minimal decreases in red cell mass parameters. Changes in clinical pathology parameters in individual animals were generally within or just outside WIL historical control ranges for the respective parameters and there were no changes in clinical chemistry or histopathology suggestive of liver injury. As such, these changes were considered to be test substance-related but nonadverse.

Based on survival, clinical pathology, organ weight, and morphologic pathology parameters, there was no dose-limiting toxicity in this study.

7. REFERENCES

Amacher, D.E.; Schomaker S.J.; Burkhardt, J.E. The relationship among microsomal enzyme induction, liver weight and histological change in rat toxicology studies. *Food and Chemical Toxicology* **1998,** *36*, 831-839.

Lund J.E. Toxicologic Effects on Blood and Bone Marrow. Chapter 8. In: Schalm's Veterinary Hematology, Fifth Edition, Feldman B.F., Zinkl J.G., Jain N.C., eds: Lippincott Williams & Wilkins, Philadelphia; **2000**; pp 44-50.

Staels B.; Dallongeville J.; Auwerx J.; Schoonjans K.; Leitersdorf E.; Fruchart J.C. Mechanism of action of fibrates on lipid and lipoprotein metabolism. *Circulation* **1998**, *98*, 2088-2093.

Williams, G.M.; Iatropoulos, M.I. Alteration of liver cell function and proliferation: Differentiation between adaptation and toxicity. *Toxicologic Pathology* **2002**, *30*, 41-53.

8. REPORT SUBMISSION

Report Submitted By:

Ellen L. Ziemer, DVM, PhD, DACVIM, DACVP
Study Clinical Pathologist

Date

Meliton N. Novilla, DVM, PhD, DACVP
Study Pathologist

20 Aug 2008
Date

Report Reviewed By:

George A. Parker, DVM, PhD, DACVP, DABT
Reviewing Pathologist

22 Aug 2008
Date

APPENDIX H

Liver Metabolic Enzyme Analyses (Sponsor-Provided Data)

DuPont-24447

TRADE SECRET

Biochemical Measurements Report for
A 28-Day Oral (Gavage) Toxicity Study of H-28397 in Rats with a 28-Day Recovery

TEST GUIDELINES: OECD Guideline for the Testing of Chemicals
Section 4 (Part 407) (1995)

AUTHOR: Suzanne I. Snajdr, B.S.

BIOCHEMICAL MEASUREMENTS REPORT COMPLETED ON: May 20, 2008

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
DuPont Haskell Global Centers for
Health & Environmental Sciences
P.O. Box 50
Newark, Delaware 19714
U.S.A.

LABORATORY PROJECT ID: DuPont-24447

WORK REQUEST NUMBER: 17568

SERVICE CODE NUMBER: 1023

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

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The work performed at DuPont Haskell was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are compatible with current OECD Good Laboratory Practices

Principal Investigator: _____ 20 May 2008
Suzanne I. Snajdr, B.S. Date
Associate Scientist

QUALITY ASSURANCE STATEMENT

Work Request Number: 17568
Service Code Number: 1023

Phase Audited	*Audit Dates*	*Date Reported to Study Director*	*Date Reported to Management*
Conduct:	March 11, 2008	March 18, 2008	March 18, 2008
Report/Records:	May 07-08, 2008	May 08, 2008	May 13, 2008

Reported by: Donna M. Johnston 20 May 2008
Donna M. Johnston
Quality Assurance Auditor
Date

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Biochemical Measurements Evaluation by: _____ 20 May 2008

Suzanne I. Snajdr, B.S.
Associate Scientist

Date

SUMMARY

H-28397 was evaluated for its ability to alter hepatic peroxisomal ß-oxidation activity, a measure of peroxisome proliferation, and for its ability to induce total hepatic microsomal cytochrome P-450 enzyme content in male and female rats following approximately 28 days of oral (gavage) administration of 0, 0.3, 3 or 30 mg/kg/day (male), 0, 3, 30 or 300 mg/kg/day (female), or after approximately 28 days of recovery.

In male rats, β-oxidation activity was statistically significantly increased at the 28-day time point at dosages of 0.3, 3 and 30 mg/kg/day H-28397 and total cytochrome P-450 content was statistically significantly increased at a dosage of 30 mg/kg/day H-28397. The increases in hepatic β-oxidation at 3 and 30 mg/kg/day H-28397 were accompanied with increases in relative liver weights. In female rats dosed with 30 and 300 mg/kg/day H-28397, β-oxidation activity was statistically significantly increased at the 28-day time point while total cytochrome P-450 content remained unaltered. The increase in hepatic β-oxidation at 300 mg/kg/day H-28397 was accompanied with an increase in relative liver weights. β-oxidation activity and relative liver weights had returned to control levels after approximately 28 days of recovery in both male and female rats. Total cytochrome P-450 content had returned to control levels after approximately 28 days of recovery in male rats.

Under the conditions of this study, H-28397 was an inducer of hepatic peroxisomal β-oxidation activity, a measure of peroxisome proliferation, in male rats after administration of 0.3, 3 and 30 mg/kg/day and in female rats after administration of 30 and 300 mg/kg/day of 28 days oral gavage. H-28397 is a peroxisome proliferator at these dosage levels. Total hepatic microsomal cytochrome P-450 enzyme content was increased at a dosage of 30 mg/kg/day of 28 days oral gavage in male rats but not in females. β-oxidation activity (male and female) and total cytochrome P-450 content (male) had returned to control levels after approximately 28 days of recovery.

MATERIALS AND METHODS

A. Biochemical Analysis

Following approximately 28 days (test day 28) of oral (gavage) administration of 0, 0.3, 3 or 30 mg/kg/day (male), 0, 3, 30 or 300 mg/kg/day (female), or after approximately 28 days of recovery (test day 56), 10 rats from each group designated for biochemical evaluation were weighed and then euthanized by CO_2 anesthesia and exsanguination at WIL Research Lab (Ashland, Ohio). The livers were removed, weighed, a portion of each was flash frozen in liquid nitrogen, stored frozen (approximately -60 ^{o}C to -80 ^{o}C) and shipped to DuPont Haskell on dry ice. The liver portions were then stored frozen (approximately -60^{o}C to -80^{o}C) until homogenized (approximately 1 gram tissue/4 mL buffer) in homogenization buffer (50 mM Tris-HCl, 50 mM Trizma-base, 0.25 M sucrose, and 5.4 mM EDTA, pH 7.4). Hepatic peroxisomes and microsomes were prepared using differential centrifugation. The resulting peroxisomal and microsomal pellets were resuspended in the homogenization buffer, aliquoted, and stored between -60 and -80°C until analyzed. The peroxisomal suspensions were diluted to a protein concentration of approximately 0.25 mg/mL, and β-oxidation activity was determined using [^{14}C]palmitoyl CoA as the substrate.(1) The microsomal suspensions were diluted to a protein concentration of approximately 1.0 mg/mL, and the total cytochrome P-450 content were measured by spectral analysis according to the method of Omura and Sato.(2) The spectra were recorded at room temperature with a spectrophotometer. The protein content of the peroxisomes and microsomes were determined before and after analysis by the Biorad method.(3) Final calculations for the rate of β-oxidation and total cytochrome P-450 content were made using the post-assay protein concentrations.

RESULTS AND DISCUSSION

Mechanistic Evaluation

(Tables 1-6, Appendix A)

H-28397 was evaluated for its ability to alter hepatic peroxisomal β-oxidation activity, a measure of peroxisome proliferation, and total hepatic microsomal cytochrome P-450 enzyme content in male and female rats following approximately 28 days of oral (gavage) administration or after approximately 28 days of recovery.

In male rats at the 28-day time point, β-oxidation activity was statistically significantly increased at dosages of 0.3, 3 and 30 mg/kg/day H-28397 (142, 374 and 873% of control, respectively). Percent liver weight relative to body weight was statistically significantly increased at dosages of 3 and 30 mg/kg/day H-28397 (119 and 156% of control, respectively). Total cytochrome P-450 content was statistically significantly increased at a dosage of 30 mg/kg/day H-28397 (123% of control). β-oxidation activity, relative liver weight and total cytochrome P-450 content had returned to the control level after approximately 28 days of recovery.

In female rats at the 28-day time point, β-oxidation activity was statistically significantly increased at dosages of 30 and 300 mg/kg/day H-28397 (149 and 298% of control, respectively). Percent liver weight relative to body weight was statistically significantly increased at a dosage of 300 mg/kg/day H-28397 (112% of control). No increase in total cytochrome P-450 content was observed. β-oxidation activity and relative liver weight had returned to the control level after approximately 28 days of recovery.

