Battelle
The Business *of* Innovation

SYN547407, SYN546308, SYN548097, SYN548012, SYN548014, SYN548102,SYN548117, SYN548098, SYN548101, SYN548013

SYN547407, SYN546308, SYN548097, SYN548012, SYN548014, SYN548102,SYN548117, SYN548098, SYN548101, and SYN548013 - Seven Day Acute Oral Gavage Toxicity Study in Female Wistar Han Rats

Final Report

DATA REQUIREMENT(S): Not Applicable

AUTHOR(S): Tessa R. Moir-Savitz, M.S.
Dawn M. Fallacara, Ph.D.
Seth Gibbs, Ph.D.

STUDY COMPLETION DATE: 19 September 2016

PERFORMING LABORATORY: Battelle Toxicology West Jefferson
Building JM10
1425 State Route 142
West Jefferson, Ohio 43162 USA

LABORATORY PROJECT ID: Report Number: 39292
Study Number: 39292
Task Number: TK0220650

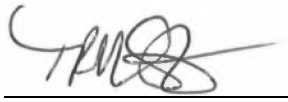
SPONSOR(S): Syngenta Ltd
Jealott's Hill International Research Centre
Bracnell, Berkshire, RG42 6EY, United Kingdom

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

This page is intentionally left blank.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

As intended, this study was not conducted in compliance with the current version of the United States Environmental Protection Agency (EPA) 40 CFR Part 160 Good Laboratory Practice (GLP) Regulations for nonclinical laboratory studies.



September 19, 2016

Tessa R. Moir-Savitz, M.S.
Study Director
Safety Assessment and Study Management

Date

Performing Laboratory: Battelle Toxicology West Jefferson
Building JM10
1425 State Route 142
West Jefferson, Ohio 43162 USA

FLAGGING STATEMENT

This page is intentionally left blank.

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Title
Tessa R. Moir-Savitz, M.S.	Study Director
Dawn M. Fallacara, Ph.D.	Toxicologist
Seth Gibbs, Ph.D.	Toxicokineticist
Kathryn Wolton	Syngenta Study Manager

Study Dates

Study initiation date: 7 January 2014
Experimental start date: 13 January 2014
Experimental termination date: 21 January 2014

Deviations from the Guidelines

None.

Retention of Samples

Reserve samples for archiving were not required.

Performing Laboratory Test Substance Reference Number

SYN547407
SYN546308
SYN548097
SYN548012
SYN548014
SYN548102
SYN548117
SYN548098
SYN548101
SYN548013

Other

All records generated by Battelle and required to reconstruct the study are maintained in labelled binders. These and all other raw data collected in this study are maintained under the direction of Battelle, along with the final report. The records will be archived as per Battelle SOPs.

TABLE OF CONTENTS

1.0 EXECUTIVE SUMMARY

The objective of this study was to determine the acute toxicity of SYN547407, SYN546308, SYN548097, SYN548012, SYN548014, SYN548102, SYN548117, SYN548098, SYN548101, and SYN548013 following a single dose by oral (gavage) administration in female Wistar Han rats. The toxicokinetic (TK) characteristics were also determined for each test substance over a seven day period.

1.1 Study Design

Two hundred and ten female Wistar Han rats were assigned to one of 42 groups (5 animals per dose group). Each animal was administered, via a single oral gavage, SYN547407, SYN546308, SYN548097, SYN548012, SYN548014, SYN548102, SYN548117, SYN548098, SYN548101, or SYN548013 at a target dose of 10, 100, 300, or 1000 mg/kg bodyweight, or the vehicle control (0.5% CMC).

The following evaluations were performed: clinical observations, body weights, triglyceride analysis, toxicokinetics, liver and adrenal gland weights, and gross necropsy, and histopathologic evaluation of the adrenal glands, duodenum, jejunum and liver.

Whole blood specimens for bioanalytical analysis were collected from the lateral tail vein into tubes containing tripotassium ethylenediaminetetraacetic acid (K_3EDTA) as the anticoagulant and diluted at a 1:1 ratio with Milli-Q water. Samples were collected for TK analysis at the following time points post-dose administration: 0.5, 1, 2, 4, 6, 12, 24, 36, 48, 72, 96, 120, 144, and 168 hours. Diluted whole blood samples were analyzed for test substance concentration which was reported as ng/mL whole blood. TK parameters evaluated included C_{max}, $C_{max/dose}$, observed T_{max}, AUC_{last}, $AUC_{last/dose}$, AUC_{∞}, and $AUC_{\infty/dose}$.

1.2 Results

1.2.1 SYN547407

All rats administered SYN547407 survived until the end of the study. Clinical observations were noted only in the 1000 mg/kg group (one rat was noted with a rough coat on Days 5 and 6; this observation resolved by Day 7). The group mean body weights increased from Day 1 to Day 8 although the body weight gains of rats administered 1000 mg/kg were less when compared to control rats. No differences were noted in triglyceride values. Treatment-related histopathological changes were noted in the adrenal glands, duodenum, and jejunum. In addition, the mean adrenal to body weight ratio increased for rats administered 1000 mg/kg.

The whole blood concentration-time curves for SYN547407 showed clear absorption and elimination phases following administration of 10 and 100 mg/kg. Blood concentrations remained similar over time without a definitive terminal elimination phase illustrated by the 300 and 1000 mg/kg groups. The group mean C_{max} when normalized for dose decreased as the dosage increased (ranging from 47.7 ng/mL (10 mg/kg) to 6.68 ng/mL (1000 mg/kg)). Group mean AUC values increased in a slightly greater than dose-proportional manner from 30 to 100

mg/kg, approximately dose-proportionally as the dose was increased from 100 to 300 mg/kg, but increased in a slightly less than dose-proportional manner after the 300 and 1000 mg/kg doses.

1.2.2 SYN546308

SYN546308 administration resulted in test substance-related changes in rats. Three rats (one each from the 10, 100, and 1000 mg/kg groups) died on Day 2 following the observation of seizure-like activity or tremors. One other 300 mg/kg rat was observed with seizure-like activity on Day 2; however, this rat survived until the end of the study. The group mean body weights increased from Day 1 to Day 8 although the body weight gains of rats administered 1000 mg/kg were less when compared to control rats. While the triglyceride values were not significantly changed 24 hours following dose administration, the triglyceride values were significantly decreased by 72 hours following administration of 1000 mg/kg. These triglyceride values were recovered by 168 hours. Treatment-related histopathological changes were noted in the adrenal glands, duodenum, and jejunum. In addition, the mean adrenal gland to body weight ratio increased for the rats administered 1000 mg/kg.

The whole blood concentration-time curves for SYN546308 showed clear absorption and elimination phases following administration of 10 and 100 mg/kg. Blood concentrations remained similar over time without a definitive terminal elimination phase illustrated by the 300 and 1000 mg/kg groups. The group mean C_{max} when normalized for dose decreased as the dosage increased (ranging from 53.7 ng/mL (10 mg/kg) to 5.81 ng/mL (1000 mg/kg)). Group mean AUC values increased proportionally as the dose was increased from 10 to 100 mg/kg, but increased in a less than dose-proportional manner after the 300 and 1000 mg/kg doses.

1.2.3 SYN548097

SYN548097 administration resulted in test substance-related changes in rats. One rat administered 10 mg/kg died on Day 4 and one rat administered 1000 mg/kg died on Day 8. Another rat administered 1000 mg/kg was observed with seizure-like activity on Day 2; however, this rat survived until the end of the study. Body weights were not significantly affected. While the triglyceride values were not significantly changed 24 hours following dose administration, the triglyceride values were significantly decreased after 72 hours following dose administration of 1000 mg/kg. These triglyceride values were recovered by 168 hours. No treatment-related changes were noted in the adrenal glands, duodenum, and jejunum. No treatment-related changes were noted in any absolute organ weights or mean organ-to-body weight ratios.

The whole blood concentration-time curves for SYN548097 showed clear absorption and elimination phases following administration of 10 and 100 mg/kg. Blood concentrations remained similar over time without a definitive terminal elimination phase illustrated by the 300 and 1000 mg/kg groups. The group mean C_{max} when normalized for dose decreased as the dosage increased (ranging from 63.0 ng/mL (10 mg/kg) to 9.35 ng/mL (1000 mg/kg)). Group mean AUC values increased in an approximately dose-proportional manner as the dose was increased from 10 to 1000 mg/kg.

1.2.4 SYN548012

All rats administered SYN548012 survived until the end of the study. There were no clinical observations at any dose level. Bodyweights were unaffected by treatment. Triglyceride values were unaffected by treatment during the course of the study. There were no noted gross or histopathological findings. No treatment-related changes were noted in any absolute organ weights or mean organ-to-body weight ratios.

The whole blood concentration-time curves for SYN548012 showed clear absorption and elimination phases following administration of 10, 100 and 1000 mg/kg. Blood concentrations remained similar over time illustrated by the 300 mg/kg group. The group mean C_{max} when normalized for dose decreased as the dosage increased (ranging from 30.8 ng/mL (10 mg/kg) to 2.85 ng/mL (1000 mg/kg)). Group mean AUC values increased in a less than dose-proportional manner as the dose was increased from 10 to 1000 mg/kg.

1.2.5 SYN548014

All rats administered SYN548014 survived until the end of the study. There were no clinical observations at any dose level. Bodyweights were unaffected by treatment. Triglyceride values were unaffected by treatment during the course of the study. There were no noted gross or histopathological findings. No treatment-related changes were noted in any absolute organ weights or mean organ-to-body weight ratios.

The whole blood concentration-time curves for SYN548014 showed clear absorption and elimination phases following administration of 10, 100, and 300 mg/kg. Blood concentrations remained similar over time without a definitive terminal elimination phase illustrated by the 1000 mg/kg group. The group mean C_{max} when normalized for dose decreased as the dosage increased (ranging from 63.3 ng/mL (10 mg/kg) to 6.88 ng/mL (1000 mg/kg)). Group mean AUC values increased proportionally as the dose was increased from 10 to 100 mg/kg, but increased in a less than dose-proportional manner after the 300 and 1000 mg/kg doses.

1.2.6 SYN548102

SYN548102 administered at 1000 mg/kg resulted in test substance-related changes. One rat given 1000 mg/kg was noted with a rough coat and died on Day 7. In addition, one rat administered 1000 mg/kg was observed with red discharge from both eyes and nostrils, hunched posture, lethargy, rough coat, thin appearance, and a wet urogenital area on Days 7 and 8. This rat was determined to be moribund on Day 8 and humanely terminated. Body weights were significantly decreased in the rats administered 1000 mg/kg when compared to controls. Triglyceride values were significantly, but transiently decreased 6 hours following administration of 100 and 1000 mg/kg. These values were not significantly decreased 24 hours following dose administration. Treatment-related histological changes were noted in the adrenal glands. In addition, the mean adrenal weight was increased for rats administered 1000 mg/kg.

The whole blood concentration-time curves for SYN548102 showed clear absorption and elimination phases following dose administration of 10 and 100 mg/kg. Blood concentrations remained similar over time without a definitive terminal elimination phase illustrated by the 300 and 1000 mg/kg groups. The group mean C_{max} when normalized for dose decreased as the

dosage increased (ranging from 79.8 ng/mL (10 mg/kg) to 6.69 ng/mL (1000 mg/kg)). Group mean AUC values increased proportionally as the dose was increased from 10 to 100 mg/kg, but increased in a less than dose-proportional manner after the 300 and 1000 mg/kg doses.

1.2.7 SYN548117

SYN548117 administration resulted in test substance-related changes in rats. Seizure-like activity was observed in a number of rats administered 300 or 1000 mg/kg, most of which survived until the end of the study; one 1000 mg/kg rat died on Day 7 and another on Day 8. In addition, other observations such as, pale skin, excess salivation, hyperactivity, hind limb weakness, labored breathing, rapid respiration, thin appearance, and a rough coat were noted. Body weights were significantly decreased in the rats administered 1000 mg/kg when compared to controls. No differences were noted in triglyceride values. Treatment-related histopathological changes were noted in the duodenum and jejunum. No treatment-related changes were noted in any absolute organ weights or mean organ-to-body weight ratios.

The whole blood concentration-time curves for SYN548117 showed clear absorption and elimination phases following administration of 10 mg/kg. Blood concentrations remained similar over time for the 100 mg/kg group. There was no definitive terminal elimination phase for the 300 and 1000 mg/kg groups. The group mean C_{max} when normalized for dose decreased as the dosage increased (ranging from 66.6 ng/mL (10 mg/kg) to 6.73 ng/mL (1000 mg/kg)). Group mean AUC values increased proportionally as the dose was increased from 10 to 100 mg/kg, but increased in a less than dose-proportional manner after the 300 and 1000 mg/kg doses.

1.2.8 SYN548098

All rats administered SYN548098 survived until the end of the study. Two rats administered 1000 mg/kg were observed with a rough coat during the course of the study. Bodyweights were unaffected by treatment. Triglyceride values were unaffected by treatment during the course of the study. There were no noted gross or histopathological findings. No treatment-related changes were noted in any absolute organ weights or mean organ-to-body weight ratios.

The whole blood concentration-time curves for SYN548098 showed clear absorption and elimination phases following administration 10 and 100 mg/kg. Blood concentrations remained similar over time without a definitive terminal elimination phase illustrated by the 300 and 1000 mg/kg groups. The group mean C_{max} when normalized for dose decreased as the dosage increased (ranging from 36.5 ng/mL (10 mg/kg) to 5.02 ng/mL (1000 mg/kg)). Group mean AUC values increased in a less than dose-proportional manner as the dose was increased. Due to the lack of a well-defined elimination phase, AUC_{∞} was not reported for the 300 and 1000 mg/kg groups.

1.2.9 SYN548101

SYN548101 administration resulted in test substance-related clinical signs in rats. One rat administered 100 mg/kg and one rat administered 300 mg/kg died on Days 2 and 7, respectively. Three rats administered 1000 mg/kg were observed with seizure-like activity and died on Days 5 or 6. In addition, other observations such as, pale skin, excess salivation, hunched posture,

lateral recumbancy, hind limb weakness, thin appearance, and a rough coat were noted. Body weights were significantly decreased in the rats administered 1000 mg/kg when compared to controls. No differences were noted in the triglyceride values. Treatment-related histopathological changes were noted in the adrenal glands. No treatment-related changes were noted in any absolute organ weights or mean organ-to-body weight ratios.

The whole blood concentration-time curves for SYN548101 showed clear absorption and elimination phases following administration of 10 mg/kg. Blood concentrations remained similar over time for the 100 mg/kg group and additionally there was no definitive terminal elimination phase for the 300 and 1000 mg/kg groups. The group mean C_{max} when normalized for dose decreased as the dosage increased (ranging from 31.4 ng/mL (10 mg/kg) to 4.39 ng/mL (1000 mg/kg)). Group mean AUC values increased in a slightly greater than dose-proportional manner as the dose was increased from 10 to 300 mg/kg, but in a less than dose-proportional manner from 300 to 1000 mg/kg.

1.2.10 SYN548013

All rats administered SYN548013 survived until the end of the study. Bodyweights were unaffected by treatment. Triglyceride values were unaffected by treatment during the course of the study. There were no noted gross or histopathological findings. No treatment-related changes were noted in any absolute organ weights or mean organ-to-body weight ratios.

The whole blood concentration-time curves for SYN548013 showed clear absorption and elimination phases following administration of 10 and 100 mg/kg. Blood concentrations remained similar over time for the 300 mg/kg group and additionally there was no definitive terminal elimination phase for the 1000 mg/kg group. The group mean C_{max} when normalized for dose decreased as the dosage increased (ranging from 69.1 ng/mL (10 mg/kg) to 7.17 ng/mL (1000 mg/kg)). Group mean AUC values increased in a less than dose-proportional manner after the 100, 300, and 1000 mg/kg doses.

1.3 Conclusions

All animals dosed with SYN547407, SYN548012, SYN548014, SYN548098 and SYN548013 survived the study following single gavage dosing at 1000 mg/kg. SYN548012, SYN548014 and SYN548013 were all well tolerated at 1000 mg/kg. There were clinical signs observed in animals dosed with 1000 mg/kg SYN547407 and SYN548098. Both SYN547407 and SYN548014 showed evidence of dose-proportionality up to 100 mg/kg, and evidence of sub proportional kinetics between the 100 and 1000 mg/kg dose groups. SYN548012, SYN548098, and SYN548013 showed evidence of sub proportionality across the dose range.

Acute toxicity was evident in animals dosed with SYN546308, SYN548097, SYN548102, SYN548117, and SYN548101 including adverse clinical signs and death in some dose groups. SYN546308, SYN548102 and SYN548117 showed evidence of dose-proportionality up to 100 mg/kg, and evidence of sub proportional kinetics between the 100 and 1000 mg/kg dose groups. Both SYN548097 and SYN548101 show evidence of dose-proportionality across the dose range.

SYN546308 was not tolerated following single dose administration with three total deaths across dose groups (one at 10 mg/kg, 30 mg/kg and 1000 mg/kg respectively). There were treatment related-transient lowering of triglycerides and histopathological changes in the adrenal glands, duodenum, and jejunum. Furthermore, clinical signs suggestive of neurological effects were observed across all dose groups.

SYN548097 was not tolerated following single dose administration with two deaths across the dose groups (one at 10 mg/kg and one at 1000 mg/kg). Furthermore, clinical signs suggestive of neurological effects were observed in the 1000 mg/kg dose group. There was a treatment-related transient lowering of triglycerides. Furthermore, clinical signs suggestive of neurological effects were observed at 1000 mg/kg.

SYN548102 was not tolerated following single dose administration with deaths and adverse clinical signs observed in the 1000 mg/kg dose group. Body weights were significantly decreased in the rats administered 1000 mg/kg. Triglyceride values were significantly, but transiently decreased following administration of 100 and 1000 mg/kg. By 24 hours, the triglyceride values had increased to a level comparable to the control group. Treatment-related histological changes were noted in the adrenal glands.

SYN548117 was not tolerated following single dose administration with deaths at 1000 mg/kg and adverse clinical signs at both 300 and 1000 mg/kg. Body weights were significantly decreased in the rats administered 1000 mg/kg when compared to controls. Treatment-related histopathological changes were noted in the duodenum and jejunum. Furthermore, clinical signs suggestive of neurological effects were observed at both 300 mg/kg and 1000 mg/kg.

SYN548101 was not tolerated following single dose administration with deaths at 100, 300, and 1000 mg/kg. Body weights were significantly decreased in the rats administered 1000 mg/kg when compared to controls. Treatment-related histopathological changes were noted in the adrenal glands. Furthermore, clinical signs suggestive of neurological effects were observed at 1000 mg/kg.

2.0 INTRODUCTION

2.1 Purpose

The objective of this study was to determine the acute toxicity of SYN547407, SYN546308, SYN548097, SYN548012, SYN548014, SYN548102, SYN548117, SYN548098, SYN548101, and SYN548013 following a single oral gavage administration in female Wistar Han rats.

3.0 MATERIALS AND METHODS

3.1 Test Substances

Test substances SYN547407 (CSCV765754), SYN548097 (CSCY970035), SYN548012 (CSCW623960), SYN548014 (CSCY735620), SYN548102 (CSCY970040), SYN548117 (CSCY970235), SYN548098 (CSCY970036), SYN548101 (CSCY970039), and SYN548013 (CSCW623960) were supplied by Syngenta Ltd. and were received on December 12, 2013.

SYN546308 (CSCD724333), also supplied by Syngenta Ltd., was received on December 13, 2013. Approximately 3 grams of each substance was received in good condition and stored at room temperature. For all test substances, the Certificates of Analysis and Test Substance Characterizations were not provided.

Due to the duration of this study being less than 4 weeks, collection of reserve samples for archiving was not required. Formulation analysis (including concentration, stability, and homogeneity) was not performed. Following the completion of in-life dose administration, the unused neat test substances were disposed per testing facility standard operating procedures (SOPs).

3.1.1 Formulation preparation

Each formulation was prepared at Battelle (West Jefferson, OH) on the day of dose administration by weighing a known quantity of test substance and combining it with the appropriate volume of vehicle [0.5% carboxymethylcellulose (CMC)] in water. A detailed description of the dose formulation method is presented in Appendix 2. Briefly, the appropriate amount of test substance was pre-weighed into each dosing container for no more than three days prior to dose administration. On the day of dose administration, the appropriate volume of the vehicle was added to the dosing container and the suspension was stirred. The suspension was then mixed with a Silverson mixer for approximately 5 minutes and stirred until visibly homogeneous. The target formulation concentrations for all test substances were 1, 10, 30, and 100 mg/mL. The formulations were stored at room temperature prior to use; residual formulations were stored in a refrigerator unit set to maintain 2 to 8°C and were appropriately discarded per testing facility SOP.

A detailed description of the formulation methods are presented in Appendix 2.

3.2 Experimental Design

3.2.1 Animals

A total of 210 female Wistar Han rats were required for this study. A sufficient number of animals were obtained from Charles River Laboratories (Raleigh, NC) to provide the required number of healthy animals for testing. The rats were approximately 6 weeks of age at the time of receipt and approximately 7 weeks of age on Day 1 of the study. The rats ranged in body weight from approximately 133 to 158 grams on Days -3 or -4.

The rat was chosen as the test system because it is an accepted species that is commonly used in safety testing and toxicity studies. At this time, studies in laboratory animals provide the best available basis for extrapolation to humans and are required to support regulatory submissions. The Battelle Institutional Animal Care and Use Committee approved the proposed activities before implementation of this study.

3.2.1.1 Animal care, housing, and environmental conditions

All animals were received, quarantined, and individually housed in polycarbonate cages with hardwood bedding according to testing facility SOPs. General procedures for animal care and

housing conformed to the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) recommendations, current requirements stated in the "Guide for Care and Use of Laboratory Animals" [National Research Council (NRC)], and to the testing facility SOP.

Rats were housed in rooms JM10-1-517 and JM10-1-519. Cages were changed at least once weekly. Racks were changed every 2 weeks in accordance with testing facility SOPs.

The environmental conditions of the animal study rooms conformed to the following: (1) the light/dark cycle was set to provide approximately 12-hour light/12-hour dark photoperiod using fluorescent lighting set to start at approximately 0600 hours each day, and lights-off at approximately 1800 hours, except when room lights were turned on during the dark cycle to accommodate blood sampling or other study procedures; (2) the room temperature and relative humidity controls were set to maintain 68 to 79°F and 30 to 70 percent, respectively, and were monitored for conformance; and (3) 100 percent fresh air (with no recirculation) was supplied to the room at a rate providing a minimum of ten changes of room air per hour. The actual temperature was within the set range 100 percent of the time and ranged from 71 to 74°F. The humidity levels were within range 100 percent of the time and ranged from 37 to 60 percent.

3.2.1.2 Diet

Rats were fed PMI Certified Rodent pellets (5002P) *ad libitum*, according to testing facility SOP.

Analytical reports of each feed lot of PMI Certified Rodent pellets were provided by the manufacturer and maintained under the direction of Battelle. Analytical reports were reviewed according to testing facility SOP to ensure acceptable standards, and freedom from levels of contaminants that may interfere with the purpose or conduct of the study. There were no known and/or reported contaminants in the certified feed that would have impacted the study results or interpretations.

3.2.1.3 Water

Fresh water from the West Jefferson municipal water supply was provided ad libitum to the rats by an automatic watering system except when an animal was removed from its cage.

The water supply was monitored as per testing facility SOP to ensure acceptable standards and freedom from levels of contaminants that may interfere with the purpose or conduct of the study. The results of these analyses are maintained under the direction of Battelle. There were no known and/or reported contaminants in the water that would have impacted the study results or interpretations.

3.2.2 Group assignment and animal identification

Animals were identified by pre-study numbers on cage cards during quarantine and acclimation. Following group assignment, rats were individually identified by ear tag displaying a number unique within the study. Each cage card contained information including the animal identification number, study number, group assignment with dosage, species, sex, and strain. Additionally, cage cards were color coded by test substance.

Two hundred and ten female rats were assigned to 42 dose groups by body weight prior to dosing using a computer program (Provantis, Version 8.6.1.2). Provantis software ensures homogeneity of group variances with respect to body weight across all groups.

The following table summarizes the group assignment, animal identification numbers, and dosing regimen (with a dose volume of 10 mL/kg):

Group	Test Substance[b]	Target Dosage (mg/kg)	Formulation Concentration (mg/mL)	Number of Rats (females)	Animal IDs
1	SYN547407	10	1	5	1-5
2		100	10	5	6-10
3		300	30	5	11-15
4		1000	100	5	16-20
5	SYN546308	10	1	5	21-25
6		100	10	5	26-30
7		300	30	5	31-35
8		1000	100	5	36-40
9	SYN548097	10	1	5	41-45
10		100	10	5	46-50
11		300	30	5	51-55
12		1000	100	5	56-60
13	SYN548012	10	1	5	61-65
14		100	10	5	66-70
15		300	30	5	71-75
16		1000	100	5	76-80
17	SYN548014	10	1	5	81-85
18		100	10	5	86-90
19		300	30	5	91-95
20		1000	100	5	96-100
21	SYN548102	10	1	5	101-105
22		100	10	5	106-110
23		300	30	5	111-115
24		1000	100	5	116-120
25	SYN548117	10	1	5	121-125
26		100	10	5	126-130
27		300	30	5	131-135
28		1000	100	5	136-140
29	SYN548098	10	1	5	141-145
30		100	10	5	146-150
31		300	30	5	151-155
32		1000	100	5	156-160
33	SYN548101	10	1	5	161-165
34		100	10	5	166-170
35		300	30	5	171-175
36		1000	100	5	176-180
37	SYN548013	10	1	5	181-185
38		100	10	5	186-190
39		300	30	5	191-195
40		1000	100	5	196-200
41	Vehicle Control (0.5% CMC)	0	0	5	201-205
42	Vehicle Control (0.5% CMC)	0	0	5	206-210

3.2.3 Dose administration

Each rat in all dose groups received a single oral (gavage) administration of the appropriate formulated test substance on Day 1. The most recent body weight (Days -3 or -4) was used in the calculation of the dose volume (10 mL/kg).

3.2.4 Clinical observations

Cage-side observations for moribundity and mortality were made twice daily, once in the morning and once in the afternoon, with at least 6 hours between observations (no later than 10:00 AM and no earlier than 2:00 PM) throughout the duration of the study.

For all surviving animals, cage-side clinical observations for evidence of toxicity were conducted and recorded at least once daily from Day 1 through Day 7. On Day 1, cage-side clinical observations were conducted 2 to 4 hours following dose administration. Detailed clinical observations were conducted prior to group assignment (Days -3 or -4) and on Day 8 prior to necropsy.

3.2.5 Body weight

Body weights were recorded on Days -3 or -4 for group assignment and dose volume calculation. Terminal body weights were recorded on the day of necropsy (Day 8).

3.2.6 Triglyceride sample collection and analysis

Whole blood samples (approximately 0.2 mL) were collected from surviving rats 6, 24, 72, and 168 hours following dose administration. Actual times were recorded. Whole blood was collected from a tail vein and placed into tubes containing no anticoagulant. Samples were processed to serum and analyzed for triglyceride values. Following analysis, residual serum was stored in a freezer set to maintain approximately -85 to -60°C until disposal.

3.2.7 Bioanalytic sample collection and analysis

3.2.7.1 Bioanalytical sample collection

Whole blood samples (approximately 0.1 mL) were collected at various time points during the 7-day study. Up to 6 specimens were collected from each animal as per the table below. Whole blood was collected from a tail vein and placed into tubes containing K_3EDTA as the anticoagulant.

Time Point (Hours Following Dose Administration)	Animal				
	A	B	C	D	E
0.5	X	-	-	-	-
1	-	X	-	-	-
2	-	-	X	-	-
4	-	-	-	X	-
6	X[(a)]	X[(a)]	X[(a)]	X[(a)]	X[(a)]
12	-	-	-	-	X
24	X[(a)]	X[(a)]	X[(a)]	X[(a)]	X[(a)]
36	X	-	-	-	-
48	-	X	-	-	-
72	X[(a)]	X[(a)]	X[(a)]	X[(a)]	X[(a)]
96	-	-	X	-	-
120	-	-	-	X	-
144	-	-	-	-	X
168	X[(a)]	X[(a)]	X[(a)]	X[(a)]	X[(a)]

X = Sample Collected; X[(a)] = Specimen collected concurrently with specimen for clinical chemistry;
- = Specimen not required.

The target blood collection time points included 0.5, 1, 2, 4, 6, 12, 24, 36, 48, 72, 96, 120, 144, and 168 hours following dose administration. Actual times were recorded.

Blood samples scheduled for collection from animals which died prior to the end of the study were collected from a surviving animal within the same group (DR-273-WJ).

Following blood collection, all whole blood specimens were diluted at a 1:1 ratio using an appropriate volume of Milli-Q water although the ratio of blood:water cannot be verified for the samples collected 120 hours following dose administration from animals 154, 159, 164, and 169 (DR-293-WJ). The volume of these samples was investigated and determined to be similar to diluted samples of known ratio, which suggested that the samples were processed appropriately at the time of collection. For calculation purposes, the blood samples were assumed to be diluted at a 1:1 ratio with water, which did not affect the overall TK conclusions.

Diluted whole blood samples were placed on dry ice until stored in a freezer set to maintain approximately -85 to -60°C. Samples were shipped to Battelle (King Avenue, Columbus, OH) on dry ice and stored in a freezer set to maintain approximately -85 to -60°C until analyzed.

3.2.7.2 Biological sample analysis

Blood samples were analyzed for test substance concentration by Battelle (King Avenue, Columbus, OH). Blood samples from animals in Groups 1 through 4 were analysed for SYN547407; from groups 5 through 8 for SYN546308; from groups 9 through 12 for SYN548097; from groups 13 through 16 for SYN548012; from groups 17 through 20 for SYN548014; from groups 21 through 24 for SYN548102; from groups 25 through 28 for

SYN548117; from groups 29 through 32 for SYN548098; from groups 33 through 36 for SYN548101; and from groups 37 through 40 for SYN548013.

Detailed descriptions of the Bioanalytical Analysis methods are presented in Appendix 4.

3.2.8 Toxicokinetics

The apparent terminal elimination phase was not considered to be adequately characterized unless the following criteria were met: (1) the coefficient of determination (r^2) for the terminal linear phase was greater than or equal to 0.85, (2) the time of the last observed concentration (T_{last}) was greater than three times the half-life, and (3) the AUC_{∞} had less than 20 percent of the area extrapolated.

Where data allowed, the toxicokinetic parameters evaluated included observed C_{max}, C_{max}/dose, observed T_{max}, and AUC using WinNonlin, Version 6.2. Nominal time points were utilized for the calculations if the actual sample collection time point was within 5 percent of the target for time points 0 to 4 hours following dose administration or within 15 minutes of the target for time points 4 hours or greater following dose administration. If the time point was outside of this range, the actual time point was utilized in the calculation. A list of all off-target collections is available in Appendix 4. The six time points outside of the acceptable range are listed below:

Test Substance	Target Time Point(s) (Hours Post-Dose Administration)	Actual Time Point (hr)
10 mg/kg SYN546308	0.5	0.617
10 mg/kg SYN548013	1	1.13
100 mg/kg SYN548013	0.5	0.533
10 mg/kg SYN548117	0.5	0.533
100 mg/kg SYN548117	0.5	0.533
300 mg/kg SYN548117	36	36.3

3.2.9 Computer systems for data management

The following computer systems are used for toxicology data management:

Computer System Name	Version No.	Manufacturer	Data Type
Analyst[b]	1.4.2	Applied Biosystems Inc.	Chromatography and Mass Spectrometry
Atlas[b]	8.2	Thermo Fisher Scientific	General Chromatography
EMCS[c]	3.10	Siemens	Animal Facility Environmental
Next Generation PATH/TOX SYSTEM[a]	1.7.2	Xybion Medical Systems Corporation	Pharmacy Data Management
Provantis[c]	8.6.1.2	Instem	Animal Toxicology and Pathology Data Management
T-Track[a]	1.0.0	Battelle	Environmental Storage
Watson LIMS[b]	7.4	Thermo Fisher Scientific	Laboratory Information Management System

a. Computer system used by the Battelle Toxicology West Jefferson Testing Facility and the Battelle Toxicology Columbus Test Site.
b. Computer systems used by the Battelle Toxicology Columbus Test Site.
c. Computer systems used by the Battelle Toxicology West Jefferson Testing Facility.

3.3 *Post Mortem* Investigations

3.3.1 Necropsy, tissue processing, and histopathology

Gross necropsies were performed according to testing facility SOP on all study rats that were found dead or terminated at an unscheduled interval. All surviving study rats were subjected to gross necropsy on Day 8 according to testing facility SOP. Rats were humanely terminated by CO_2 inhalation. Each necropsy included: examination of the external surface of the body; all orifices; the cranial, thoracic, abdominal, and pelvic cavities and their contents; and collection of the adrenal glands, duodenum, jejunum, liver, brain, and spinal cord. The adrenal glands, duodenum, jejunum, and liver were evaluated microscopically.

For all rats surviving until Day 8 [including the Day 8 moribund sacrificed rats (DR-272-WJ)], the adrenal glands and liver were weighed and placed in 10 percent neutral buffered formalin (NBF). The brain, spinal cord, duodenum, and jejunum were placed in 10 percent NBF (Protocol Amendment 1). The adrenal glands, liver, duodenum, and jejunum were processed to slides and stained with hematoxylin and eosin in accordance with testing facility SOPs for histopathologic evaluation. The brain and spinal cord was not processed (Protocol Amendment 1). Non-neoplastic lesions were semiquantitatively graded across a 4-point scale, where Grade 1 (minimal) referred to a minor change of negligible significance, and which affected less than 10 percent of the presented tissue area; Grade 2 (mild) referred to a slight change which affected 10 to 19 percent of the tissue area; Grade 3 (moderate) referred to a change of biologic relevance, or

which affected at least 20 percent of the tissue area; and Grade 4 (marked) was reserved for lesions considered to be of maximal morphologic change.

Slides were examined microscopically by a board-certified veterinary pathologist. An internal peer review was performed according to testing facility SOP.

Comparisons of the results were made between individual test substances and the appropriate control group.

3.4 Data Evaluation

All appropriate quantitative in-life data collected at Battelle using the Provantis system were analyzed for test substance effects by parametric or nonparametric analysis of variance (ANOVA). For parametric analysis, normality was determined by the Shapiro-Wilks test and homogeneity of variances will be determined by Levene's test. Both tests were conducted at the 0.05 level of significance. Data may have been log-transformed to meet parametric assumptions. For data determined to be normally distributed and homogenous among groups, an ANOVA F-test was used to determine whether there are differences among the group means. If the ANOVA F-test was significant at the 0.05 level, then tests for differences between the control and each of the comparison groups were conducted using Dunnett's test, which adjusts for multiple comparisons. For data that were not normally-distributed and/or non-homogenous, analogous nonparametric methods were used. For the nonparametric analysis, a Kruskal-Wallis test was used to determine whether there were differences among the group means. If the Kruskal-Wallis test was significant at the 0.05 level, then tests for differences between the control and each of the comparison groups were conducted using Wilcoxon tests and the Bonferroni-Holm method to correct for multiple comparisons. Statistically significant values for each comparison are reported at the 0.05 level after accounting for multiple comparisons.

Two vehicle control groups were included in the study design, as dose administration occurred over two days. Comparisons occurred between a single control (vehicle) group (either Group 41 or Group 42) and each comparison group by day of dose administration. The following test substance groups were compared to Group 41: SYN547407, SYN546308, SYN548097, SYN548012, and SYN548014. The following test substance groups were compared to Group 42: SYN548102, SYN548117, SYN548098, SYN548101, and SYN548013. The vehicle control groups used in each analysis are specified in the report and in report tables.

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Survival

The mortality results are portrayed in the table below:

Test Substance	Target Dosage	Number of Early Deaths	Day(s) of Death
SYN547407	10	0	N/A
	100	0	N/A
	300	0	N/A
	1000	0	N/A
SYN546308	10	1	2
	100	1	2
	300	0	N/A
	1000	1	2
SYN548097	10	1	4
	100	0	N/A
	300	0	N/A
	1000	1	8
SYN548012	10	0	N/A
	100	0	N/A
	300	0	N/A
	1000	0	N/A
SYN548014	10	0	N/A
	100	0	N/A
	300	0	N/A
	1000	0	N/A
SYN548102	10	0	N/A
	100	0	N/A
	300	0	N/A
	1000	2	7, 8
SYN548117	10	0	N/A
	100	0	N/A
	300	0	N/A
	1000	2	7, 8
SYN548098	10	0	N/A
	100	0	N/A
	300	0	N/A
	1000	0	N/A
SYN548101	10	0	N/A
	100	1	2
	300	1	7
	1000	3	5, 6, 6
SYN548013	10	0	N/A
	100	0	N/A
	300	0	N/A
	1000	0	N/A

N/A = Not applicable.

4.1.2 Clinical observations

Group summaries of the clinical abnormalities are presented in Table 3. All individual animal clinical observations are presented in Appendix 3.