Under the conditions of this study, H-28397 was an inducer of hepatic peroxisomal β-oxidation activity, a measure of peroxisome proliferation, in male rats after administration of 0.3, 3 and 30 mg/kg/day and in female rats after administration of 30 and 300 mg/kg/day of 28 days oral gavage. H-28397 is a peroxisome proliferator at these dosage levels. Total hepatic microsomal cytochrome P-450 enzyme content was increased at a dosage of 30 mg/kg/day of 28 days oral gavage in male rats but not in females. β-oxidation activity (male and female) and total cytochrome P-450 content (male) had returned to control levels after approximately 28 days of recovery.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at DuPont Haskell, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware.

Laboratory-specific raw data such as personnel files, instrument, equipment, refrigerator and/or freezer raw data will be retained at the facility where the work was done.

REFERENCES

1. Lazarow, P.B. (1981). Assay of Peroxisomal Beta-Oxidation of Fatty Acids. *Methods in Enzymology* **72**, 315-319.

2. Omura, T., and Sato, R. (1964). The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem.* **239**, 2370-2378.

3. Bradford, M.M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* **72**, 248-254.

TABLES

EXPLANATORY NOTES

ABBREVIATIONS:

n number of samples
NA not applicable
SD standard deviation

Table 1
Summary of Hepatic Peroxisomal Beta-Oxidation Activity in Male Rats

		Hepatic Peroxisomal Beta-Oxidation Activity (nmol/min/mg protein)			
	Dosage	28-Day		28-Day Recovery	
Group[a]	(mg/kg/day)	Mean	SD	Mean	SD
1	0	8.8	1.8	7.5	1.3
2	0.3	12.5[b]	3.7	--[c]	--
3	3	32.9[b]	13.7	--	--
4	30	76.8[b]	8.3	7.5	1.6

a n=10.
b Statistically significant difference from control at $p < 0.05$ by Dunnett's test.
c Group not analyzed.

Table 2
Summary of Hepatic Peroxisomal Beta-Oxidation Activity in Female Rats

		Hepatic Peroxisomal Beta-Oxidation Activity (nmol/min/mg protein)			
	Dosage	28-Day		28-Day Recovery	
Group[a]	(mg/kg/day)	Mean	SD	Mean	SD
1	0	4.7	1.4	9.5	1.6
2	3	6.1	2.6	--[b]	--
3	30	7.0[c]	1.6	--	--
4	300	14.0[c]	1.7	10.5	1.8

a n=10.
b Group not analyzed.
c Statistically significant difference from control at $p < 0.05$ by Dunnett's test.

Table 3
Summary of Percent Liver Weight Relative to Body Weight in Male Rats

		Relative Liver Weight (%)[a]			
	Dosage	28-Day		28-Day Recovery	
Group[b]	(mg/kg/day)	Mean	SD	Mean	SD
1	0	3.199	0.1782	2.982	0.1841
2	0.3	3.251	0.2536	--[c]	--
3	3	3.794[d]	0.2937	--	--
4	30	4.975[d]	0.4315	2.944	0.1654

a Data supplied by WIL Research Laboratories, LLC project number WIL-189205.
b n=10.
c Group not analyzed.
d Statistically significant difference from control at $p < 0.05$ by Dunnett's test.

Table 4
Summary of Percent Liver Weight Relative to Body Weight in Female Rats

		Relative Liver Weight (%)[a]			
	Dosage	28-Day		28-Day Recovery	
Group[b]	(mg/kg/day)	Mean	SD	Mean	SD
1	0	3.409	0.1199	3.096	0.1673
2	3	3.393	0.1681	--[c]	--
3	30	3.391	0.2883	--	--
4	300	3.822[d]	0.1864	3.098	0.1876

a Data supplied by WIL Research Laboratories, LLC project number WIL-189205.
b n=10.
c Group not analyzed.
d Statistically significant difference from control at $p < 0.05$ by Dunnett's test.

Table 5
Summary of Hepatic Microsomal Total Cytochrome P450 Content in Male Rats

		Hepatic Microsomal Total Cytochrome P450 Content (nmol/mg protein)			
	Dosage	28-Day		28-Day Recovery	
Group[a]	(mg/kg/day)	Mean	SD	Mean	SD
1	0	0.670	0.078	0.756	0.097
2	0.3	0.700	0.097	--[b]	--
3	3	0.755	0.130	--	--
4	30	0.822[c]	0.091	0.746	0.096

a n=10.
b Group not analyzed.
c Statistically significant difference from control at $p < 0.05$ by Dunnett's test.

Table 6
Summary of Hepatic Microsomal Total Cytochrome P450 Content in Female Rats

		Hepatic Microsomal Total Cytochrome P450 Content (nmol/mg protein)			
	Dosage	28-Day		28-Day Recovery	
Group[a]	(mg/kg/day)	Mean	SD	Mean	SD
1	0	0.519	0.072	--[b]	--
2	3	0.530	0.089	--	--
3	30	0.554	0.079	--	--
4	300	0.512	0.091	--	--

a n=10.
b Group not analyzed.

There were no statistically significant differences from control at $p < 0.05$ by Dunnett's or Dunn's test.

Appendix A
Individual Animal Data

INDIVIDUAL ANIMAL DATA

EXPLANATORY NOTES

ABBREVIATIONS:

NA - not analyzed

FOOTNOTES:
a Data supplied by WIL Research Laboratories, LLC project number WIL-189205.

28-Day Male Rats

Animal Number	Group	Dose (mg/kg)	Hepatic Peroxisomal Beta-Oxidation Rate (nmol/mg-min)	Relative Liver Weight (%)[a]	Hepatic Microsomal Total Cytochrome P450 Content (nmol/mg protein)
90108	1	0	10.7	3.239	0.628
90123	1	0	6.7	3.283	0.565
90125	1	0	7.3	3.544	0.591
90139	1	0	7.7	2.927	0.691
90145	1	0	10.7	3.194	0.707
90152	1	0	7.1	3.207	0.818
90159	1	0	8.4	3.126	0.753
90163	1	0	12.1	3.272	0.695
90166	1	0	7.8	3.261	0.639
90173	1	0	9.1	2.935	0.614
90118	2	0.3	12.7	3.312	0.634
90124	2	0.3	7.5	3.315	0.625
90127	2	0.3	6.8	3.186	0.589
90134	2	0.3	10.3	3.015	0.643
90138	2	0.3	16.0	3.161	0.721
90144	2	0.3	16.1	3.325	0.848
90148	2	0.3	11.0	3.812	0.786
90151	2	0.3	18.3	3.190	0.840
90157	2	0.3	13.3	2.841	0.712
90167	2	0.3	13.1	3.355	0.606
90110	3	3	11.3	3.676	0.573
90121	3	3	15.0	3.232	0.618
90126	3	3	54.5	3.540	0.731
90128	3	3	43.1	4.227	0.658
90141	3	3	44.4	3.979	0.806
90146	3	3	22.3	4.020	0.908
90161	3	3	41.8	3.967	0.897
90165	3	3	30.1	3.970	0.889
90170	3	3	33.3	3.744	0.623
90174	3	3	33.4	3.580	0.842
90119	4	30	81.6	4.547	0.698
90130	4	30	70.8	5.014	0.800
90131	4	30	85.8	4.570	0.852
90132	4	30	90.2	5.890	0.789
90135	4	30	64.4	4.881	0.700
90136	4	30	74.0	5.103	0.808
90142	4	30	76.5	4.874	1.007
90143	4	30	68.4	5.269	0.865
90162	4	30	83.8	5.195	0.801
90171	4	30	72.3	4.410	0.898

28-Day Female Rats

Animal Number	Group	Dose (mg/kg)	Hepatic Peroxisomal Beta-Oxidation Rate (nmol/mg-min)	Relative Liver Weight (%)[a]	Hepatic Microsomal Total Cytochrome P450 Content (nmol/mg protein)
90175	1	0	7.1	3.338	0.464
90177	1	0	4.0	3.573	0.533
90192	1	0	5.1	3.558	0.404
90204	1	0	3.8	3.232	0.525
90209	1	0	3.5	3.452	0.416
90214	1	0	4.8	3.410	0.531
90224	1	0	3.4	3.488	0.629
90225	1	0	4.9	3.391	0.547
90232	1	0	3.5	3.222	0.550
90235	1	0	7.1	3.430	0.594
90193	2	3	2.7	3.346	0.472
90199	2	3	8.0	3.468	0.441
90206	2	3	4.5	3.181	0.570
90210	2	3	5.3	3.523	0.432
90217	2	3	1.9	3.712	0.408
90221	2	3	9.9	3.469	0.682
90230	2	3	5.6	3.381	0.620
90234	2	3	7.7	3.255	0.533
90236	2	3	8.6	3.156	0.580
90244	2	3	6.4	3.436	0.560
90178	3	30	5.3	3.269	0.565
90181	3	30	6.8	3.190	0.666
90191	3	30	4.8	3.460	0.573
90195	3	30	7.4	3.954	0.453
90202	3	30	7.5	3.261	0.439
90205	3	30	7.9	3.689	0.515
90212	3	30	7.5	2.965	0.578
90215	3	30	9.9	3.251	0.654
90219	3	30	4.7	3.259	0.609
90228	3	30	7.8	3.608	0.488
90176	4	300	14.1	3.727	0.489
90182	4	300	12.2	4.063	0.463
90183	4	300	11.2	3.820	0.428
90187	4	300	15.8	3.676	0.596
90190	4	300	13.1	3.850	0.581
90213	4	300	15.8	3.698	0.521
90220	4	300	15.6	3.917	0.374
90227	4	300	15.1	3.487	0.543
90231	4	300	15.2	4.113	0.443
90242	4	300	12.3	3.868	0.677

28-Day Recovery Male Rats

Animal Number	Group	Dose (mg/kg)	Hepatic Peroxisomal Beta-Oxidation Rate (nmol/mg-min)	Relative Liver Weight (%)[a]	Hepatic Microsomal Total Cytochrome P450 Content (nmol/mg protein)
90105	1	0	6.4	3.017	0.613
90116	1	0	8.1	3.227	0.963
90117	1	0	8.1	3.105	0.742
90120	1	0	5.3	2.906	0.683
90129	1	0	6.8	3.111	0.748
90140	1	0	7.1	2.972	0.793
90153	1	0	7.9	2.640	0.834
90156	1	0	8.7	3.177	0.710
90158	1	0	6.8	2.887	0.791
90164	1	0	10.0	2.777	0.683
90109	4	30	8.4	3.225	0.833
90111	4	30	8.6	2.724	0.880
90112	4	30	8.8	3.186	0.696
90114	4	30	8.4	2.960	0.568
90115	4	30	4.1	2.798	0.789
90133	4	30	6.9	2.904	0.738
90149	4	30	8.4	2.777	0.754
90160	4	30	8.3	2.917	0.839
90168	4	30	7.6	2.919	0.732
90172	4	30	5.5	3.032	0.635

28-Day Recovery Female Rats

Animal Number	Group	Dose (mg/kg)	Hepatic Peroxisomal Beta-Oxidation Rate (nmol/mg-min)	Relative Liver Weight (%)[a]	Hepatic Microsomal Total Cytochrome P450 Content (nmol/mg protein)
90179	1	0	10.6	2.956	NA
90185	1	0	9.8	3.106	NA
90194	1	0	10.7	3.123	NA
90196	1	0	6.7	2.964	NA
90197	1	0	7.2	3.166	NA
90198	1	0	7.9	3.146	NA
90203	1	0	10.0	3.492	NA
90222	1	0	11.1	3.124	NA
90229	1	0	11.2	2.920	NA
90233	1	0	9.3	2.962	NA
90186	4	300	11.1	3.205	NA
90188	4	300	13.5	3.344	NA
90200	4	300	10.9	2.993	NA
90201	4	300	7.3	3.175	NA
90207	4	300	12.3	2.991	NA
90216	4	300	8.9	3.241	NA
90223	4	300	8.6	3.339	NA
90226	4	300	10.6	2.883	NA
90237	4	300	11.1	2.813	NA
90240	4	300	10.4	2.996	NA

APPENDIX I

Study Protocol

Study Number: WIL-189205

PROTOCOL AMENDMENT VII

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

A. Title of Study:

A 28-Day Oral (Gavage) Toxicity Study of H-28397 in Rats with a 28-Day Recovery

B. Protocol Modifications:

1) **12 WORK PRODUCT:**

The first paragraph of this section of the protocol is revised to read as follows:

Sponsor will have title to all documentation records, raw data, slides, specimens, or other work product generated during the performance of the study. All work product including slides, specimens, raw paper data, pertinent electronic storage media, and leftover test substance will be returned to the Sponsor at the address on page 2 of this protocol at the time of issuance of the final report. Unless otherwise indicated, all remaining formulation and clinical pathology samples will not be sent to Archives and will be discarded at the time of the issuance of the final report or earlier.

WIL-189205
Protocol Amendment VII
Page 2 of 2

C. Reasons for Protocol Modification:

1) Clarification of final disposition of study work product by request of the Sponsor.

Sponsor approval received via e-mail on 8/13/08.
Date

E.I. du Pont de Nemours and Company

Carol Carpenter — 8/14/08
Carol Carpenter, Sponsor Representative — Date

WIL Research Laboratories, LLC

Matthew C. Haas, BA, LAT, Study Director — 8/13/08 Date

Christopher P. Chengelis, PhD, DABT, Director, Toxicology — 8/12/08 Date

Study Number: WIL-189205

PROTOCOL AMENDMENT VI

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

A. Title of Study:

A 28-Day Oral (Gavage) Toxicity Study of H-28397 in Rats with a 28-Day Recovery

B. Protocol Modifications:

1) **8.7.4 Microscopic Examination:**

The microscopic slides for this study will be shipped to the Sponsor at the address below for Sponsor's internal review, including taking of photomicrographs of the slides. This Sponsor review is only for Sponsor's internal use, is not to be considered a peer review and will not be conducted under GLP compliance. Results of this internal review and/or the photomicrographs of slides will not be included in the Final Report for this study.

Shipping address:
Carolyn Lloyd
Haskell Laboratory for Health & Environmental Sciences
1090 Newark Elkton Road
Bldg S320
Newark, DE 19714

WIL-189205
Protocol Amendment VI
Page 2 of 2

C. Reasons for Protocol Modification:

1) Slide shipment and Sponsor's internal review scheduled by request of the Sponsor.

Sponsor approval received via e-mail on 6/9/08.
Date

E.I. du Pont de Nemours and Company

Carol Carpenter — 6/12/08
Carol Carpenter, Sponsor Representative — Date

WIL Research Laboratories, LLC

6/10/08
Matthew C. Haas, BA, LAT, Study Director — Date

for Christopher P. Chengelis, PhD, DABT, Director, Toxicology — 17 June 2008
Date

Study Number: WIL-189205

PROTOCOL AMENDMENT V

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

A. Title of Study:

A 28-Day Oral (Gavage) Toxicity Study of H-28397 in Rats with a 28-Day Recovery

B. Protocol Modifications:

1) **1** **OBJECTIVE:**

The United States Environmental Protection Agency (EPA) Good Laboratory Practice Regulations that this study will be conducted in compliance with should be 40 CFR Part 792, September 18, 1989.

2) **3** **STUDY SCHEDULE:**

Proposed Experimental Termination Date: April 7, 2008

3) **3** **STUDY SCHEDULE:**

In accordance with Test Article Formulations Instructions approved by the Study Director and dated 12/4/07, a stock dilution (solution) of the test article (15% purity) was prepared for use in preparing future formulations for this study. Four 1-mL samples were collected from the middle stratum of the stock dilution (solution) on 12/5/07 per the Study Director. In accordance with Study Director Notification dated 12/7/07, the four 1-mL samples collected from the stock dilution (solution) on 12/5/07 were sent to the Department of Analytical Chemistry at WIL to be analyzed for concentration. In addition, per e-mails sent on 12/13/08 and 1/3/08 from the Study Director to the Formulations Department at WIL, the stock dilution (solution) was sampled on 12/14/07 and 1/7/08 and stored frozen until possible future analysis.

C. Reasons for Protocol Modification:

1) Clarification of EPA Regulation study conducted in compliance with.

2) The Proposed Experimental Termination Date was added based upon the last histopathological examination.

WIL-189205
Protocol Amendment V
Page 2 of 2

3) Addition of the sampling, storage and/or analysis of the test article stock dilution (solution).