4.1.2.1 SYN547407

One rat (dosed at 1000 mg/kg) was observed with a rough coat on Days 5 and 6.

4.1.2.2 SYN546308

Rats administered 10 to 1000 mg/kg SYN546308 were observed with labored respiration, tremors, opaque eyes, red nasal discharge, seizure-like activity, rough coat, and/or thin appearance.

Target Dosage (mg/kg)	Observation	Number of Animals Affected	First Day of Observation	Last Day of Observation	Total Number of Observations
10	Respiration Abnormality/ Labored	1	2	2	1
	Tremors	1	2	2	1
100	Eye Abnormality/ Both-Opacity	1	2	2	1
	Nasal Discharge/Both-Red	1	2	2	1
	Seizure	1	2	2	1
300	Seizure	1	2	2	1
1000	Eye Abnormality/ Both-Opacity	1	2	2	1
	Rough Coat	3	5	8	11
	Seizure	1	2	2	1
	Thin	1	8	8	1

4.1.2.3 SYN548097

Rats administered 1000 mg/kg SYN548097 were observed with a rough coat and/or seizure-like activity.

Target Dosage (mg/kg)	Observation	Number of Animals Affected	First Day of Observation	Last Day of Observation	Total Number of Observations
1000	Rough Coat	2	5	8	7
	Seizure	1	2	2	1

4.1.2.4 SYN548012

No clinical observations were noted for the rats administered SYN548012.

4.1.2.5 SYN548014

No clinical observations were noted for the rats administered SYN548014.

4.1.2.6 SYN548102

Rats administered 1000 mg/kg SYN548102 were observed with red eye discharge, hunched posture, lethargy, red nasal discharge, rough coat, thin appearance, and/or a wet urogenital area.

Target Dosage (mg/kg)	Observation	Number of Animals Affected	First Day of Observation	Last Day of Observation	Total Number of Observations
1000	Eye Discharge/ Both-Red	1	7	8	2
	Hunched	1	8	8	1
	Lethargy	1	8	8	1
	Nasal Discharge/Both-Red	1	7	8	2
	Rough Coat	2	7	8	3
	Thin	1	7	8	2
	Wet Urogenital Area	1	8	8	1

4.1.2.7 SYN548117

Rats administered 100 to 1000 mg/kg SYN548117 were observed with seizure-like activity, thin appearance, pale skin, excess salivation, hyperactivity, hind limb weakness, labored respiration, rapid respiration, and/or rough coat.

Target Dosage (mg/kg)	Observation	Number of Animals Affected	First Day of Observation	Last Day of Observation	Total Number of Observations
100	Seizure	1	2	2	1
300	Seizure	2	2	6	2
	Thin	1	8	8	1
1000	Discolored Skin/Pale	1	5	5	1
	Excess Salivation	1	8	8	1
	Hyperactivity	1	7	7	1
	Limb Weakness/Both Hind limbs/Legs	1	4	5	3
	Respiration Abnormality/ Labored	1	5	5	1
	Respiration Abnormality/ Rapid	1	4	4	1
	Rough Coat	5	4	8	20
	Seizure	3	4	8	5
	Thin	1	8	8	1

4.1.2.8 SYN548098

Rats administered 10 or 1000 mg/kg SYN548098 were observed with an abrasion on the head/neck area and/or rough coat. The abrasion is not attributed to the administration of the test substance.

Target Dosage (mg/kg)	Observation	Number of Animals Affected	First Day of Observation	Last Day of Observation	Total Number of Observations
10	Skin Abrasion/Head/ Neck	1	8	8	1
1000	Rough Coat	2	4	7	7

4.1.2.9 SYN548101

Rats administered 300 or 1000 mg/kg SYN548101 were observed with pale skin, rough coat, excess salivation, hunched posture, hind limb weakness, lateral recumbency, seizure-like activity, and/or thin appearance.

Target Dosage (mg/kg)	Observation	Number of Animals Affected	First Day of Observation	Last Day of Observation	Total Number of Observations
300	Discolored Skin/Pale	1	4	4	1
	Rough Coat	2	4	7	7
1000	Discolored Skin/Pale	3	5	6	5
	Excess Salivation	3	5	6	4
	Hunched	1	8	8	1
	Limb Weakness/ Both Hind limbs/Legs	1	5	5	1
	Recumbent/Lateral	1	6	6	2
	Rough Coat	4	4	8	10
	Seizure	3	5	6	8
	Thin	1	8	8	1

4.1.2.10 SYN548013

No clinical observations were noted for the rats administered SYN548013.

4.1.3 Body weight

Group mean absolute body weights and group mean body weight change per day is presented in Tables 1 and 2, respectively, and Figures 1 through 10. The individual animal body weight and individual animal body weight change per day is presented in Appendix 3.

The following table summarizes the group mean body weights for the rats administered SYN547407, SYN546308, SYN548097, SYN548012, and SYN548014 and percent body weight difference when compared to the vehicle group:

Test Substance	Target Dosage	Day -3 Group Mean Body Weight (g)	Day -3 % Difference from Vehicle Control	Day 8 Group Mean Body Weight (g)	Day 8 % Difference from Vehicle Control
Vehicle (Group 41)	N/A	145.390	N/A	161.862	N/A
SYN547407	10	145.134	-0.18	168.600	4.16
	100	144.982	-0.28	172.092	6.32
	300	144.458	-0.64	165.612	2.32
	1000	144.236	-0.79	149.058	-7.91
SYN546308	10	145.356	-0.02	169.498	4.72
	100	143.826	-1.08	170.295	5.21
	300	145.256	-0.09	174.074	7.54
	1000	145.544	0.11	150.040	-7.30
SYN548097	10	145.390	0.00	171.743	6.10
	100	145.046	-0.24	172.588	6.63
	300	144.924	-0.32	168.928	4.37
	1000	146.072	0.47	159.854	-1.24
SYN548012	10	145.534	0.10	171.902	6.20
	100	144.274	-0.77	169.600	4.78
	300	144.392	-0.69	172.584	6.62
	1000	144.400	-0.68	171.936	6.22
SYN548014	10	144.546	-0.58	170.500	5.34
	100	143.750	-1.13	171.744	6.11
	300	145.386	0.00	171.580	6.00
	1000	143.754	-1.13	166.536	2.89

N/A = Not applicable; this is the comparison group.

On Day 8, the group mean body weight of the rats administered 10, 100, and 300 mg/kg SYN547407 were 6.7, 10.2, and 3.8 g more, respectively, whereas the rats administered 1000 mg/kg were 12.8 g less when compared to the rats administered vehicle only.

On Day 8, the group mean body weight of the rats administered 10, 100, and 300 mg/kg SYN546308 were 7.6, 8.4, and 12.2 g more, respectively, whereas the rats administered 1000 mg/kg were 11.8 g less when compared to the rats administered vehicle only.

On Day 8, the group mean body weight of the rats administered 10, 100, and 300 mg/kg SYN548097 were 9.9, 10.7, and 7.1 g more, respectively, whereas the rats administered 1000 mg/kg were 2.0 g less when compared to the rats administered vehicle only.

On Day 8, the group mean body weight of the rats administered 10, 100, 300, and 1000 mg/kg SYN548012 were 10.0, 7.7, 10.7, and 10.1 g more, respectively, when compared to the rats administered vehicle only.

On Day 8, the group mean body weight of the rats administered 10, 100, 300, and 1000 mg/kg SYN548014 were 8.6, 9.9, 9.7, and 4.7 g more, respectively, when compared to the rats administered vehicle only.

Though without statistical significance, the administration of 1000 mg/kg SYN547407, SYN546308, and SYN548097 caused a decrease in the group mean body weight when compared to the rats administered vehicle. Administration of SYN548012 and SYN548014 had no effect on the group mean body weight at any dosage.

The following table summarizes the group mean body weights for the rats administered SYN548102, SYN548117, SYN548098, SYN548101, and SYN548013 and percent body weight difference when compared to the vehicle group:

Test Substance	Target Dosage	Day -4 Group Mean Body Weight (g)	Day -4 % Difference from Vehicle Control	Day 8 Group Mean Body Weight (g)	Day 8 % Difference from Vehicle Control
Vehicle (Group 42)	N/A	144.236	N/A	174.640	N/A
SYN548102	10	144.996	0.53	174.718	0.04
	100	144.582	0.24	167.502	-4.09
	300	144.522	0.20	173.230	-0.81
	1000	145.178	0.65	**138.007***	-20.98
SYN548117	10	145.416	0.82	176.746	1.21
	100	146.148	1.33	175.868	0.70
	300	145.730	1.04	161.718	-7.40
	1000	143.588	-0.45	**139.748***	-19.98
SYN548098	10	144.890	0.45	175.078	0.25
	100	145.342	0.77	173.074	-0.90
	300	145.500	0.88	170.866	-2.16
	1000	144.238	0.00	166.286	-4.78
SYN548101	10	145.832	1.11	172.730	-1.09
	100	144.700	0.32	168.683	-3.41
	300	145.848	1.12	161.313	-7.63
	1000	145.670	0.99	**140.550***	-19.52
SYN548013	10	145.106	0.60	170.682	-2.27
	100	144.712	0.33	172.568	-1.19
	300	146.024	1.24	175.434	0.45
	1000	143.656	-0.40	171.660	-1.71

N/A = Not applicable.
***** Denotes statistical significance when compared to the vehicle group.

On Day 8, the group mean body weight of the rats administered 10 mg/kg SYN548102 was 0.1 grams more, whereas the rats administered 100, 300, and 1000 mg/kg were 7.1, 1.4, and 36.6 g less when compared to the rats administered vehicle only.

On Day 8, the group mean body weight of the rats administered 10 and 100 mg/kg SYN548117 were 2.1 and 1.2 g more, respectively, whereas the rats administered 300 and 1000 mg/kg were 12.9 and 34.9 g less when compared to the rats administered vehicle only.

On Day 8, the group mean body weight of the rats administered 10 mg/kg SYN548098 was 0.4 grams more, whereas the rats administered 100, 300, and 1000 mg/kg were 1.6, 3.8, and 8.4 g less when compared to the rats administered vehicle only.

On Day 8, the group mean body weight of the rats administered 10, 100, 300, and 1000 mg/kg SYN548101 were 1.9, 6.0, 13.3, and 34.1 g less, respectively, when compared to the rats administered vehicle only.

On Day 8, the group mean body weight of the rats administered 300 mg/kg SYN548013 was 0.8 grams more, whereas the rats administered 10, 100, and 1000 mg/kg were 4.0, 2.1, and 3.0 g less when compared to the rats administered vehicle only.

The administration of 1000 mg/kg SYN548102, SYN548117, and SYN548101 caused a statistically significant decrease in the group mean body weight when compared to the rats administered vehicle. Administration SYN548098 and SYN548013 had no effect on the group mean body weight at any dosage.

There were no statistically significant changes in the group mean terminal body weights for all test substances, when compared to the respective control groups.

4.1.4 Triglyceride analysis

A detailed description of the triglyceride analysis results including individual animal results is provided in Appendix 5. Samples were collected at 6, 24, 72, and 168 hours following dose administration. Actual times were recorded.

The administration of 100 and 1000 mg/kg SYN548102 caused a statistically significant decrease in the serum triglycerides 6 hours following dose administration, when compared to the rats administered vehicle. The administration of 1000 mg/kg SYN546308 and SYN548097 caused as statistically significant decrease in the serum triglycerides 72 hours following dose administration, when compared to the rats administered vehicle. These decreases were considered test substance related. There were no statistically significant differences noted in the serum triglycerides in any group 24 hours and 168 hours following dose administration.

4.1.5 Bioanalytical analysis

A detailed description of the bioanalytical analysis results is provided in Appendix 4.

4.1.6 Toxicokinetics

Concentrations of SYN547407, SYN546308, SYN548097, SYN548012, SYN548014, SYN548102, SYN548117, SYN548098, SYN548101, and SYN548013 were determined in blood samples taken from animals at 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, and 168 hours following dose administration. The blood concentrations and concentration time-profiles are presented in Figures 11 through 30.

The kinetic parameters that were evaluated for the test substances were observed C_{max}, C_{max}/dose, observed T_{max}, and AUC_{last}, AUC_{last}/dose, AUC_{∞}, and AUC_{∞}/dose. Individual animal toxicokinetic parameter data are presented in Appendix 4.

4.1.6.1 SYN547407

SYN547407 was detectable in blood following a single oral administration for up to 120 (10 mg/kg) to 168 hours (100, 300, and 1000 mg/kg) following dose administration. The derived TK parameters are summarized in the following table. Due to the lack of a well-defined elimination phase, AUC_{∞} was not reported for the 300 and 1000 mg/kg groups.

Target Time (hr)	Blood Concentration (ng/mL) ± SE			
	10 mg/kg	100 mg/kg	300 mg/kg	1000 mg/kg
0.5	65.1	407	701	622
1	331	1110	1150	1270
2	359	1020	2950	3310
4	477	3710	3780	4360
6	383 ± 31	2960 ± 310	3560 ± 410	3950 ± 350
12	209	3930	4180	5870
24	118 ± 17	2630 ± 410	4800 ± 980	5140 ± 370
36	69.2	2240	5350	5740
48	21.4	1330	1020	5300
72	9.91 ± 4.59	629 ± 135	3720 ± 950	6680 ± 570
96	BLOQ<2.02	33.3	3690	6290
120	2.80	57.4	3700	5340
144	BLOQ<2.02	36.1	928	2030
168	BLOQ<2.02	16.8 ± 6.1	386 ± 127	3670 ± 1310
C_{max} (ng/mL)	477	3930	5350	6680
C_{max}/Dose ((ng/mL)/(mg/kg))	47.7	39.3	17.8	6.68
T_{max} (hr)	4.00	12.0	36.0	72.0
AUC_{last} (hr*ng/mL)	8240	158,000	499,000	842,000
AUC_{last}/Dose ((hr*ng/mL)/(mg/kg))	824	1580	1660	842
AUC_{∞} (hr*ng/mL)	8340	159,000	NR	NR
AUC_{∞}/Dose ((hr*ng/mL)/(mg/kg))	834	1590	NR	NR

NA = Not applicable.
NR = Not reported.

4.1.6.2 SYN546308

SYN546308 was detectable in blood following a single oral administration for up 72 (10 mg/kg) to 168 hours (100, 300, and 1000 mg/kg) following dose administration. The derived TK parameters are summarized in the following table. Due to the lack of a well-defined elimination phase, AUC_{∞} was not reported for the 300 and 1000 mg/kg groups.

Target Time (hr)	Blood Concentration (ng/mL) ± SE			
	10 mg/kg	100 mg/kg	300 mg/kg	1000 mg/kg
0.5	77.5[a]	450	336	396
1	138	623	944	1610
2	NA	1600	2280	1720
4	537	2530	2370	2930
6	500 ± 88	2380 ± 290	2240 ± 320	2920 ± 360
12	494	3270	2970	4770
24	197 ± 38	2540 ± 290	2690 ± 500	4360 ± 700
36	138	1740	1670	2750
48	10.6	991	582	5810
72	8.68 ± 0.33	634 ± 96	2290 ± 750	4710 ± 720
96	BLOQ<2.06	183	2110	4820
120	BLOQ<2.06	57.9	613	4950
144	BLOQ<2.06	37.6	1580	76.0
168	BLOQ<2.06	5.76 ± NA	158 ± 112	3570 ± 1250
C_{max} (ng/mL)	537	3270	2970	5810
C_{max}/Dose ((ng/mL)/(mg/kg))	53.7	32.7	9.90	5.81
T_{max} (hr)	4.00	12.0	12.0	48.0
AUC_{last} (hr*ng/mL)	12,400	138,000	268,000	647,000
AUC_{last}/Dose ((hr*ng/mL)/(mg/kg))	1240	1380	893	647
AUC_{∞} (hr*ng/mL)	12,500	138,000	NR	NR
AUC_{∞}/Dose ((hr*ng/mL)/(mg/kg))	1250	1380	NR	NR

NA = Not applicable.
NR = Not reported.
Actual time point = 0.617 hr.

4.1.6.3 SYN548097

SYN548097 was detectable in blood following a single oral administration for up to 96 (10 mg/kg) to 168 hours (100, 300, and 1000 mg/kg) following dose administration. The derived TK parameters are summarized in the following table. Due to the lack of a well-defined elimination phase, AUC_{∞} was not reported for the 300 and 1000 mg/kg groups.

Target Time (hr)	Blood Concentration (ng/mL) ± SE			
	10 mg/kg	100 mg/kg	300 mg/kg	1000 mg/kg
0.5	52.2	227	231	587
1	103	893	753	1350
2	134	1490	580	2480
4	630	2840	4100	BLOQ<2.18
6	517 ± 58	3430 ± 130	4460 ± 540	4310 ± 610
12	403	3840	4510	6720
24	161 ± 18	2070 ± 150	3510 ± 220	6280 ± 290
36	46.0	1150	4310	NA
48	32.2	251	3510	9350
72	6.85 ± 0.99	121 ± 47	2370 ± 1360	8240 ± 2070
96	2.27	13.9	6480	4840
120	BLOQ<2.18	8.34	389	BLOQ<2.18
144	BLOQ<2.18	BLOQ<2.18	2.22	5200
168	BLOQ<2.18	2.86	299 ± 187	840 ± 383
C_{max} (ng/mL)	630	3840	6480	9350
C_{max}/Dose ((ng/mL)/(mg/kg))	63.0	38.4	21.6	9.35
T_{max} (hr)	4.00	12.0	96.0	48.0
AUC_{last} (hr*ng/mL)	10,500	104,000	451,000	996,000
AUC_{last}/Dose ((hr*ng/mL)/(mg/kg))	1050	1040	1500	996
AUC_{∞} (hr*ng/mL)	10,600	104,000	NR	NR
AUC_{∞}/Dose ((hr*ng/mL)/(mg/kg))	1060	1040	NR	NR

NA = Not applicable.
NR = Not reported.

4.1.6.4 SYN548012

SYN548012 was detectable in blood following a single oral administration for up to 168 hours following dose administration. The derived TK parameters are summarized in the following table.

Target Time (hr)	Blood Concentration (ng/mL) ± SE			
	10 mg/kg	**100 mg/kg**	**300 mg/kg**	**1000 mg/kg**
0.5	11.9	85.9	110	248
1	88.2	313	195	518
2	188	578	475	700
4	308	1100	1400	1800
6	306 ± 14	1330 ± 40	1690 ± 110	1990 ± 100
12	159	1140	1130	2850
24	117 ± 10	705 ± 112	1800 ± 480	1560 ± 250
36	110	444	642	838
48	57.9	260	2020	1060
72	25.8 ± 8.5	281 ± 54	823 ± 184	597 ± 85
96	12.5	180	350	344
120	2.55	31.3	502	233
144	BLOQ<1.85	67.3	142	286
168	5.44	23.6 ± 3.5	109 ± 28	91.2 ± 15.7
C_{max} (ng/mL)	308	1330	2020	2850
C_{max}/Dose ((ng/mL)/(mg/kg))	30.8	13.3	6.73	2.85
T_{max} (hr)	4.00	6.00	48.0	12.0
AUC_{last} (hr*ng/mL)	8530	51,100	131,000	123,000
AUC_{last}/Dose ((hr*ng/mL)/(mg/kg))	853	511	437	123
AUC_{∞} (hr*ng/mL)	8610	52,100	137,000	130,000
AUC_{∞}/Dose ((hr*ng/mL)/(mg/kg))	861	521	457	130

NA = Not applicable.
NR = Not reported.

4.1.6.5 SYN548014

SYN548014 was detectable in blood following a single oral administration for up to 72 (10 mg/kg) to 168 hours (300 and 1000 mg/kg) following dose administration. The derived TK parameters are summarized in the following table. The AUC values increased proportionally as the dose was increased from 10 to 100 mg/kg, but increased in a less than dose-proportional manner at the 300 and 1000 mg/kg doses. There was apparent saturation of elimination at the 1000 mg/kg dose. The 1000 mg/kg group had an aberrantly low concentration at 96 hours. The analysis was performed with and without this time point with the resulting PK parameters from the profile without the 96 hour time point presented in this report.

Target Time (hr)	Blood Concentration (ng/mL) ± SE			
	10 mg/kg	100 mg/kg	300 mg/kg	1000 mg/kg[a]
0.5	27.9	307	541	705
1	246	1650	1470	1160
2	458	2140	2000	3120
4	633	3770	2600	3200
6	476 ± 102	3590 ± 160	4830 ± 420	5020 ± 640
12	393	2350	4780	6030
24	112 ± 20	2150 ± 520	4690 ± 660	5220 ± 850
36	24.9	1090	2150	6880
48	30.3	509	1810	6080
72	6.52 ± 1.14	178 ± 63	684 ± 216	4650 ± 1150
96	BLOQ<2.14	21.3	180	69.4
120	BLOQ<2.14	25.6	8.13	638
144	BLOQ<2.14	7.31	16.1	595
168	BLOQ<2.14	BLOQ<2.14	3.13 ± NA	77.7 ± 31.8
C_{max} (ng/mL)	633	3770	4830	6880
C_{max}/Dose ((ng/mL)/(mg/kg))	63.3	37.7	16.1	6.88
T_{max} (hr)	4.00	4.00	6.00	36.0
AUC_{last} (hr*ng/mL)	9860	101,000	208,000	547,000
AUC_{last}/Dose ((hr*ng/mL)/(mg/kg))	986	1010	693	547
AUC_{∞} (hr*ng/mL)	9940	101,000	208,000	551,000
AUC_{∞}/Dose ((hr*ng/mL)/(mg/kg))	994	1010	693	551

NA = Not applicable.
NR = Not reported.
Kinetic parameters determined without the 96 hour time point.

4.1.6.6 SYN548102

SYN548102 was detectable in blood following a single oral for up to 168 hours following dose administration. The derived TK parameters are summarized in the following table. Due to the lack of a well-defined elimination phase, AUC_{∞} was not reported for the 300 and 1000 mg/kg groups.

Target Time (hr)	Blood Concentration (ng/mL) ± SE			
	10 mg/kg	100 mg/kg	300 mg/kg	1000 mg/kg
0.5	157	101	274	445
1	318	826	1050	1360
2	432	1840	1210	1330
4	798	2500	1340	1270
6	683 ± 125	2960 ± 330	1820 ± 100	2170 ± 290
12	168	3840	2180	3500
24	205 ± 43	2670 ± 410	3010 ± 200	3030 ± 250
36	133	3690	3970	5720
48	94.5	1520	1310	4750
72	33.7 ± 9.9	922 ± 158	2360 ± 410	3890 ± 430
96	27.0	266	2300	6690
120	10.2	64.8	850	3720
144	BLOQ<2.02	126	499	3280
168	2.11	31.0 ± 9.4	575 ± 262	2070 ± NA
C_{max} (ng/mL)	798	3840	3970	6690
C_{max}/Dose ((ng/mL)/(mg/kg))	79.8	38.4	13.2	6.69
T_{max} (hr)	4.00	12.0	36.0	96.0
AUC_{last} (hr*ng/mL)	14,400	192,000	291,000	683,000
AUC_{last}/Dose ((hr*ng/mL)/(mg/kg))	1440	1920	970	683
AUC_{∞} (hr*ng/mL)	14,500	193,000	NR	NR
AUC_{∞}/Dose ((hr*ng/mL)/(mg/kg))	1450	1930	NR	NR

NA = Not applicable.
NR = Not reported.

4.1.6.7 SYN548117

SYN548117 was detectable in blood following a single oral for up to 168 hours following dose administration. The derived TK parameters are summarized in the following table. Due to the lack of a well-defined elimination phase, AUC_{∞} was not reported for the 300 and 1000 mg/kg groups.

Target Time (hr)	Blood Concentration (ng/mL) ± SE			
	10 mg/kg	100 mg/kg	300 mg/kg	1000 mg/kg
0.5	16.8[a]	272[b]	494	689
1	167	430	468	532
2	440	1860	1550	2620
4	666	2050	2020	1580
6	647 ± 66	1880 ± 170	2130 ± 160	2640 ± 250
12	645	2790	3280	3490
24	321 ± 10	2510 ± 60	3090 ± 90	3100 ± 240
36	331	3040	2760[c]	3840
48	152	4380	4790	1380
72	66.6 ± 9.8	1640 ± 340	3100 ± 690	3610 ± 140
96	46.9	1670	1410	3870
120	BLOQ<2.02	269	3080	3890
144	2.31	363	265	6730
168	4.05 ± 0.92	151 ± 39	802 ± 318	6270 ± 200
C_{max} (ng/mL)	666	4380	4790	6730
C_{max}/Dose ((ng/mL)/(mg/kg))	66.6	43.8	16.0	6.73
T_{max} (hr)	4.00	48.0	48.0	144
AUC_{last} (hr*ng/mL)	24,500	282,000	399,000	668,000
AUC_{last}/Dose ((hr*ng/mL)/(mg/kg))	2450	2820	1330	668
AUC_{∞} (hr*ng/mL)	24,600	288,000	NR	NR
AUC_{∞}/Dose ((hr*ng/mL)/(mg/kg))	2460	2880	NR	NR

NA = Not applicable.
NR = Not reported.
a. Actual time point = 0.533 hr.
b. Actual time point = 0.533 hr.
c. Actual time point = 36.3 hr.

4.1.6.8 SYN548098

SYN548098 was detectable in blood following a single oral for up to 168 hours following dose administration. The derived TK parameters are summarized in the following table. AUC values increased in a less than dose-proportional manner following the 100, 300, and 1000 mg/kg dosages. This suggests a saturation of elimination beginning at a dose of 100 mg/kg. Due to the lack of a well-defined elimination phase, AUC_{∞} was not reported for the 300 and 1000 mg/kg groups. In addition, the 100 mg/kg group had an aberrantly high concentration at 96 hours. The analysis was performed with and without this time point with the resulting PK parameters from the profile without the 96 hour time point presented in this report.

Target Time (hr)	Blood Concentration (ng/mL) ± SE			
	10 mg/kg	100 mg/kg[a]	300 mg/kg	1000 mg/kg
0.5	25.2	9.05	106	479
1	76.7	222	516	1220
2	124	648	799	2410
4	302	882	1440	1820
6	365 ± 21	1130 ± 110	1850 ± 160	5020 ± 1480
12	272	969	1610	3770
24	208 ± 39	797 ± 55	1550 ± 330	4030 ± 290
36	171	514	3530	4070
48	67.9	460	574	2950
72	50.8 ± 9.4	734 ± 412	750 ± 141	3720 ± 570
96	24.0	1310	946	1010
120	22.1	135	230	4910
144	3.58	50.3	195	2880
168	5.83	75.7 ± 48.2	372 ± 102	2050 ± 680
C_{max} (ng/mL)	365	1130	3530	5020
C_{max}/Dose ((ng/mL)/(mg/kg))	36.5	11.3	11.8	5.02
T_{max} (hr)	6.00	6.00	36.0	6.00
AUC_{last} (hr*ng/mL)	13,000	73,600	153,000	538,000
AUC_{last}/Dose ((hr*ng/mL)/(mg/kg))	1300	736	510	538
AUC_{∞} (hr*ng/mL)	13,200	76,700	NR	NR
AUC_{∞}/Dose ((hr*ng/mL)/(mg/kg))	1320	767	NR	NR

NA = Not applicable.
NR = Not reported.
Kinetic parameters determined without the 96 hour time point.

4.1.6.9 SYN548101

SYN548101 was detectable in blood following a single oral for up to 72 (10 mg/kg) to 168 hours (100, 300, and 1000 mg/kg) following dose administration. The derived TK parameters are summarized in the following table. Due to the lack of a well-defined elimination phase, AUC_{∞} was not reported for the 300 and 1000 mg/kg groups.

Target Time (hr)	Blood Concentration (ng/mL) ± SE			
	10 mg/kg	100 mg/kg	300 mg/kg	1000 mg/kg
0.5	102	307	438	407
1	266	859	997	1780
2	221	1440	1630	1780
4	314	981	1620	2190
6	284 ± 63	1830 ± 160	1740 ± 120	1830 ± 100
12	54.8	1860	3290	3440
24	78.9 ± 16.6	2100 ± 160	2930 ± 190	2880 ± 170
36	74.5	1470	4850	3380
48	25.4	1840	3020	3810
72	5.60 ± 1.28	930 ± 137	3420 ± 400	3590 ± 220
96	BLOQ<1.83	800	2780	3830
120	BLOQ<1.83	313	5590	4390
144	BLOQ<1.83	33.9	3380	3090
168	BLOQ<1.83	41.0 ± 11.5	2000 ± 380	3570 ± 2050
C_{max} (ng/mL)	314	2100	5590	4390
C_{max}/Dose ((ng/mL)/(mg/kg))	31.4	21	18.6	4.39
T_{max} (hr)	4.00	24.0	120	120
AUC_{last} (hr*ng/mL)	5200	155,000	579,000	591,000
AUC_{last}/Dose ((hr*ng/mL)/(mg/kg))	520	1550	1930	591
AUC_{∞} (hr*ng/mL)	5280	156,000	NR	NR
AUC_{∞}/Dose ((hr*ng/mL)/(mg/kg))	528	1560	NR	NR

NA = Not applicable.
NR = Not reported.

4.1.6.10 SYN548013

SYN548013 was detectable in blood following a single oral administration for up 168 hours following dose administration. The derived TK parameters are summarized in the following table. Due to the lack of a well-defined elimination phase, AUC_{∞} was not reported for the 1000 mg/kg group.

Target Time (hr)	Blood Concentration (ng/mL) ± SE			
	10 mg/kg	100 mg/kg	300 mg/kg	1000 mg/kg
0.5	60.4	38.6[a]	313	466
1	275[b]	540	779	1500
2	497	2140	2250	2100
4	627	3140	2810	4400
6	691 ± 31	3100 ± 310	3850 ± 450	5080 ± 500
12	425	3020	3600	5490
24	302 ± 35	2040 ± 160	4180 ± 880	7170 ± 1050
36	214	1900	4100	6930
48	134	1600	2210	4180
72	48.0 ± 10.0	786 ± 94	2730 ± 620	4360 ± 630
96	37.7[c]	222	3440	2430
120	2.27	218	1430	1270
144	2.95	117	478	2570
168	5.76 ± 3.05	39.3 ± 8.6	481 ± 201	1040 ± 320
C_{max} (ng/mL)	691	3140	4180	7170
C_{max}/Dose ((ng/mL)/(mg/kg))	69.1	31.4	13.9	7.17
T_{max} (hr)	6.00	4.00	24.0	24.0
AUC_{last} (hr*ng/mL)	19,700	158,000	396,000	595,000
AUC_{last}/Dose ((hr*ng/mL)/(mg/kg))	1970	1580	1320	595
AUC_{∞} (hr*ng/mL)	19,700	160,000	409,000	NR
AUC_{∞}/Dose ((hr*ng/mL)/(mg/kg))	1970	1600	1360	NR

NA = Not applicable.
NR = Not reported.
a. Actual time point = 0.533 hr.
b. Actual time point = 1.13 hr.
c. Actual time point = 96.5 hr.

4.1.7 *Post mortem* investigations

The anatomical pathology results are presented in Appendix 6.

4.1.7.1 Organ weights

When compared to the respective control groups, a few statistically significant organ weight changes were noted:

- SYN548102 (1000 mg/kg): increased mean adrenal absolute gland weight
- SYN547407 (1000 mg/kg): increased mean adrenal gland-to-body weight ratio

- SYN546308 (1000 mg/kg): increased mean adrenal gland-to-body weight ratio.

The increased mean adrenal gland weights (absolute weight and adrenal gland-body weight ratio) were deemed attributable to cytoplasm vacuolation, predominantly to involve the zona fasiculata region.

4.1.7.2 Necropsy

Gross lesions were noted across test substance groups and are portrayed in the table below. These findings were interpreted to be spontaneous in occurrence and unrelated to test substance administration. A proximate cause for early death (or moribund sacrificed) rats 24, 29, 40, 44, 59, 118, 120, 136, 166, 174, 176, 177, and 179 was not ascertained in the limited tissues examined. Microscopic examination of the tissues from moribund sacrificed rats 119 and 137 did not reveal a proximate cause of moribundity.

Test Substance	Target Dosage (mg/kg)	Animal ID	Finding	Microscopic Correlate
SYN546308	100	29	Eye, bilateral, discoloration, opaque	Not examined
	1000	40	Eye, bilateral, discoloration, opaque	Not examined
SYN548102	1000	119	Brain, meninges, discoloration, dark	Peracute meningeal haemorrhage
SYN548117	1000	137	Brain, meninges, discoloration, dark	Peracute meningeal haemorrhage
			Liver, discoloration, dark	Congestion, acute
SYN548098	100	149	Liver, left, deformity (G1 = agenesis)	No microscopic correlate (lobe = missing)
			Liver, median, small, 0.5X	No microscopic correlate (lobe = normal)
			Liver, right posterior, enlarged, 2X	Congestion, acute
			Liver, right anterior, enlarged, 2X	Congestion, acute
			Liver, caudate, enlarged, 2X	Congestion, acute

4.1.7.3 Histopathology

The adrenal glands and/or duodenum/jejunum appeared as target tissues in five of the test substances administered at the 100 mg/kg target dosage. The results of each individual test substances are detailed below. In addition to these findings, a few additional microscopic findings were rendered in the summary and individual animal data tables within Appendix 6. These additional findings were deemed incidental and spontaneous changes of neither biologic nor toxicological significance.

SYN547407

Treatment-related changes, limited to the highest dose group (1000 mg/kg), were observed in the adrenal glands, duodenum, and jejunum. In the adrenal gland, fine (microvesicular) cytoplasm vacuolization which primarily involved cells within the zona fasiculata region was observed. Due to the subtlety of this microscopic finding, cytoplasm vacuolization was uniformly graded as "minimal". Cytoplasm vacuolization was also evident in the mucosal epithelium (enterocytes) of the duodenum and jejunum; primarily involving enterocytes lining the mid and distal portion of villi. Affected villi often appeared elongate; giving the mucosa a hypercellular appearance.

SYN546308

Treatment-related changes, limited to the highest dose group (1000 mg/kg), were observed in the adrenal glands, duodenum, and jejunum. In the adrenal gland, fine (microvesicular) cytoplasm vacuolization primarily involving cells within the zona fasiculata region was present. Cytoplasm vacuolization was also evident in the mucosal epithelium (enterocytes) of the duodenum and jejunum; primarily involving enterocytes lining the mid and distal portion of villi. Affected villi often appeared elongate; giving the mucosa a hypercellular appearance.

SYN548097

There were no findings associated with SYN548097 administration.

SYN548012

There were no findings associated with SYN548012 administration.

SYN548014

There were no findings associated with SYN548014 administration.

SYN548102

A treatment-related change, limited to the highest dose group (1000 mg/kg), was observed in the adrenal glands to consist of fine (microvesicular) cytoplasm vacuolization primarily involving cells within the zona fasiculata region.

SYN548117

Treatment-related changes, limited to the highest dose group (1000 mg/kg), were observed in the duodenum and jejunum to consist of cytoplasm vacuolization of mucosal epithelium (enterocytes); primarily involving enterocytes lining the mid and distal portion of villi. Affected villi often appeared elongate; giving the mucosa a hypercellular appearance.

SYN548098

There were no findings associated with SYN548098 administration.

SYN548101

A treatment-related change, limited to the highest dose group (1000 mg/kg), was observed in the adrenal glands to consist of fine (microvesicular) cytoplasm vacuolization primarily involving cells within the zona fasiculata region.

SYN548013

There were no findings associated with SYN548013 administration.

4.2 Discussion

4.2.1 SYN547407

All rats administered SYN547407 survived until the end of the study. Clinical observations were noted only in the 1000 mg/kg group (one rat was noted with a rough coat on Days 5 and 6; this observation resolved by Day 7). . The group mean body weights increased from Day 1 to Day 8 although the body weight gains of rats administered 1000 mg/kg were less when compared to control rats. No differences were noted in triglyceride values. Treatment-related histopathological changes were noted in the adrenal glands, duodenum, and jejunum. In addition, the mean adrenal to body weight ratio increased for rats administered 1000 mg/kg.

The whole blood concentration-time curves for SYN547407 showed clear absorption and elimination phases following administration of 10 and 100 mg/kg. Blood concentrations remained similar over time without a definitive terminal elimination phase illustrated by the 300 and 1000 mg/kg groups. The group mean Cmax when normalized for dose decreased as the dosage increased (ranging from 47.7 ng/mL (10 mg/kg) to 6.68 ng/mL (1000 mg/kg)). Group mean AUC values increased in a slightly greater than dose-proportional manner from 30 to 100 mg/kg, approximately dose-proportionally as the dose was increased from 100 to 300 mg/kg, but increased in a slightly less than dose-proportional manner after the 300 and 1000 mg/kg doses.