Sponsor approval received via e-mail on 5/21/08.
Date

E.I. du Pont de Nemours and Company

Carol Carpenter — 22-May-2008
Carol Carpenter, Sponsor Representative — Date

WIL Research Laboratories, LLC

Matthew C. Haas, BA, LAT, Study Director — 5/21/08 Date

Christopher P. Chengelis, PhD, DABT, Director, Toxicology — 21 May 2008 Date

Study Number: WIL-189205

PROTOCOL AMENDMENT IV

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

A. Title of Study:

A 28-Day Oral (Gavage) Toxicity Study of H-28397 in Rats with a 28-Day Recovery

B. Protocol Modifications:

1) 3 **STUDY SCHEDULE:**

The fifth item in this section will be revised to read as follows:

Proposed Audited Report Date: May 21, 2008

C. Reasons for Protocol Modification:

1) The Proposed Audited Draft Report Date was re-scheduled following scheduling of the target tissue histopath and for an earlier available date per request of the Sponsor.

Sponsor approval received via e-mail on 4/3/08.
Date

E.I. du Pont de Nemours and Company

Carol Carpenter — 1 Apr 2008
Carol Carpenter, Sponsor Representative — Date

WIL Research Laboratories, LLC

Matthew C. Haas, BA, LAT, Study Director — 4/3/08 Date

Christopher P. Chengelis, PhD, DABT, Director, Toxicology — 3 April 08 Date

Study Number: WIL-189205

PROTOCOL AMENDMENT III

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

A. Title of Study:

A 28-Day Oral (Gavage) Toxicity Study of H-28397 in Rats with a 28-Day Recovery

B. Protocol Modifications:

1) **8.7.4 Microscopic Examination:**

Microscopic examination will be extended (at additional cost) to the livers in the low- and mid-dose groups euthanized at the primary necropsy and the control and high-dose groups euthanized at the recovery necropsy since the liver was identified as a potential target organ based on histopathological examination of tissues from the control and high dose groups or other parameters (organ weights, clinical pathology, etc.).

C. Reasons for Protocol Modification:

1) Additional histopathological examination on potential target organs requested by Sponsor.

Sponsor approval received via e-mail on 3/12/08.
Date

E.I. du Pont de Nemours and Company

Carol Carpenter — 25 Mar 2008
Carol Carpenter — Date
Sponsor Representative

WIL Research Laboratories, LLC

3/12/08
Matthew C. Haas, BA, LAT — Date
Study Director

12 Mar 2008
Christopher P. Chengelis, PhD, DABT — Date
Director, Toxicology

Study Number: WIL-189205

PROTOCOL AMENDMENT II

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

A. Title of Study:

A 28-Day Oral (Gavage) Toxicity Study of H-28397 in Rats with a 28-Day Recovery

B. Protocol Modifications:

1) **2.2** **WIL Study Director:**

Effective February 15, 2008 this section will be revised to read as follows:

Matthew C. Haas, BA, LAT
Staff Toxicologist
Tel: (419) 289-8700
Fax: (419) 289-3650
Email: mhaas@wilresearch.com

2) **2.6** **Principal Investigator – Pathology:**

This section will be revised to read as follows:

Meliton Novilla, DVM, PhD, DACVP
Senior Pathologist
Biotechnics, Inc.
Tel: (317) 467-0836
Email: mnovilla@biotechnics-inc.com

3) **3** **STUDY SCHEDULE:**

The fifth item in this section will be revised to read as follows:

Proposed Audited Report Date: May 30, 2008

4) **7.4.1** **Organization of Test Groups:**

In accordance with the Study Director Notifications dated December 10, 2007 and January 3, 2008 the dosage chart will be revised to read as follows:

WIL-189205
Protocol Amendment II
Page 2

Group Number	Treatment	Dosage Level[a] (mg/kg/day)	Dose Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Animals
Males					
1	Vehicle[b]	0	0	10[d]	20[c]
2	H-28397	0.3	0.03	10[d]	10[c]
3	H-28397	3	0.3	10[d]	10[c]
4	H-28397	30	3	10[d]	20[c]
Females					
1	Vehicle[b]	0	0	10[d]	20[c]
2	H-28397	3	0.3	10[d]	10[c]
3	H-28397	30	3	10[d]	10[c]
4	H-28397	300	30	10[d]	20[c]

a = Dosage levels will be corrected for purity using a correction factor of 1.14.
b = Deionized (DI) water
c = 10 animals/sex/group will be submitted for euthanasia at the primary necropsy on Day 28. The remaining animals (≤ 10 animals/sex/group) in Groups 1 and 4 will be euthanized at the recovery necropsy on Day 56.
d = Dose volume increased to 12 mL/kg from December 10, 2007 through January 3, 2008.

7.5.2 Homogeneity, Resuspension Homogeneity, Stability and Concentration Determination of Test Article Formulations:

5) In accordance with the Study Director Notification dated December 3 and 7, 2007 the following text will be added as a new paragraph between the first and second paragraphs of this section:

In the event that the concentration, homogeneity, stability or resuspension homogeneity of the formulations cannot be established prior to the preparation of the dosing formulations these parameters will be established from the first preparation of the dosing formulations. In that event the 0.03 and 30 mg/mL bulk preparations will be sampled to analyze concentration and homogeneity, and two daily dosing aliquots from both the low and high concentration formulation will be set aside to establish stability and resuspension homogeneity at two time points. Store one of the daily dosing aliquots from each concentration at room temperature for 5 hours and the other under refrigerated conditions for 10 days. Collect and store the samples as indicated in the preceding paragraph.

6) In accordance with the Study Director Notifications dated December 14, 2007 the new third paragraph of this section will be revised to read as follows:

Four 1-mL samples will be collected from the middle stratum of each dose concentration (including controls) of the Week 0, 1, 2 and 3 dosing formulations for analysis of test article concentration. The samples from all dose concentrations (including controls) of the Week 0 and 3 preparations will be analyzed for confirmation of concentration at WIL Research Laboratories, LLC according to a validated method. The samples from Weeks 1 and 2 will be stored for possible future analysis. Unanalyzed samples will be discarded upon finalization of the study report.

7) **8.7.3 Liver Metabolic Enzyme Analysis:**

In accordance with the Study Director Notification dated January 8, 2008 the first sentence of this section will be revised to read as follows:

Following collection of the organ weights for all animals euthanized at the scheduled necropsies, an approximate 3-gram section of liver (median lobe and caudate lobe, if necessary) will be collected.

8) **8.7.4 Microscopic Examination:**

The first paragraph of this section will be revised to read as follows:

Histologic preparation will be conducted at the WIL Research Laboratory site in Ashland, Ohio.

C. Reasons for Protocol Modification:

1) Change in Study Director.

2) The information became available.

3) The Proposed Audited Draft Report Date was postponed to allow for incorporation of all ancillary reports into the main report prior to issuance.

4) Analysis of Week 0 dosing formulation concentrations revealed the formulations concentrations were at the low end, or below, the acceptable range for a suspension formulation. Thus dosage volumes were increased from 10 mL/kg to 12 mL/kg to ensure dosage at the protocol-specified dosage levels. Analysis of Week 3 dosing formulation concentrations revealed the formulations concentrations were within the acceptable range thus the dose volume reverted to 10 mL/kg to ensure dosage at the protocol-specified dosage levels.

5) Due to the inability to establish concentration, homogeneity, stability or

resuspension homogeneity of the formulations prior to the preparation of the dosing formulations these parameters will have to be established from the first preparation of the dosing formulations.

6) Due to difficulties in preparing pre-initiation formulations and Week 0 formulations, samples from the Week 1 and Week 2 formulations were collected for possible future analysis.

7) The median lobe of the female rat may not be large enough to obtain approximately 3 gram of liver from the median lobe alone. Thus, when the entire median lobe of an animal is not enough to fulfill the requirements of the 3 gram liver tissue sample additional tissue may be taken from the caudate lobe of the same animal.

8) Histological preparation was performed at WIL Research in Ashland, OH.

Sponsor approval received via email on 11 Feb 2008
Date

E.I. du Pont de Nemours and Company

Carol Carpenter 15-Feb-2008
Carol Carpenter Date
Sponsor Representative

WIL Research Laboratories, LLC

13 Feb 2008
Michael S. Koch, PhD Date
Study Director

13 Feb 2008
Christopher P. Chengelis, PhD, DABT Date
Director, Toxicology

Study Number: WIL-189205

PROTOCOL AMENDMENT I

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

A. Title of Study:

A 28-Day Oral (Gavage) Toxicity Study of H-28397 in Rats with a 28-Day Recovery

B. Protocol Modifications:

1) **4.4 Purity:**

In accordance with the Study Director Notification dated November 27, 2007 this section will be revised to read as follows:

The Certificate of Analysis (C of A) provided by the Sponsor indicates the test article is 88% pure. A copy of the C of A will be maintained in the study records. A factor of 1.14 will be used to correct for purity.

2) **7.4.1 Organization of Test Groups:**

In accordance with the Study Director Notification dated November 27, 2007 the first sentence of this section will be revised to read as follows:

The dose levels were supplied by the Sponsor, and will be adjusted using a correction factor of 1.14 to correct for purity.

3) **7.4.1 Organization of Test Groups:**

In accordance with the Study Director Notification dated November 27, 2007 footnote "a" in this section will be revised to read as follows:

a - Dosage levels will be adjusted for purity using a correction factor of 1.14.

4) **7.5.1 Storage and Method of Preparation of the Test Article:**

In accordance with the Study Director Notification dated November 27, 2007 the last sentence of this section will be revised to read as follows:

The dose formulations will be adjusted for purity using a correction factor of 1.14.

WIL-189205
Protocol Amendment I
Page 2

C. Reasons for Protocol Modification:

1) The purity of H-28397 was determined by the Sponsor and provided in a C of A. Once the purity of the test article was known, the correction factor to be applied to formulations was determined to be 1.14 (i.e., 100%/88%).

2) To include the correction factor.

3) To include the correction factor.

4) To include the correction factor.

Sponsor approval received via email on 28 Nov 2007.
Date

E.I. du Pont de Nemours and Company

Carol Carpenter — 29-NOV-2007
Carol Carpenter, Sponsor Representative — Date

WIL Research Laboratories, LLC

28 Nov 2007
Michael S. Koch, PhD, Study Director — Date

Christopher P. Chengelis, PhD, DABT, Director, Toxicology — Date

WIL-189205
November 16, 2007

PROTOCOL

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY OF H-28397 IN RATS WITH A 28-DAY RECOVERY

OECD 407 Guidelines

Sponsor:

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

Work Request, Service Code: **WR 17568, SC 1023**
DuPont study number: DuPont-24447

Performing Laboratory:

WIL Research Laboratories, LLC
1407 George Road
Ashland, OH 44805-8946

1 OBJECTIVE:

The objective of this study is to evaluate the potential toxicity, and recovery therefrom, of H-28397 when administered to rats by oral gavage for 28 consecutive days.

This study will be conducted in compliance with the United States Environmental Protection Agency (EPA) Good Laboratory Practice Regulations (40 CFR Part 160), October 16, 1989, the Organization for Economic Co-operation and Development Principles of Good Laboratory Practice [(C/97 186/Final], the Standard Operating Procedures of WIL Research Laboratories, LLC, and the protocol as approved by the Sponsor.

This study was designed in accordance with the OECD Guideline for the Testing of Chemicals 407 (Repeated Dose 28-Day Oral Toxicity Study in Rodents).

2 PERSONNEL INVOLVED IN THE STUDY:

2.1 Sponsor Representative:

Carol Carpenter
Senior Staff Toxicologist
DuPont Haskell Global Centers for Health and Environmental Sciences
1090 Elkton Rd, P.O. Box 50
Newark, DE 19714
Tel: (302) 366-5201
Fax: (302) 366-5207
Email: Carol.Carpenter@usa.dupont.com

2.2 WIL Study Director:

Michael S. Koch, PhD
Staff Toxicologist
Tel: (419) 289-8700
Fax: (419) 289-3650
Email: mkoch@wilresearch.com

2.3 WIL Deputy Director:

Jason M. Roper, PhD
Staff Toxicologist
E-mail: jroper@wilresearch.com

2.4 WIL Departmental Responsibilities:

Christopher P. Chengelis, PhD, DABT
Director, Toxicology

Jozef J.W.M. Mertens, PhD, DABT
Associate Director, General Toxicology

George A. Parker, DVM, PhD, DACVP, DABT
Director, Pathology

Daniel W. Sved, PhD
Director, Metabolism and Analytical Chemistry

Philip L. Stetson, MD, PhD
Associate Director, Analytical Chemistry

Walter R. Miller, Jr., BS, DVM
Clinical Veterinarian, Head of Surgery
and Experimental Medicine

Susan C. Haley, BS
Manager, Clinical Pathology

Sally A. Keets, AS
Senior Operations Manager, Vivarium

Carol A. Kopp, BS, LAT
Manager, Gross Pathology and
Developmental Toxicology Laboratory

Teresa D. Morris, BS
Senior Operations Manager, Toxicology

Heather L. Johnson, BS, RQAP-GLP
Manager, Quality Assurance

Theresa M. Rafeld
Group Manager, Formulations Laboratory

Michael Safron, AS, HT (ASCP)
Manager, Histology

Robert A. Wally, BS, RAC
Manager, Reporting and Regulatory
Technical Services

Ronald E. Wilson, BS
Director, Informational Systems

2.5 Principal Investigator – Clinical Pathology Data Analysis:

Ellen L. Ziemer, DVM, MS, PhD, DACVP, DACVIM
Senior Clinical Pathologist
Biotechnics LLC
310 Millstone Drive
Hillsborough, NC 27278
Tel: (919) 245-3114
Email: eziemer@wilresearch.com

2.6 Principal Investigator – Pathology:

To be added by protocol amendment.

3 STUDY SCHEDULE:

Proposed Animal Receipt Date:	November 27, 2007
Proposed Experimental Start Date:	December 10 & 11, 2007
Proposed Necropsy Dates:	
Primary Necropsy	January 7 & 8, 2008
Recovery Necropsy	February 4 & 5, 2008
Proposed Experimental Termination Date:	To be added by amendment
Proposed Audited Report Date:	April 30, 2008

4 TEST ARTICLE DATA:

4.1 Identification:

FRD-902 (or H-28397)

4.2 Haskell Test Substance Number:

H-28397

4.3 Lot Number:

E1131181-19-B

4.4 Purity:

To be determined, purity will be added by protocol amendment. An as-yet-to-be-determined correction factor will be used to correct for purity.

4.5 Stability:

The analysis will be performed by the Sponsor, and documented on the Certificate of Analysis.

4.6 Physical Description:

To be documented by WIL Research Laboratories, LLC.

4.7 Storage Conditions:

Controlled room temperature and humidity (approximately 18° to 24°C and 20% to 70% relative humidity)

4.8 Reserve Samples:

Retention samples will be collected and stored in accordance with WIL Standard Operating Procedures.

4.9 Personnel Safety:

MSDS to be provided by the Sponsor.

4.10 Test Article Disposition:

With the exception of the reserve sample for each batch of test article, which will be archived as described, all neat test article remaining at study completion will be returned to the Sponsor.

5 TEST SYSTEM:

5.1 Species:

Rat

5.2 Strain:

Crl:CD(SD)

5.3 Source:

Charles River Laboratories
(Facility to be documented in the raw data.)

5.4 Number of Animals:

Seventy males and 70 females will be ordered and 60 of each sex placed on study. Animals not utilized on study will be deemed as part of the stock colony or euthanized by CO_2 inhalation and discarded without necropsy.

5.5 Approximate Age and Weight:

Each animal will be approximately 5 to 6 weeks of age at receipt. Animals will be approximately 7 to 8 weeks of age at initiation of dosing. The males are expected to weigh approximately 190-290 grams and the females approximately 150-250 grams at initiation of dosing. Females will be nulliparous and non-pregnant.

5.6 Identification System:

Each animal will be uniquely identified by a metal ear tag displaying the animal number. Individual cage cards will be affixed to each cage and will display the animal number, sex, group number and study number.

5.7 Justification for Selection:

This species and strain of animal is recognized as appropriate for subchronic toxicity studies. The Crl:CD(SD) rat will be utilized because it is a widely used strain for which historical control data are available. The number of animals selected is the minimum needed to yield statistically and scientifically meaningful data, and is consistent with regulatory guidelines.

6 SPECIFIC MAINTENANCE SCHEDULE:

6.1 Animal Housing:

Animals will be housed individually in an environmentally controlled room in clean, suspended, wire-mesh cages. The cages will be elevated above cage-board or other suitable material. The cages will be subject to routine cleaning at a frequency consistent with maintaining good animal health.