4.2.2 SYN546308

SYN546308 administration resulted in test substance-related changes in rats. Three rats (one each from the 10, 100, and 1000 mg/kg groups) died on Day 2 following the observation of seizure-like activity or tremors. One other 300 mg/kg rat was observed with seizure-like activity on Day 2; however, this rat survived until the end of the study. The group mean body weights increased from Day 1 to Day 8 although the body weight gains of rats administered 1000 mg/kg were less when compared to control rats. While the triglyceride values were not significantly changed 24 hours following dose administration, the triglyceride values were significantly decreased by 72 hours following administration of 1000 mg/kg. These triglyceride values were recovered by 168 hours. Treatment-related histopathological changes were noted in the adrenal glands, duodenum, and jejunum. In addition, the mean adrenal gland to body weight ratio increased for the rats administered 1000 mg/kg.

The whole blood concentration-time curves for SYN546308 showed clear absorption and elimination phases following administration of 10 and 100 mg/kg. Blood concentrations remained similar over time without a definitive terminal elimination phase illustrated by the 300 and 1000 mg/kg groups. The group mean C_{max} when normalized for dose decreased as the

dosage increased (ranging from 53.7 ng/mL (10 mg/kg) to 5.81 ng/mL (1000 mg/kg)). Group mean AUC values increased proportionally as the dose was increased from 10 to 100 mg/kg, but increased in a less than dose-proportional manner after the 300 and 1000 mg/kg doses.

4.2.3 SYN548097

SYN548097 administration resulted in test substance-related changes in rats. One rat administered 10 mg/kg died on Day 4 and one rat administered 1000 mg/kg died on Day 8. Another rat administered 1000 mg/kg was observed with seizure-like activity on Day 2; however, this rat survived until the end of the study. Body weights were not significantly affected. While the triglyceride values were not significantly changed 24 hours following dose administration, the triglyceride values were significantly decreased after 72 hours following dose administration of 1000 mg/kg. These triglyceride values were recovered by 168 hours. No treatment-related changes were noted in the adrenal glands, duodenum, and jejunum. No treatment-related changes were noted in any absolute organ weights or mean organ-to-body weight ratios.

The whole blood concentration-time curves for SYN548097 showed clear absorption and elimination phases following administration of 10 and 100 mg/kg. Blood concentrations remained similar over time without a definitive terminal elimination phase illustrated by the 300 and 1000 mg/kg groups. The group mean C_{max} when normalized for dose decreased as the dosage increased (ranging from 63.0 ng/mL (10 mg/kg) to 9.35 ng/mL (1000 mg/kg)). Group mean AUC values increased in an approximately dose-proportional manner as the dose was increased from 10 to 1000 mg/kg.

4.2.4 SYN548012

All rats administered SYN548012 survived until the end of the study. There were no clinical observations at any dose level. Bodyweights were unaffected by treatment. Triglyceride values were unaffected by treatment during the course of the study. There were no noted gross or histopathological findings. No treatment-related changes were noted in any absolute organ weights or mean organ-to-body weight ratios.

The whole blood concentration-time curves for SYN548012 showed clear absorption and elimination phases following administration of 10, 100 and 1000 mg/kg. Blood concentrations remained similar over time illustrated by the 300 mg/kg group. The group mean Cmax when normalized for dose decreased as the dosage increased (ranging from 30.8 ng/mL (10 mg/kg) to 2.85 ng/mL (1000 mg/kg)). Group mean AUC values increased in a less than dose-proportional manner as the dose was increased from 10 to 1000 mg/kg.

4.2.5 SYN548014

All rats administered SYN548014 survived until the end of the study. There were no clinical observations at any dose level. Bodyweights were unaffected by treatment. Triglyceride values were unaffected by treatment during the course of the study. There were no noted gross or histopathological findings. No treatment-related changes were noted in any absolute organ weights or mean organ-to-body weight ratios.

The whole blood concentration-time curves for SYN548014 showed clear absorption and elimination phases following administration of 10, 100, and 300 mg/kg. Blood concentrations remained similar over time without a definitive terminal elimination phase illustrated by the 1000 mg/kg group. The group mean C_{max} when normalized for dose decreased as the dosage increased (ranging from 63.3 ng/mL (10 mg/kg) to 6.88 ng/mL (1000 mg/kg)). Group mean AUC values increased proportionally as the dose was increased from 10 to 100 mg/kg, but increased in a less than dose-proportional manner after the 300 and 1000 mg/kg doses.

4.2.6 SYN548102

SYN548102 administered at 1000 mg/kg resulted in test substance-related changes. One rat given 1000 mg/kg was noted with a rough coat and died on Day 7. In addition, one rat administered 1000 mg/kg was observed with red discharge from both eyes and nostrils, hunched posture, lethargy, rough coat, thin appearance, and a wet urogenital area on Days 7 and 8. This rat was determined to be moribund on Day 8 and humanely terminated. Body weights were significantly decreased in the rats administered 1000 mg/kg when compared to controls. Triglyceride values were significantly, but transiently decreased 6 hours following administration of 100 and 1000 mg/kg. These values were not significantly decreased 24 hours following dose administration. Treatment-related histological changes were noted in the adrenal glands. In addition, the mean adrenal weight was increased for rats administered 1000 mg/kg.

The whole blood concentration-time curves for SYN548102 showed clear absorption and elimination phases following dose administration of 10 and 100 mg/kg. Blood concentrations remained similar over time without a definitive terminal elimination phase illustrated by the 300 and 1000 mg/kg groups. The group mean C_{max} when normalized for dose decreased as the dosage increased (ranging from 79.8 ng/mL (10 mg/kg) to 6.69 ng/mL (1000 mg/kg)). Group mean AUC values increased proportionally as the dose was increased from 10 to 100 mg/kg, but increased in a less than dose-proportional manner after the 300 and 1000 mg/kg doses.

4.2.7 SYN548117

SYN548117 administration resulted in test substance-related changes in rats. Seizure-like activity was observed in a number of rats administered 300 or 1000 mg/kg, most of which survived until the end of the study; one 1000 mg/kg rat died on Day 7 and another on Day 8. In addition, other observations such as, pale skin, excess salivation, hyperactivity, hind limb weakness, labored breathing, rapid respiration, thin appearance, and a rough coat were noted. Body weights were significantly decreased in the rats administered1000 mg/kg when compared to controls. No differences were noted in triglyceride values. Treatment-related histopathological changes were noted in the duodenum and jejunum. No treatment-related changes were noted in any absolute organ weights or mean organ-to-body weight ratios.

The whole blood concentration-time curves for SYN548117 showed clear absorption and elimination phases following administration of 10 mg/kg. Blood concentrations remained similar over time for the 100 mg/kg group. There was no definitive terminal elimination phase for the 300 and 1000 mg/kg groups. The group mean C_{max} when normalized for dose decreased as the dosage increased (ranging from 66.6 ng/mL (10 mg/kg) to 6.73 ng/mL (1000 mg/kg)). Group mean AUC values increased proportionally as the dose was increased from 10 to 100

mg/kg, but increased in a less than dose-proportional manner after the 300 and 1000 mg/kg doses.

4.2.8 SYN548098

All rats administered SYN548098 survived until the end of the study. Two rats administered 1000 mg/kg were observed with a rough coat during the course of the study. Bodyweights were unaffected by treatment. Triglyceride values were unaffected by treatment during the course of the study. There were no noted gross or histopathological findings. No treatment-related changes were noted in any absolute organ weights or mean organ-to-body weight ratios.

The whole blood concentration-time curves for SYN548098 showed clear absorption and elimination phases following administration 10 and 100 mg/kg. Blood concentrations remained similar over time without a definitive terminal elimination phase illustrated by the 300 and 1000 mg/kg groups. The group mean C_{max} when normalized for dose decreased as the dosage increased (ranging from 36.5 ng/mL (10 mg/kg) to 5.02 ng/mL (1000 mg/kg)). Group mean AUC values increased in a less than dose-proportional manner as the dose was increased. Due to the lack of a well-defined elimination phase, AUC_{∞} was not reported for the 300 and 1000 mg/kg groups.

4.2.9 SYN548101

SYN548101 administration resulted in test substance-related clinical signs in rats. One rat administered 100 mg/kg and one rat administered 300 mg/kg died on Days 2 and 7, respectively. Three rats administered 1000 mg/kg were observed with seizure-like activity and died on Days 5 or 6. In addition, other observations such as, pale skin, excess salivation, hunched posture, lateral recumbancy, hind limb weakness, thin appearance, and a rough coat were noted. Body weights were significantly decreased in the rats administered 1000 mg/kg when compared to controls. No differences were noted in the triglyceride values. Treatment-related histopathological changes were noted in the adrenal glands. No treatment-related changes were noted in any absolute organ weights or mean organ-to-body weight ratios.

The whole blood concentration-time curves for SYN548101 showed clear absorption and elimination phases following administration of 10 mg/kg. Blood concentrations remained similar over time for the 100 mg/kg group and additionally there was no definitive terminal elimination phase for the 300 and 1000 mg/kg groups. The group mean C_{max} when normalized for dose decreased as the dosage increased (ranging from 31.4 ng/mL (10 mg/kg) to 4.39 ng/mL (1000 mg/kg)). Group mean AUC values increased in a slightly greater than dose-proportional manner as the dose was increased from 10 to 300 mg/kg, but in a less than dose-proportional manner from 300 to 1000 mg/kg.

4.2.10 SYN548013

All rats administered SYN548013 survived until the end of the study. Bodyweights were unaffected by treatment. Triglyceride values were unaffected by treatment during the course of the study. There were no noted gross or histopathological findings. No treatment-related changes were noted in any absolute organ weights or mean organ-to-body weight ratios.

The whole blood concentration-time curves for SYN548013 showed clear absorption and elimination phases following administration of 10 and 100 mg/kg. Blood concentrations remained similar over time for the 300 mg/kg group and additionally there was no definitive terminal elimination phase for the 1000 mg/kg group. The group mean C_{max} when normalized for dose decreased as the dosage increased (ranging from 69.1 ng/mL (10 mg/kg) to 7.17 ng/mL (1000 mg/kg)). Group mean AUC values increased in a less than dose-proportional manner after the 100, 300, and 1000 mg/kg doses.

5.0 CONCLUSIONS

All animals dosed with SYN547407, SYN548012, SYN548014, SYN548098 and SYN548013 survived the study following single gavage dosing at 1000 mg/kg. SYN548012, SYN548014 and SYN548013 were all well tolerated at 1000 mg/kg. There were clinical signs observed in animals dosed with 1000 mg/kg SYN547407 and SYN548098. Both SYN547407 and SYN548014 showed evidence of dose-proportionality up to 100 mg/kg, and evidence of sub proportional kinetics between the 100 and 1000 mg/kg dose groups. SYN548012, SYN548098, and SYN548013 showed evidence of sub proportionality across the dose range.

Acute toxicity was evident in animals dosed with SYN546308, SYN548097, SYN548102, SYN548117, and SYN548101 including adverse clinical signs and death in some dose groups. SYN546308, SYN548102 and SYN548117 showed evidence of dose-proportionality up to 100 mg/kg, and evidence of sub proportional kinetics between the 100 and 1000 mg/kg dose groups. Both SYN548097 and SYN548101 show evidence of dose-proportionality across the dose range.

SYN546308 was not tolerated following single dose administration with three total deaths across dose groups (one at 10 mg/kg, 30 mg/kg and 1000 mg/kg respectively). There were treatment related-transient lowering of triglycerides and histopathological changes in the adrenal glands, duodenum, and jejunum. Furthermore, clinical signs suggestive of neurological effects were observed across all dose groups.

SYN548097 was not tolerated following single dose administration with two deaths across the dose groups (one at 10 mg/kg and one at 1000 mg/kg). Furthermore, clinical signs suggestive of neurological effects were observed in the 1000 mg/kg dose group. There was a treatment-related transient lowering of triglycerides. Furthermore, clinical signs suggestive of neurological effects were observed at 1000 mg/kg.

SYN548102 was not tolerated following single dose administration with deaths and adverse clinical signs observed in the 1000 mg/kg dose group. Body weights were significantly decreased in the rats administered 1000 mg/kg. Triglyceride values were significantly, but transiently decreased following administration of 100 and 1000 mg/kg. By 24 hours, the triglyceride values had increased to a level comparable to the control group. Treatment-related histological changes were noted in the adrenal glands.

SYN548117 was not tolerated following single dose administration with deaths at 1000 mg/kg and adverse clinical signs at both 300 and 1000 mg/kg. Body weights were significantly decreased in the rats administered 1000 mg/kg when compared to controls. Treatment-related

histopathological changes were noted in the duodenum and jejunum. Furthermore, clinical signs suggestive of neurological effects were observed at both 300 mg/kg and 1000 mg/kg.

SYN548101 was not tolerated following single dose administration with deaths at 100, 300, and 1000 mg/kg. Body weights were significantly decreased in the rats administered 1000 mg/kg when compared to controls. Treatment-related histopathological changes were noted in the adrenal glands. Furthermore, clinical signs suggestive of neurological effects were observed at 1000 mg/kg.

TABLES SECTION

TABLE 1 Group Mean Body Weight (g) Data – Female

Body Weight (g)

Sex: Female		Day(s) Relative to Start Date			
		-3	2	4	8
SYN547407	Mean	145.134	-	-	168.600
10 mg/kg	SD	6.148	-	-	6.630
	N	5	-	-	5
SYN547407	Mean	144.982	-	-	172.092
100 mg/kg	SD	5.543	-	-	8.806
	N	5	-	-	5
SYN547407	Mean	144.458	-	-	165.612
300 mg/kg	SD	6.548	-	-	4.429
	N	5	-	-	5
SYN547407	Mean	144.236	-	-	149.058
1000 mg/kg	SD	7.945	-	-	14.058
	N	5	-	-	5
SYN546308	Mean	145.356	-	-	169.498
10 mg/kg	SD	6.989	-	-	4.513
	N	5	-	-	4

Statistical Test: Generalised Anova/Ancova Test Transformation: Auto

TABLE 1 Group Mean Body Weight (g) Data – Female (Continued)

Body Weight (g)

Sex: Female		Day(s) Relative to Start Date			
		-3	2	4	8
SYN546308	Mean	143.826	140.350	-	170.295
100 mg/kg	SD	7.744	-	-	11.587
	N	5	1	-	4
SYN546308	Mean	145.256	-	-	174.074
300 mg/kg	SD	8.286	-	-	12.100
	N	5	-	-	5
SYN546308	Mean	145.544	131.140	-	150.040
1000 mg/kg	SD	7.190	-	-	25.933
	N	5	1	-	4
SYN548097	Mean	145.390	-	151.650	171.743
10 mg/kg	SD	5.902	-	-	9.711
	N	5	-	1	4
SYN548097	Mean	145.046	-	-	172.588
100 mg/kg	SD	7.570	-	-	8.160
	N	5	-	-	5

Statistical Test: Generalised Anova/Ancova Test Transformation: Auto

TABLE 1 Group Mean Body Weight (g) Data – Female (Continued)

Body Weight (g)

Sex: Female		Day(s) Relative to Start Date			
		-3	2	4	8
SYN548097	Mean	144.924	-	-	168.928
300 mg/kg	SD	7.389	-	-	13.243
	N	5	-	-	5
SYN548097	Mean	146.072	-	-	159.854
1000 mg/kg	SD	7.163	-	-	24.950
	N	5	-	-	5
SYN548012	Mean	145.534	-	-	171.902
10 mg/kg	SD	6.627	-	-	5.304
	N	5	-	-	5
SYN54012	Mean	144.274	-	-	169.600
100 mg/kg	SD	6.286	-	-	6.331
	N	5	-	-	5
SYN548012	Mean	144.392	-	-	172.584
300 mg/kg	SD	7.230	-	-	9.997
	N	5	-	-	5

Statistical Test: Generalised Anova/Ancova Test Transformation: Auto

TABLE 1 Group Mean Body Weight (g) Data – Female (Continued)

Body Weight (g)

Sex: Female		Day(s) Relative to Start Date			
		-3	2	4	8
SYN548012	Mean	144.400	-	-	171.936
1000 mg/kg	SD	7.096	-	-	4.354
	N	5	-	-	5
SYN548014	Mean	144.546	-	-	170.500
10 mg/kg	SD	6.345	-	-	5.579
	N	5	-	-	5
SYN548014	Mean	143.750	-	-	171.744
100 mg/kg	SD	7.281	-	-	6.781
	N	5	-	-	5
SYN548014	Mean	145.386	-	-	171.580
300 mg/kg	SD	7.251	-	-	6.718
	N	5	-	-	5
SYN548014	Mean	143.754	-	-	166.536
1000 mg/kg	SD	6.879	-	-	8.752
	N	5	-	-	5

Statistical Test: Generalised Anova/Ancova Test Transformation: Auto

TABLE 1 Group Mean Body Weight (g) Data – Female (Continued)

Body Weight (g)

Sex: Female		Day(s) Relative to Start Date			
		-3	2	4	8
Control	Mean	145.390 R[1]	-	-	161.862 R[1]
0 mg/kg	SD	7.252	-	-	8.641
	N	5	-	-	5

Statistical Test: Generalised Anova/Ancova Test Transformation: Auto

1 [R - Auto Transformation: Rank]

TABLE 1 Group Mean Body Weight (g) Data – Female (Continued)

Body Weight (g)

Sex: Female		Day(s) Relative to Start Date		
		-4	7	8
SYN548102	Mean	144.996	-	174.718
10 mg/kg	SD	5.923	-	9.971
	N	5	-	5
SYN548102	Mean	144.582	-	167.502
100 mg/kg	SD	6.238	-	6.368
	N	5	-	5
SYN548102	Mean	144.522	-	173.230
300 mg/kg	SD	5.637	-	7.683
	N	5	-	5
SYN548102	Mean	145.178	121.070	138.007 ddd[1]
1000 mg/kg	SD	5.619	-	22.271
	N	5	1	3
SYN548117	Mean	145.416	-	176.746
10 mg/kg	SD	5.992	-	4.063
	N	5	-	5

Statistical Test: Generalised Anova/Ancova Test Transformation: Auto

1 [ddd - Test: Dunnett 2 Sided p < 0.001]

TABLE 1 Group Mean Body Weight (g) Data – Female (Continued)

Body Weight (g)

Sex: Female		Day(s) Relative to Start Date		
		-4	7	8
SYN548117	Mean	146.148	-	175.868
100 mg/kg	SD	6.216	-	4.681
	N	5	-	5
SYN548117	Mean	145.730	-	161.718
300 mg/kg	SD	7.004	-	15.867
	N	5	-	5
SYN548117	Mean	143.588	113.460	139.748 ddd[1]
1000 mg/kg	SD	7.447	-	12.198
	N	5	1	4
SYN548098	Mean	144.890	-	175.078
10 mg/kg	SD	6.697	-	11.102
	N	5	-	5
SYN548098	Mean	145.342	-	173.074
100 mg/kg	SD	6.279	-	7.826
	N	5	-	5

Statistical Test: Generalised Anova/Ancova Test Transformation: Auto

1 [ddd - Test: Dunnett 2 Sided p < 0.001]

TABLE 1 Group Mean Body Weight (g) Data – Female (Continued)

Body Weight (g)

Sex: Female		Day(s) Relative to Start Date		
		-4	7	8
SYN548098	Mean	145.500	-	170.866
300 mg/kg	SD	6.369	-	7.127
	N	5	-	5
SYN548098	Mean	144.238	-	166.286
1000 mg/kg	SD	7.155	-	11.274
	N	5	-	5
SYN548101	Mean	145.832	-	172.730
10 mg/kg	SD	6.118	-	6.142
	N	5	-	5
SYN548101	Mean	144.700	-	168.683
100 mg/kg	SD	6.263	-	11.484
	N	5	-	4
SYN548101	Mean	145.848	124.920	161.313
300 mg/kg	SD	6.999	-	9.850
	N	5	1	4

Statistical Test: Generalised Anova/Ancova Test Transformation: Auto

TABLE 1 Group Mean Body Weight (g) Data – Female (Continued)

Body Weight (g)

Sex: Female		Day(s) Relative to Start Date		
		-4	7	8
SYN548101	Mean	145.670	-	140.550 dd[1]
1000 mg/kg	SD	7.558	-	21.227
	N	5	-	2
SYN548013	Mean	145.106	-	170.682
10 mg/kg	SD	6.025	-	7.208
	N	5	-	5
SYN548013	Mean	144.712	-	172.568
100 mg/kg	SD	5.719	-	7.299
	N	5	-	5
SYN548013	Mean	146.024	-	175.434
300 mg/kg	SD	7.544	-	5.813
	N	5	-	5
SYN548013	Mean	143.656	-	171.660
1000 mg/kg	SD	6.659	-	7.568
	N	5	-	5

Statistical Test: Generalised Anova/Ancova Test Transformation: Auto

1 [dd - Test: Dunnett 2 Sided p < 0.01]

TABLE 1 Group Mean Body Weight (g) Data – Female (Continued)

Body Weight (g)

Sex: Female		Day(s) Relative to Start Date		
		-4	7	8
Control	Mean	144.236 R[1]	-	174.640 [2]
0 mg/kg	SD	8.925	-	8.996
	N	5	-	5

Statistical Test: Generalised Anova/Ancova Test Transformation: Auto

1 [R - Auto Transformation: Rank] 2 [I,aaa - Auto Transformation: Identity, Group Factor Test: Analysis of Variance $p < 0.001$]

TABLE 2 Group Mean Body Weight (g) Change per Day Data – Female

Mean Weight Gain (g/Day)

Sex: Female		Day(s) Relative to Start Date		
		-3 → 2	-3 → 4	-3 → 8
SYN547407	Mean	-	-	2.133
10 mg/kg	SD	-	-	0.726
	N	-	-	5
SYN547407	Mean	-	-	2.465
100 mg/kg	SD	-	-	0.308
	N	-	-	5
SYN547407	Mean	-	-	1.923
300 mg/kg	SD	-	-	0.340
	N	-	-	5
SYN547407	Mean	-	-	0.438
1000 mg/kg	SD	-	-	0.742
	N	-	-	5
SYN546308	Mean	-	-	2.210
10 mg/kg	SD	-	-	0.345
	N	-	-	4

Statistical Test: Generalised Anova/Ancova Test Transformation: Auto

TABLE 2 Group Mean Body Weight (g) Change per Day Data – Female (Continued)

Mean Weight Gain (g/Day)

Sex: Female		Day(s) Relative to Start Date		
		-3 → 2	-3 → 4	-3 → 8
SYN546308	Mean	0.234	-	2.301
100 mg/kg	SD	-	-	0.350
	N	1	-	4
SYN546308	Mean	-	-	2.620
300 mg/kg	SD	-	-	0.749
	N	-	-	5
SYN546308	Mean	-1.034	-	0.199
1000 mg/kg	SD	-	-	2.201
	N	1	-	4
SYN548097	Mean	-	1.379	2.319
10 mg/kg	SD	-	-	0.757
	N	-	1	4
SYN548097	Mean	-	-	2.504
100 mg/kg	SD	-	-	0.418
	N	-	-	5

Statistical Test: Generalised Anova/Ancova Test Transformation: Auto

TABLE 2 Group Mean Body Weight (g) Change per Day Data – Female (Continued)

Mean Weight Gain (g/Day)

Sex: Female		Day(s) Relative to Start Date		
		-3 → 2	-3 → 4	-3 → 8
SYN548097	Mean	-	-	2.182
300 mg/kg	SD	-	-	0.914
	N	-	-	5
SYN548097	Mean	-	-	1.253
1000 mg/kg	SD	-	-	2.046
	N	-	-	5
SYN548012	Mean	-	-	2.397
10 mg/kg	SD	-	-	0.917
	N	-	-	5
SYN54012	Mean	-	-	2.302
100 mg/kg	SD	-	-	0.724
	N	-	-	5
SYN548012	Mean	-	-	2.563
300 mg/kg	SD	-	-	0.667
	N	-	-	5

Statistical Test: Generalised Anova/Ancova Test Transformation: Auto

TABLE 2 Group Mean Body Weight (g) Change per Day Data – Female (Continued)

Mean Weight Gain (g/Day)

Sex: Female		Day(s) Relative to Start Date		
		-3 → 2	-3 → 4	-3 → 8
SYN548012	Mean	-	-	2.503
1000 mg/kg	SD	-	-	0.539
	N	-	-	5
SYN548014	Mean	-	-	2.359
10 mg/kg	SD	-	-	0.634
	N	-	-	5
SYN548014	Mean	-	-	2.545
100 mg/kg	SD	-	-	0.259
	N	-	-	5
SYN548014	Mean	-	-	2.381
300 mg/kg	SD	-	-	0.367
	N	-	-	5
SYN548014	Mean	-	-	2.071
1000 mg/kg	SD	-	-	0.862
	N	-	-	5

Statistical Test: Generalised Anova/Ancova Test Transformation: Auto

TABLE 2 Group Mean Body Weight (g) Change per Day Data – Female (Continued)

Mean Weight Gain (g/Day)

Sex: Female		Day(s) Relative to Start Date		
		-3 → 2	-3 → 4	-3 → 8
Control	Mean	-	-	1.497 R[1]
0 mg/kg	SD	-	-	0.580
	N	-	-	5

Statistical Test: Generalised Anova/Ancova Test Transformation: Auto

1 [R - Auto Transformation: Rank]

TABLE 2 Group Mean Body Weight (g) Change per Day Data – Female (Continued)

Mean Weight Gain (g/Day)

Sex: Female		Day(s) Relative to Start Date	
		-4 → 7	-4 → 8
SYN548102	Mean	-	2.477
10 mg/kg	SD	-	0.367
	N	-	5
SYN548102	Mean	-	1.910
100 mg/kg	SD	-	0.436
	N	-	5
SYN548102	Mean	-	2.392
300 mg/kg	SD	-	0.537
	N	-	5
SYN548102	Mean	-1.785	-0.903
1000 mg/kg	SD	-	2.134
	N	1	3
SYN548117	Mean	-	2.611
10 mg/kg	SD	-	0.404
	N	-	5

Statistical Test: Generalised Anova/Ancova Test Transformation: Auto

TABLE 2 Group Mean Body Weight (g) Change per Day Data – Female (Continued)

Mean Weight Gain (g/Day)

Sex: Female		Day(s) Relative to Start Date	
		-4 → 7	-4 → 8
SYN548117	Mean	-	2.477
100 mg/kg	SD	-	0.521
	N	-	5
SYN548117	Mean	-	1.332
300 mg/kg	SD	-	1.691
	N	-	5
SYN548117	Mean	-2.458	-0.384
1000 mg/kg	SD	-	0.869
	N	1	4
SYN548098	Mean	-	2.516
10 mg/kg	SD	-	0.745
	N	-	5
SYN548098	Mean	-	2.311
100 mg/kg	SD	-	0.590
	N	-	5

Statistical Test: Generalised Anova/Ancova Test Transformation: Auto

TABLE 2 Group Mean Body Weight (g) Change per Day Data – Female (Continued)

Mean Weight Gain (g/Day)

Sex: Female		Day(s) Relative to Start Date	
		-4 → 7	-4 → 8
SYN548098	Mean	-	2.114
300 mg/kg	SD	-	0.226
	N	-	5
SYN548098	Mean	-	1.837
1000 mg/kg	SD	-	0.542
	N	-	5
SYN548101	Mean	-	2.242
10 mg/kg	SD	-	0.655
	N	-	5
SYN548101	Mean	-	1.990
100 mg/kg	SD	-	0.485
	N	-	4
SYN548101	Mean	-1.225	1.134
300 mg/kg	SD	-	0.480
	N	1	4

Statistical Test: Generalised Anova/Ancova Test Transformation: Auto

TABLE 2 Group Mean Body Weight (g) Change per Day Data – Female (Continued)

Mean Weight Gain (g/Day)

Sex: Female		Day(s) Relative to Start Date	
		-4 → 7	-4 → 8
SYN548101	Mean	-	-0.526
1000 mg/kg	SD	-	0.565
	N	-	2
SYN548013	Mean	-	2.131
10 mg/kg	SD	-	0.583
	N	-	5
SYN548013	Mean	-	2.321
100 mg/kg	SD	-	0.170
	N	-	5
SYN548013	Mean	-	2.451
300 mg/kg	SD	-	0.793
	N	-	5
SYN548013	Mean	-	2.334
1000 mg/kg	SD	-	0.332
	N	-	5

Statistical Test: Generalised Anova/Ancova Test Transformation: Auto

TABLE 2 Group Mean Body Weight (g) Change per Day Data – Female (Continued)

Mean Weight Gain (g/Day)

Sex: Female		Day(s) Relative to Start Date	
		-4 → 7	-4 → 8
Control	Mean	-	2.534 [1]
0 mg/kg	SD	-	0.350
	N	-	5

Statistical Test: Generalised Anova/Ancova Test Transformation: Auto

1 [R,kk - Auto Transformation: Rank, Group Factor Test: Kruskal-Wallis $p < 0.01$]