The facilities at WIL Research Laboratories, LLC are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

6.2 Environmental Conditions:

Controls will be set to maintain an average daily temperature at 71 ± 5°F (22 ± 3°C) and an average daily relative humidity at approximately 30-70%. Temperature and relative humidity will be monitored continuously. Data for these two parameters will be scheduled for automatic collection on an hourly basis. Fluorescent lighting will provide illumination for a 12-hour light/dark photoperiod. Temporary adjustments to the light/dark cycles may be made to accommodate protocol-specified activities. The ventilation rate will be set at a minimum of 10 room air changes per hour, 100% fresh air.

6.3 Drinking Water:

Reverse osmosis-treated tap water will be available *ad libitum*. Filters servicing the automatic watering system will be changed regularly according to Standard Operating Procedures. Municipal water supplying the laboratory will be analyzed for contaminants to ascertain that none are present at concentrations that would be expected to affect the outcome of the study according to WIL Standard Operating Procedures.

6.4 Diet:

PMI Nutrition International, LLC Certified Rodent LabDiet® 5002 (Meal) will be offered *ad libitum* during the study, except during fasting prior to clinical pathology blood collection. Each lot utilized will be identified and recorded. Standard Operating Procedures provide specifications for acceptable levels of heavy metals and pesticides that are reasonably expected to be present in the diet without interfering with the purpose or conduct of the study. Each lot of feed has been analyzed to assure specifications are met. Copies of lot appropriate analyses will be included in the study records. Feeders will be changed and sanitized once per week.

7 EXPERIMENTAL DESIGN:

7.1 Animal Receipt and Acclimation:

Each animal will be inspected by qualified personnel upon receipt. Animals judged to be in good health will be placed immediately in acclimation for at least 10 days (including the pretest period). During the acclimation period, each animal will be assigned a permanent animal number and observed twice daily for changes in general appearance and behavior. The animals will be allowed a

pretest week (as part of the acclimation period) during which body weights and food consumption will be determined and general health will be monitored, but they will not receive test article. All animals will receive a detailed physical examination during the pretest period and at the time of selection for randomization.

7.2 Randomization:

Near the end of the pretest period, animals judged to be suitable for testing will be assigned to groups at random based on body weight stratification into a block design using a computer program. A printout containing the animal numbers and individual group assignments will be generated. Animals will then be arranged into the groups according to the printout. Body weights at randomization will be within ± 20% of the mean for each sex. If, after randomization, significant differences between groups exist, new randomizations will be generated until group mean body weights are not statistically significant between groups.

Following randomization but before dosing on Day 0, it may be necessary to replace individual animals. The replacement animal(s) will be arbitrarily selected from the remaining pre-test animals. The reason(s) for replacement will be appropriately documented in the study records.

7.3 Route and Rationale of Test Article Administration:

The route of administration will be oral, by gavage, since one of the study objectives is to determine the potential toxicity of the test article when administered by the oral route and further potential testing will be by the oral route.

7.4 Organization of Test Groups, Dosage Levels and Treatment Regimen:

7.4.1 Organization of Test Groups:

The dose levels were supplied by the Sponsor, and will be adjusted to correct for purity. The following table presents the study group arrangement:

Group Number	Treatment	Dosage Level[a] (mg/kg/day)	Dose Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Animals
Males					
1	Vehicle[b]	0	0	10	20[c]
2	H-28397	0.3	0.03	10	10[c]
3	H-28397	3	0.3	10	10[c]
4	H-28397	30	3	10	20[c]
Females					
1	Vehicle[b]	0	0	10	20[c]
2	H-28397	3	0.3	10	10[c]
3	H-28397	30	3	10	10[c]
4	H-28397	300	30	10	20[c]

a - Dosage levels will be corrected for purity using a correction factor to be added by amendment.
b - Deionized (DI) water.
c - 10 animals/sex/group will be submitted for euthanasia at the primary necropsy on Day 28. The remaining animals (≤ 10 animals/sex/group) in Groups 1 and 4 will be euthanized at the recovery necropsy on Day 56.

7.4.2 Justification of Dosage Levels:

The dosage levels used on this study were selected by the Sponsor based upon existing toxicity data for this test article.

7.4.3 Treatment Regimen:

Vehicle or H-28397 formulations will be administered orally by gavage once daily for a minimum of 28 consecutive days (until the day prior to the scheduled necropsy). The first day of dosing will be designated as Day 0. Day 28 is the first day of the primary necropsy and the first day of recovery. Recovery animals will be held without dosing for a minimum of an additional 28 days. Day 56 is the day of the recovery necropsy. Group 1 animals will receive the vehicle and serve as controls.

7.4.4 Method of Administration:

The test article and vehicle formulations will be administered via stainless steel ball-tipped metal and PTFE plastic dosing cannulae and plastic syringes of appropriate size. Vehicle and test article formulations will be stirred continuously after preparation and throughout the dosing period.

7.4.5 Adjustment of Dosages:

Individual doses will be adjusted for the duration of the study, based on the most recent body weights. Adjusted doses will become effective on the day new body weights are recorded.

7.5 Preparation and Analysis of Test Article Dosing Formulations:

7.5.1 Storage and Method of Preparation of the Test Article:

Formulations of the test article in the vehicle will be prepared approximately weekly, with daily aliquots stored refrigerated (approximately 2° to 8°C), and used within 10 days of preparation. The dose formulations will be placed on a stir plate for continuous stirring during sample collection and prior to dose administration. The dose formulations will be adjusted for purity.

7.5.2 Homogeneity, Resuspension Homogeneity, Stability and Concentration Determination of Test Article Formulations:

Pre-initiation batches of H-28397 with concentrations of 0.01 mg/mL (batch size 400 mL) and 30 mg/mL (batch size 675 mL) will be prepared in a volume large enough to dose a group of animals for approximately one week. These concentrations are anticipated to bracket the concentrations to be used in this and any subsequent studies. On the day of formulation, four 1-mL samples will be collected from each stratum (top, middle and bottom) of these formulations to determine the homogeneity of the batches. The samples from the middle stratum will also serve as confirmation of concentration samples. To assess resuspension homogeneity and stability of the pre-initiation formulations, two aliquots similar in size to the amount required for dosing a group of animals for one day (i.e., 60 mL of the 0.01 mg/mL formulation and 100 mL of the 30 mg/mL formulation) will be drawn from each of the bulk preparations. One aliquot will be stored at room temperature (approximately 18° to 24°C) for 5 hours and the second aliquot will be stored refrigerated (approximately 2° to 8°C) for 10 days. After remixing at room temperature for a minimum of ten minutes using a magnetic stirrer, four 1-mL samples will be collected from the top and bottom strata of the formulations to assess resuspension homogeneity and stability. Samples will be analyzed at WIL Research Laboratories, LLC according to a validated method.

Four 1-mL samples will be collected from the middle stratum of each dose concentration (including controls) of the Week 0 and Week 3 dosing formulations for analysis of test article concentration. Samples

from all dose concentrations (including controls) will be analyzed for confirmation of concentration at WIL Research Laboratories, LLC according to a validated method.

7.5.3 Analysis of Test Article Formulations:

Samples will be transferred to the Department of Analytical Chemistry at WIL Research Laboratories, LLC for analysis. Analyses of test article formulations will be performed using a method developed and validated by WIL Research Laboratories, LLC. Initially, two of each set of four replicate, 1-mL samples will be analyzed; the remaining two 1-mL samples will be stored frozen (approximately -20°C) at WIL and will function as back-up samples. Back-up samples will be analyzed if requested by the Sponsor or Study Director or may be discarded if the results are within specifications.

Results of the analyses will be provided to the Study Director, and included in the WIL Research Final Report.

8 PARAMETERS TO BE EVALUATED:

8.1 Viability Observations:

All animals will be observed for mortality/moribundity twice daily, once in the morning and once in the afternoon. Moribund animals will be euthanized and necropsied. Found dead animals will be necropsied as soon as possible to minimize the possibility of tissues being lost due to autolysis.

8.2 Animals to be Euthanized *in Extremis*:

All animals to be euthanized *in extremis* will receive a detailed physical examination and have a final body weight collected prior to release for necropsy. Additionally, an attempt will be made to collect blood samples for evaluation of hematology (excluding coagulation parameters) and serum chemistry parameters (see Clinical Pathology section), to aid in determining the cause of the animal's moribund condition. The animal will then be released for euthanasia by CO_2 inhalation and subsequent necropsy (see Anatomic Pathology section).