FIGURES SECTION

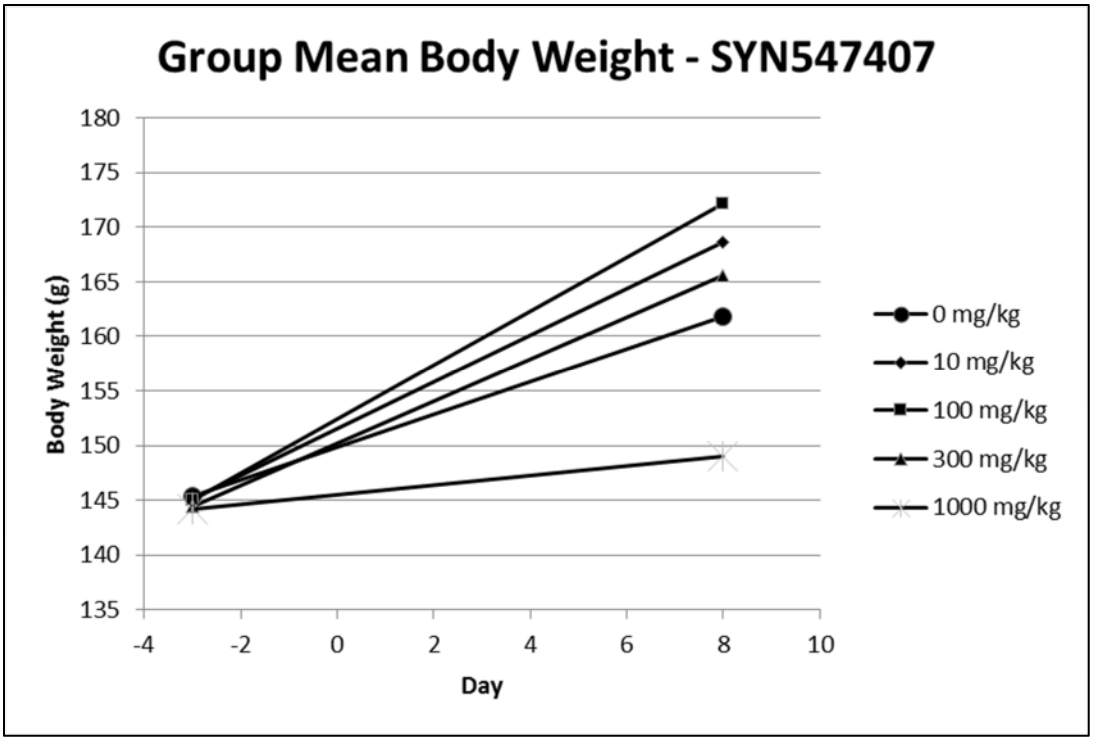


FIGURE 1 **Group Mean Body Weight for Rats Administered SYN547407 or Vehicle**

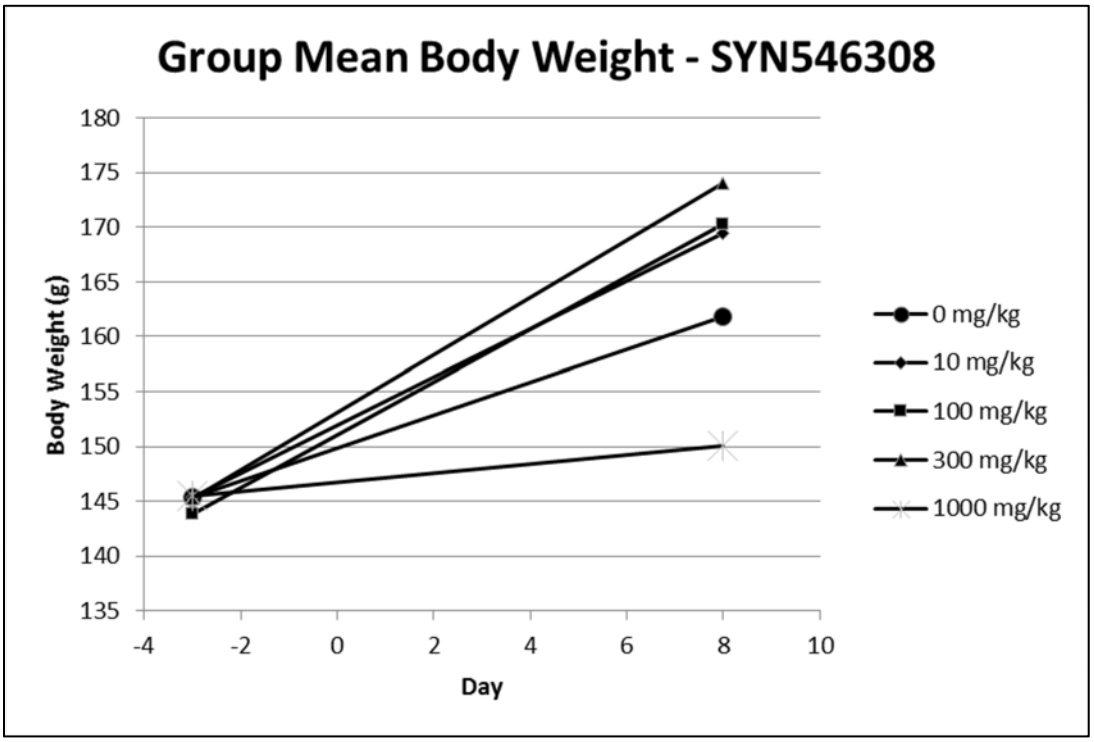


FIGURE 2 **Group Mean Body Weight for Rats Administered SYN546308 or Vehicle**

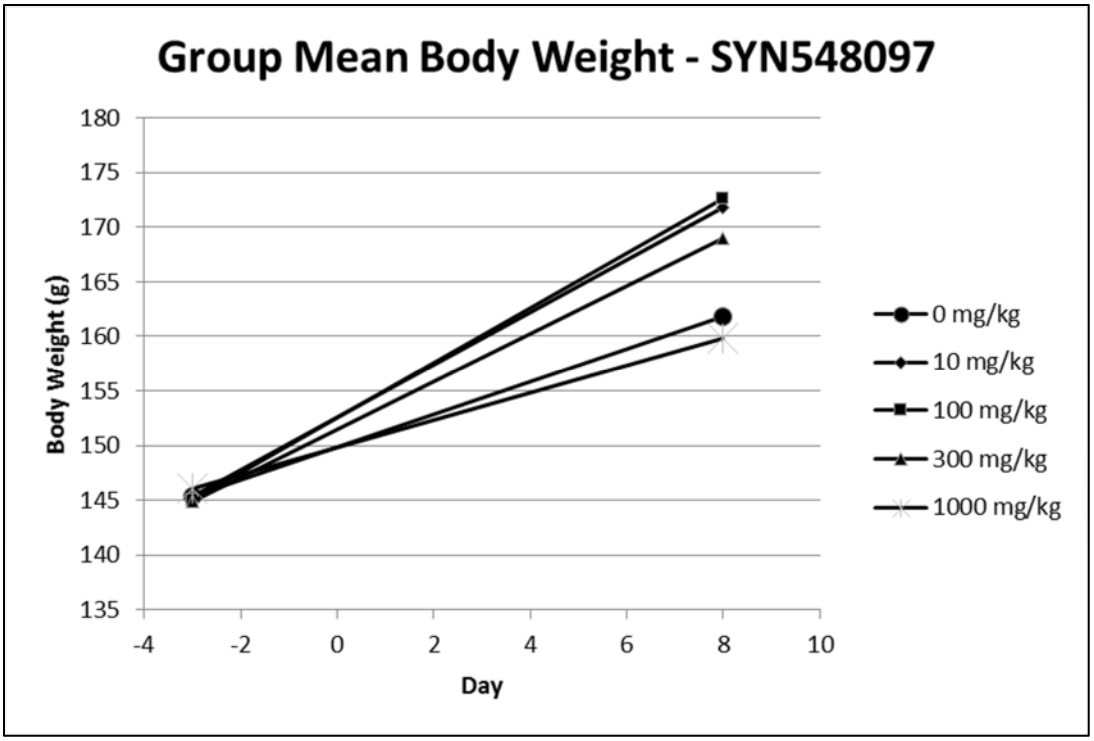


FIGURE 3 **Group Mean Body Weight for Rats Administered SYN548097 or Vehicle**

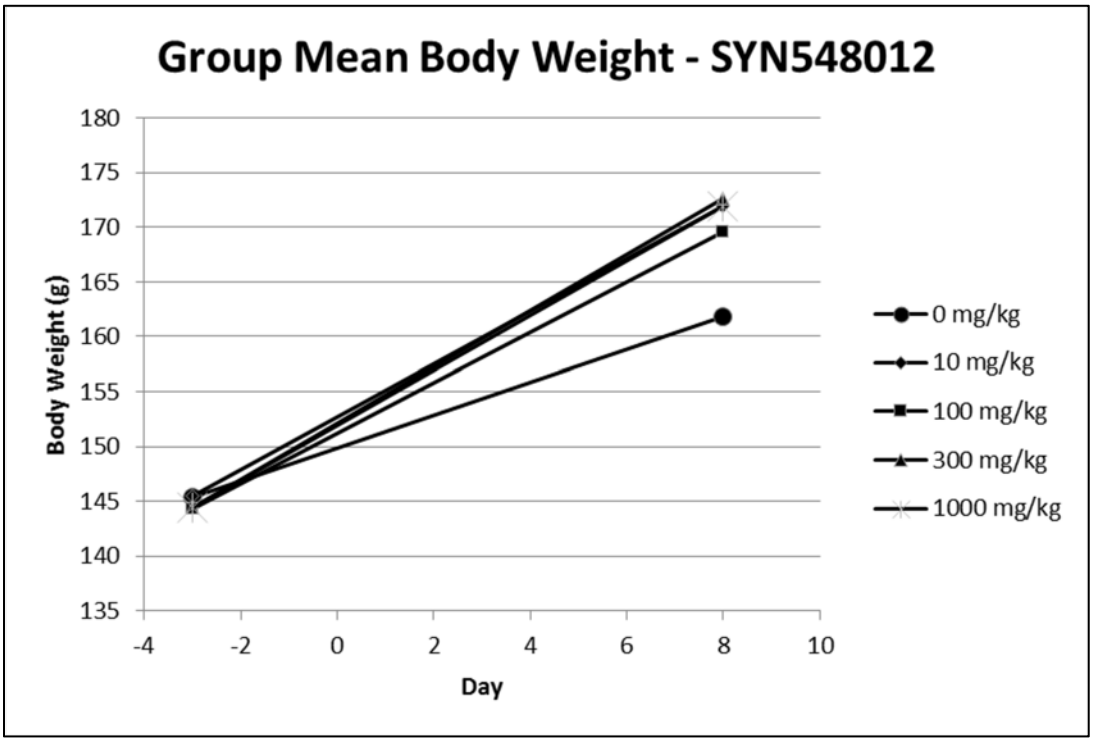


FIGURE 4 **Group Mean Body Weight for Rats Administered SYN548012 or Vehicle**

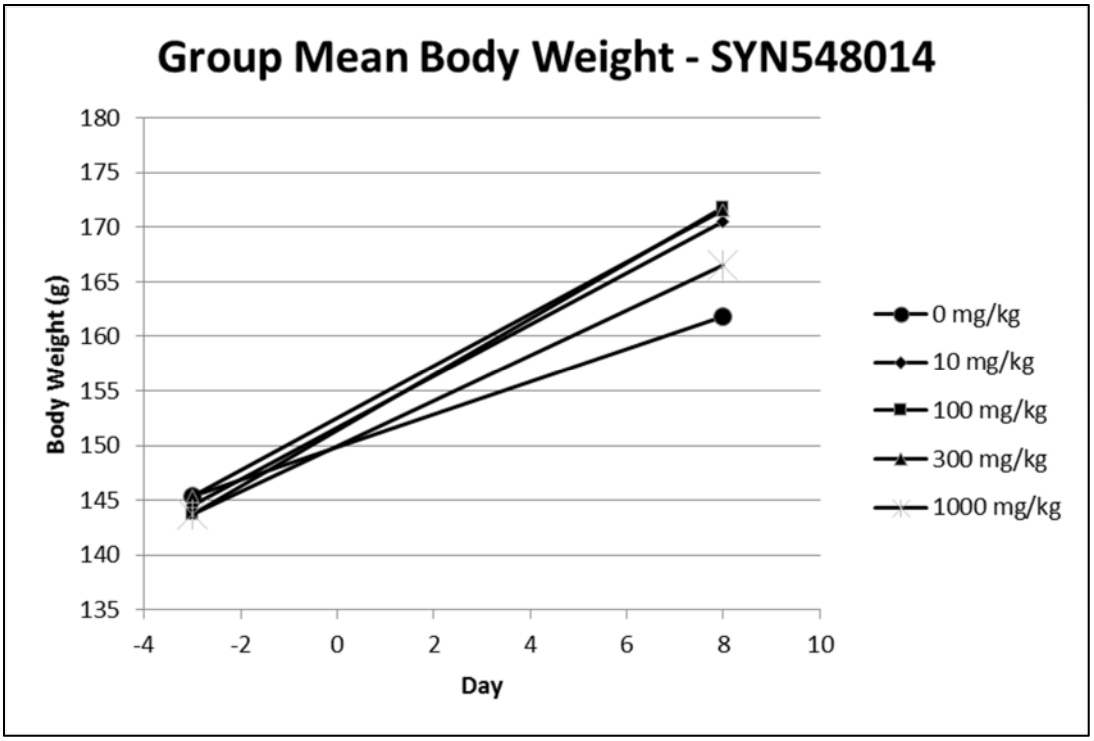


FIGURE 5 **Group Mean Body Weight for Rats Administered SYN548014 or Vehicle**

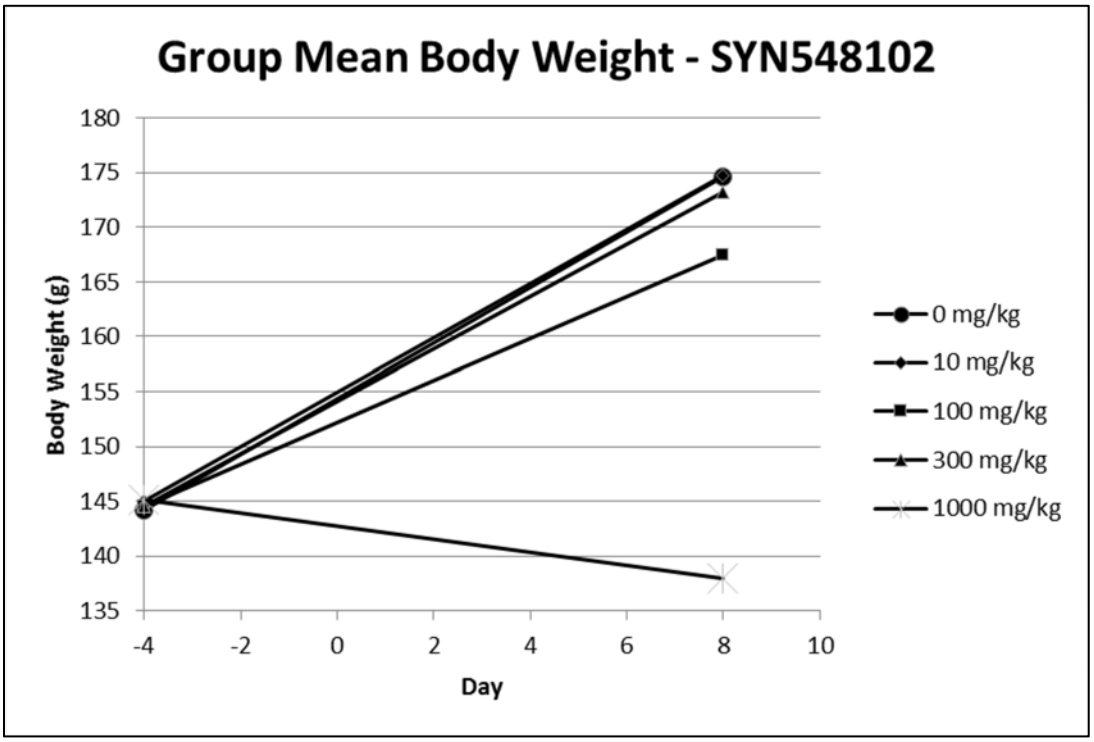


FIGURE 6 **Group Mean Body Weight for Rats Administered SYN548102 or Vehicle**

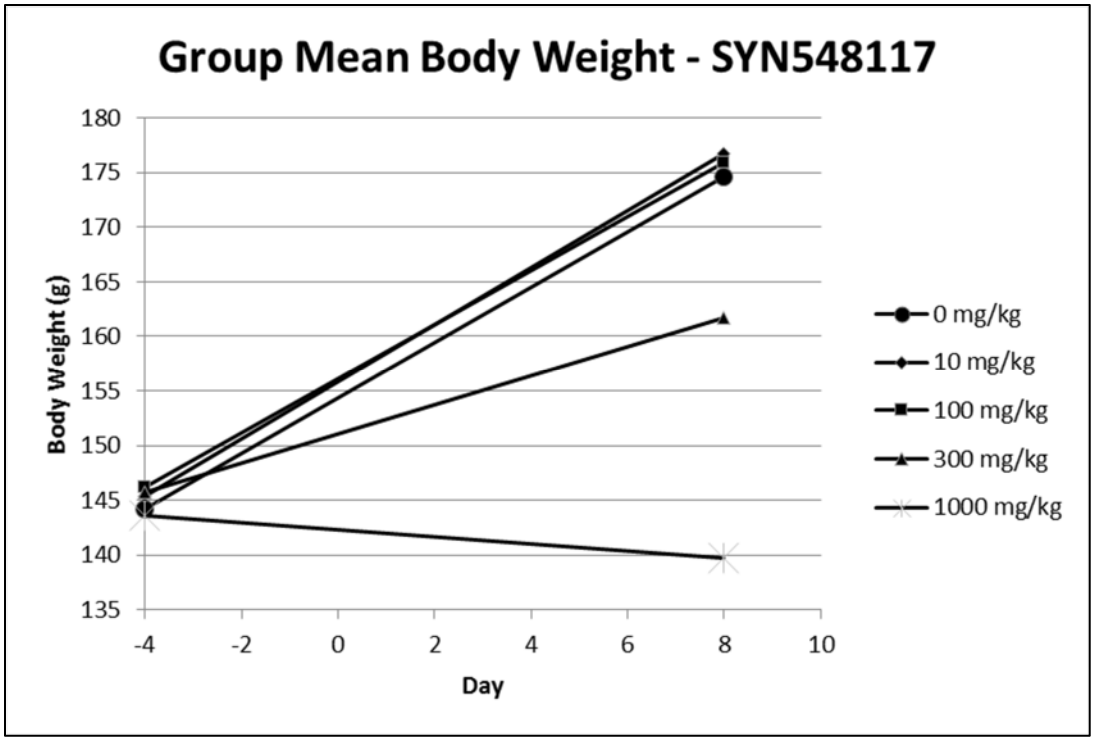


FIGURE 7 **Group Mean Body Weight for Rats Administered SYN548117 or Vehicle**

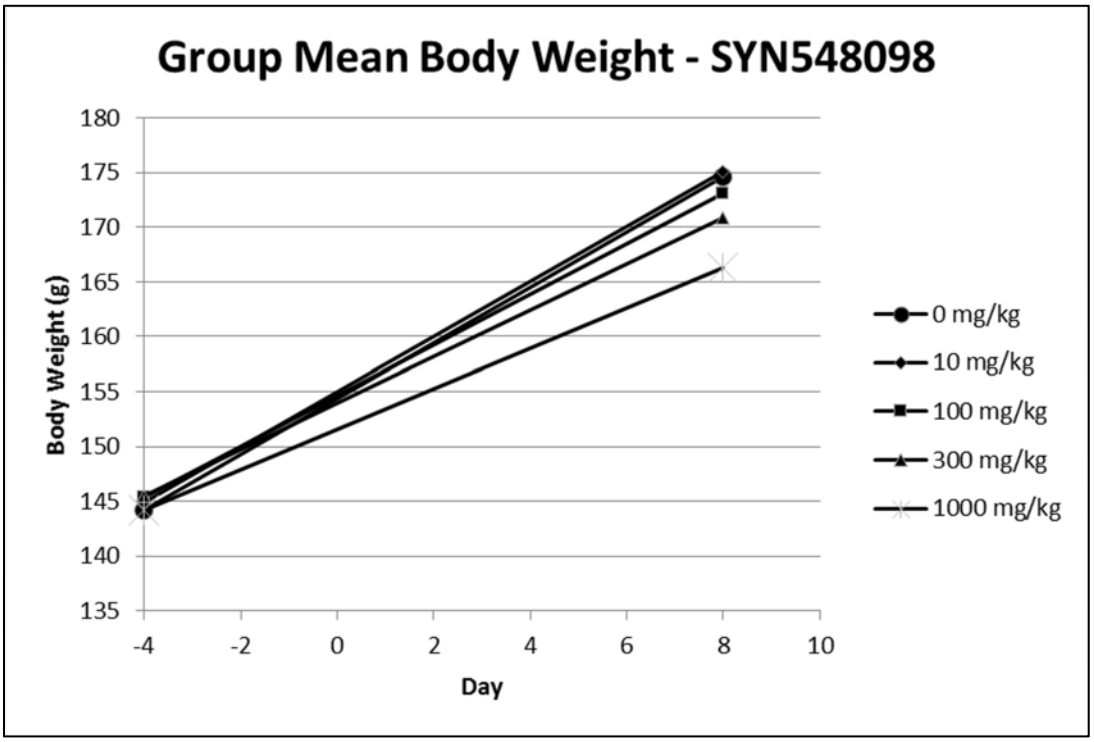


FIGURE 8 **Group Mean Body Weight for Rats Administered SYN548098 or Vehicle**

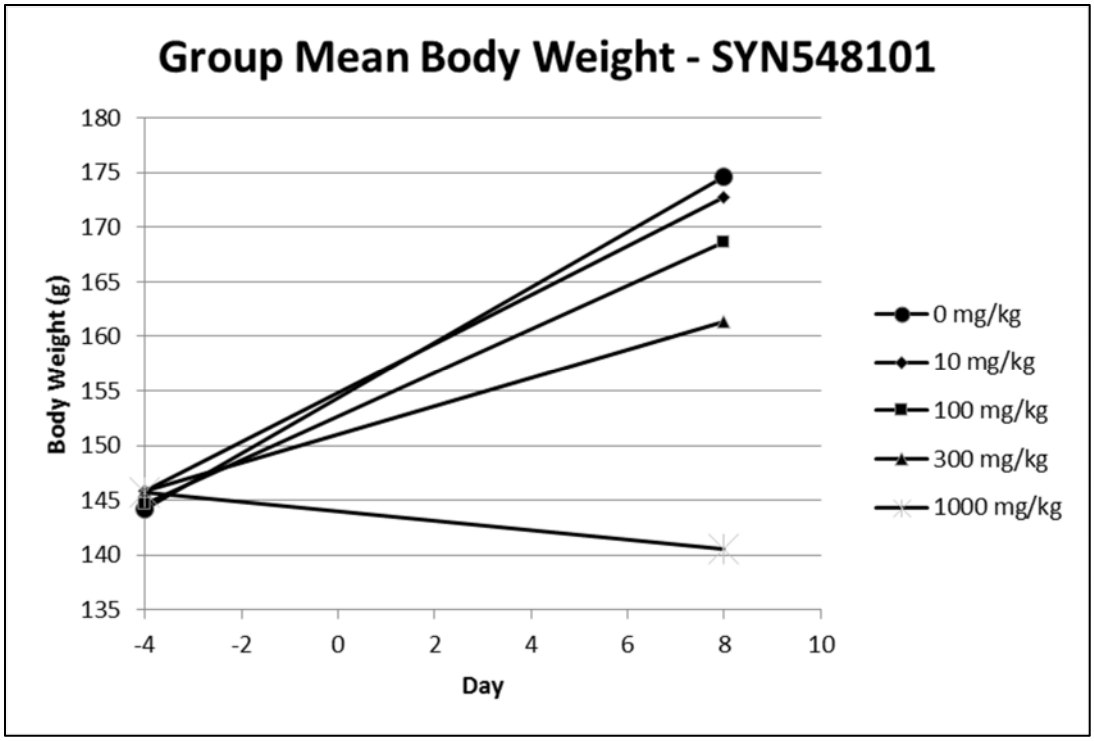


FIGURE 9 **Group Mean Body Weight for Rats Administered SYN548101 or Vehicle**

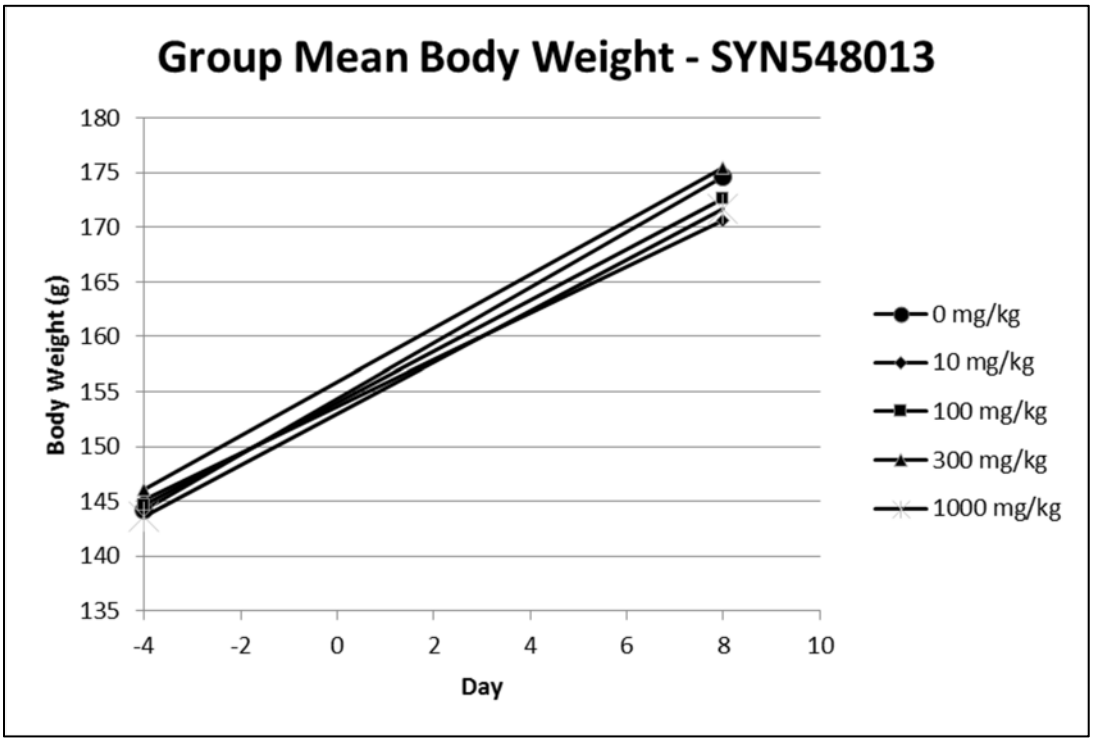


FIGURE 10 **Group Mean Body Weight for Rats Administered SYN548013 or Vehicle**

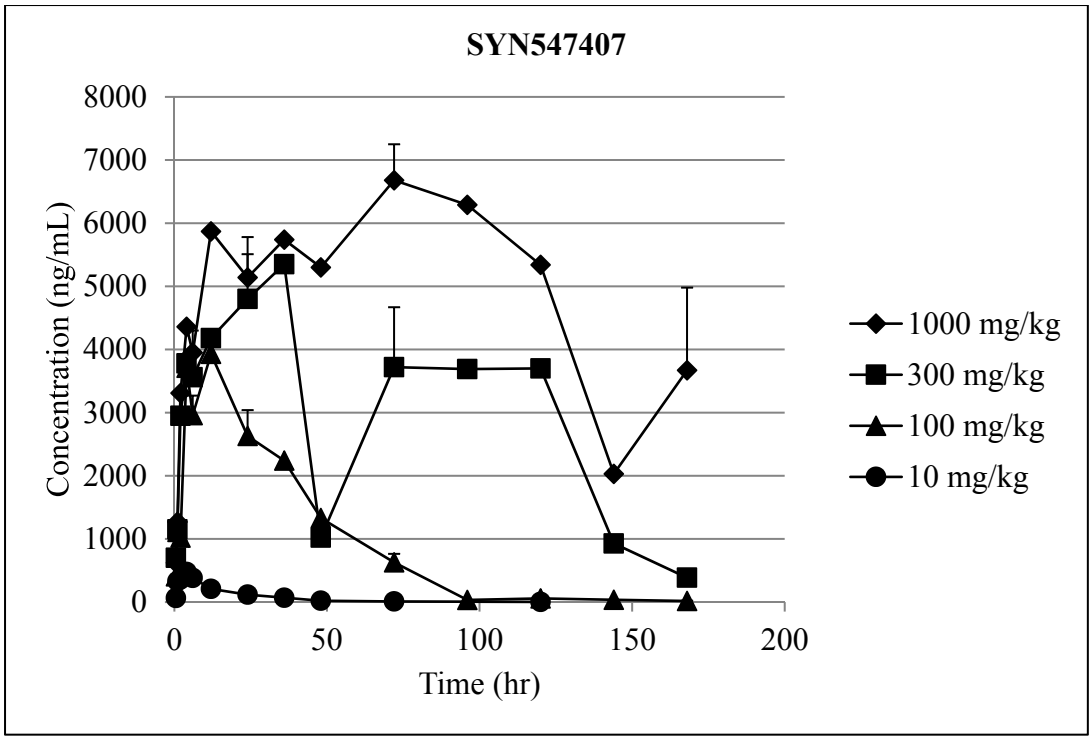


FIGURE 11 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN547407

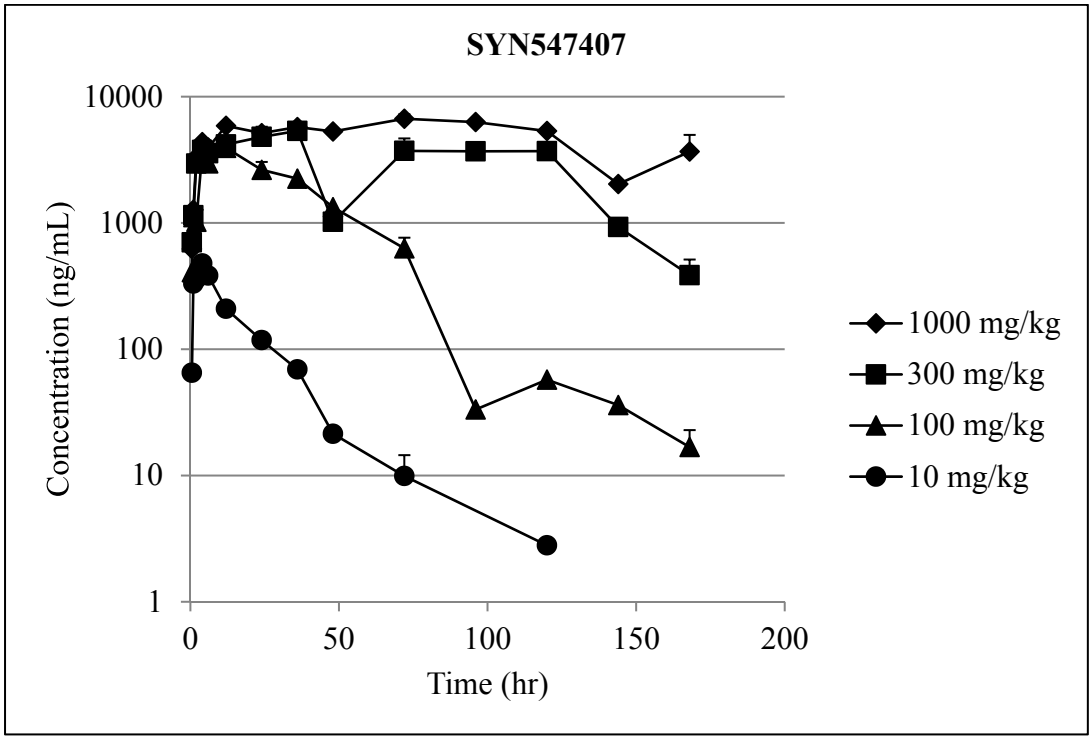


FIGURE 12 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN547407 – Logarithmic Scale

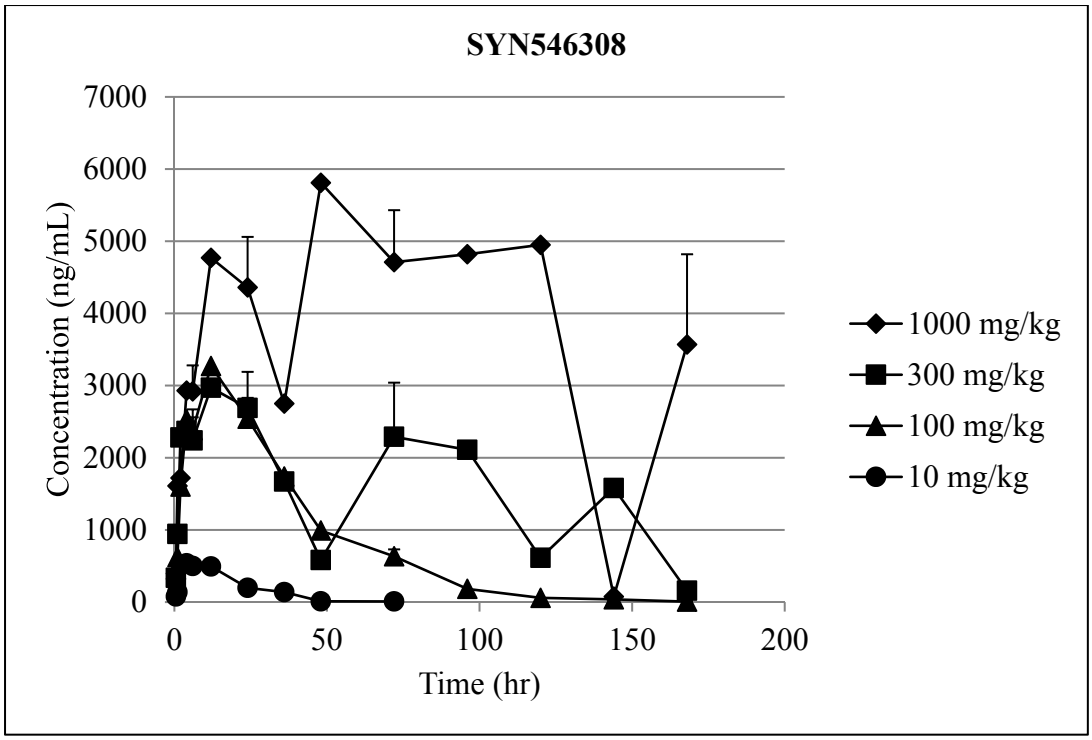


FIGURE 13 **Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN546308**

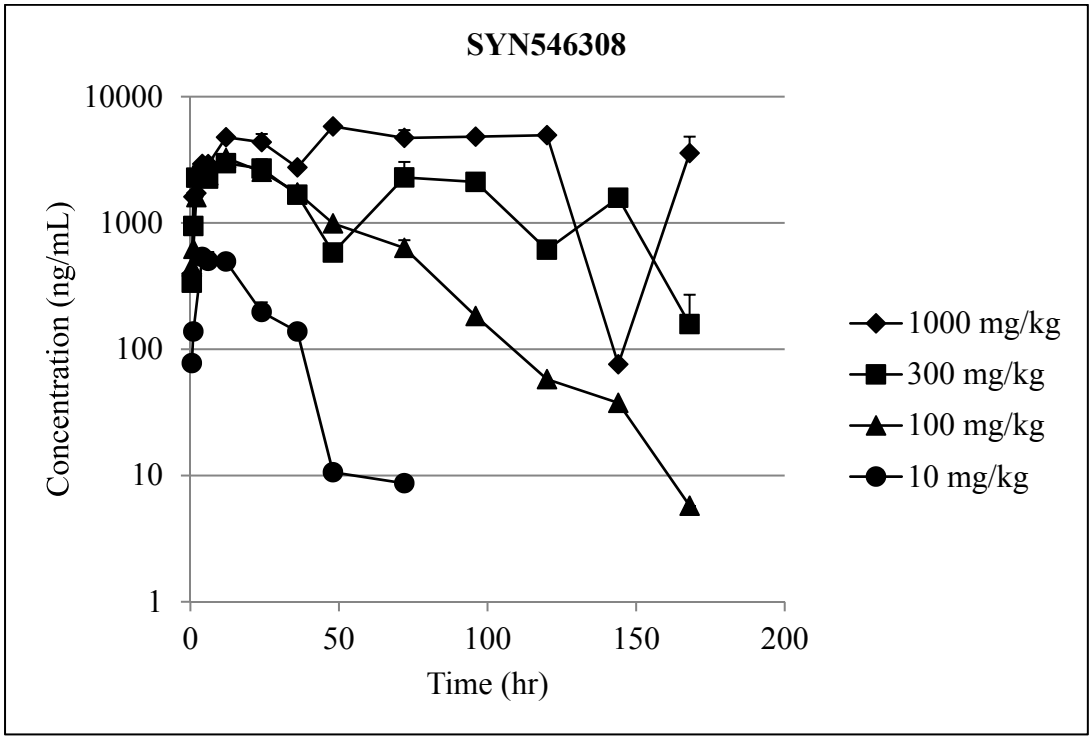


FIGURE 14 **Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN546308 – Logarithmic Scale**

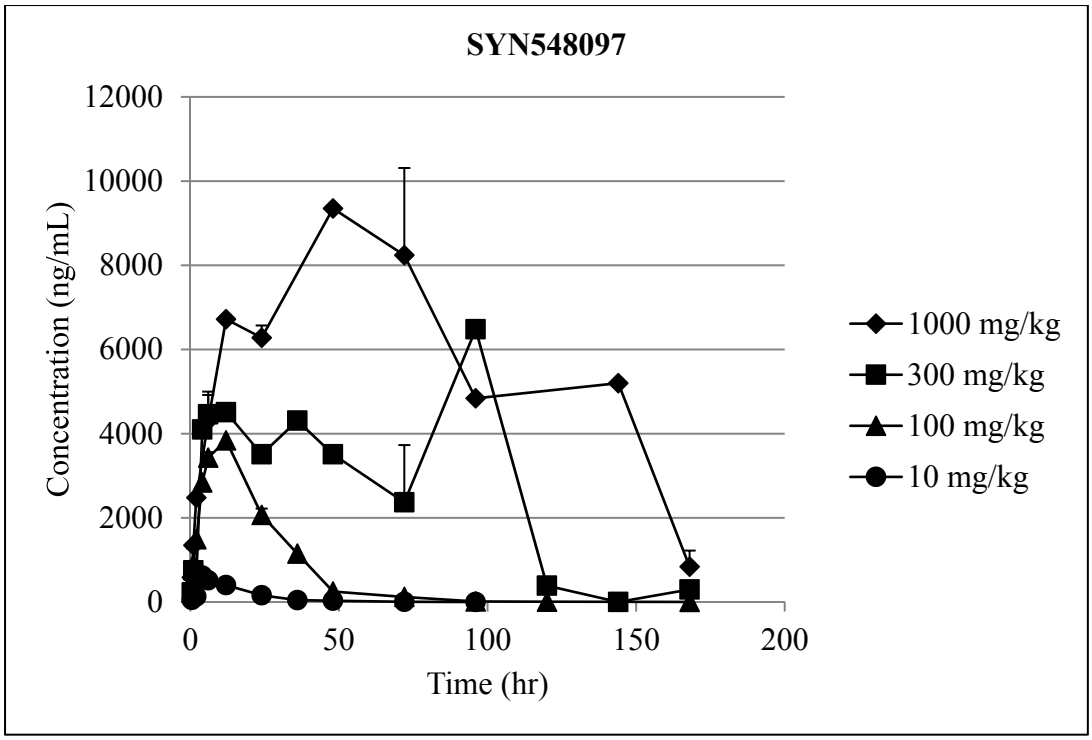


FIGURE 15 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548097

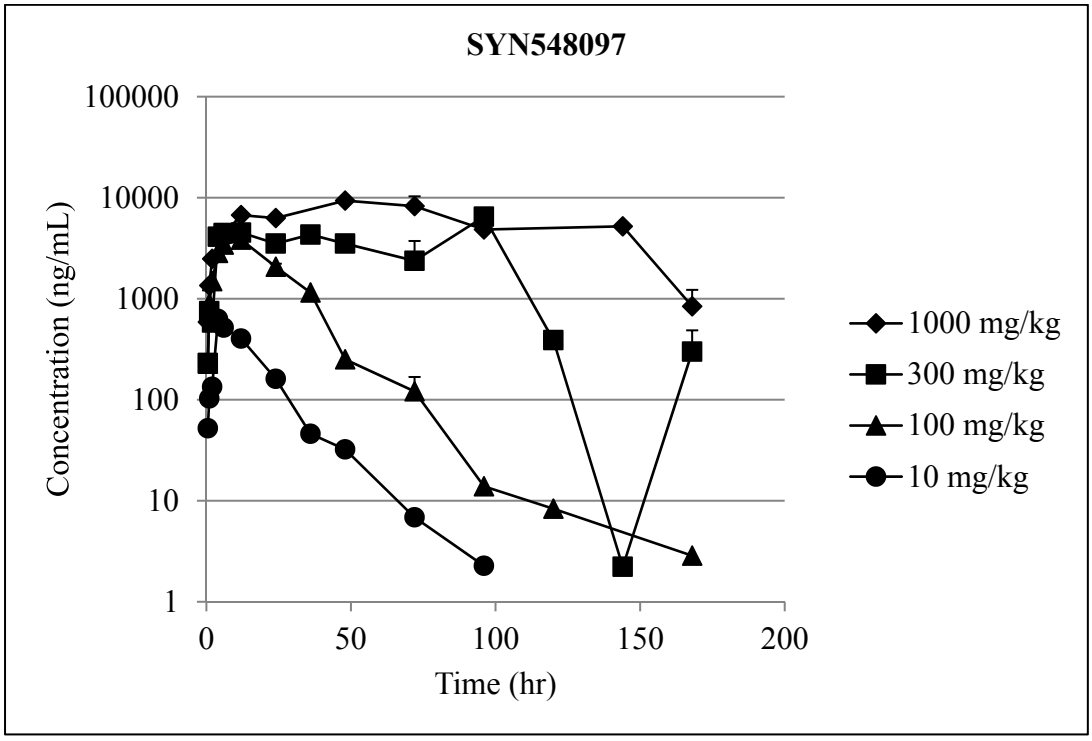


FIGURE 16 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548097 – Logarithmic Scale

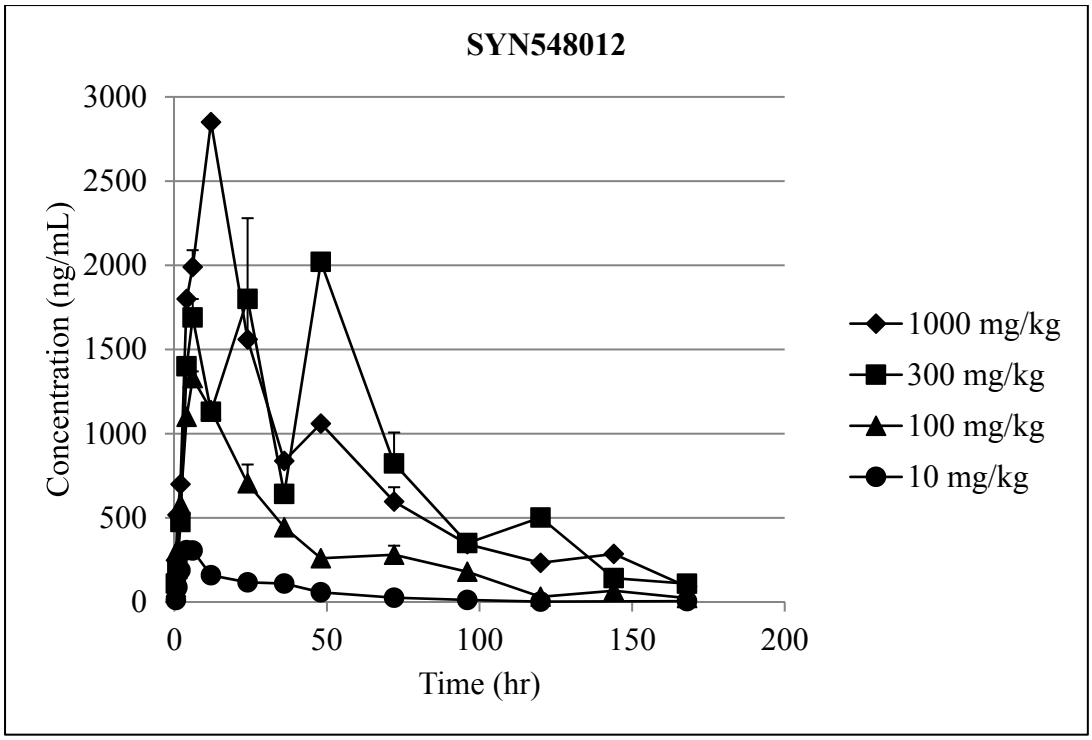


FIGURE 17 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548012

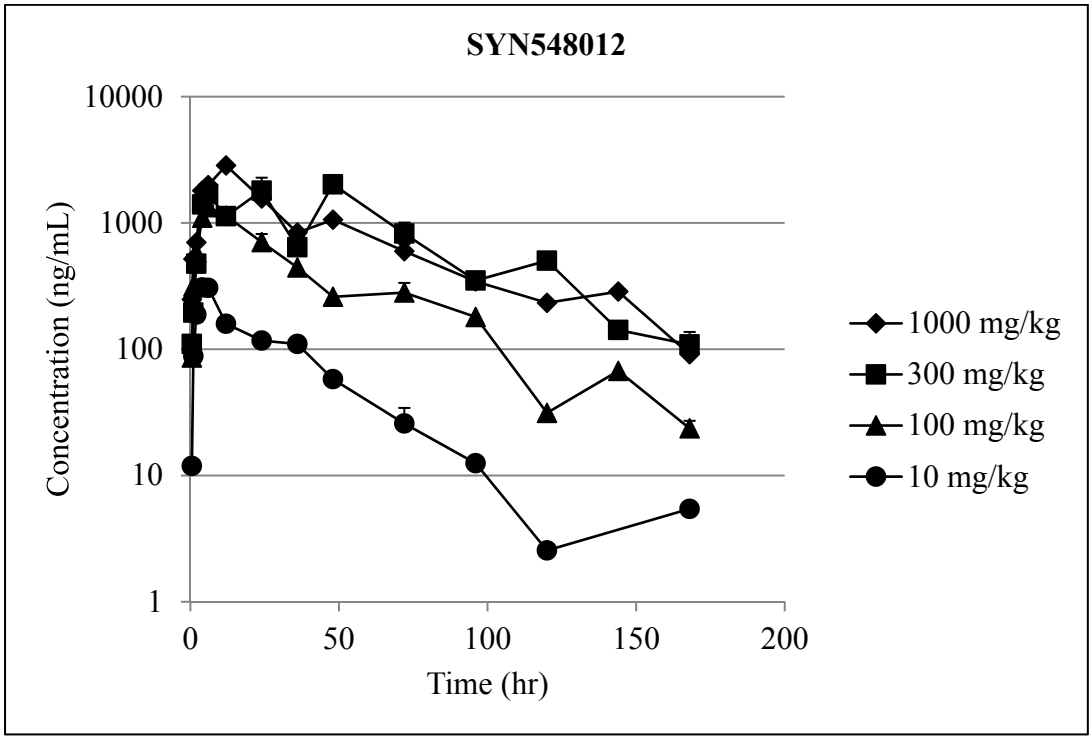


FIGURE 18 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548012 – Logarithmic Scale

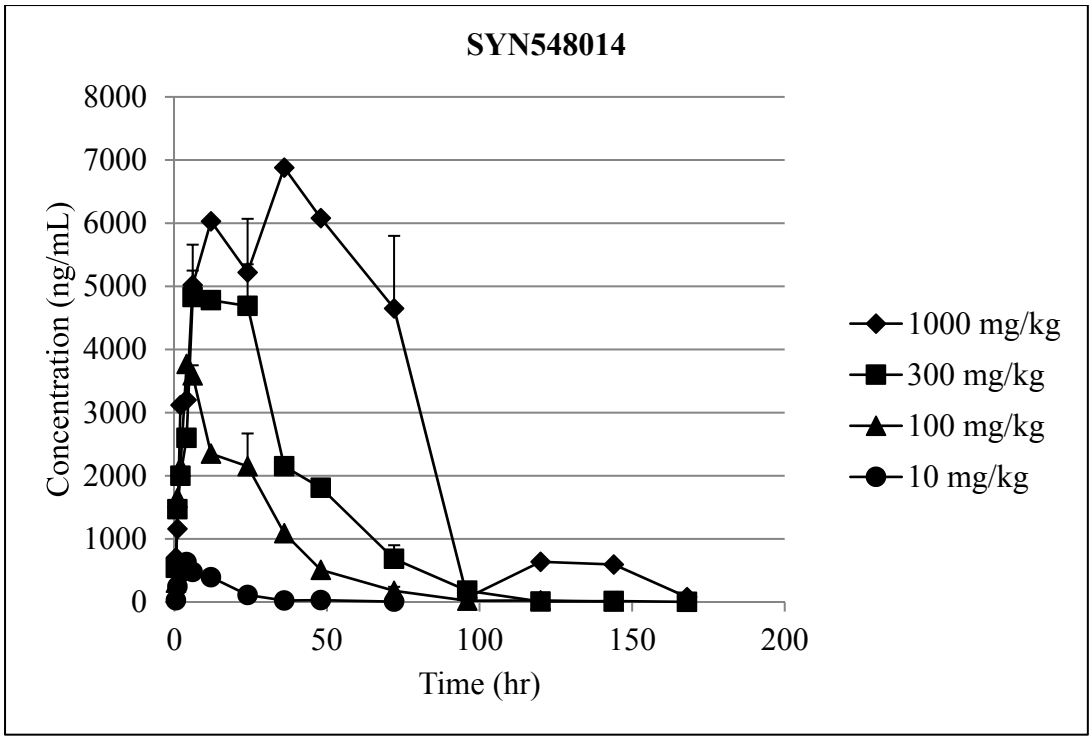


FIGURE 19 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548014

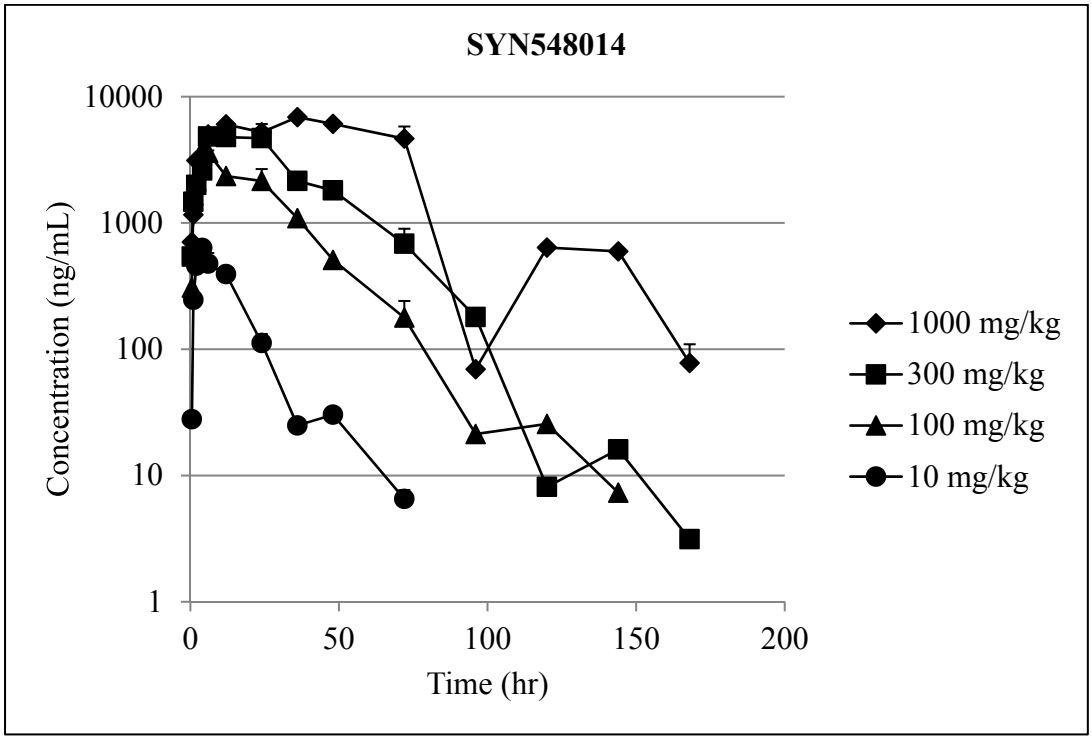


FIGURE 20 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548014 – Logarithmic Scale

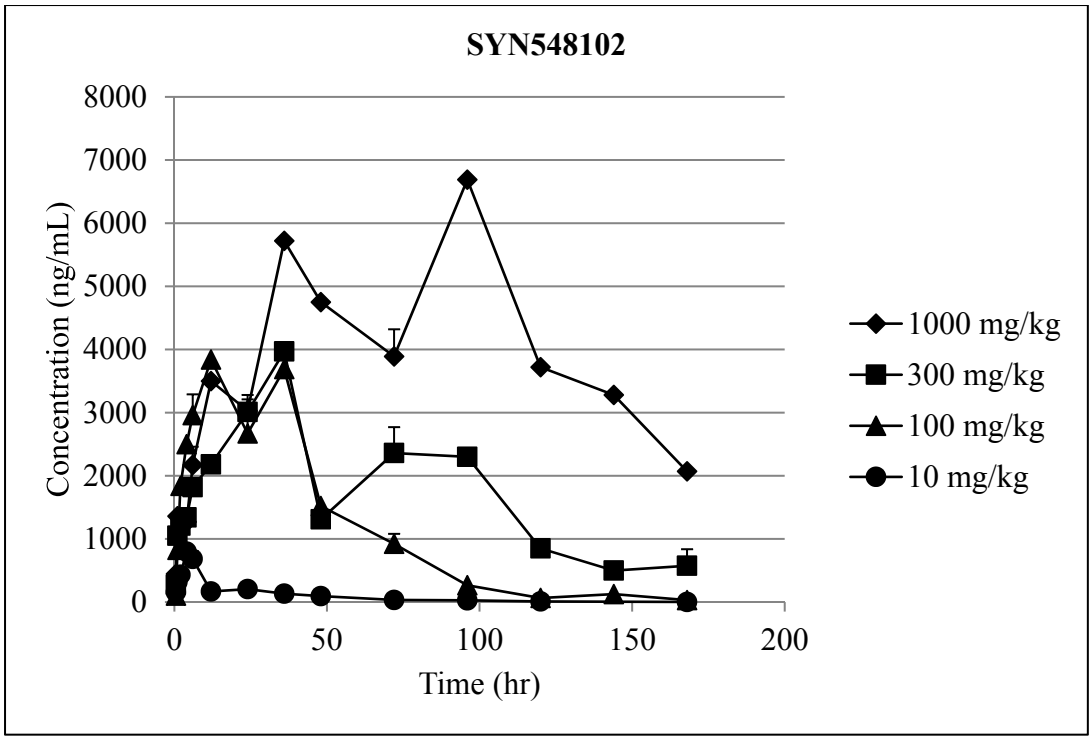


FIGURE 21 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548102

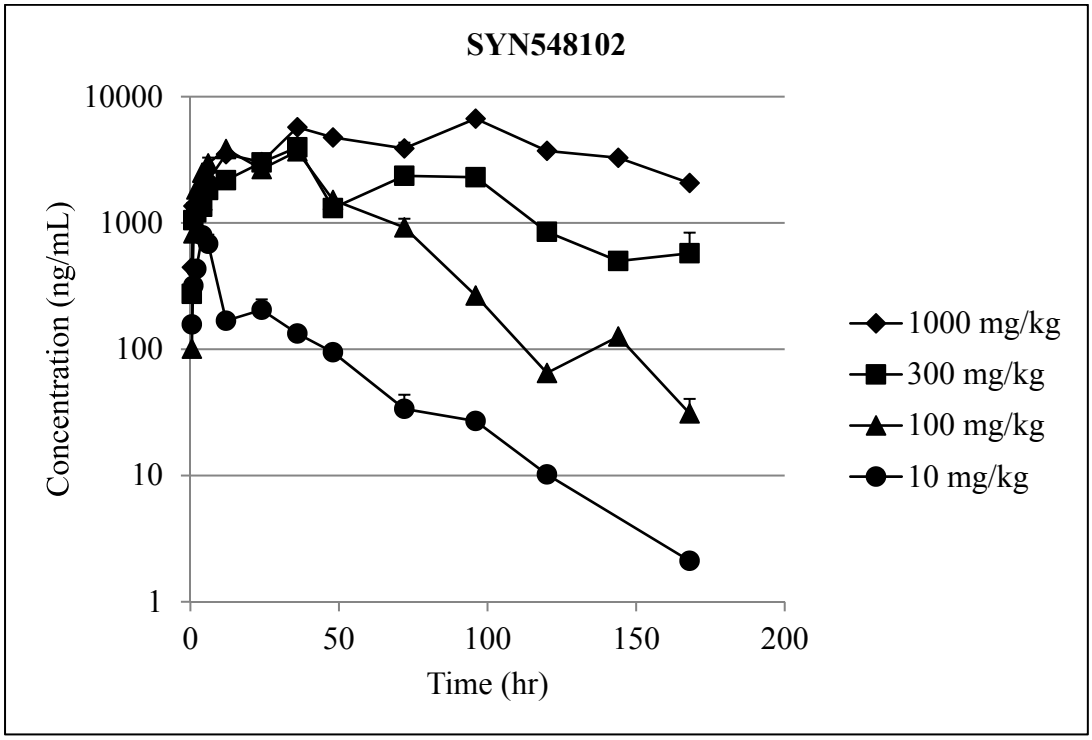


FIGURE 22 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548102 – Logarithmic Scale

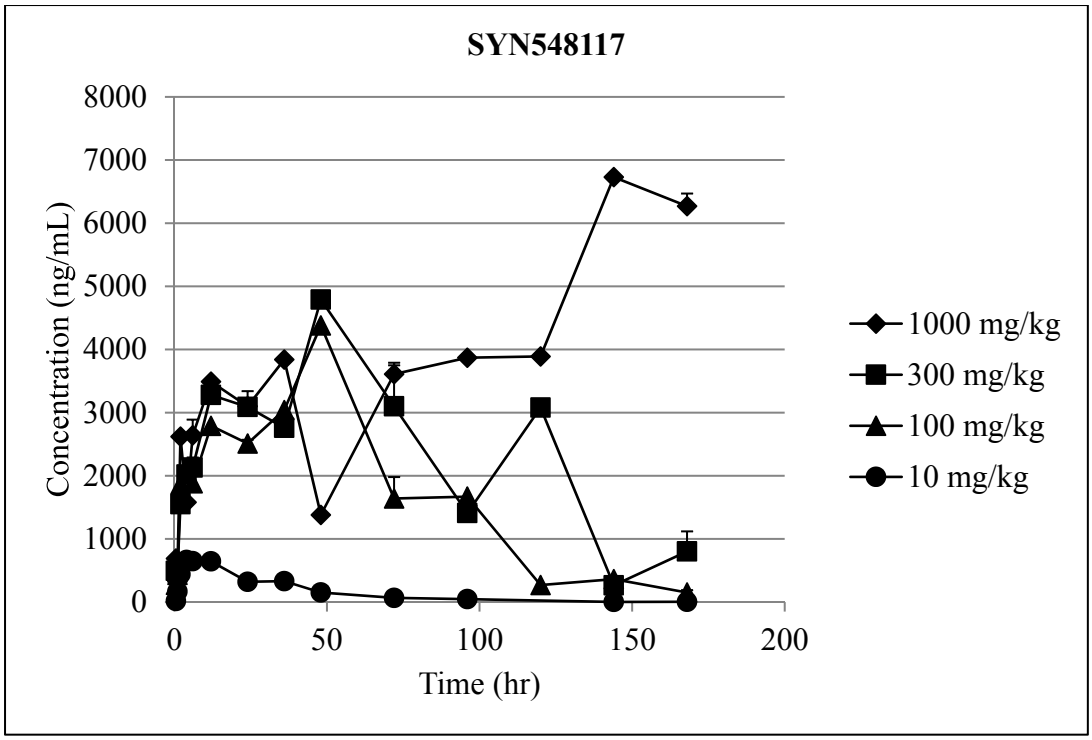


FIGURE 23 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548117

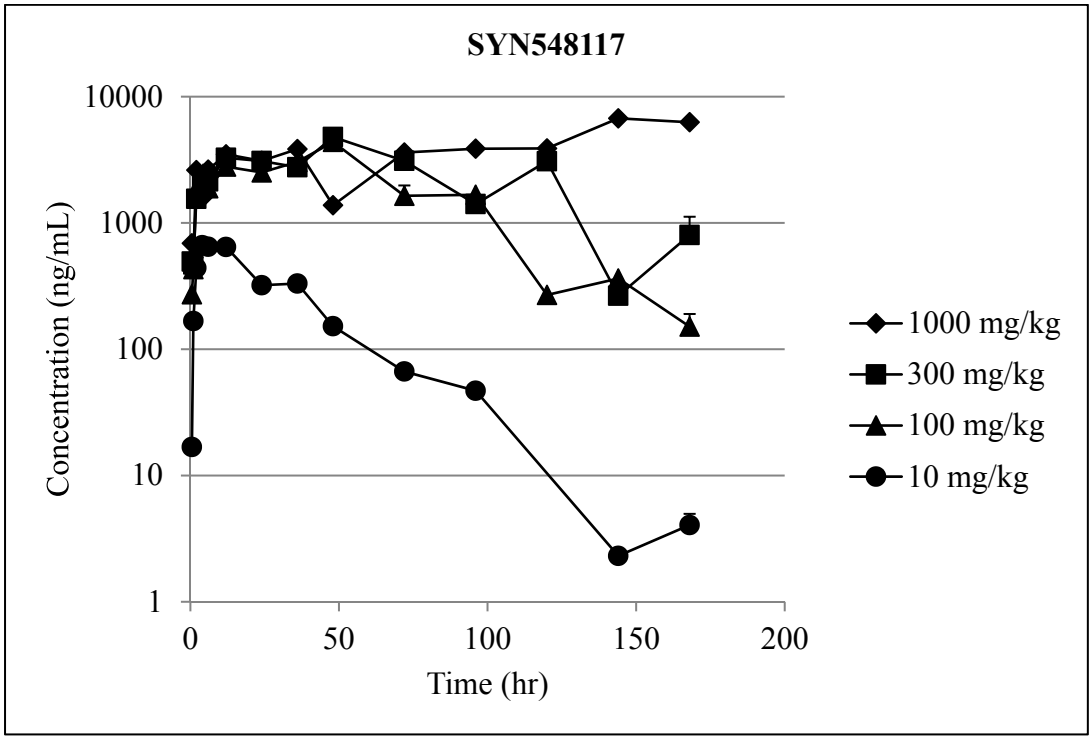


FIGURE 24 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548117 – Logarithmic Scale

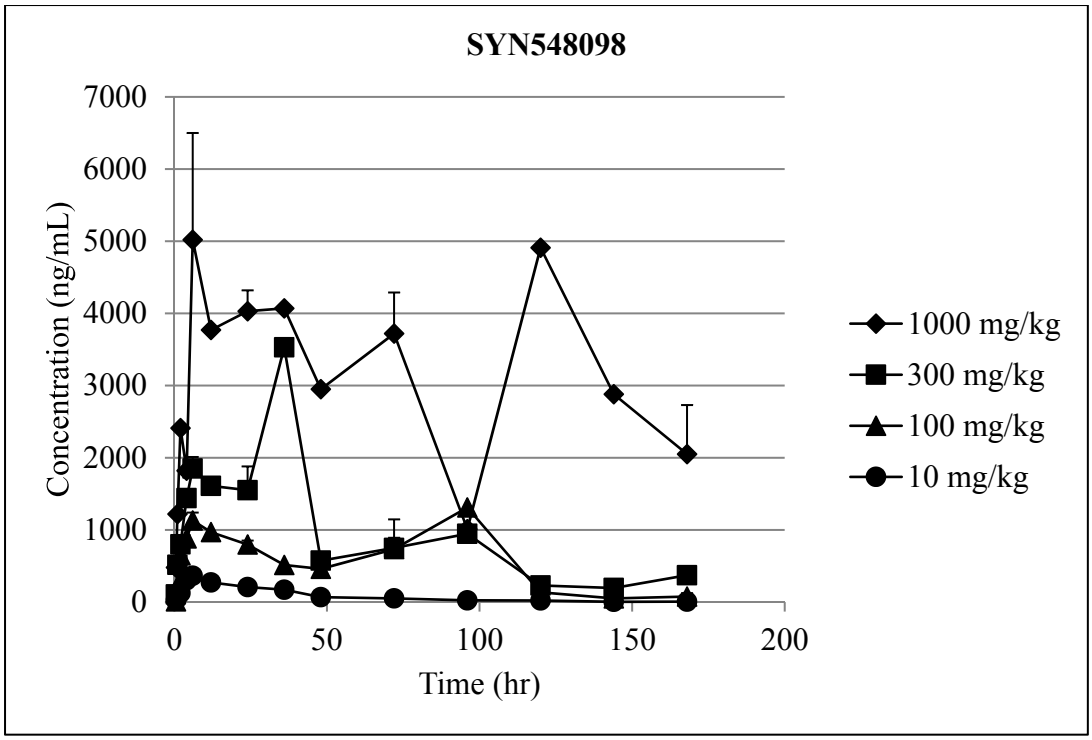


FIGURE 25 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548098

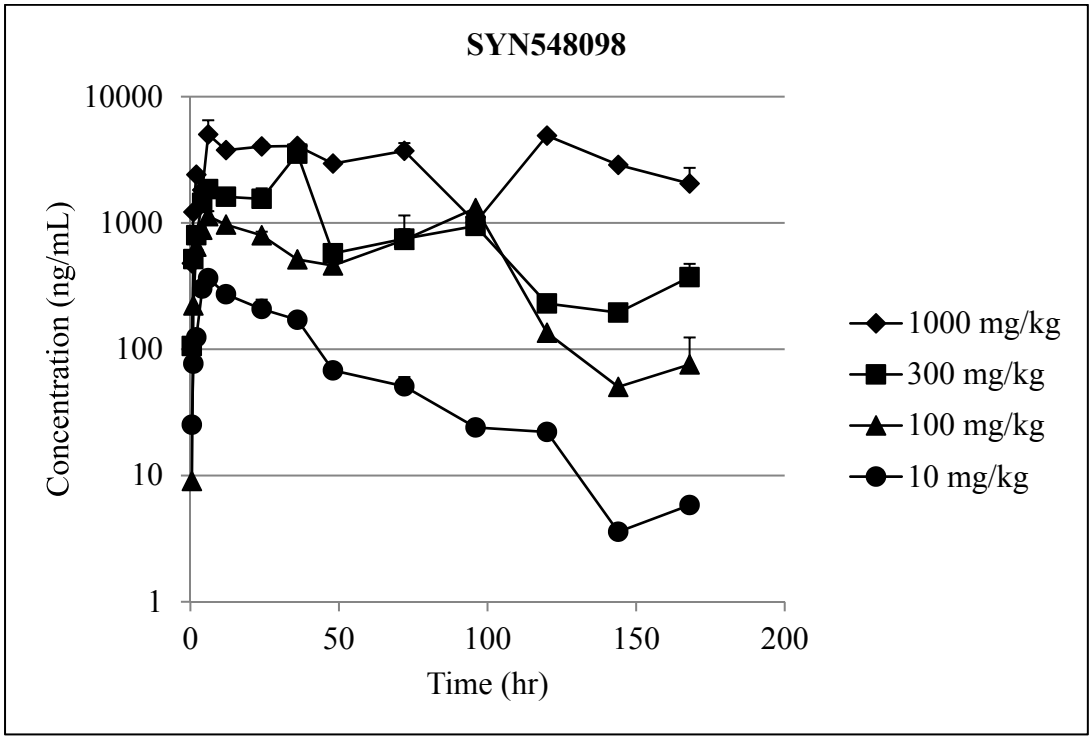


FIGURE 26 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548098 – Logarithmic Scale

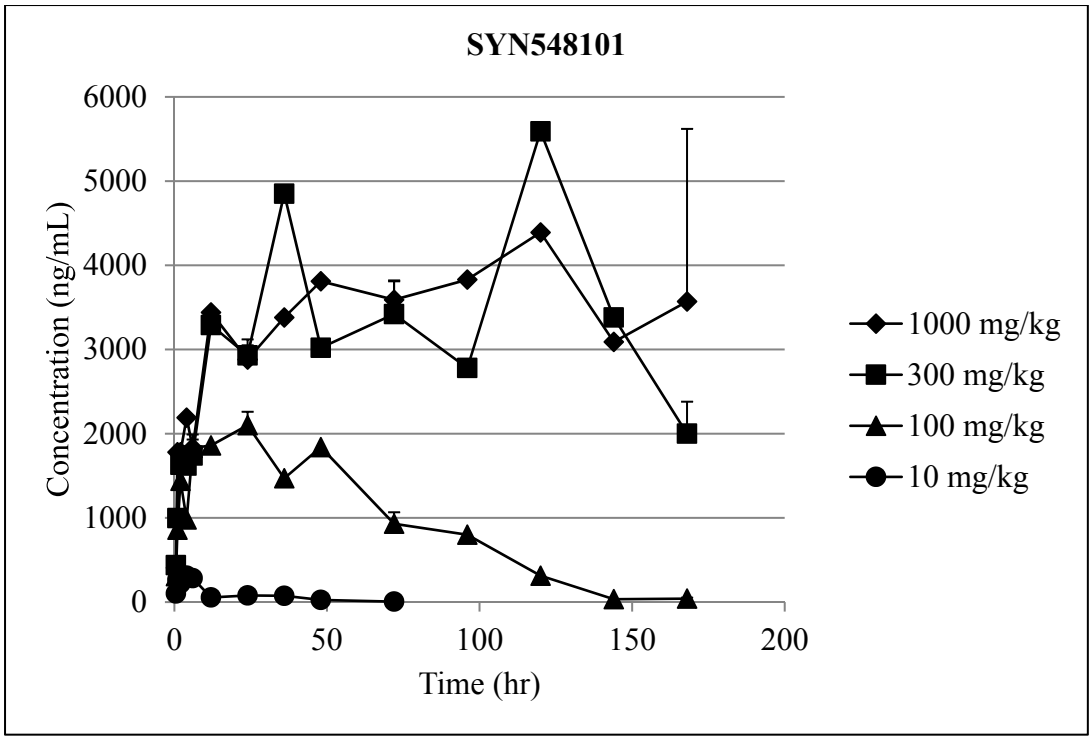


FIGURE 27 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548101

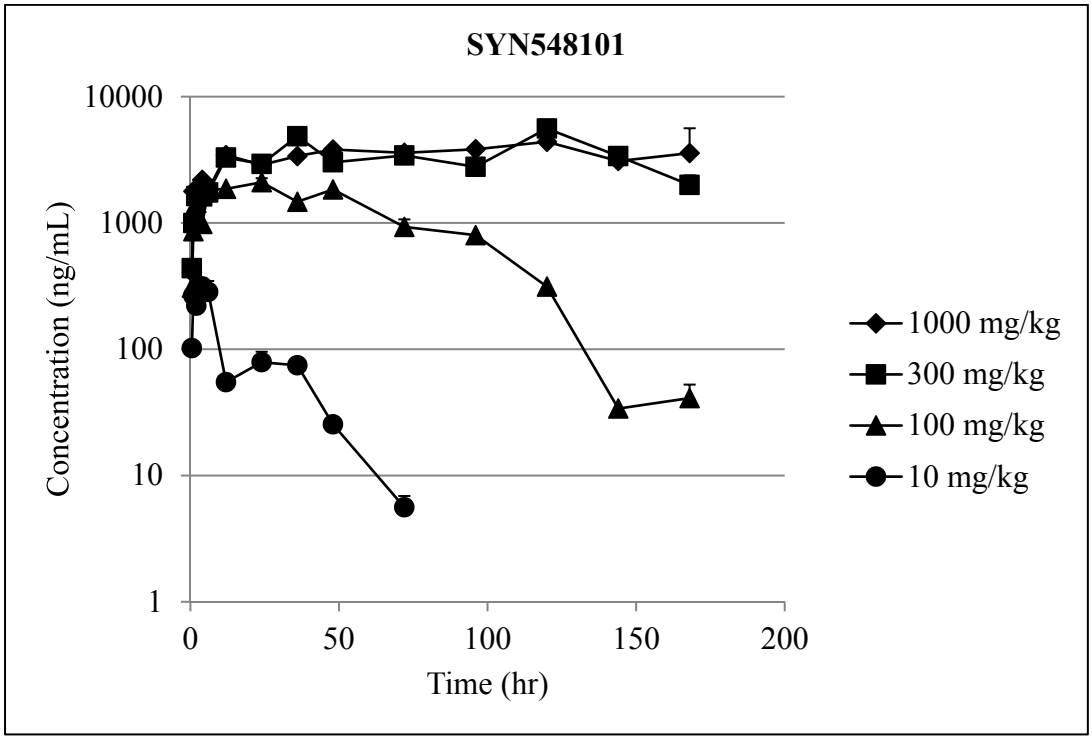


FIGURE 28 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548101– Logarithmic Scale

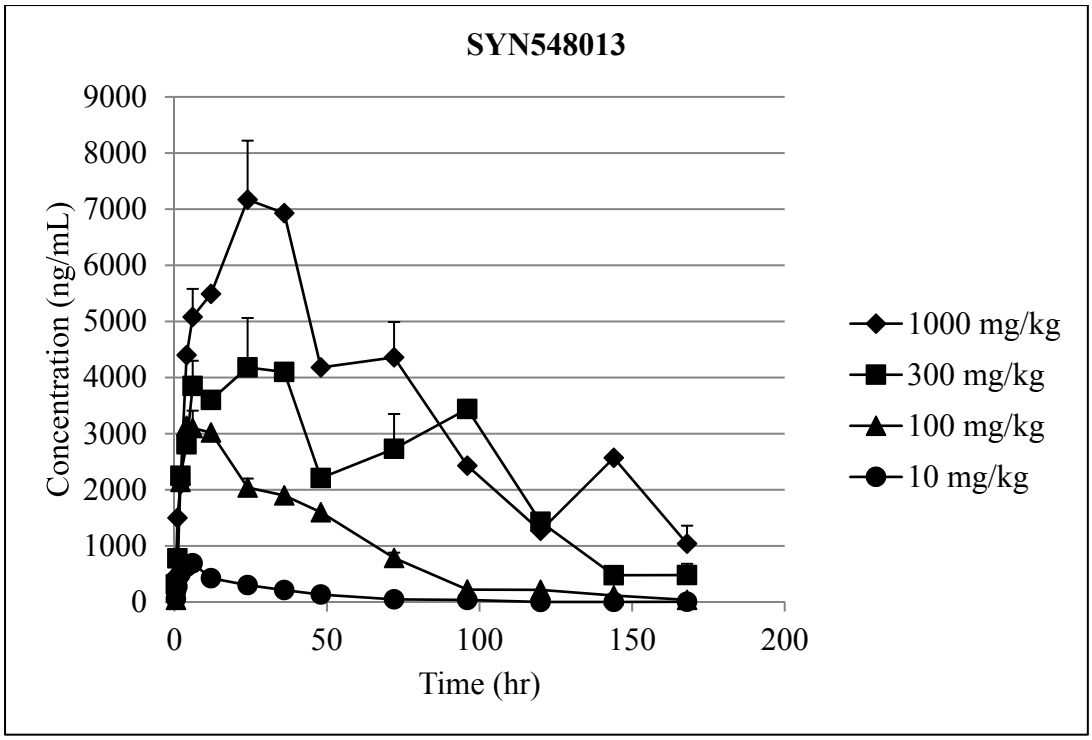


FIGURE 29 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548013

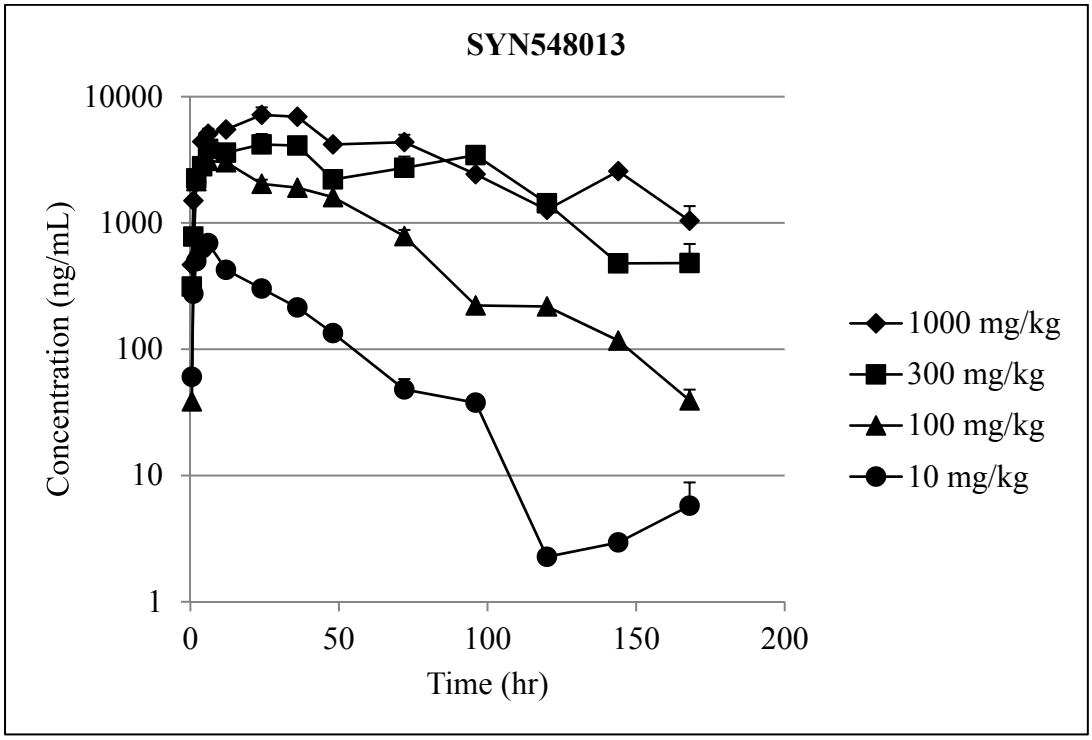


FIGURE 30 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548013– Logarithmic Scale

APPENDICES SECTION

APPENDIX 1 Protocol, Amendments, and Deviations

STUDY PROTOCOL

SYN547407, SYN546308, SYN548097, SYN548012, SYN548014, SYN548102, SYN548117, SYN548098, SYN548101, AND SYN548013 - SEVEN DAY ACUTE ORAL GAVAGE TOXICITY STUDY IN FEMALE WISTAR HAN RATS

TESTING FACILITY:
BATTELLE TOXICOLOGY WEST JEFFERSON
BLDG JM10
1425 PLAIN CITY-GEORGESVILLE ROAD
WEST JEFFERSON, OHIO 43162

SPONSOR:
SYNGENTA LTD.
BRACKNELL, BERKSHIRE, RG42 6EY UNITED KINGDOM

This protocol was approved by the Sponsor Study Monitor on January 7, 2014 SM.
Date Initials

APPROVED, BATTELLE:

Tessa R. Moir-Savitz, M.S. Study Director	January 7, 2014 Date
Dawn M. Fallacara, Ph.D., D.A.B.T. Toxicologist	Date
Barney R. Sparrow, Ph.D., D.A.B.T. Manager of Safety Assessment	Date
Katherine M. Gideon Katherine M. Gideon, METM Testing Facility Management, LSR	1-7-14 Date

APPROVED, SPONSOR:

Rizwan Nisar, Ph.D. Study Monitor	Date

To the best of our knowledge, this study does not unnecessarily duplicate any previous experiments.

This protocol was approved by the Sponsor Study Monitor on January 8, 2014 TAM.
Date Initials

APPROVED, BATTELLE:

Tessa R. Moir-Savitz, M.S.
Study Director
January 7 2014
Date

Dawn M. Fallacara, Ph.D., D.A.B.T.
Toxicologist
1/7/14
Date

Barney R. Sparrow, Ph.D., D.A.B.T.
Manager of Safety Assessment
01/07/14
Date

Katherine M. Gideon
Katherine M. Gideon, METM
Testing Facility Management, LSR
1-7-14
Date

APPROVED, SPONSOR:

Rizwan Nisar
Rizwan Nisar, Ph.D.
Study Monitor
08 - JANUARY 2014
Date

To the best of our knowledge, this study does not unnecessarily duplicate any previous experiments.

1.0 OBJECTIVE

The objective of this study is to determine the acute toxicity of SYN547407, SYN546308, SYN548097, SYN548012, SYN548014, SYN548102, SYN548117, SYN548098, SYN548101, and SYN548013 following a single oral (gavage) administration in female Wistar Han rats.

2.0 STUDY DESIGN RATIONALE

The test substance will be administered by oral gavage because the oral route may be the potential route of exposure of these compounds in humans.

3.0 REGULATORY COMPLIANCE

As intended, this study will not be conducted in compliance with the current version of the United States Environmental Protection Agency (EPA) 40 CFR Part 160 Good Laboratory Practice (GLP) Regulations for nonclinical laboratory studies.

All portions of this study performed at Battelle will adhere to the study protocol, protocol amendment(s), and applicable Battelle testing facility standard operating procedures (SOPs).

Any deviations from the study protocol or amendments will be documented in the study file and final report. Deviations from SOP will be documented in the study file.