8.3 Clinical Observations:

8.3.1 Daily Observations:

A clinical examination will be performed daily for all animals at the time of dosing and approximately 1-2 hours after dosing, or once daily

during the recovery period. Clinical examinations during the recovery period may be omitted on days of detailed physical examinations. Observations will include, but are not limited to, changes in the skin, fur, eyes and mucous membranes; respiratory, circulatory, autonomic and central nervous systems function; somatomotor activity and behavior patterns. The absence or presence of clinical findings at each scheduled observation period will be recorded for individual animals. Findings noted for individual animals outside of the specified observation periods will also be recorded.

8.3.2 Detailed Physical Examination:

All animals will receive a detailed physical examination at least once during the pretreatment period, at randomization, at least weekly thereafter, and just prior to the scheduled necropsy. The animals will be removed from their home cages and placed in a standard arena for observations. Observations will be detailed and carefully recorded. Where appropriate, explicitly defined scoring systems will be used if, in the opinion of the Study Director, doing so increases the utility of the data. Signs noted shall include, but not be limited to, changes in the skin, fur, eyes, and mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g., lacrimation, piloerection, pupil size, and unusual respiratory pattern). Changes in gait, posture and response to handling, as well as presence of clonic or tonic movements, stereotypic behavior (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backwards) will be recorded. Signs such as skin lesions and hair loss will also be recorded at this time. The absence or presence of findings will be recorded for individual animals.

8.4 Individual Body Weights:

Individual body weights will be recorded at pretest initiation, at randomization, at least weekly during the treatment and recovery periods, on the day prior to necropsy (non-fasted), and on the day of euthanasia (fasted).

8.5 Individual Food Consumption:

Individual food consumption will be recorded approximately weekly during the pretest period, prior to randomization, at least weekly during the treatment and recovery periods, and on the day prior to necropsy.

8.6 Clinical Pathology:

Blood samples for clinical pathology will be collected from all surviving animals on the day of their scheduled necropsy (i.e., 10 animals/sex/group at the primary necropsy and ≤ 10 animals/sex/group for Groups 1 and 4 at the recovery necropsy). If possible, clinical pathology parameters will be analyzed for animals euthanized *in extremis* (see Animals to be Euthanized *in Extremis* section).

Blood samples for serum chemistry and hematology will be collected from the retro-orbital sinus of animals anesthetized by inhalation of isoflurane. The animals sampled at the scheduled clinical pathology intervals will be fasted overnight prior to blood collection. Urine will be collected overnight using metabolism caging.

Blood samples for analysis of coagulation parameters will be collected by necropsy personnel from the vena cava at the time of scheduled euthanasia from animals euthanized by inhalation of CO_2.

Anticoagulants will be potassium EDTA for the hematology and sodium citrate for the coagulation tests. Samples for serum chemistry will be collected without anticoagulants.

8.6.1 Hematology:

Blood smears[a]	MCHC
Differential leukocyte count	MCV
Erythrocyte count	Platelet count
Hematocrit	Reticulocyte count
Hemoglobin	Total leukocyte count
MCH	

a - Blood smears will be made for all animals receiving a hematology evaluation as per WIL Research Laboratories, LLC SOPs T5-027, T5-085 and T5-139. The blood smear will only be evaluated if scientifically warranted (at additional cost). Parameters evaluated from these smears will include a differential leukocyte count, platelet estimates and RBC morphology.

8.6.2 Coagulation Parameters:

Activated partial thromboplastin time	Prothrombin time

8.6.3 Serum Chemistry:

A/G ratio	Glucose
Alanine aminotransferase	Phosphorus
Albumin	Potassium
Alkaline phosphatase	Sodium
Aspartate aminotransferase	Sorbitol dehydrogenase
Calcium	Total bilirubin
Chloride	Total cholesterol
Creatinine	Total protein
Gamma glutamyltransferase	Triglycerides
Globulin	Urea nitrogen

8.6.4 Urinalysis:

Color and clarity	Occult blood
Specific gravity	Leukocytes
pH	Nitrites
Protein	Urobilinogen
Glucose	Volume
Ketones	Microscopy of sediment
Bilirubin	Osmolality

8.7 Anatomic Pathology:

8.7.1 Macroscopic Examination:

A complete necropsy will be conducted on all animals dying spontaneously, euthanized *in extremis* or at the scheduled necropsies. Animals euthanized *in extremis* or at study termination will be euthanized by carbon dioxide inhalation and exsanguinated. Necropsy will include examination of the external surface, all orifices and the cranial, thoracic, abdominal and pelvic cavities including viscera. At the time of necropsy the following tissues and organs will be collected and placed in 10% neutral-buffered formalin (except as noted):

Adrenals (2)
Aorta
Bone with marrow
  Femur
  Sternum
Bone marrow smear [a]
Brain
  Cerebrum Level 1
  Cerebrum Level 2
  Cerebellum with medulla/pons
Cervix
Epididymides (2)[c]
Exorbital lacrimal glands (2)
Eyes with optic nerve (2)[b]
Gastrointestinal tract
  Esophagus
  Stomach
  Duodenum
  Jejunum
  Ileum
  Peyer's patches
  Cecum
  Colon
  Rectum
Heart
Kidneys (2)
Larynx
Liver (sections of two lobes)
Lungs (including bronchi, fixed by inflation with fixative)
Lymph node
  Mandibular
  Mesenteric
Nasal cavity[d]
Ovaries (2) with oviducts[e]
Pancreas
Peripheral nerve (sciatic)
Pharynx
Pituitary
Prostate
Salivary glands [mandibular (2)]
Seminal vesicles (2)
Skeletal muscle (rectus femoris)
Skin with mammary gland[f]
Spinal cord
  Cervical
  Thoracic
  Lumbar
Spleen
Testes (2)[c]
Thymus
Thyroid (with parathyroids) (2)[e]
Tongue
Trachea
Urinary bladder
Uterus
Vagina
All gross lesions and masses (when possible)

a - Not taken from animals found dead, not placed in formalin, only evaluated if scientifically warranted.
b - To be placed in Davidson's solution.
c - To be placed in Bouin's solution.
d - Levels I and III according to the method of Young (Young, 1981) will be examined.
e - Oviducts and parathyroids will be examined microscopically if in the plane of section and in all cases where a gross lesion of the organ is present.
f - For females; a corresponding section of skin will be taken from the same anatomic area for males.

8.7.2 Organ Weights:

The following organs from all animals euthanized at the scheduled necropsies will be weighed:

Adrenals	Ovaries (with oviducts)
Brain	Spleen
Epididymides	Testes
Heart	Thymus
Kidneys	Uterus
Liver	

Paired organs will be weighed together. Organ-to-body-weight and organ-to-brain-weight ratios will be calculated from animals euthanized at the scheduled necropsy.

8.7.3 Liver Metabolic Enzyme Analysis

Following collection of the organ weights for all animals euthanized at the scheduled necropsies, an approximate 3-gram section of liver (median lobe) will be collected. The tissue will then be rinsed in chilled saline, placed in a plastic bag, flash frozen in liquid nitrogen and stored frozen (approximately -60° to -80°C) until shipped to DuPont Haskell for analysis. A liver tissue sample for metabolic enzyme analysis will not be collected from any animal euthanized *in extremis* or found dead. Frozen tissue samples will be shipped on dry ice, by overnight courier, to the address:

Carol Carpenter
Senior Staff Toxicologist
DuPont Haskell Global Centers for Health and Environmental Sciences
1090 Elkton Rd, P.O. Box 50
Newark, DE 19714
Tel: (302) 366-5201
Fax: (302) 366-5207
Email: Carol.Carpenter@usa.dupont.com

Liver samples will be analyzed for the following parameters according to DuPont Haskell SOPs:

Total cytochrome P450 content
Beta oxidation activity

The results of these analyses will be provided to WIL Research Laboratories, LLC in the form of a GLP-compliant report, which will be appended to the final report.

8.7.4 Microscopic Examination:

Histologic preparation will be conducted at either the WIL Research Laboratory site in Ashland, Ohio or at the WIL Research Laboratories subsidiary (Biotechnics) in Hillsborough, North Carolina, and documented in the raw data. If the tissues are shipped to North Carolina, an appropriate protocol amendment will be issued prior to such shipment.

Microscopic examination of hematoxylin-eosin stained paraffin sections will be performed on the tissues/organs listed in the Macroscopic Examination Section from all animals found dead, euthanized *in extremis* and in the control and high-dose groups euthanized at the scheduled primary necropsy. Gross lesions will be examined from animals in the low- and mid-dose groups euthanized at the primary necropsy and animals in the control and high-dose groups euthanized at the recovery necropsy. Microscopic examination may be extended to other organs/tissues in the low- and mid-dose groups euthanized at the primary necropsy and the control and high-dose groups euthanized at the recovery necropsy (by protocol amendment, at additional cost) if a potential target organ is noted based on histopathological examination of tissues from the control and high dose groups or other parameters (organ weights, clinical pathology, etc.). Special stains may be used at the discretion of the pathologist to further characterize lesions and changes. Any special stains used will be documented in the individual animal data and interpretation of results will be included in the final report.

9 STATISTICAL METHODS:

All analyses except for liver metabolic analysis will be two-tailed for significance levels of 5% and 1%. Significance for liver metabolic analysis will be judged at $p < 0.05$. Statistical analysis will not be performed on groups with an N of two (2) or less. Separate analyses will be performed on the data collected for each sex. All means will be presented with standard deviations. All statistical tests will be performed using appropriate computing devices or programs. Body weights, body weight changes and food consumption as well as clinical pathology values (except gamma glutamyltransferase), and absolute and relative organ weights will be subjected to a one-way analysis of variance (Snedecor and Cochran, 1980). If a statistically significant difference ($p<0.05$) is present in this ANOVA, a comparison of the control group to each treated group by Dunnett's test (Dunnett, 1964) will be

performed. Gamma glutamyltransferase values that fall under the detectable range will be assigned a value of 0.1 (half the lower limit of quantitation) for statistical analysis and reporting. Non-parametric statistical analysis will be conducted on gamma glutamyltransferase.

Cytochrome P450 and beta oxidation data will be subjected to a preliminary test of homogeneity (Levene, 1960) and normality (Shapiro and Wilk, 1965). If the preliminary test is not significant, one-way analysis of variance (Snedecor and Cochran, 1980) followed by Dunnett's test (Dunnett, 1964; Tamhane, 1979) will be performed. If the preliminary test is significant, a Kruskal-Wallis test (Kruskal and Wallis, 1952) followed by Dunn's test (Dunn, 1964) will be performed.

10 QUALITY ASSURANCE:

The study will be audited by the WIL Quality Assurance Unit while in progress to assure compliance with Good Laboratory Practice regulations, adherence to the protocol and to WIL Research Laboratories, LLC Standard Operating Procedures. The raw data and draft report will be audited by the WIL Quality Assurance Unit to assure that the final report accurately describes the conduct and the findings of the study. Unless requested by the Sponsor, the WIL QAU will not audit the work performed by subcontractors or Sponsor. It is assumed that these organizations have independent QAUs and that they will be responsible for GLP compliance of their work.

This study is a GLP-compliant study and will be included on the WIL Research Laboratories, LLC master list of regulated studies.

Liver metabolic enzyme analysis will be audited by the DuPont Haskell Quality Assurance Unit.

11 RECORDS TO BE MAINTAINED:

All original raw data records, as defined by WIL SOPs and the applicable GLPs, will be returned to the Sponsor, as described in protocol section 12.

12 WORK PRODUCT:

Sponsor will have title to all documentation records, raw data, slides, specimens, or other work product generated during the performance of the study. All work product including raw paper data, pertinent electronic storage media, and leftover test substance will be returned to the Sponsor at the address on page 2 of this protocol. All specimens will be shipped directly to EPL Archives, Inc., Sterling, VA. Unless otherwise indicated, all remaining formulation and clinical pathology samples will not be sent to Archives and will be discarded at the time of the issuance of the final report.

Any work product, including documents, specimens, and samples, that are required by this protocol, its amendments, or other written instructions of the Sponsor, to be shipped by WIL Research Laboratories, LLC to another location will be appropriately packaged and labeled as defined by WIL's SOPs and delivered to a common carrier for shipment. WIL Research Laboratories, LLC will not be responsible for shipment following delivery to the common carrier.

13 REPORTS:

The final report will contain a summary, test substance data, methods and procedures, appropriate individual animal and summary data tables, a copy of the protocol and amendments (if any) and an interpretation and discussion of the study results. The report will contain all information necessary to conform to current OECD specifications. The report will be comprehensive and shall attempt to define the level(s) inducing toxic effects, as well as "no-effect" level(s) under the conditions of this investigation.

WIL Research Laboratories, LLC will provide one (1) copy of an Audited Draft Report, submitted in a timely manner upon completion of the study phase and prior to issuance of the final report. One (1) revision will be permitted as part of the cost of the study, from which Sponsor's reasonable revisions and suggestions will be incorporated into the Final Report as appropriate. Additional changes or revisions may be made, at extra cost. It is expected that the Sponsor will review the draft report and provide comments to WIL within a two (2) month time frame following submission. WIL will submit the Final Report within one (1) month following receipt of comments. If the Sponsor's comments and/or authorization to finalize the report have not been received at WIL within one year of submission of the draft report, WIL may elect to finalize the report following appropriate written notification to the Sponsor. The Final Report will be provided as a PDF (electronic) copy and in MS Word format (electronic copy).

14 PROTOCOL MODIFICATION:

Modification of the protocol may be accomplished during the course of this investigation. However, no changes will be made in the study design without the verbal or written permission of the Sponsor. In the event that the Sponsor verbally requests or approves changes in the protocol, such changes will be made by appropriate documentation in the form of protocol amendments. All alterations of the protocol and reasons for the modification(s) will be signed by the Study Director and the Sponsor Representative.

15 ANIMAL WELFARE ACT COMPLIANCE:

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR). The Sponsor should make particular note of the following:

- The Sponsor signature on this protocol documents for the Study Director the Sponsor's assurance that the study described does not unnecessarily duplicate previous experiments
- Whenever possible, procedures used in this study have been designed to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study protocol or in written laboratory Standard Operating Procedures.
- Animals that experience severe or chronic pain or distress that cannot be relieved will be painlessly euthanized, as deemed appropriate by the veterinary staff and Study Director. The Sponsor will be advised by the Study Director of all circumstances which could lead to this action, in as timely a manner as possible.
- Methods of euthanasia used during this study are in conformance with the above-referenced regulation.
- The Sponsor/Study Director has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals and has provided a written narrative description (AWA covered species) of the methods and sources used to determine that alternatives are not available.

16 REFERENCES:

Dunn, O.J. Multiple contrasts using rank sums. *Technometrics* **1964**, *6*, pp. 241-252.

Dunnett, C.W. New tables for multiple comparisons with a control. *Biometrics* **1964**, *20*, pp. 482-491.

Kruskal, W.H. and Wallis, W.A. Use of ranks in one-criterion analysis of variance. *J. Amer. Statist. Assoc.* **1952**, *47*, pp. 583-621.

Levene, H. Robust test for equality of variances. In *Contributions to Probability and Statistics*, J. Olkin, ed.; Stanford University Press: Palo Alto, CA, **1960**; pp 278-292.

Shapiro, S.S. and Wilk, M.B. An analysis of variance test for normality (complete samples). *Biometrika* **1965**, *52*, pp. 591-611.

Snedecor, G.W.; Cochran, W.G. One Way Classifications; Analysis of Variance. In *Statistical Methods,* 7th ed.; The Iowa State University Press: Ames, IA, **1980;** pp. 215-237.

Tamhane, A.C. A comparison of procedures for multiple comparison of means with unequal variances. *J. Amer. Statist. Assoc.* **1979**, *74*, pp. 471-480.

Young, J.T. Histopathological examination of the rat nasal cavity. *Fundamental and Applied Toxicology* **1981,** *1,* 309-312.

17 PROTOCOL APPROVAL:

Sponsor approval via email on 16 Nov 2007.

E.I. du Pont de Nemours and Company

Carol Carpenter — 26-NOV-2007
Carol Carpenter
Sponsor Representative
Date

WIL Research Laboratories, LLC

16 Nov 2007
Michael S. Koch, PhD
Study Director
Date

16 Nov 2007
Christopher P. Chengelis, PhD, DABT
Director, Toxicology
Date