4.0 SPONSOR AND STUDY MONITOR

4.1 Sponsor

Syngenta Ltd.
Jealott's Hill International Research Centre
Bracknell, Berkshire
RG42 6EY
United Kingdom

4.2 Sponsor's Study Monitor

Rizwan Nisar, Ph.D.
Telephone (office): +44 (0)1344 414786
E-mail: rizwan.nisar@SYNGENTA.COM

5.0 TESTING FACILITY AND TEST SITE

5.1 Testing Facility

Battelle Toxicology West Jefferson
Bldg JM10
1425 Plain City-Georgesville Road
West Jefferson, Ohio 43162

5.2 Testing Site

Battelle
Toxicology Battelle Columbus
505 King Avenue
Columbus, Ohio 43201

6.0 STUDY PERSONNEL

6.1 Study Personnel, Battelle

Study Director: Tessa R. Moir-Savitz, M.S.
Telephone: (614) 642-5320
Fax: (614) 427-6320
E-mail: moir-savitzt@battelle.org

Toxicologist: Dawn M. Fallacara, Ph.D., D.A.B.T.
Telephone: (614) 424-4591
Fax: (614) 458-5036
E-mail: fallacarad@battelle.org

Principle Investigator (Biological Sample Analysis and Toxicokinetic Evaluation):
Natalie South, B.S.
Telephone: (614) 424-7246
Fax: (614) 458-7246
Email: southn@battelle.org

7.0 PROPOSED STUDY SCHEDULE

The proposed dates for the following study events are listed below. The actual dates will be included in the study file.

Anticipated Animal Receipt:	January 7, 2014
Proposed Experimental Initiation Date:	Week of January 13, 2014
Necropsy:	Week of January 20, 2014

8.0 TEST SYSTEM

Species:	Rats
Strain:	Wistar Han
Source:	Charles River
Anticipated Age of Animals at Study Start:	Approximately 7 weeks
Number of Animals Required for Study:	210 female rats. A sufficient number of extra rats will be obtained to provide the required number of rats for the study.

8.1 Test System Justification

The rat is an accepted species that is commonly used in safety testing and toxicity studies. At this time, studies in laboratory animals provide the best available basis for extrapolation to humans and are required to support regulatory submissions. The number of animals to be used is the minimum number necessary to yield meaningful results.

9.0 ANIMAL CARE, HOUSING, AND ENVIRONMENTAL CONDITIONS

General procedures for animal care and housing will meet current AAALAC recommendations, current requirements stated in the "Guide for Care and Use of Laboratory Animals" (National Research Council, Current Edition), and will conform to the testing facility Standard Operating Procedures (SOP).

9.1 Quarantine and Acclimation

The rats will be quarantined for a minimum of seven days and acclimated in accordance with testing facility SOP. Only healthy rats will be placed on study.

9.2 Animal Housing

All animal housing and environmental conditions will follow testing facility SOP. The rats will be individually housed in polycarbonate cages with hardwood bedding.

9.3 Feed

Rats will be fed Purina Certified Rodent Diet (LabDiet 5002) *ad libitum*, according to testing facility SOP, except when removed from the home cage.

Analytical reports of each feed lot of Purina Certified Rodent Diet will be provided by the manufacturer. Analytical reports will be reviewed according to testing facility SOP to ensure acceptable standards, and freedom from levels of contaminants that may interfere with the purpose or conduct of the study. Analytical results will be retained in facility records.

9.4 Water

The rats will be provided fresh water *ad libitum* from the West Jefferson municipal water supply using an automatic watering system according to testing facility SOP except when removed from the cage. Water bottles may be supplemented as needed in accordance with testing facility SOP.

The water supply is analyzed periodically to ensure acceptable standards and freedom from levels of contaminants that may interfere with the purpose or conduct of the study. Analytical results will be retained in facility records.

10.0 TEST SUBSTANCES AND VEHICLES

For this study, the control substance will be referred to as the vehicle. Records of receipt and use of the test substances will be maintained. The receipt of the vehicle will not be documented.

10.1 Test Substances

Names:	SYN547407, SYN546308, SYN548097, SYN548012, SYN548014, SYN548102, SYN548117, SYN548098, SYN548101, and SYN548013
Supplier:	Syngenta Ltd.
Characterization:	Due to the discovery nature of this study, complete characteristics and/or stability of the test substances will be limited to known characteristics provided by the sponsor.
Stability:	Due to the discovery nature of this study, complete characteristics and/or stability of the test substances will be limited to known characteristics provided by the sponsor.
Storage Conditions:	Ambient temperature

10.2 Vehicle

Name:	0.5% Carboxymethylcellulose (CMC) in water
Lot Number:	To be documented in the study file and final report
Expiration Date:	To be documented in the study file and final report
Supplier:	Sigma or equivalent
Characterization:	As a commercially available material, the CMC will be characterized by its labeling.
Storage Conditions:	Ambient temperature

10.3 Reserve Samples

Reserve samples for archiving are not required on this study because the duration is less than 4 weeks. Disposition of any remaining unused bulk test substance will be conducted as the testing facility SOP. Disposition of the CMC will not be documented.

10.4 Formulation Preparation

Each formulation will be prepared at Battelle (West Jefferson) on the day of dosing by weighing a known quantity of test substance and combining it with the appropriate volume of vehicle. The target formulation concentrations are 1, 10, 30 and 100 mg/mL. The formulations will be stored at room temperature prior to use. Residual formulations will be stored in a refrigerator unit set to maintain 2 to 8°C and appropriately discarded per testing facility SOP.

10.5 Formulation Stability and Homogeneity

Homogeneity and formulation stability will not be confirmed.

10.6 Formulation Analysis

Formulation analysis (including concentration and homogeneity analysis) will not be performed.

10.7 Disposition of Unused Test Substances

Following the completion of in-life dose administration, unused neat test substances will be disposed of per testing facility SOP.

11.0 EXPERIMENTAL DESIGN

11.1 Group Assignment and Animal Identification

Two hundred and ten (210) female rats will be assigned to one of forty-two dose groups by body weight prior to dosing using a computer program (Provantis) which ensures similar group mean body weights.

Prior to group assignment, the rats will be identified by pre-test identification numbers (ID) on cage cards. After assignment groups, each rat will be identified by study cage card and ear tag with an animal identification number unique within the study. Each cage card will contain information including, but not limited to, study number, group assignment, and animal identification number.

If replacement rats are used, they will be assigned according to testing facility SOP. Animal replacement may only occur prior to dose administration.

The following table summarizes the groups, animal identification numbers, and dosing regimen.

Group	Test Substance[b]	Target Dosage (mg/kg)	Formulation Concentration (mg/mL)[a]	Number of Rats (females)	Animal IDs
1	SYN547407	10	1	5	1-5
2		100	10	5	6-10
3		300	30	5	11-15
4		1000	100	5	16-20
5	SYN546308	10	1	5	21-25
6		100	10	5	26-30
7		300	30	5	31-35
8		1000	100	5	36-40
9	SYN548097	10	1	5	41-45
10		100	10	5	46-50
11		300	30	5	51-55
12		1000	100	5	56-60
13	SYN548012	10	1	5	61-65
14		100	10	5	66-70
15		300	30	5	71-75
16		1000	100	5	76-80
17	SYN548014	10	1	5	81-85
18		100	10	5	86-90
19		300	30	5	91-95
20		1000	100	5	96-100
21	SYN548102	10	1	5	101-105
22		100	10	5	106-110
23		300	30	5	111-115
24		1000	100	5	116-120
25	SYN548117	10	1	5	121-125
26		100	10	5	126-130
27		300	30	5	131-135
28		1000	100	5	136-140
29	SYN548098	10	1	5	141-145
30		100	10	5	146-150
31		300	30	5	151-155
32		1000	100	5	156-160
33	SYN548101	10	1	5	161-165
34		100	10	5	166-170
35		300	30	5	171-175
36		1000	100	5	176-180

(table continued)

Group	Test Substance[b]	Target Dosage (mg/kg)	Formulation Concentration (mg/mL)[a]	Number of Rats (females)	Animal IDs
37	SYN548013	10	1	5	181-185
38		100	10	5	186-190
39		300	30	5	191-195
40		1000	100	5	196-200
41	Vehicle Control (0.5% CMC)	0	0	5	201-205
42	Vehicle Control (0.5% CMC)	0	0	5	206-210

a. Dose volume is 10 mL/kg.
b. Dose administration will occur over two days (five test substances per day). Five vehicle control rats will be administered 0.5% CMC on each day of dose administration.

11.2 Dose Administration

Each rat in all dose groups will receive a single oral administration of formulated test substance on Day 1. Dose volumes will be based on each rat's most recent individual body weight. Dose administration will occur over two days with five test substances administered per day. Five vehicle control rats will be administered 0.5% CMC on each day of dose administration.

11.3 Clinical Observations

Observations for moribundity and mortality will be performed on all rats in the study room twice daily, according to testing facility SOP. Detailed clinical observations will be conducted prior to group assignment and on Day 8 prior to necropsy.

For all surviving rats, cage-side clinical observations for evidence of toxicity will be conducted and recorded at least once daily from Day 1 through Day 7. On Day 1, cage-side clinical observations will be conducted at a target of 2 to 4 hours after dosing. Observations may be collected more often as clinical signs warrant.

11.4 Body Weight

Body weights will be recorded during pre-test for group assignment. Terminal body weights will be recorded on the day of necropsy (Day 8).

11.5 Specimen Collection for Triglyceride Analysis

Whole blood specimens (target volume of 0.2 mL) will be collected from all study rats 6, 24, 72, and 168 hours following dose administration from the tail vein and placed into tubes containing no anticoagulant (but may contain clot activators and/or serum separator gel). Actual blood collection times will be recorded. In the event that blood cannot be collected (a specimen cannot be obtained, the rat is moribund), analysis will be limited to those rats from which specimens have been successfully collected at that time point.

The whole blood specimens will be processed to serum and analyzed for triglycerides as per testing facility SOPs. Following analysis, the serum will be stored in a freezer set to maintain approximately -85 to -60°C until final disposition.

A clinical pathology report will be provided to the Study Director for inclusion in the final study report. This report will contain a comparison of the results from the individual test substances to the appropriate control group. A comparison between the different test substances will not be conducted.

11.6 Whole Blood Test Substance Level Determination

Whole blood specimens (target volume of 0.1 mL) will be collected from rats within Groups 1 through 40 at various time points during the 7-day study. Whole blood will be collected from the tail vein and placed into tubes containing tripotassium ethylenediaminetetraacetic acid (K_3·EDTA) as the anticoagulant. The target blood collection time points include 0.5, 1, 2, 4, 6, 12, 24, 36, 48, 72, 96, 120, 144, and 168 hours following dose administration according to the collection schedule below (per dose group, excluding the vehicle control groups). Actual times will be recorded. Immediately following blood collection, all whole blood specimens will be diluted at a 1:1 ratio using an appropriate volume of MilliQ water. Diluted whole blood specimens will be placed on dry ice until stored in a freezer set to maintain approximately -85 to -60°C. In the event that blood cannot be collected (a specimen cannot be obtained, the rat is moribund), analysis will be limited to those rats from which specimens have been successfully collected at that time point. Samples will be shipped to Battelle (King Avenue) on dry ice and stored in a freezer set to maintain approximately -80 to -60°C until analyzed.

Time Point (Hours Following Dose Administration)	Animal				
	A	B	C	D	E
0.5	X	-	-	-	-
1	-	X	-	-	-
2	-	-	X	-	-
4	-	-	-	X	-
6	$X^{(a)}$	$X^{(a)}$	$X^{(a)}$	$X^{(a)}$	$X^{(a)}$
12	-	-	-	-	X
24	$X^{(a)}$	$X^{(a)}$	$X^{(a)}$	$X^{(a)}$	$X^{(a)}$
36	X	-	-	-	-
48	-	X	-	-	-
72	$X^{(a)}$	$X^{(a)}$	$X^{(a)}$	$X^{(a)}$	$X^{(a)}$
96	-	-	X	-	-
120	-	-	-	X	-
144	-	-	-	-	X
168	$X^{(a)}$	$X^{(a)}$	$X^{(a)}$	$X^{(a)}$	$X^{(a)}$

X = Sample Collected; $X^{(a)}$ = Specimen collected concurrently with specimen for clinical chemistry; - = Specimen not required.

Specimens will be analyzed for test substance concentration by Battelle. All dose levels will be analyzed for the respective parent compound only.

11.7 Toxicokinetics

The toxicokinetic (TK) parameters determined will be observed C_{max}, observed T_{max}, and AUC only. Statistical analysis will be limited to simple descriptive statistics. TK data tables will be generated for inclusion in the overall report.

11.8 Necropsy

11.8.1 Unscheduled Necropsy

Gross necropsies will be performed according to testing facility SOP on all study rats that die or are terminated at an unscheduled interval. Moribund rats will be terminated using CO_2. All study rats found dead will be refrigerated, if needed, until necropsy. Blood will not be collected and tissues will not be weighed or processed from rats that are found dead or terminated prior to the end of the study.

11.8.2 Scheduled Necropsy

All surviving study rats will be subjected to gross necropsy on Day 8 according to

testing facility SOP. Rats will be humanely terminated by CO_2 inhalation according to facility SOP. Each necropsy will include examination of the external surface of the body and all orifices; the cranial, thoracic, abdominal and pelvic cavities, and their contents; and the collection of tissues.

Tissues listed below, when present, will be collected from all rats undergoing scheduled necropsy as per testing facility SOP. The adrenal glands and liver will be weighed and placed in 10 percent neutral buffered formalin (NBF). The duodenum and jejunum will be placed in 10 percent NBF.

Animal identification[a]	Jejunum
Adrenal glands (2)	Liver
Duodenum	

a. Collected but not processed.

11.9 Organ Weights

The following organs, when present, will be weighed for all scheduled necropsies. Paired organs will be weighed together. Absolute organ weight and organ-to-body weight ratios will be reported.

Adrenal glands (2)	Liver

11.10 Tissue Processing

The fixed tissues listed in section 11.8.2 will be processed to slides and stained with hematoxylin and eosin according to testing facility SOP for histopathologic examination.

11.11 Histopathologic Evaluation

The above tissue slides will be examined microscopically by a board-certified veterinary pathologist. An internal peer review will be performed according to testing facility SOP.

An anatomic pathology narrative will be included in the study file and final report. This report will contain a comparison of the results from the individual test substances to the appropriate control group. A comparison between the different test substances will not be conducted.

11.11 Computer Systems for Data Management

The following computer systems are used for toxicology data management:

Computer System	Version Number (or higher)	Manufacturer	Data Type
EMCS	3.10	Siemens	Animal Facility Environmental
T-Track	1.0.0	Battelle	Environmental Storage
Provantis	8.6.1.2	Instem	Animal Toxicology and Pathology Data Management

Note: Actual version numbers will be included in the final report

12.0 STATISTICAL ANALYSIS

All appropriate quantitative in-life data collected at Battelle using the Provantis system will be analyzed for test article effects by parametric or nonparametric analysis of variance (ANOVA). For parametric analysis, normality will be determined by the Shapiro-Wilks test and homogeneity of variances will be determined by Levene's test. Both tests will be conducted at the 0.05 level of significance. Data may be log-transformed to meet parametric assumptions. For data determined to be normally distributed and homogenous among groups, an ANOVA F-test will be used to determine whether there are differences among the group means. If the ANOVA F-test is significant at the 0.05 level, then tests for differences between the control and each of the comparison groups will be conducted using Dunnett's test, which adjusts for multiple comparisons. For data that are not normally-distributed and/or non-homogenous, analogous nonparametric methods will be used. For the nonparametric analysis, a Kruskal-Wallis test will be used to determine whether there are differences among the group means. If the Kruskal-Wallis test is significant at the 0.05 level, then tests for differences between the control and each of the comparison groups will be conducted using Wilcoxon tests and the Bonferroni-Holm method to correct for multiple comparisons. Statistical significance for each comparison will be reported at the 0.05 level after accounting for multiple comparisons.

Two vehicle control groups are included in the study design, as dose administration occurs over two days. Comparisons will occur between a single control (vehicle) group (either Group 41 or Group 42) and each comparison group by day of dose administration. The vehicle control groups used in each analysis will be specified in the report and in report tables.

13.0 REPORTING

A draft final report will be prepared and submitted to the Sponsor. The Sponsor shall submit final comments, if any, on the draft report to the Study Director. Battelle will submit a final report to the Sponsor following receipt of the Sponsor's final comments.

14.0 STORAGE OF STUDY MATERIALS, AND RECORDS RETENTION

All records required to reconstruct the study and the final report will be maintained under the director of Battelle according to testing facility SOP.

Wet tissues, embedded tissues, and histopathology slides will be archived under the direction of Battelle.

PROTOCOL AMENDMENT NUMBER 1

SYN547407, SYN546308, SYN548097, SYN548012, SYN548014, SYN548102, SYN548117, SYN548098, SYN548101, AND SYN548013 - SEVEN DAY ACUTE ORAL GAVAGE TOXICITY STUDY IN FEMALE WISTAR HAN RATS

1. **Part to be changed:** Section 6.1 Study Personnel, Battelle

   Effective date: January 20, 2014

   Add: Principle Investigator (Histopathology):
   Tara P. Arndt, D.V.M, D.A.C.V.P.
   Telephone: (614) 642-5220
   Fax: (614) 458-5220
   Email: arndtt@battelle.org

   Justification: The tissue processing and histopathologic examination of the tissues will be conducted at the testing site listed in section 5.2 of the protocol (505 King Avenue; Columbus, Ohio 43201).

2. **Part to be changed:** Section 11.8.2 Scheduled Necropsy

   Effective date: January 20, 2014

   Add to table: Brain, Spinal Cord (thoracolumbar segment)

   Justification: Due to the number of animals that were observed with seizure-like activity, the brain and spinal cord will be collected and preserved at the time of scheduled necropsy.

3. **Part to be changed:** Section 11.8.2 Scheduled Necropsy

   Effective date: January 20, 2014

   Change from: The duodenum and jejunum will be placed in 10 percent NBF.

   Change (in bold) to: The **brain, spinal cord,** duodenum, and jejunum will be placed in 10 percent NBF.

Justification: Due to the number of animals that were observed with seizure-like activity, the brain and spinal cord will be collected and preserved at the time of scheduled necropsy.

4. **Part to be changed:** Section 11.10 Tissue Processing

Effective date: January 20, 2014

Change from: The fixed tissues listed in section 11.8.2 will be processed to slides and stained with hematoxylin and eosin according to testing facility SOP for histopathologic examination.

Change (in bold) to: The fixed tissues **(adrenal glands, duodenum, jejunum, and liver)** will be processed to slides and stained with hematoxylin and eosin according to testing facility SOP for histopathologic examination. **The brain and spinal cord will be processed and evaluated at the request of the Sponsor and added by amendment. If the brain and spinal cord are not processed or evaluated, these tissues will be discarded at the discretion of the Sponsor.**

Justification: The brain and spinal cord will be retained for histopathologic evaluation upon request by the Sponsor.

APPROVED, BATTELLE:

Tessa R. Moir-Savitz, M.S.
Study Director — Date: 1-20-14

Barney R. Sparrow, Ph.D., D.A.B.T.
Manager — Date: 01/22/14

APPROVED, SPONSOR:

Rizwan Nisar, Ph.D.
Study Monitor — Date: 22-01-14

SUMMARY OF PROTOCOL DEVIATIONS

DR-272-WJ:

As per the protocol, only gross necropsy was to be performed on any animal that was moribund or found dead prior to the end of the study. Animals #119 and #137 were observed as moribund on the day of scheduled necropsy; however, the adrenal glands and liver were weighed. In addition, the adrenal glands, duodenum, jejunum, brain, spinal cord (thoracolumbar segment), and liver were placed into 10% neutral buffered formalin (NBF).

This deviation had a positive impact on the study. Significant findings were identified during the gross necropsy of these animals and the histopathological evaluation of these tissues was reported.

DR-273-WJ:

The following animals were bled for whole blood test substance level determinations at time points that were not assigned to their subset:

Animal No.	Additional Collection Timepoint	Replacement for Animal No.
169 (Subset D)	36 hour post-dose	166 (Subset A)
21 (Subset A)	120 hour post-dose	24 (Subset D)
26 (Subset A)	120 hour post-dose	29 (Subset D)
41 (Subset A)	120 hour post-dose	44 (Subset D)
180 (Subset E)	120 hour post-dose	179 (Subset D)
36 (Subset A)	144 hour post-dose	40 (Subset E)
116 (Subset A)	144 hour post-dose	120 (Subset E)

Animals 166, 24, 29, 44, 179, 40, and 120 died prior to their scheduled blood collection. In order to not lose entire time points needed to build the TK profiles, blood was collected from another animal with the same group.

This deviation had a positive impact on the study as blood was successfully collected at these time points from the replacement animals and submitted for TK analysis.

DR-293-WJ:

As per the protocol, immediately following blood collection, all whole blood specimens were to be diluted at a 1:1 ratio using an appropriate volume of MilliQ water. The dilution of the specimens collected 120 hours post dose administration from animals 154, 159, 164, and 169 cannot be verified.

This deviation had no impact on the scientific integrity of the study. The volume of these specimens was investigated and determined to be similar to diluted specimens of known blood:water ratio suggesting that the specimens were processed appropriately at the time of collection though the actual volumes were not documented. For calculation purposes, the whole blood specimens were assumed to be diluted at a 1:1 ratio with MilliQ water, which does not affect the overall TK conclusions.

APPENDIX 2 Dose Formulation Methods

DOCUMENTATION FORM FOR THE FORMULATION OF VARIOUS SYN COMPOUNDS IN 0.5% CARBOXYMETHYLCELLULOSE SODIUM SALT IN DI WATER

1.0 PROCEDURES

1.1 General Instructions

Document all materials and equipment on the DOC Form.

Initial and date the top of the page on the day that the work for that page was begun. Other entries made by the formulation technician on a later date or entries made by another person will be initialed and dated near the data entry.

1.2 Preformulation Activities

Transfer any required materials and equipment to a low hazard laboratory.

1.3 Labeling

The formulation technician will prepare labels containing all necessary information for the samples. Each label will be marked with the appropriate color code and indicated in Table 1.

Table 1. Study Color Codes

Test Substance	Target Concentration (mg/mL)	Color Code (Top/Bottom)
Control (Vehicle)	0	White
SYN547407	1	White / Black
SYN547407	10	Blue / Black
SYN547407	30	Yellow / Black
SYN547407	100	Red / Black
SYN546308	1	White / Grey
SYN546308	10	Blue / Grey
SYN546308	30	Yellow / Grey
SYN546308	100	Red / Grey
SYN548097	1	White / Tan
SYN548097	10	Blue / Tan
SYN548097	30	Yellow / Tan
SYN548097	100	Red / Tan
SYN548012	1	White / Lilac
SYN548012	10	Blue / Lilac
SYN548012	30	Yellow / Lilac
SYN548012	100	Red / Lilac
SYN548014	1	White / Blue
SYN548014	10	Blue / Blue
SYN548014	30	Yellow / Blue
SYN548014	100	Red / Blue

Attach one label to one 30-mL clear glass straight sided jar with Teflon-lined cap.

1.4 Material

See Table 2 for all required chemicals and vehicles. Check the labels carefully to ensure that they are not expired and are the proper purity/grade. Document the supplier and lot number as appropriate on the DOC FORM.

Table 2. Materials

Chemical	Use	Supplier	Lot Number	Expiration Date
SYN547407	Test Substance	Syngenta		None Given
SYN546308	Test Substance	Syngenta		None Given
SYN548097	Test Substance	Syngenta		None Given
SYN548012	Test Substance	Syngenta		None Given
SYN548014	Test Substance	Syngenta		None Given
Carboxymethylcellulose Sodium Salt, Medium Viscosity (Cat# C4888)	Vehicle	Sigma		
Deionized Water (DI)	Vehicle	Battelle	RM JM10-1-418	NA

1.5 Equipment

See Table 3 for all required major pieces of equipment. Check calibration of all equipment requiring calibration (e.g., balances) to ensure it is current. Document the identity of each piece of equipment on the Doc Form.

Table 3. Equipment

Equipment	Use	ID	Calibration Due
Analytical Balance	Weigh Test substance		
Analytical Balance	Weigh Test substance		
Top Loading Balance	Weigh Test substance/Vehicle		
Weight Set	Calibrate Balance		
Weight Set	Calibrate Balance		
Thermometer	Prepare Vehicle		
Silverston Mixer	Prepare Formulation		
Spatulas	Prepare Formulation	NA	NA
Beaker or Carboy	Prepare Vehicle	NA	NA
Weighing Containers	Weigh Test Material	NA	NA
Stirbar/Stirplate/Vortex	Prepare Formulation	NA	NA
Mixing/Dosing Container (30-mL clear glass straight-sided jar with Teflon-lined lid)	Dosing Container	NA	NA

1.6 Verification of Equipment

Calibrate the balances according to the current SOP on using balances. Document the actual balance calibration weights in Table 4. Proceed with the formulation only if the balance passes calibration.

Table 4. Verification

Top Loading Balance		
Nominal Weight (g)	**Acceptance Range (g)**	**Actual Weights (g)**
1	0.98 to 1.02	
5000	4900 to 5100	
Analytical Balance		
Nominal Weight (g)	**Acceptance Range (g)**	**Actual Weights (g)**
0.05	0.0490 to 0.0510	
100	98.0000 to 102.0000	

1.7 Preparation of Vehicle

Prepare the formulation by following the instructions in this section.

Table 5. Vehicle Formulation

Target Conc. (%)	Target Amount of carboxymethylcellulose sodium salt (g)	Actual Amount of carboxymethylcellulose sodium salt (g)	Final Volume with DI Water (mL)
0.5% carboxymethylcellulose sodium salt in DI Water	10.00 ± 0.10		2000

Weigh the amounts of sodium carboxyl methylcellulose in a weighing container according to Table 5.

Heat approximately half of the final volume of deionized water to approximately 60ºC.

Transfer the heated water into the calibrated carboy.

Add the weighed sodium carboxyl methylcellulose into the calibrated carboy with the heated water and rinse at least three times with heated DI water.

Dilute to ~98% of volume with DI water.

Mix with an over head stirrer until a solution is formed. Overnight stirring may be necessary.

Once a solution is formed, dilute to final volune with deionized water (DI) and stir for approximately 10 minutes.

If previously prepared reference study number and date: _____.

Store at ~5°C for one month.

1.8 Dispensing of Milli-Q Water

Dispense approximately 200 mL of Milli-Q water into twenty 250-mL amber glass bottles and label with an expiration of 30 days from the date aliquoted.

1.9 Formulations

Prepare the formulation by following the instructions in this section. Prepare each test substance separately from low to high concentration and decontaminate between substances.

The test substance will be pre-weighed into each dosing container for each batch prior to the actual addition of the vehicle. The vehicle will be added on the day of dosing and mixed.

Weigh the bulk bottle of test substance on a top loading balance and record the weight in Table 7.

Zero/tare a dosing container on an appropriate balance.

Weigh the appropriate amount of test substance according to Table 6.

Table 6. Gavage Formulation

Target Conc. mg/mL	Test Substance	Batch		Target Test substance Range (g)	Actual Test substance Weighed (g)	Volume of Vehicle (mL)
1	SYN547407	___-SYN-	1	0.02000 ± 0.00020		20
10			2	0.2000 ± 0.0020		20
30			3	0.6000 ± 0.0060		20
100			4	2.0000 ± 0.0200		20
1	SYN546308		5	0.02000 ± 0.00020		20
10			6	0.2000 ± 0.0020		20
30			7	0.6000 ± 0.0060		20
100			8	2.0000 ± 0.0200		20
1	SYN548097		9	0.02000 ± 0.00020		20
10			10	0.2000 ± 0.0020		20
30			11	0.6000 ± 0.0060		20
100			12	2.0000 ± 0.0200		20
1	SYN548012		13	0.02000 ± 0.00020		20
10			14	0.2000 ± 0.0020		20
30			15	0.6000 ± 0.0060		20
100			16	2.0000 ± 0.0200		20
1	SYN548014		17	0.02000 ± 0.00020		20
10			18	0.2000 ± 0.0020		20
30			19	0.6000 ± 0.0060		20
100			20	2.0000 ± 0.0200		20

Date Test Substance Weighed:_____.

Date Vehicle added and mixed:_____.

Table 7. Gavage Test substance Accountability

Target Conc. (mg/mL)	Batch/ Group	Initial Weight of Bulk TA Container (g)	Final Weight of Bulk TA Container (g)	B (Calculated Difference) (g)	A (Target Weight)[a](g)	% Difference[b]
1	1				0.02000	
10	2				0.2000	
30	3				0.6000	
100	4				2.0000	
1	5				0.02000	
10	6				0.2000	
30	7				0.6000	
100	8				2.0000	
1	9				0.02000	
10	10				0.2000	
30	11				0.6000	
100	12				2.0000	
1	13				0.02000	
10	14				0.2000	
30	15				0.6000	
100	16				2.0000	
1	17				0.02000	
10	18				0.2000	
30	19				0.6000	
100	20				2.0000	

a. From Table 6.
b. Calculate the absolute value of the % difference of test substance used for each formulation by the following equation: $\left|([B-A]/A)\right| * 100$.

Weigh the bulk bottle of test substance on a top loading balance and record the final weight in Table 7.

Calculate the absolute value of the percent difference. If it is 1% or less, proceed with the formulation. If it is greater than 1%, notify the formulation supervisor or designee immediately. By signing the technical review, the technical reviewer has

determined the percent differences are acceptable. A comment will be made if they are not.

Measure the appropriate final volume of vehicle using a syringe and add the vehicle slowly to the container with test substance according to Table 6 .

Stir slowly on a stir plate to incorporate the test substance into the vehicle. Sonicate if necessary.

Homogenize for approximately 5 minutes using a Silverson Mixer at a setting of 7000 rpm using the appropriate probe for the size batch.

Note probe size used for each batch:_____

Stir until visibly a homogeneous suspension is achieved.

Leave stir bar in each container.

1.10 0.5% CMC Control Formulation

Aliquot 20 mL of vehicle into a clear glass dosing bottle. Add a stir bar and cap.

Batch: __-CTRL-41.

1.11 Decontamination of Low Hazard Formulation Work Area and Equipment

Use Acetone as the decontamination solvent.

Rinse all contaminated glassware and utensils with solvent at least three times or until there is no visible contamination. Dispose of the rinse into the hazardous waste can.

Wash all rinsed glassware and utensils in hot soapy water. Rinse with hot water then deionized water.

Wipe down the balance and test substance container with solvent-dampened paper towels and remove them from the hood. It may be necessary to remove the balance pan.

Dispose of all contaminated paper towels and absorbent paper appropriately.

Wipe down the counter and walls of the hood with solvent-dampened paper towels. The acetone may be applied directly to the hood surfaces before wiping down with paper towels.

1.12 Distribution of Formulations

Place labels identifying the doses on the current form and complete the form.

These samples are to be released at room temperature and stored 2-8ºC for 1 day.

2.0 SIGNATURES

Documentation
Form Approved by: _____ Date: _____

Technical Review: _____ Date: _____

DOCUMENTATION FORM FOR THE FORMULATION OF VARIOUS SYN COMPOUNDS IN 0.5% CARBOXYMETHYLCELLULOSE SODIUM SALT IN DI WATER

1.0 PROCEDURES

1.1 General Instructions

Document all materials and equipment on the DOC Form.

Initial and date the top of the page on the day that the work for that page was begun. Other entries made by the formulation technician on a later date or entries made by another person will be initialed and dated near the data entry.

1.2 Preformulation Activities

Transfer any required materials and equipment to a low hazard laboratory.

1.3 Labeling

The formulation technician will prepare labels containing all necessary information for the samples. Each label will be marked with the appropriate color code and indicated in Table 1.

Table 1. Study Color Codes

Test Substance	Target Concentration (mg/mL)	Color Code (Top/Bottom)
Control (Vehicle)	0	White
SYN548102	1	White / Green
SYN548102	10	Blue / Green
SYN548102	30	Yellow / Green
SYN548102	100	Red / Green
SYN548117	1	White / Yellow
SYN548117	10	Blue / Yellow
SYN548117	30	Yellow / Yellow
SYN548117	100	Red / Yellow
SYN548098	1	White / Orange
SYN548098	10	Blue / Orange
SYN548098	30	Yellow / Orange
SYN548098	100	Red / Orange
SYN548101	1	White / Red
SYN548101	10	Blue / Red
SYN548101	30	Yellow / Red
SYN548101	100	Red / Red
SYN548103	1	White / Black
SYN548103	10	Blue / Black
SYN548103	30	Yellow / Black
SYN548103	100	Red / Black

Attach one label to one 30-mL clear glass straight-sided jar with Teflon-lined cap.

1.4 Material

See Table 2 for all required chemicals and vehicles. Check the labels carefully to ensure that they are not expired and are the proper purity/grade. Document the supplier and lot number as appropriate on the DOC FORM.

Table 2. Materials

Chemical	Use	Supplier	Lot Number	Expiration Date
SYN548102	Test Substance	Syngenta		None Given
SYN548117	Test Substance	Syngenta		None Given
SYN548098	Test Substance	Syngenta		None Given
SYN548101	Test Substance	Syngenta		None Given
SYN548103	Test Substance	Syngenta		None Given
Carboxymethylcellulose Sodium Salt, Medium Viscosity (Cat# C4888)	Vehicle	Sigma		
Deionized Water (DI)	Vehicle	Battelle	RM JM-10-1-418	NA

1.5 Equipment

See Table 3 for all required major pieces of equipment. Check calibration of all equipment requiring calibration (e.g., balances) to ensure it is current. Document the identity of each piece of equipment on the Doc Form.

Table 3. Equipment

Equipment	Use	ID	Calibration Due
Analytical Balance	Weigh Test substance		
Analytical Balance	Weigh Test substance		
Top Loading Balance	Weigh Test substance/Vehicle		
Weight Set	Calibrate Balance		
Weight Set	Calibrate Balance		
Thermometer	Prepare Vehicle		
Silverson Mixer	Prepare Formulation		
Spatulas	Prepare Formulation	NA	NA
Beaker or Carboy	Prepare Vehicle	NA	NA
Weighing Containers	Weigh Test Material	NA	NA
Stirbar/Stirplate/Vortex	Prepare Formulation	NA	NA
Mixing/Dosing Container (30-mL clear glass straight-sided jar with Teflon-lined lid)	Dosing Container	NA	NA

1.6 Verification of Equipment

Calibrate the balances according to the current SOP on using balances. Document the actual balance calibration weights in Table 4. Proceed with the formulation only if the balance passes calibration.

Table 4. Verification

Top Loading Balance		
Nominal Weight (g)	**Acceptance Range (g)**	**Actual Weights (g)**
1	0.98 to 1.02	
5000	4900 to 5100	
Analytical Balance		
Nominal Weight (g)	**Acceptance Range (g)**	**Actual Weights (g)**
0.05	0.0490 to 0.0510	
100	98.0000 to 102.0000	
Analytical Balance		
Nominal Weight (g)	**Acceptance Range (g)**	**Actual Weights (g)**
0.05	0.0490 to 0.0510	
100	98.0000 to 102.0000	

1.7 Preparation of Vehicle

Prepare the formulation by following the instructions in this section.

Table 5. Vehicle Formulation

Target Conc. (%)	Target Amount of carboxymethylcellulose sodium salt (g)	Actual Amount of carboxymethylcellulose sodium salt (g)	Final Volume with DI Water (mL)
0.5% carboxymethylcellulose sodium salt in DI Water	10.00 ± 0.10		2000

Weigh the amounts of sodium carboxyl methylcellulose in a weighing container according to Table 5.

Heat approximately half of the final volume of deionized water to approximately 60ºC.

Transfer the heated water into the calibrated carboy.

Add the weighed sodium carboxyl methylcellulose into the calibrated carboy with the heated water and rinse at least three times with heated DI water.

Dilute to ~98% of volume with DI water.

Mix with an over head stirrer until a solution is formed. Overnight stirring may be necessary.

Once a solution is formed, dilute to final volune with deionized water (DI) and stir for approximately 10 minutes.

If previously prepared reference study number and date: _____.

Store at ~5°C for one month.

1.8 Dispensing of Milli-Q Water

Dispense approximately 200 mL of Milli-Q water into twenty 250-mL amber glass bottles and label with an expiration of 30 days from the date aliquoted.

1.9 Formulations

Prepare the formulation by following the instructions in this section. Prepare each test substance separately from low to high concentration and decontaminate between substances.

The test substance will be pre-weighed into each dosing container for each batch prior to the actual addition of the vehicle. The vehicle will be added on the day of dosing and mixed.

Weigh the bulk bottle of test substance on a top loading balance and record the weight in Table 7.

Zero/tare a dosing container on an appropriate balance.

Weigh the appropriate amount of test substance according to Table 6.

Table 6. Gavage Formulation

Target Conc. mg/mL	Test Substance	Batch		Target Test Article Range (g)	Actual Test Article Weighed (g)	Volume of Vehicle (mL)
1	SYN548102	___-SYN-	21	0.02000 ± 0.00020		20
10			22	0.2000 ± 0.0020		20
30			23	0.6000 ± 0.0060		20
100			24	2.0000 ± 0.0200		20
1	SYN548117		25	0.02000 ± 0.00020		20
10			26	0.2000 ± 0.0020		20
30			27	0.6000 ± 0.0060		20
100			28	2.0000 ± 0.0200		20
1	SYN548098		29	0.02000 ± 0.00020		20
10			30	0.2000 ± 0.0020		20
30			31	0.6000 ± 0.0060		20
100			32	2.0000 ± 0.0200		20
1	SYN548101		33	0.02000 ± 0.00020		20
10			34	0.2000 ± 0.0020		20
30			35	0.6000 ± 0.0060		20
100			36	2.0000 ± 0.0200		20
1	SYN548103		37	0.02000 ± 0.00020		20
10			38	0.2000 ± 0.0020		20
30			39	0.6000 ± 0.0060		20
100			40	2.0000 ± 0.0200		20

Date Test Substance Weighed:_____.

Date Vehicle added and mixed:_____.

Table 7. Gavage Test Article Accountability

Target Conc. (mg/mL)	Batch/ Group	Initial Weight of Bulk TA Container (g)	Final Weight of Bulk TA Container (g)	B (Calculated Difference) (g)	A (Target Weight)[a](g)	% Difference[b]
1	21				0.02000	
10	22				0.2000	
30	23				0.6000	
100	24				2.0000	
1	25				0.02000	
10	26				0.2000	
30	27				0.6000	
100	28				2.0000	
1	29				0.02000	
10	30				0.2000	
30	31				0.6000	
100	32				2.0000	
1	33				0.02000	
10	34				0.2000	
30	35				0.6000	
100	36				2.0000	
1	37				0.02000	
10	38				0.2000	
30	39				0.6000	
100	40				2.0000	

a. From Table 6.
b. Calculate the absolute value of the % difference of test article used for each formulation by the following equation: $\left|([B-A]/A)\right| * 100$.

Weigh the bulk bottle of test article on a top loading balance and record the final weight in Table 7.

Calculate the absolute value of the percent difference. If it is 1% or less, proceed with the formulation. If it is greater than 1%, notify the formulation supervisor or designee immediately. By signing the technical review, the technical reviewer has

determined the percent differences are acceptable. A comment will be made if they are not.

Measure the appropriate final volume of vehicle using a syringe and add the vehicle slowly to the container with test substance according to Table 6 .

Stir slowly on a stir plate to incorporate the test substance into the vehicle. Sonicate if necessary.

Homogenize for approximately 5 minutes using a Silverson Mixer at a setting of 7000 rpm using the appropriate probe for the size batch.

Note probe size used for each batch:_____

Stir until visibly a homogeneous suspension is achieved.

Leave stir bar in each container.

1.10 0.5% CMC Control Formulation

Aliquot the appropriate 20 mL of vehicle into a clear glass dosing bottle.

Batch: __-CTRL-42.

1.11 Decontamination of Low Hazard Formulation Work Area and Equipment

Use Acetone as the decontamination solvent.

Rinse all contaminated glassware and utensils with solvent at least three times or until there is no visible contamination. Dispose of the rinse into the hazardous waste can.

Wash all rinsed glassware and utensils in hot soapy water. Rinse with hot water then deionized water.

Wipe down the balance and test article container with solvent-dampened paper towels and remove them from the hood. It may be necessary to remove the balance pan.

Dispose of all contaminated paper towels and absorbent paper appropriately.

Wipe down the counter and walls of the hood with solvent-dampened paper towels. The acetone may be applied directly to the hood surfaces before wiping down with paper towels.

1.12 Distribution of Formulations

Place labels identifying the doses on the current form and complete the form.

These samples are to be released at room temperature and stored 2-8ºC for 1 day.

2.0 SIGNATURES

Documentation
Form Approved by: _____ Date: _____

Technical Review: _____ Date: _____

APPENDIX 3 Individual Animal Data

TABLE 3-1 Individual Body Weights (g)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN547407 10 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
1	-	145.190	-	-	-	157.290
2	-	137.570	-	-	-	168.530 >
3	-	141.890	-	-	-	172.760 >
4	-	146.930	-	-	-	170.700 >
5	-	154.090	-	-	-	173.720 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN547407 100 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
6	-	138.590	-	-	-	162.330 >
7	-	143.150	-	-	-	168.230 >
8	-	142.530	-	-	-	167.760 >
9	-	147.520	-	-	-	177.910 >
10	-	153.120	-	-	-	184.230 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN547407 300 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
11	-	148.800	-	-	-	167.600 >
12	-	143.450	-	-	-	163.840 >
13	-	152.510	-	-	-	172.330 >
14	-	135.450	-	-	-	163.190 >
15	-	142.080	-	-	-	161.100 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN547407 1000 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
16	-	144.730	-	-	-	152.930
17	-	133.440	-	-	-	124.440
18	-	152.940	-	-	-	157.780
19	-	139.620	-	-	-	151.820
20	-	150.450	-	-	-	158.320

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN546308 10 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
21	-	135.710	-	-	-	164.330 >
22	-	141.690	-	-	-	168.070 >
23	-	153.750	-	-	-	175.100 >
24	-	146.030	-	-	-	-
25	-	149.600	-	-	-	170.490 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN546308 100 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
26	-	147.760	-	-	-	177.760 >
27	-	154.200	-	-	-	180.640 >
28	-	143.920	-	-	-	167.770 >
29	-	139.180	140.350	-	-	-
30	-	134.070	-	-	-	155.010 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN546308 300 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
31	-	139.770	-	-	-	174.560 >
32	-	135.760	-	-	-	167.540 >
33	-	143.550	-	-	-	159.400 >
34	-	151.130	-	-	-	176.930 >
35	-	156.070	-	-	-	191.940 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN546308 1000 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
36	-	150.300	-	-	-	180.280 >
37	-	154.430	-	-	-	139.090
38	-	141.080	-	-	-	120.430 <
39	-	145.600	-	-	-	160.360 >
40	-	136.310	131.140	-	-	-

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548097 10 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
41	-	152.450	-	-	-	179.580 >
42	-	143.960	-	-	-	157.950
43	-	150.330	-	-	-	177.320 >
44	-	142.000	-	151.650	-	-
45	-	138.210	-	-	-	172.120 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548097 100 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
46	-	156.080	-	-	-	181.740 >
47	-	146.730	-	-	-	170.250 >
48	-	144.830	-	-	-	173.040 >
49	-	135.160	-	-	-	160.280 >
50	-	142.430	-	-	-	177.630 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548097 300 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
51	-	155.220	-	-	-	181.600 >
52	-	145.100	-	-	-	173.720 >
53	-	141.140	-	-	-	147.380
54	-	135.430	-	-	-	166.210 >
55	-	147.730	-	-	-	175.730 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548097 1000 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
56	-	155.470	-	-	-	182.420 >
57	-	136.990	-	-	-	155.010 >
58	-	150.390	-	-	-	176.640 >
59	-	145.340	-	-	-	119.360 <
60	-	142.170	-	-	-	165.840 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548012 10 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
61	-	137.510	-	-	-	177.970 >
62	-	154.860	-	-	-	171.630 >
63	-	142.460	-	-	-	175.880 >
64	-	149.000	-	-	-	169.470 >
65	-	143.840	-	-	-	164.560 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN54012 100 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
66	-	137.110	-	-	-	160.640 >
67	-	147.800	-	-	-	169.860 >
68	-	152.460	-	-	-	167.080
69	-	139.090	-	-	-	172.900 >
70	-	144.910	-	-	-	177.520 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548012 300 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
71	-	140.220	-	-	-	179.640 >
72	-	148.430	-	-	-	168.880 >
73	-	134.750	-	-	-	157.810 >
74	-	145.120	-	-	-	173.160 >
75	-	153.440	-	-	-	183.430 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548012 1000 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
76	-	155.470	-	-	-	174.720 >
77	-	137.700	-	-	-	165.530 >
78	-	139.250	-	-	-	171.180 >
79	-	142.900	-	-	-	177.030 >
80	-	146.680	-	-	-	171.220 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548014 10 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
81	-	151.580	-	-	-	169.320 >
82	-	137.540	-	-	-	162.260 >
83	-	143.360	-	-	-	169.670 >
84	-	139.630	-	-	-	176.620 >
85	-	150.620	-	-	-	174.630 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548014 100 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
86	-	146.000	-	-	-	174.020 >
87	-	133.700	-	-	-	162.250 >
88	-	148.500	-	-	-	179.690 >
89	-	139.000	-	-	-	167.830 >
90	-	151.550	-	-	-	174.930 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548014 300 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
91	-	136.180	-	-	-	169.090 >
92	-	150.600	-	-	-	175.750 >
93	-	141.480	-	-	-	164.500 >
94	-	154.430	-	-	-	181.070 >
95	-	144.240	-	-	-	167.490 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548014 1000 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
96	-	141.480	-	-	-	170.160 >
97	-	133.630	-	-	-	165.810 >
98	-	151.240	-	-	-	179.210 >
99	-	143.610	-	-	-	156.420
100	-	148.810	-	-	-	161.080

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548102 10 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
101	136.790	-	-	-	-	162.530 >
102	152.540	-	-	-	-	188.790 >
103	145.800	-	-	-	-	177.810 >
104	142.150	-	-	-	-	168.410 >
105	147.700	-	-	-	-	176.050 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548102 100 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
106	145.820	-	-	-	-	163.760 >
107	147.050	-	-	-	-	168.660 >
108	141.520	-	-	-	-	160.680 >
109	152.580	-	-	-	-	177.500 >
110	135.940	-	-	-	-	166.910 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548102 300 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
111	152.050	-	-	-	-	186.090 >
112	148.130	-	-	-	-	169.930 >
113	143.800	-	-	-	-	165.980 >
114	140.470	-	-	-	-	170.560 >
115	138.160	-	-	-	-	173.590 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548102 1000 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
116	145.790	-	-	-	-	153.890
117	148.230	-	-	-	-	147.580
118	138.640	-	-	-	-	-
119	152.520	-	-	-	-	112.550 <
120	140.710	-	-	-	121.070 <	-

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548117 10 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
121	142.200	-	-	-	-	179.440 >
122	148.890	-	-	-	-	180.310 >
123	143.700	-	-	-	-	171.490 >
124	138.490	-	-	-	-	173.250 >
125	153.800	-	-	-	-	179.240 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548117 100 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
126	153.660	-	-	-	-	176.720 >
127	138.190	-	-	-	-	170.270 >
128	150.910	-	-	-	-	174.710 >
129	142.810	-	-	-	-	174.530 >
130	145.170	-	-	-	-	183.110 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548117 300 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
131	137.810	-	-	-	-	157.340 >
132	156.360	-	-	-	-	136.700 <
133	144.270	-	-	-	-	169.460 >
134	142.140	-	-	-	-	166.730 >
135	148.070	-	-	-	-	178.360 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548117 1000 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
136	140.500	-	-	-	113.460 <	-
137	133.110	-	-	-	-	125.600
138	153.310	-	-	-	-	137.400 <
139	146.050	-	-	-	-	155.230
140	144.970	-	-	-	-	140.760

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548098 10 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
141	141.890	-	-	-	-	185.150 >
142	143.330	-	-	-	-	175.320 >
143	153.250	-	-	-	-	176.840 >
144	136.250	-	-	-	-	156.480 >
145	149.730	-	-	-	-	181.600 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548098 100 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
146	139.000	-	-	-	-	174.750 >
147	138.980	-	-	-	-	168.350 >
148	150.100	-	-	-	-	172.790 >
149	152.690	-	-	-	-	185.090 >
150	145.940	-	-	-	-	164.390 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548098 300 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
151	142.670	-	-	-	-	168.390 >
152	137.360	-	-	-	-	161.190 >
153	147.170	-	-	-	-	177.130 >
154	154.730	-	-	-	-	178.650 >
155	145.570	-	-	-	-	168.970 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548098 1000 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
156	144.430	-	-	-	-	161.110 >
157	154.090	-	-	-	-	186.260 >
158	134.680	-	-	-	-	159.090 >
159	141.150	-	-	-	-	161.500 >
160	146.840	-	-	-	-	163.470 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548101 10 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
161	138.590	-	-	-	-	171.650 >
162	154.010	-	-	-	-	178.160 >
163	142.420	-	-	-	-	179.630 >
164	149.870	-	-	-	-	169.270 >
165	144.270	-	-	-	-	164.940 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548101 100 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
166	144.280	-	-	-	-	-
167	147.860	-	-	-	-	166.450 >
168	152.810	-	-	-	-	184.990 >
169	135.950	-	-	-	-	158.060 >
170	142.600	-	-	-	-	165.230 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548101 300 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
171	147.720	-	-	-	-	167.800 >
172	156.710	-	-	-	-	171.420
173	141.620	-	-	-	-	155.150
174	138.400	-	-	-	124.920	-
175	144.790	-	-	-	-	150.880

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548101 1000 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
176	141.770	-	-	-	-	-
177	145.880	-	-	-	-	-
178	136.650	-	-	-	-	125.540
179	146.970	-	-	-	-	-
180	157.080	-	-	-	-	155.560

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548013 10 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
181	153.360	-	-	-	-	179.600 >
182	138.060	-	-	-	-	174.670 >
183	147.850	-	-	-	-	166.780 >
184	145.590	-	-	-	-	171.500 >
185	140.670	-	-	-	-	160.860 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548013 100 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
186	141.940	-	-	-	-	167.550 >
187	152.800	-	-	-	-	181.700 >
188	143.890	-	-	-	-	171.540 >
189	137.620	-	-	-	-	164.000 >
190	147.310	-	-	-	-	178.050 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548013 300 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
191	142.670	-	-	-	-	183.100 >
192	157.130	-	-	-	-	172.270
193	148.800	-	-	-	-	174.910 >
194	144.630	-	-	-	-	178.840 >
195	136.890	-	-	-	-	168.050 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

SYN548013 1000 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
196	134.690	-	-	-	-	165.190 >
197	147.530	-	-	-	-	169.840 >
198	142.960	-	-	-	-	169.000 >
199	140.860	-	-	-	-	169.490 >
200	152.240	-	-	-	-	184.780 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

Control 0 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
201	-	150.660	-	-	-	176.300 >
202	-	144.270	-	-	-	157.610
203	-	135.450	-	-	-	155.510 >
204	-	154.020	-	-	-	163.440
205	-	142.550	-	-	-	156.450

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Body Weight (g)

Control 0 mg/kg	Day(s) Relative to Start Date					
	-4	-3	2	4	7	8
206	147.180	-	-	-	-	178.240 >
207	134.040	-	-	-	-	168.620 >
208	139.200	-	-	-	-	163.230 >
209	143.130	-	-	-	-	176.620 >
210	157.630	-	-	-	-	186.490 >

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Comments and Markers

Page	Measurement	Group	Sex	Subject	Day	Type	Marker
1	Body Weight	1	Female	2	8	Out of Range	>
1	Body Weight	1	Female	3	8	Out of Range	>
1	Body Weight	1	Female	4	8	Out of Range	>
1	Body Weight	1	Female	5	8	Out of Range	>
2	Body Weight	2	Female	6	8	Out of Range	>
2	Body Weight	2	Female	7	8	Out of Range	>
2	Body Weight	2	Female	8	8	Out of Range	>
2	Body Weight	2	Female	9	8	Out of Range	>
2	Body Weight	2	Female	10	8	Out of Range	>
3	Body Weight	3	Female	11	8	Out of Range	>
3	Body Weight	3	Female	12	8	Out of Range	>
3	Body Weight	3	Female	13	8	Out of Range	>
3	Body Weight	3	Female	14	8	Out of Range	>
3	Body Weight	3	Female	15	8	Out of Range	>
5	Body Weight	5	Female	21	8	Out of Range	>
5	Body Weight	5	Female	22	8	Out of Range	>
5	Body Weight	5	Female	23	8	Out of Range	>
5	Body Weight	5	Female	25	8	Out of Range	>
6	Body Weight	6	Female	26	8	Out of Range	>
6	Body Weight	6	Female	27	8	Out of Range	>
6	Body Weight	6	Female	28	8	Out of Range	>
6	Body Weight	6	Female	30	8	Out of Range	>
7	Body Weight	7	Female	31	8	Out of Range	>
7	Body Weight	7	Female	32	8	Out of Range	>
7	Body Weight	7	Female	33	8	Out of Range	>
7	Body Weight	7	Female	34	8	Out of Range	>
7	Body Weight	7	Female	35	8	Out of Range	>
8	Body Weight	8	Female	36	8	Out of Range	>
8	Body Weight	8	Female	38	8	Out of Range	<

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Comments and Markers

Page	Measurement	Group	Sex	Subject	Day	Type	Marker
8	Body Weight	8	Female	39	8	Out of Range	>
9	Body Weight	9	Female	41	8	Out of Range	>
9	Body Weight	9	Female	43	8	Out of Range	>
9	Body Weight	9	Female	45	8	Out of Range	>
10	Body Weight	10	Female	46	8	Out of Range	>
10	Body Weight	10	Female	47	8	Out of Range	>
10	Body Weight	10	Female	48	8	Out of Range	>
10	Body Weight	10	Female	49	8	Out of Range	>
10	Body Weight	10	Female	50	8	Out of Range	>
11	Body Weight	11	Female	51	8	Out of Range	>
11	Body Weight	11	Female	52	8	Out of Range	>
11	Body Weight	11	Female	54	8	Out of Range	>
11	Body Weight	11	Female	55	8	Out of Range	>
12	Body Weight	12	Female	56	8	Out of Range	>
12	Body Weight	12	Female	57	8	Out of Range	>
12	Body Weight	12	Female	58	8	Out of Range	>
12	Body Weight	12	Female	59	8	Out of Range	<
12	Body Weight	12	Female	60	8	Out of Range	>
13	Body Weight	13	Female	61	8	Out of Range	>
13	Body Weight	13	Female	62	8	Out of Range	>
13	Body Weight	13	Female	63	8	Out of Range	>
13	Body Weight	13	Female	64	8	Out of Range	>
13	Body Weight	13	Female	65	8	Out of Range	>
14	Body Weight	14	Female	66	8	Out of Range	>
14	Body Weight	14	Female	67	8	Out of Range	>
14	Body Weight	14	Female	69	8	Out of Range	>
14	Body Weight	14	Female	70	8	Out of Range	>
15	Body Weight	15	Female	71	8	Out of Range	>
15	Body Weight	15	Female	72	8	Out of Range	>
15	Body Weight	15	Female	73	8	Out of Range	>

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Comments and Markers

Page	Measurement	Group	Sex	Subject	Day	Type	Marker
15	Body Weight	15	Female	74	8	Out of Range	>
15	Body Weight	15	Female	75	8	Out of Range	>
16	Body Weight	16	Female	76	8	Out of Range	>
16	Body Weight	16	Female	77	8	Out of Range	>
16	Body Weight	16	Female	78	8	Out of Range	>
16	Body Weight	16	Female	79	8	Out of Range	>
16	Body Weight	16	Female	80	8	Out of Range	>
17	Body Weight	17	Female	81	8	Out of Range	>
17	Body Weight	17	Female	82	8	Out of Range	>
17	Body Weight	17	Female	83	8	Out of Range	>
17	Body Weight	17	Female	84	8	Out of Range	>
17	Body Weight	17	Female	85	8	Out of Range	>
18	Body Weight	18	Female	86	8	Out of Range	>
18	Body Weight	18	Female	87	8	Out of Range	>
18	Body Weight	18	Female	88	8	Out of Range	>
18	Body Weight	18	Female	89	8	Out of Range	>
18	Body Weight	18	Female	90	8	Out of Range	>
19	Body Weight	19	Female	91	8	Out of Range	>
19	Body Weight	19	Female	92	8	Out of Range	>
19	Body Weight	19	Female	93	8	Out of Range	>
19	Body Weight	19	Female	94	8	Out of Range	>
19	Body Weight	19	Female	95	8	Out of Range	>
20	Body Weight	20	Female	96	8	Out of Range	>
20	Body Weight	20	Female	97	8	Out of Range	>
20	Body Weight	20	Female	98	8	Out of Range	>
21	Body Weight	21	Female	101	8	Out of Range	>
21	Body Weight	21	Female	102	8	Out of Range	>
21	Body Weight	21	Female	103	8	Out of Range	>
21	Body Weight	21	Female	104	8	Out of Range	>
21	Body Weight	21	Female	105	8	Out of Range	>

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Comments and Markers

Page	Measurement	Group	Sex	Subject	Day	Type	Marker
22	Body Weight	22	Female	106	8	Out of Range	>
22	Body Weight	22	Female	107	8	Out of Range	>
22	Body Weight	22	Female	108	8	Out of Range	>
22	Body Weight	22	Female	109	8	Out of Range	>
22	Body Weight	22	Female	110	8	Out of Range	>
23	Body Weight	23	Female	111	8	Out of Range	>
23	Body Weight	23	Female	112	8	Out of Range	>
23	Body Weight	23	Female	113	8	Out of Range	>
23	Body Weight	23	Female	114	8	Out of Range	>
23	Body Weight	23	Female	115	8	Out of Range	>
24	Body Weight	24	Female	119	8	Out of Range	<
24	Body Weight	24	Female	120	7	Out of Range	<
25	Body Weight	25	Female	121	8	Out of Range	>
25	Body Weight	25	Female	122	8	Out of Range	>
25	Body Weight	25	Female	123	8	Out of Range	>
25	Body Weight	25	Female	124	8	Out of Range	>
25	Body Weight	25	Female	125	8	Out of Range	>
26	Body Weight	26	Female	126	8	Out of Range	>
26	Body Weight	26	Female	127	8	Out of Range	>
26	Body Weight	26	Female	128	8	Out of Range	>
26	Body Weight	26	Female	129	8	Out of Range	>
26	Body Weight	26	Female	130	8	Out of Range	>
27	Body Weight	27	Female	131	8	Out of Range	>
27	Body Weight	27	Female	132	8	Out of Range	<
27	Body Weight	27	Female	133	8	Out of Range	>
27	Body Weight	27	Female	134	8	Out of Range	>
27	Body Weight	27	Female	135	8	Out of Range	>
28	Body Weight	28	Female	136	7	Out of Range	<
28	Body Weight	28	Female	138	8	Out of Range	<
29	Body Weight	29	Female	141	8	Out of Range	>

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Comments and Markers

Page	Measurement	Group	Sex	Subject	Day	Type	Marker
29	Body Weight	29	Female	142	8	Out of Range	>
29	Body Weight	29	Female	143	8	Out of Range	>
29	Body Weight	29	Female	144	8	Out of Range	>
29	Body Weight	29	Female	145	8	Out of Range	>
30	Body Weight	30	Female	146	8	Out of Range	>
30	Body Weight	30	Female	147	8	Out of Range	>
30	Body Weight	30	Female	148	8	Out of Range	>
30	Body Weight	30	Female	149	8	Out of Range	>
30	Body Weight	30	Female	150	8	Out of Range	>
31	Body Weight	31	Female	151	8	Out of Range	>
31	Body Weight	31	Female	152	8	Out of Range	>
31	Body Weight	31	Female	153	8	Out of Range	>
31	Body Weight	31	Female	154	8	Out of Range	>
31	Body Weight	31	Female	155	8	Out of Range	>
32	Body Weight	32	Female	156	8	Out of Range	>
32	Body Weight	32	Female	157	8	Out of Range	>
32	Body Weight	32	Female	158	8	Out of Range	>
32	Body Weight	32	Female	159	8	Out of Range	>
32	Body Weight	32	Female	160	8	Out of Range	>
33	Body Weight	33	Female	161	8	Out of Range	>
33	Body Weight	33	Female	162	8	Out of Range	>
33	Body Weight	33	Female	163	8	Out of Range	>
33	Body Weight	33	Female	164	8	Out of Range	>
33	Body Weight	33	Female	165	8	Out of Range	>
34	Body Weight	34	Female	167	8	Out of Range	>
34	Body Weight	34	Female	168	8	Out of Range	>
34	Body Weight	34	Female	169	8	Out of Range	>
34	Body Weight	34	Female	170	8	Out of Range	>
35	Body Weight	35	Female	171	8	Out of Range	>
37	Body Weight	37	Female	181	8	Out of Range	>

TABLE 3-1 Individual Body Weights (g) (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Comments and Markers

Page	Measurement	Group	Sex	Subject	Day	Type	Marker
37	Body Weight	37	Female	182	8	Out of Range	>
37	Body Weight	37	Female	183	8	Out of Range	>
37	Body Weight	37	Female	184	8	Out of Range	>
37	Body Weight	37	Female	185	8	Out of Range	>
38	Body Weight	38	Female	186	8	Out of Range	>
38	Body Weight	38	Female	187	8	Out of Range	>
38	Body Weight	38	Female	188	8	Out of Range	>
38	Body Weight	38	Female	189	8	Out of Range	>
38	Body Weight	38	Female	190	8	Out of Range	>
39	Body Weight	39	Female	191	8	Out of Range	>
39	Body Weight	39	Female	193	8	Out of Range	>
39	Body Weight	39	Female	194	8	Out of Range	>
39	Body Weight	39	Female	195	8	Out of Range	>
40	Body Weight	40	Female	196	8	Out of Range	>
40	Body Weight	40	Female	197	8	Out of Range	>
40	Body Weight	40	Female	198	8	Out of Range	>
40	Body Weight	40	Female	199	8	Out of Range	>
40	Body Weight	40	Female	200	8	Out of Range	>
41	Body Weight	41	Female	201	8	Out of Range	>
41	Body Weight	41	Female	203	8	Out of Range	>
42	Body Weight	42	Female	206	8	Out of Range	>
42	Body Weight	42	Female	207	8	Out of Range	>
42	Body Weight	42	Female	208	8	Out of Range	>
42	Body Weight	42	Female	209	8	Out of Range	>
42	Body Weight	42	Female	210	8	Out of Range	>

TABLE 3-1 Individual Body Weights (g) (Continued)

Key Page

Measurement Descriptions

Headings Used	Description
Body Weight	Body Weight

Group Information

Short Name	Long Name	Report Headings 1-4	
1	Group 1	SYN547407	10 mg/kg
2	Group 2	SYN547407	100 mg/kg
3	Group 3	SYN547407	300 mg/kg
4	Group 4	SYN547407	1000 mg/kg
5	Group 5	SYN546308	10 mg/kg
6	Group 6	SYN546308	100 mg/kg
7	Group 7	SYN546308	300 mg/kg
8	Group 8	SYN546308	1000 mg/kg
9	Group 9	SYN548097	10 mg/kg
10	Group 10	SYN548097	100 mg/kg
11	Group 11	SYN548097	300 mg/kg
12	Group 12	SYN548097	1000 mg/kg
13	Group 13	SYN548012	10 mg/kg
14	Group 14	SYN54012	100 mg/kg
15	Group 15	SYN548012	300 mg/kg
16	Group 16	SYN548012	1000 mg/kg
17	Group 17	SYN548014	10 mg/kg
18	Group 18	SYN548014	100 mg/kg
19	Group 19	SYN548014	300 mg/kg
20	Group 20	SYN548014	1000 mg/kg
21	Group 21	SYN548102	10 mg/kg

TABLE 3-1 Individual Body Weights (g) (Continued)

Key Page

Group Information (Continued)

Short Name	Long Name	Report Headings 1-4	
22	Group 22	SYN548102	100 mg/kg
23	Group 23	SYN548102	300 mg/kg
24	Group 24	SYN548102	1000 mg/kg
25	Group 25	SYN548117	10 mg/kg
26	Group 26	SYN548117	100 mg/kg
27	Group 27	SYN548117	300 mg/kg
28	Group 28	SYN548117	1000 mg/kg
29	Group 29	SYN548098	10 mg/kg
30	Group 30	SYN548098	100 mg/kg
31	Group 31	SYN548098	300 mg/kg
32	Group 32	SYN548098	1000 mg/kg
33	Group 33	SYN548101	10 mg/kg
34	Group 34	SYN548101	100 mg/kg
35	Group 35	SYN548101	300 mg/kg
36	Group 36	SYN548101	1000 mg/kg
37	Group 37	SYN548013	10 mg/kg
38	Group 38	SYN548013	100 mg/kg
39	Group 39	SYN548013	300 mg/kg
40	Group 40	SYN548013	1000 mg/kg
41	Group 41	Control	0 mg/kg
42	Group 42	Control	0 mg/kg

TABLE 3-2 Individual Body Weight Changes – Females

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN547407 10 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
1	-	-	-	1.100	-
2	-	-	-	2.815	-
3	-	-	-	2.806	-
4	-	-	-	2.161	-
5	-	-	-	1.785	-

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN547407 100 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
6	-	-	-	2.158	-
7	-	-	-	2.280	-
8	-	-	-	2.294	-
9	-	-	-	2.763	-
10	-	-	-	2.828	-

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN547407 300 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
11	-	-	-	1.709	-
12	-	-	-	1.854	-
13	-	-	-	1.802	-
14	-	-	-	2.522	-
15	-	-	-	1.729	-

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN547407 1000 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
16	-	-	-	0.745	-
17	-	-	-	-0.818	-
18	-	-	-	0.440	-
19	-	-	-	1.109	-
20	-	-	-	0.715	-

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN546308 10 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
21	-	-	-	2.602	-
22	-	-	-	2.398	-
23	-	-	-	1.941	-
25	-	-	-	1.899	-

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN546308 100 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
26	-	-	-	2.727	-
27	-	-	-	2.404	-
28	-	-	-	2.168	-
29	0.234	-	-	-	-
30	-	-	-	1.904	-

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN546308 300 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
31	-	-	-	3.163	-
32	-	-	-	2.889	-
33	-	-	-	1.441	-
34	-	-	-	2.345	-
35	-	-	-	3.261	-

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN546308 1000 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
36	-	-	-	2.725	-
37	-	-	-	-1.395	-
38	-	-	-	-1.877	-
39	-	-	-	1.342	-
40	-1.034	-	-	-	-

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548097 10 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
41	-	-	-	2.466	-
42	-	-	-	1.272	-
43	-	-	-	2.454	-
44	-	1.379	-	-	-
45	-	-	-	3.083	-

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548097 100 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
46	-	-	-	2.333	-
47	-	-	-	2.138	-
48	-	-	-	2.565	-
49	-	-	-	2.284	-
50	-	-	-	3.200	-

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548097 300 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
51	-	-	-	2.398	-
52	-	-	-	2.602	-
53	-	-	-	0.567	-
54	-	-	-	2.798	-
55	-	-	-	2.545	-

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548097 1000 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
56	-	-	-	2.450	-
57	-	-	-	1.638	-
58	-	-	-	2.386	-
59	-	-	-	-2.362	-
60	-	-	-	2.152	-

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548012 10 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
61	-	-	-	3.678	-
62	-	-	-	1.525	-
63	-	-	-	3.038	-
64	-	-	-	1.861	-
65	-	-	-	1.884	-

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN54012 100 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
66	-	-	-	2.139	-
67	-	-	-	2.005	-
68	-	-	-	1.329	-
69	-	-	-	3.074	-
70	-	-	-	2.965	-

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548012 300 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
71	-	-	-	3.584	-
72	-	-	-	1.859	-
73	-	-	-	2.096	-
74	-	-	-	2.549	-
75	-	-	-	2.726	-

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548012 1000 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
76	-	-	-	1.750	-
77	-	-	-	2.530	-
78	-	-	-	2.903	-
79	-	-	-	3.103	-
80	-	-	-	2.231	-

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548014 10 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
81	-	-	-	1.613	-
82	-	-	-	2.247	-
83	-	-	-	2.392	-
84	-	-	-	3.363	-
85	-	-	-	2.183	-

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548014 100 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
86	-	-	-	2.547	-
87	-	-	-	2.595	-
88	-	-	-	2.835	-
89	-	-	-	2.621	-
90	-	-	-	2.125	-

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548014 300 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
91	-	-	-	2.992	-
92	-	-	-	2.286	-
93	-	-	-	2.093	-
94	-	-	-	2.422	-
95	-	-	-	2.114	-

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548014 1000 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
96	-	-	-	2.607	-
97	-	-	-	2.925	-
98	-	-	-	2.543	-
99	-	-	-	1.165	-
100	-	-	-	1.115	-

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548102 10 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
101	-	-	-	-	2.145
102	-	-	-	-	3.021
103	-	-	-	-	2.668
104	-	-	-	-	2.188
105	-	-	-	-	2.363

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548102 100 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
106	-	-	-	-	1.495
107	-	-	-	-	1.801
108	-	-	-	-	1.597
109	-	-	-	-	2.077
110	-	-	-	-	2.581

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548102 300 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
111	-	-	-	-	2.837
112	-	-	-	-	1.817
113	-	-	-	-	1.848
114	-	-	-	-	2.508
115	-	-	-	-	2.953

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548102 1000 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
116	-	-	-	-	0.675
117	-	-	-	-	-0.054
119	-	-	-	-	-3.331
120	-	-	-1.785	-	-

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548117 10 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
121	-	-	-	-	3.103
122	-	-	-	-	2.618
123	-	-	-	-	2.316
124	-	-	-	-	2.897
125	-	-	-	-	2.120

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548117 100 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
126	-	-	-	-	1.922
127	-	-	-	-	2.673
128	-	-	-	-	1.983
129	-	-	-	-	2.643
130	-	-	-	-	3.162

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548117 300 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
131	-	-	-	-	1.628
132	-	-	-	-	-1.638
133	-	-	-	-	2.099
134	-	-	-	-	2.049
135	-	-	-	-	2.524

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548117 1000 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
136	-	-	-2.458	-	-
137	-	-	-	-	-0.626
138	-	-	-	-	-1.326
139	-	-	-	-	0.765
140	-	-	-	-	-0.351

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548098 10 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
141	-	-	-	-	3.605
142	-	-	-	-	2.666
143	-	-	-	-	1.966
144	-	-	-	-	1.686
145	-	-	-	-	2.656

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548098 100 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
146	-	-	-	-	2.979
147	-	-	-	-	2.448
148	-	-	-	-	1.891
149	-	-	-	-	2.700
150	-	-	-	-	1.538

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548098 300 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
151	-	-	-	-	2.143
152	-	-	-	-	1.986
153	-	-	-	-	2.497
154	-	-	-	-	1.993
155	-	-	-	-	1.950

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548098 1000 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
156	-	-	-	-	1.390
157	-	-	-	-	2.681
158	-	-	-	-	2.034
159	-	-	-	-	1.696
160	-	-	-	-	1.386

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548101 10 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
161	-	-	-	-	2.755
162	-	-	-	-	2.013
163	-	-	-	-	3.101
164	-	-	-	-	1.617
165	-	-	-	-	1.723

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548101 100 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
167	-	-	-	-	1.549
168	-	-	-	-	2.682
169	-	-	-	-	1.843
170	-	-	-	-	1.886

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548101 300 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
171	-	-	-	-	1.673
172	-	-	-	-	1.226
173	-	-	-	-	1.128
174	-	-	-1.225	-	-
175	-	-	-	-	0.508

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548101 1000 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
178	-	-	-	-	-0.926
180	-	-	-	-	-0.127

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548013 10 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
181	-	-	-	-	2.187
182	-	-	-	-	3.051
183	-	-	-	-	1.578
184	-	-	-	-	2.159
185	-	-	-	-	1.683

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548013 100 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
186	-	-	-	-	2.134
187	-	-	-	-	2.408
188	-	-	-	-	2.304
189	-	-	-	-	2.198
190	-	-	-	-	2.562

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548013 300 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
191	-	-	-	-	3.369
192	-	-	-	-	1.262
193	-	-	-	-	2.176
194	-	-	-	-	2.851
195	-	-	-	-	2.597

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

SYN548013 1000 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
196	-	-	-	-	2.542
197	-	-	-	-	1.859
198	-	-	-	-	2.170
199	-	-	-	-	2.386
200	-	-	-	-	2.712

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

Control 0 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
201	-	-	-	2.331	-
202	-	-	-	1.213	-
203	-	-	-	1.824	-
204	-	-	-	0.856	-
205	-	-	-	1.264	-

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Generalised Results - Animals by Time - Fixed Parameter

39292 - Seven Day Acute Oral Gavage Toxicity Study In Female Wistar Han Rats

Sex: Female Mean Weight Gain (g/Day)

Control 0 mg/kg	Day(s) Relative to Start Date				
	-3 → 2	-3 → 4	-4 → 7	-3 → 8	-4 → 8
206	-	-	-	-	2.588
207	-	-	-	-	2.882
208	-	-	-	-	2.003
209	-	-	-	-	2.791
210	-	-	-	-	2.405

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Key Page

Measurement Descriptions

Headings Used	Description
Mean Weight Gain	Mean Weight Gain

Group Information

Short Name	Long Name	Report Headings 1-4	
1	Group 1	SYN547407	10 mg/kg
2	Group 2	SYN547407	100 mg/kg
3	Group 3	SYN547407	300 mg/kg
4	Group 4	SYN547407	1000 mg/kg
5	Group 5	SYN546308	10 mg/kg
6	Group 6	SYN546308	100 mg/kg
7	Group 7	SYN546308	300 mg/kg
8	Group 8	SYN546308	1000 mg/kg
9	Group 9	SYN548097	10 mg/kg
10	Group 10	SYN548097	100 mg/kg
11	Group 11	SYN548097	300 mg/kg
12	Group 12	SYN548097	1000 mg/kg
13	Group 13	SYN548012	10 mg/kg
14	Group 14	SYN54012	100 mg/kg
15	Group 15	SYN548012	300 mg/kg
16	Group 16	SYN548012	1000 mg/kg
17	Group 17	SYN548014	10 mg/kg
18	Group 18	SYN548014	100 mg/kg
19	Group 19	SYN548014	300 mg/kg
20	Group 20	SYN548014	1000 mg/kg
21	Group 21	SYN548102	10 mg/kg

TABLE 3-2 Individual Body Weight Changes – Females (Continued)

Key Page

Group Information (Continued)

Short Name	Long Name	Report Headings 1-4	
22	Group 22	SYN548102	100 mg/kg
23	Group 23	SYN548102	300 mg/kg
24	Group 24	SYN548102	1000 mg/kg
25	Group 25	SYN548117	10 mg/kg
26	Group 26	SYN548117	100 mg/kg
27	Group 27	SYN548117	300 mg/kg
28	Group 28	SYN548117	1000 mg/kg
29	Group 29	SYN548098	10 mg/kg
30	Group 30	SYN548098	100 mg/kg
31	Group 31	SYN548098	300 mg/kg
32	Group 32	SYN548098	1000 mg/kg
33	Group 33	SYN548101	10 mg/kg
34	Group 34	SYN548101	100 mg/kg
35	Group 35	SYN548101	300 mg/kg
36	Group 36	SYN548101	1000 mg/kg
37	Group 37	SYN548013	10 mg/kg
38	Group 38	SYN548013	100 mg/kg
39	Group 39	SYN548013	300 mg/kg
40	Group 40	SYN548013	1000 mg/kg
41	Group 41	Control	0 mg/kg
42	Group 42	Control	0 mg/kg

TABLE 3-3 Individual Animal Clinical Abnormalities – Females

Group	Animal ID	Observation	Observed			
			First Day	Last Day	Interval	Total Number
SYN547407 1000 mg/kg	17	Rough Coat	5	6	2	2
SYN546308 10 mg/kg	24	Respiration Abnormality/Labored	2	2	1	1
SYN546308 10 mg/kg	24	Tremors	2	2	1	1
SYN546308 100 mg/kg	29	Eye Abnormality/Both-Opacity	2	2	1	1
SYN546308 100 mg/kg	29	Nasal Discharge/Both-Red	2	2	1	1
SYN546308 100 mg/kg	29	Seizure	2	2	1	1[a]
SYN546308 300 mg/kg	35	Seizure	2	2	1	1[a,b]
SYN546308 1000 mg/kg	37	Rough Coat	5	8	4	4
SYN546308 1000 mg/kg	38	Rough Coat	5	8	4	4
SYN546308 1000 mg/kg	38	Thin	8	8	1	1
SYN546308 1000 mg/kg	39	Rough Coat	6	8	3	3
SYN546308 1000 mg/kg	40	Eye Abnormality/Both-Opacity	2	2	1	1
SYN546308 1000 mg/kg	40	Seizure	2	2	1	1[a,b]
SYN548097 1000 mg/kg	56	Seizure	2	2	1	1[c]
SYN548097 1000 mg/kg	58	Rough Coat	6	8	3	3
SYN548097 1000 mg/kg	59	Rough Coat	5	8	4	4
SYN548102 1000 mg/kg	119	Eye Discharge/Both-Red	7	8	2	2
SYN548102 1000 mg/kg	119	Hunched	8	8	1	1
SYN548102 1000 mg/kg	119	Lethargy	8	8	1	1
SYN548102 1000 mg/kg	119	Nasal Discharge/Both-Red	7	8	2	2
SYN548102 1000 mg/kg	119	Rough Coat	7	8	2	2
SYN548102 1000 mg/kg	119	Thin	7	8	2	2
SYN548102 1000 mg/kg	119	Wet Urogenital Area	8	8	1	1
SYN548102 1000 mg/kg	120	Rough Coat	7	7	1	1

a. Animal began seizing while in warming box for 24 hour TK blood collection.
b. Cool water gently rubbed on animal's coat to cool it immediately following seizure.
c. Animal began seizing within 2 minutes of being placed in warming box for 36 hour TK blood collection.

TABLE 3-3 Individual Animal Clinical Abnormalities – Females (Continued)

Group	Animal ID	Observation	Observed			
			First Day	Last Day	Interval	Total Number
SYN548117 100 mg/kg	126	Seizure	2	2	1	1[d]
SYN548117 300 mg/kg	131	Seizure	2	2	1	1[d]
SYN548117 300 mg/kg	132	Seizure	6	6	1	1[e]
SYN548117 300 mg/kg	132	Thin	8	8	1	1
SYN548117 1000 mg/kg	136	Rough Coat	4	7	4	4
SYN548117 1000 mg/kg	137	Excess Salivation	8	8	1	1
SYN548117 1000 mg/kg	137	Rough Coat	4	8	5	5
SYN548117 1000 mg/kg	137	Seizure	8	8	1	1[f]
SYN548117 1000 mg/kg	137	Thin	8	8	1	1
SYN548117 1000 mg/kg	138	Hyperactivity	7	7	1	1
SYN548117 1000 mg/kg	138	Rough Coat	8	8	1	1
SYN548117 1000 mg/kg	138	Seizure	7	7	1	1[g]
SYN548117 1000 mg/kg	139	Rough Coat	4	8	5	5
SYN548117 1000 mg/kg	140	Discolored Skin/Pale	5	5	1	1
SYN548117 1000 mg/kg	140	Limb Weakness/Both Hindlimbs/Legs	4	5	2	3
SYN548117 1000 mg/kg	140	Respiration Abnormality/Labored	5	5	1	1
SYN548117 1000 mg/kg	140	Respiration Abnormality/Rapid	4	4	1	1
SYN548117 1000 mg/kg	140	Rough Coat	4	8	5	5
SYN548117 1000 mg/kg	140	Seizure	4	5	2	3[h]
SYN548098 10 mg/kg	142	Skin Abrasion/Head/Neck	8	8	1	1
SYN548098 1000 mg/kg	159	Rough Coat	4	7	4	4
SYN548098 1000 mg/kg	160	Rough Coat	5	7	3	3
SYN548101 300 mg/kg	174	Rough Coat	4	6	3	3

d. Animal began seizing while in warming box for 36 hour TK blood collection.
e. Seizure lasted 12 minutes.
f. Seizure lasted until termination.
g. Seizure lasted approximately 10 seconds.
h. Two seizures on Day 4 occurred at least 1.5 hours prior to placement into warming box. One seizure on Day 5 lasted 4 minutes.

TABLE 3-3 Individual Animal Clinical Abnormalities – Females (Continued)

Group	Animal ID	Observation	Observed			
			First Day	Last Day	Interval	Total Number
SYN548101 300 mg/kg	175	Discolored Skin/Pale	4	4	1	1
SYN548101 300 mg/kg	175	Rough Coat	4	7	4	4
SYN548101 1000 mg/kg	176	Discolored Skin/Pale	6	6	1	1
SYN548101 1000 mg/kg	176	Excess Salivation	6	6	1	2
SYN548101 1000 mg/kg	176	Seizure	6	6	1	3
SYN548101 1000 mg/kg	177	Discolored Skin/Pale	5	5	1	2
SYN548101 1000 mg/kg	177	Excess Salivation	5	5	1	1
SYN548101 1000 mg/kg	177	Limb Weakness/Both Hindlimbs/Legs	5	5	1	1
SYN548101 1000 mg/kg	177	Rough Coat	4	5	2	2
SYN548101 1000 mg/kg	177	Seizure	5	5	1	2[i]
SYN548101 1000 mg/kg	178	Hunched	8	8	1	1
SYN548101 1000 mg/kg	178	Rough Coat	4	8	5	5
SYN548101 1000 mg/kg	178	Thin	8	8	1	1
SYN548101 1000 mg/kg	179	Discolored Skin/Pale	6	6	1	2
SYN548101 1000 mg/kg	179	Excess Salivation	6	6	1	1
SYN548101 1000 mg/kg	179	Recumbent/Lateral	6	6	1	2
SYN548101 1000 mg/kg	179	Rough Coat	4	5	2	2
SYN548101 1000 mg/kg	179	Seizure	6	6	1	3[j]
SYN548101 1000 mg/kg	180	Rough Coat	4	4	1	1

i. Seizure lasted through two observation periods at which time the animal was terminated.
j. Seizure noted upon entry to the study room; seizure lasted through three observation periods.

APPENDIX 4 Bioanalytical and Toxicokinetic Analysis

RAT BLOOD ANALYSIS SUMMARY

Diluted rat blood samples from the study "SYN547407, SYN546308, SYN548097, SYN548012, SYN548014, SYN548102, SYN548117, SYN548098, SYN548101 and SYN548013 - Seven Day Acute Oral Gavage Toxicity Study in Female Wistar Han Rats" were received frozen on dry ice from Battelle Toxicology West Jefferson on 01/22/14 to determine blood concentration levels of SYN547407.

A summary of the method is as follows: Whole Blood:Water (1:1) calibration standards were prepared from one stock solution. The calibration standards, blanks, Quality Control (QC) samples, and study samples were processed by protein precipitation. The extracts were analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS). SYN547407 concentrations were calculated using peak area and a regression line constructed from the concentrations and peak area response of the calibration standards.

All samples were successfully analyzed. Samples that yielded SYN547407 concentrations higher than that of the highest calibration standards in their initial analyses were diluted and reanalyzed in a separate run. The lower limit of quantitation (LLOQ) for the 1:1 diluted blood method is approximately 1 ng/mL and the upper limit of quantitation (ULOQ) is approximately 500 ng /mL extracted from whole blood:water (1:1) using a 20-µL aliquot of sample. The final results were converted to whole blood concentrations by correcting for the 1:1 dilution factor.

RAT BLOOD ANALYSIS SUMMARY

Diluted rat blood samples from the study "SYN547407, SYN546308, SYN548097, SYN548012, SYN548014, SYN548102, SYN548117, SYN548098, SYN548101 and SYN548013 - Seven Day Acute Oral Gavage Toxicity Study in Female Wistar Han Rats" were received frozen on dry ice from Battelle Toxicology West Jefferson on 01/22/14 to determine blood concentration levels of SYN546308.

A summary of the method is as follows: Whole Blood:Water (1:1) calibration standards were prepared from one stock solution. The calibration standards, blanks, Quality Control (QC) samples, and study samples were processed by protein precipitation. The extracts were analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS). SYN546308 concentrations were calculated using peak area and a regression line constructed from the concentrations and peak area response of the calibration standards.

All samples were successfully analyzed. Samples that yielded SYN546308 concentrations higher than that of the highest calibration standards in their initial analyses were diluted and reanalyzed in a separate run. The lower limit of quantitation (LLOQ) for the 1:1 diluted blood method is approximately 1 ng/mL and the upper limit of quantitation (ULOQ) is approximately 500 ng /mL extracted from whole blood:water (1:1) using a 20-µL aliquot of sample. The final results were converted to whole blood concentrations by correcting for the 1:1 dilution factor.

RAT BLOOD ANALYSIS SUMMARY

Diluted rat blood samples from the study "SYN547407, SYN546308, SYN548097, SYN548012, SYN548014, SYN548102, SYN548117, SYN548098, SYN548101 and SYN548013 - Seven Day Acute Oral Gavage Toxicity Study in Female Wistar Han Rats" were received frozen on dry ice from Battelle Toxicology West Jefferson on 01/22/14 to determine blood concentration levels of SYN548097.

A summary of the method is as follows: Whole Blood:Water (1:1) calibration standards were prepared from one stock solution. The calibration standards, blanks, Quality Control (QC) samples, and study samples were processed by protein precipitation. The extracts were analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS). SYN548097 concentrations were calculated using peak area and a regression line constructed from the concentrations and peak area response of the calibration standards.

All samples were successfully analyzed. Samples that yielded SYN548097 concentrations higher than that of the highest calibration standards in their initial analyses were diluted and reanalyzed in a separate run. The lower limit of quantitation (LLOQ) for the 1:1 diluted blood method is approximately 1 ng/mL and the upper limit of quantitation (ULOQ) is approximately 500 ng /mL extracted from whole blood:water (1:1) using a 20-µL aliquot of sample. The final results were converted to whole blood concentrations by correcting for the 1:1 dilution factor.

RAT BLOOD ANALYSIS SUMMARY

Diluted rat blood samples from the study "SYN547407, SYN546308, SYN548097, SYN548012, SYN548014, SYN548102, SYN548117, SYN548098, SYN548101 and SYN548013 - Seven Day Acute Oral Gavage Toxicity Study in Female Wistar Han Rats" were received frozen on dry ice from Battelle Toxicology West Jefferson on 01/22/14 to determine blood concentration levels of SYN548012.

A summary of the method is as follows: Whole Blood:Water (1:1) calibration standards were prepared from one stock solution. The calibration standards, blanks, Quality Control (QC) samples, and study samples were processed by protein precipitation. The extracts were analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS). SYN548012 concentrations were calculated using peak area and a regression line constructed from the concentrations and peak area response of the calibration standards.

All samples were successfully analyzed. Samples that yielded SYN548012 concentrations higher than that of the highest calibration standards in their initial analyses were diluted and reanalyzed in a separate run. The lower limit of quantitation (LLOQ) for the 1:1 diluted blood method is approximately 1 ng/mL and the upper limit of quantitation (ULOQ) is approximately 500 ng /mL extracted from whole blood:water (1:1) using a 20-µL aliquot of sample. The final results were converted to whole blood concentrations by correcting for the 1:1 dilution factor.

RAT BLOOD ANALYSIS SUMMARY

Diluted rat blood samples from the study "SYN547407, SYN546308, SYN548097, SYN548012, SYN548014, SYN548102, SYN548117, SYN548098, SYN548101 and SYN548013 - Seven Day Acute Oral Gavage Toxicity Study in Female Wistar Han Rats" were received frozen on dry ice from Battelle Toxicology West Jefferson on 01/22/14 to determine blood concentration levels of SYN548014.

A summary of the method is as follows: Whole Blood:Water (1:1) calibration standards were prepared from one stock solution. The calibration standards, blanks, Quality Control (QC) samples, and study samples were processed by protein precipitation. The extracts were analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS). SYN548014 concentrations were calculated using peak area and a regression line constructed from the concentrations and peak area response of the calibration standards.

All samples were successfully analyzed. Samples that yielded SYN548014 concentrations higher than that of the highest calibration standards in their initial analyses were diluted and reanalyzed in a separate run. The lower limit of quantitation (LLOQ) for the 1:1 diluted blood method is approximately 1 ng/mL and the upper limit of quantitation (ULOQ) is approximately 500 ng /mL extracted from whole blood:water (1:1) using a 20-µL aliquot of sample. The final results were converted to whole blood concentrations by correcting for the 1:1 dilution factor.

RAT BLOOD ANALYSIS SUMMARY

Diluted rat blood samples from the study "SYN547407, SYN546308, SYN548097, SYN548012, SYN548014, SYN548102, SYN548117, SYN548098, SYN548101 and SYN548013 - Seven Day Acute Oral Gavage Toxicity Study in Female Wistar Han Rats" were received frozen on dry ice from Battelle Toxicology West Jefferson on 01/22/14 to determine blood concentration levels of SYN548102.

A summary of the method is as follows: Whole Blood:Water (1:1) calibration standards were prepared from one stock solution. The calibration standards, blanks, Quality Control (QC) samples, and study samples were processed by protein precipitation. The extracts were analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS). SYN548102 concentrations were calculated using peak area and a regression line constructed from the concentrations and peak area response of the calibration standards.

All samples were successfully analyzed. Samples that yielded SYN548102 concentrations higher than that of the highest calibration standards in their initial analyses were diluted and reanalyzed in a separate run. The lower limit of quantitation (LLOQ) for the 1:1 diluted blood method is approximately 1 ng/mL and the upper limit of quantitation (ULOQ) is approximately 500 ng /mL extracted from whole blood:water (1:1) using a 20-µL aliquot of sample. The final results were converted to whole blood concentrations by correcting for the 1:1 dilution factor.

RAT BLOOD ANALYSIS SUMMARY

Diluted rat blood samples from the study "SYN547407, SYN546308, SYN548097, SYN548012, SYN548014, SYN548102, SYN548117, SYN548098, SYN548101 and SYN548013 - Seven Day Acute Oral Gavage Toxicity Study in Female Wistar Han Rats" were received frozen on dry ice from Battelle Toxicology West Jefferson on 01/22/14 to determine blood concentration levels of SYN548117.

A summary of the method is as follows: Whole Blood:Water (1:1) calibration standards were prepared from one stock solution. The calibration standards, blanks, Quality Control (QC) samples, and study samples were processed by protein precipitation. The extracts were analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS). SYN548117 concentrations were calculated using peak area and a regression line constructed from the concentrations and peak area response of the calibration standards.

All samples were successfully analyzed. Samples that yielded SYN548117 concentrations higher than that of the highest calibration standards in their initial analyses were diluted and reanalyzed in a separate run. The lower limit of quantitation (LLOQ) for the 1:1 diluted blood method is approximately 1 ng/mL and the upper limit of quantitation (ULOQ) is approximately 500 ng /mL extracted from whole blood:water (1:1) using a 20-µL aliquot of sample. The final results were converted to whole blood concentrations by correcting for the 1:1 dilution factor.

RAT BLOOD ANALYSIS SUMMARY

Diluted rat blood samples from the study "SYN547407, SYN546308, SYN548097, SYN548012, SYN548014, SYN548102, SYN548117, SYN548098, SYN548101 and SYN548013 - Seven Day Acute Oral Gavage Toxicity Study in Female Wistar Han Rats" were received frozen on dry ice from Battelle Toxicology West Jefferson on 01/22/14 to determine blood concentration levels of SYN548098.

A summary of the method is as follows: Whole Blood:Water (1:1) calibration standards were prepared from one stock solution. The calibration standards, blanks, Quality Control (QC) samples, and study samples were processed by protein precipitation. The extracts were analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS). SYN548098 concentrations were calculated using peak area and a regression line constructed from the concentrations and peak area response of the calibration standards.

All samples were successfully analyzed. Samples that yielded SYN548098 concentrations higher than that of the highest calibration standards in their initial analyses were diluted and reanalyzed in a separate run. The lower limit of quantitation (LLOQ) for the 1:1 diluted blood method is approximately 1 ng/mL and the upper limit of quantitation (ULOQ) is approximately 500 ng /mL extracted from whole blood:water (1:1) using a 20-µL aliquot of sample. The final results were converted to whole blood concentrations by correcting for the 1:1 dilution factor.

RAT BLOOD ANALYSIS SUMMARY

Diluted rat blood samples from the study "SYN547407, SYN546308, SYN548097, SYN548012, SYN548014, SYN548102, SYN548117, SYN548098, SYN548101 and SYN548013 - Seven Day Acute Oral Gavage Toxicity Study in Female Wistar Han Rats" were received frozen on dry ice from Battelle Toxicology West Jefferson on 01/22/14 to determine blood concentration levels of SYN548101.

A summary of the method is as follows: Whole Blood:Water (1:1) calibration standards were prepared from one stock solution. The calibration standards, blanks, Quality Control (QC) samples, and study samples were processed by protein precipitation. The extracts were analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS). SYN548101 concentrations were calculated using peak area and a regression line constructed from the concentrations and peak area response of the calibration standards.

All samples were successfully analyzed. Samples that yielded SYN548101 concentrations higher than that of the highest calibration standards in their initial analyses were diluted and reanalyzed in a separate run. The lower limit of quantitation (LLOQ) for the 1:1 diluted blood method is approximately 1 ng/mL and the upper limit of quantitation (ULOQ) is approximately 500 ng /mL extracted from whole blood:water (1:1) using a 20-µL aliquot of sample. The final results were converted to whole blood concentrations by correcting for the 1:1 dilution factor.

RAT BLOOD ANALYSIS SUMMARY

Diluted rat blood samples from the study "SYN547407, SYN546308, SYN548097, SYN548012, SYN548014, SYN548102, SYN548117, SYN548098, SYN548101 and SYN548013 - Seven Day Acute Oral Gavage Toxicity Study in Female Wistar Han Rats" were received frozen on dry ice from Battelle Toxicology West Jefferson on 01/22/14 to determine blood concentration levels of SYN548013.

A summary of the method is as follows: Whole Blood:Water (1:1) calibration standards were prepared from one stock solution. The calibration standards, blanks, Quality Control (QC) samples, and study samples were processed by protein precipitation. The extracts were analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS). SYN548013 concentrations were calculated using peak area and a regression line constructed from the concentrations and peak area response of the calibration standards.

All samples were successfully analyzed. Samples that yielded SYN548013 concentrations higher than that of the highest calibration standards in their initial analyses were diluted and reanalyzed in a separate run. The lower limit of quantitation (LLOQ) for the 1:1 diluted blood method is approximately 1 ng/mL and the upper limit of quantitation (ULOQ) is approximately 500 ng /mL extracted from whole blood:water (1:1) using a 20-µL aliquot of sample. The final results were converted to whole blood concentrations by correcting for the 1:1 dilution factor.

METHOD FOR THE ANALYSIS OF SYN547407 IN RAT WHOLEBLOOD:WATER (1:1) BY TANDEM LC-MS

1.0 PROCEDURE

1.1 General Instructions

USE TWO PAIR OF DISSIMILAR GLOVES DURING NEAT CHEMICAL HANDLING.

Calibrate all required balances according to the SOP on balance usage.

Make equivalent dilutions when the volume needed varies from the volume stated in the method.

Label all standard and reagent solutions as specified in the appropriate SOP.

Document all materials, equipment, and the chromatographic parameters. Initial and date on the top of each page of this document to signify that you have followed the instructions as written, all materials and reagents are current, and all equipment has been properly calibrated.

Initial and date the top of the page on the day that the work for that page was begun. Other entries made by the analyst on a later date or entries made by another person will be initialed and dated near the data entry.

The procedures are written in general chronological order. However, it is not essential that all sections be performed sequentially. The analyst may determine the order for conducting the task in the most efficient manner, unless the order for certain activities is specified.

Line through or "NA" any section that is not needed for a specific task. No formal explanation is required.

1.2 Samples

Reference the Chain of Custody or Sample Inventory Forms.

1.3 Materials

See Table 1 for all required chemicals, reagents, matrix, and solvents. Use Table 1 for documentation. Check the labels carefully to ensure that materials with expiration dates are current and are the proper grade/purity.

Table 1. Materials

Chemical	Supplier	Grade/Purity	Lot Number	Exp Date
SYN547407	See Analytical Standards Section 1.6			
Nifedipine	Sigma Aldrich	≥98%		
Water	Aqua Solutions Model 2121BL	ASTM Type 1	NA	NA
Acetonitrile	Sigma Aldrich	HPLC		
Methanol	Sigma Aldrich	HPLC		
Formic Acid	Fisher	HPLC		
Blank 1:1 Rat Wholeblood:Water[a]	Bioreclamation	Wistar Hannover (K3EDTA)		

a. Will be referred to as Blank blood throughout the document.

1.4 Equipment

All major pieces of equipment used for this method are listed in Table 2 and in the bulleted list below the table. Check the calibration of all equipment requiring calibration (e.g. balances and pipets) to ensure it is current. Document the actual piece of equipment, X or SN, and calibration due date in Table 2.

Table 2. Equipment

Equipment	Equipment ID	Calibration Due Date
Analytical Balance		
Weight Set		
Positive Displacement Pipet		
Positive Displacement Pipet		
Positive Displacement Pipet		
Positive Displacement Pipet		
Repeater Pipet		
Aqua Solutions Water Filtration	09011-21BL	NA
Refrigerator (2 to 8ºC)		NA
Freezer (~-70ºC)		NA

Balance Calibrated by: _____

The following lists the additional general equipment and supplies that are used throughout this method:

- Volumetric flasks, Class A
- Volumetric Pipets, Class A
- Sonicator
- Centrifuge
- Vortex
- Micro-centrifuge Tubes
- Polypropylene Centrifuge Tubes

1.5 Preparation of Solutions

1.5.1 Mobile Phase A, 0.1% Formic acid in Water

Add 1 mL of formic acid to 1 L of water. Cap and mix well. Store at room temperature. May be used for up to one month.

Date Prepared: _____

1.5.2 Mobile Phase B, 0.1% Formic acid in Methanol

Add 1 mL of formic acid to 1 L of methanol. Cap and mix well. Store at room temperature. May be used for up to one month.

Date Prepared: _____

1.6 Preparation of Analytical Standard Solutions

1.6.1 Analytical Standard Information

Document the analytical standard information in Table 3.

Table 3. Analytical Standard Information (SYN547407)

Identification	SYN547407
Lot Number	12DEC13
Storage Location at Time of Use	
Supplier	Battelle
Grade or Purity	NA
Storage Conditions	RT
Expiration Date	12/12/18

1.6.2 Preparation of Stock Analytical Standard Solutions

Preparation of Stock Analytical Standard Solution

Weigh the amounts of analytical standard shown in Table 4 and transfer into a volumetric flask. Add 1 mL of acetonitrile. Cap and mix well. Sonicate for ~5 minutes if necessary. Dilute to volume with acetonitrile and mix thoroughly. Store refrigerated. May be used for up to one year.

Table 4. Preparation of Stock SYN547407 Analytical Standard Solution

ID	Target Conc. (µg/mL)	Target Weight (mg)	Actual Weight (mg)[a]	Final Volume (mL)
A	1000	2 ± 0.2		2

a. Weigh to at least the nearest 0.01 mg. Document weight to at least four significant figures.

Date Prepared: _____

1.6.3 Preparation of Solvent Standard Spiking Solution

Using an adjustable volume pipet, transfer the volumes of standard indicated in Table 5 into volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to one year or the expiration date of the source solution, whichever is the shortest.

Table 5. Preparation of Solvent Standard Spiking Solutions

ID	Target Conc. (ng/mL)	Source	Source Volume (mL) each	Final Volume (mL)
SS1	10000	A	0.10	10
SS2	5000	A	0.05	10
SS3	2000	SS1	1.00	5
SS4	500.0	SS2	0.50	5
SS5	200.0	SS3	0.50	5
SS6	50.00	SS4	0.50	5
SS7	20.00	SS5	0.50	5

Date Prepared: _____

1.7 Preparation of Blood Standards

1.7.1 Preparation of Blood Calibration Standards

Using an adjustable volume pipet transfer the volumes of spiking solution and blank blood indicated in Table 6 into the appropriate micro-centrifuge tubes. **Take care to avoid coagulated clumps.** Cap and mix vigorously by vortexing for approximately 1 minute. Prepare fresh each day of extraction. Process duplicate aliquots at each concentration level with each extraction set.

Table 6. Preparation of SYN547407 Blood Calibration Standards

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
CS1	500.0	SS1	0.05	0.95	1
CS2	250.0	SS2	0.05	0.95	1
CS3	100.0	SS3	0.05	0.95	1
CS4	25.00	SS4	0.05	0.95	1
CS5	10.00	SS5	0.05	0.95	1
CS6	2.500	SS6	0.05	0.95	1
CS7	1.000	SS7	0.05	0.95	1

1.8 Preparation of Blanks

On each day of extraction, process duplicate blood blanks and duplicate blanks + IS.

1.9 Preparation of Internal Standard Solutions

1.9.1 Preparation of Stock Internal Standard Solution

Weigh the amount of Nifedipine analytical standard shown in Table 7 and transfer into a volumetric flask. Dilute to volume with acetonitrile. Cap and mix well. Sonicate for ~5 minutes. If needed, add acetonitrile drop wise and sonicate until dissolved. Store refrigerated. May be used for up to six months.

Table 7. Preparation of Stock Internal Standard Solution

ID	Target Conc. (µg/mL)	Target Weight (mg)	Actual Weight (mg)[a]	Final Volume (mL)
Stock IS	1000	5 ± 0.5		5

b. Weigh to at least the nearest 0.01 mg. Document weight to at least four significant figures.

Date Prepared: _____

1.9.2 Preparation of Intermediate Internal Standard Solution

Using an adjustable volume pipet, transfer the volume of stock internal standard listed in Table 8 into a volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to six months or the expiration date of the stock internal standard solution, whichever is the shortest.

Table 8. Preparation of Intermediate Internal Standard Solution

ID	Target Conc. (µg/mL)	Source	Source Volume (mL)	Final Volume (mL)
Int IS	10	Stock IS	0.25	25

Date Prepared: _____

1.9.3 Preparation of Working Internal Standard (WIS) Solution

Using an adjustable volume pipet, transfer the volume of intermediate internal standard listed in Table 9 into a volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to six months or the expiration date of the stock internal standard solution, whichever is the shortest.

Table 9. Preparation of Working Internal Standard Solution

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Final Volume (mL)
WIS	250	Int IS	1.25	50

Date Prepared: _____

1.10 Preparation of Quality Control (QC) Samples

1.10.1 Preparation of QC Spiking Solutions

Using an adjustable volume pipet, transfer the volume of solutions indicated in Table 10 into volumetric flasks. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to one year or the expiration date of the stock internal standard solution, whichever is the shortest of the two.

Table 10. Preparation of QC Spiking Solution

ID	Target Conc. (ng/mL)	Source	Source Volume (mL)	Final Volume (mL)
QC SS High	8000	A	0.08	10
QC SS Mid	4000	QC SS High	5.00	10
QC SS Low	60.00	QC SS Mid	0.15	10

Date Prepared: _____

1.10.2 Preparation of Blood QC Samples

Using an adjustable volume pipet, transfer the volumes of solutions indicated in Table 11 into individual micro-centrifuge tubes. **Take care to avoid coagulated clumps.** Cap and vortex to mix. Transfer ~0.5 mL aliquots to micro-centrifuge tubes for storage. Store frozen at ~ -70°C. May be used for up to three months.. Process four replicates at each concentration for each extraction set

Table 11. Preparation of Blood QC Samples

ID	Target Conc. (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
QC High	400.0	QC SS High	0.1	1.9	2
QC Mid	200.0	QC SS Mid	0.1	1.9	2
QC Low	3.000	QC SS Low	0.1	1.9	2

Date Prepared: _____

1.11 Preparation of System Suitability (SYS)

Using a positive displacement pipet, transfer the volume of blank blood indicated in Table 12 into a micro-centrifuge tube. Using an adjustable volume pipet, transfer the volume of spiking solution indicated in Table 12 into the micro-centrifuge tube. Cap and vortex to mix. Prepare fresh on each day of extraction. Process four replicates with each extraction set and combine the extracts.

Table 12. Preparation of System Suitability Sample

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
SYS	12.5	CS2	0.05	0.95	1

1.12 Preparation of Blood Standards, Blanks, QCs, and Samples

- Allow blank blood, and samples to thaw at room temperature if previously frozen.

- Document any sample irregularities.

- A second analyst will vortex each study sample thoroughly and open each container one at a time while the primary extracting analyst removes the appropriate volume of sample from the container.

  Second Analyst Initials/Date: _____

- Using an adjustable volume pipet, transfer 20 µL of each calibration standard, blood blank, and study sample into individual micro-centrifuge tubes. Document any samples requiring dilution in Table 13. Use blank blood to bring any samples to 20 µL.

Table 13. Sample Dilution Table

Sample ID	Sample Volume (µL)	Sample ID	Sample Volume (µL)

Reference the sample list at the front of the data binder (if available) for any dilutions not recorded in Table 13.

- Add 100 µL of WIS to all samples, except the blanks without IS. For the blanks without IS, add 100 µL of Acetonitrile instead of WIS.

- Vortex for ~1 minute to mix.

- Centrifuge at a maximum speed for ~10 minutes.

- Transfer supernatant to auto-sampler vials.

- Submit for analysis by LC-MS.

- Sample extracts are stored at the temperature of the auto-sampler if not analyzed on the day of extraction. Storage location of the extracts until injection if not stored on the auto-sampler: _____

1.13 Analysis of Standards, Blanks, QC, and Samples

Use the LC-MS system conditions specified in Table 14. The conditions, which are designated, may be modified by the analyst to produce acceptable chromatography. Record the actual values inside the parentheses.

Set up the automated chromatography data software (CDS) system to collect the electronic output from each injection, acquiring the data.

If the instrument has not been previously conditioned, condition the instrument by injecting the SYS sample for approximately 1 hour prior to starting the system suitability injections. Conditioning run performed: _____ Yes _____ No

Make a minimum of three injections of the appropriate SYS sample prior to starting the sample run to ensure optimal operation of the system.

Make single injections of all calibration standards, blanks, QCs, and samples. Inject one calibration curve near the beginning of the run and one near the end of the run. The calibration standards near the beginning of the run will be injected from high to low concentration. The calibration standards near the end of the run will be injected from low to high concentration. The first replicate of the blank and blank with IS will be injected prior to the first curve. The second replicate of the blank and blank with IS will be injected immediately following the second calibration curve. Distribute the QCs throughout the run.

Make duplicate injections of a post-run SYS sample after the run to assess drift.

Table 14. LC-MS System

Data System	Analyst, Version 1.6.1
MRM	SYN547407 548 > 418 amu Nifedipine 347 > 254 amu
HPLC System	Pump[a]:_____ X/SN:_____ Autosampler[a]:_____ X/SN:_____
MS System	Sciex API _____(Toronto, Ontario Canada) SN:_____
Ionization Source	Heated Nebulizer, Positive Ion Mode
Analytical Column	Phenomenex Luna C18, 5 µm, 50 x 2 mm SN/Lot#:_____
Guard Column	Phenomenex C18 4 x 2 mm
Column Temperature	40ºC
Auto-Sampler Temperature	RT
Mobile Phase	A: 0.1% Formic Acid in Water B: 0.1% Formic Acid in Methanol
Mobile Phase Gradient[b]	Time (minutes) %B 0.0 35 0.2 90 2.2 90 2.3 35 4.5 35
Flow Rate (µL/min)[b]	500 (_____) µL/min
Injection Volume[b]	10 µL (_____) µL
Run Time[b]	4.5 minutes (_____) minutes

a. Agilent (Santa Clara, CA), Shimadzu (Kyoto, Japan), Leap CTC Analytics (Carrboro, NC).
b. Parameters which may be modified by analyst.

Run ID: _____

2.0 CALCULATIONS

Determine the optimal automatic integration parameters for the run. Examine the integration of the analyte and the internal standard by the chromatography system. Modify, if necessary, to obtain optimum consistent integration. Document the rationale of any manual or automated reintegration of peaks.

Use Microsoft Excel to perform the calculations as follows:

Calculate the individual and average percent area of each blank and blank with IS compared with the average area response of the Lower Limit of Quantitation (LLOQ) standard.

Calculate the individual percent area of the blank following the high standard compared with the area response of the nearest LLOQ standard.

Calculate the SYS average ratio and rsd. Calculate the Drift average area ratio. Compare the average ratio of the SYS to the average ratio of the Drift.

Calculate the exact concentrations of the plasma standards and plasma QCs. Enter the values into the Watson Bioanalytical LIMS.

Use the Watson Bioanalytical LIMS, version 7.4, to perform the calculations as follows:

Calculate the peak area response ratio (Analyte/IS) for each injection.

Determine optimal regression parameters and record the model and weighting in Table 15 to calculate the regression equation.

Table 15. Regression Parameters

Model	
Weighting	
y-intercept	Calculate, Do not force through origin
y-values	Instrument Response
x-values	Nominal Concentration

Using the peak area response of the standards and the regression equation, calculate their determined concentrations.

Using the peak area response ratios of the QCs and samples, the regression equation, and any dilution factor, if applicable, calculate their individual determined concentrations.

Using the determined concentration, calculate the individual relative error (%RE) for each standard and QC concentration.

3.0 ACCEPTANCE CRITERIA

There are no specific acceptance criteria for a this assay. The following will be used to assess each individual run, however the task leader may technically decide to revise these criteria or accept criteria outside these limits.

- System Suitability – At least three consecutive injections of the SYS immediately preceding the run must have a RSD of ≤ 10.0% for the run to be valid using the area ratio.

- Drift – Average area ratio within 15.0% of the pre-run system suitability average area ratio.

- Carryover - The blank immediately following the second curve must have an area response of no greater than 20.0% of the area response of the nearest acceptable low standard.

- Linearity – The coefficient of determination will be ≥ 0.98.

- Calibration Standards – Standards will have determined concentrations within 15.0% of nominal as measured by %RE, except for the LLOQ, where it should be within 20.0% of nominal to be acceptable. If a standard is not acceptable, it will not be used to generate the calibration curve. The standards that are not acceptable will be removed one-by-one with the standard deviating furthest from nominal concentration as measured by %RE removed first; regression will be performed again after each removal. Up to 3 standards falling outside these limits can be discarded (deactivated), provided they do not change the established model. At least 75% (11 of 14) of the standards must be acceptable for the calibration curve to be acceptable. A minimum of six concentration levels must define the curve.

- Selectivity – The blanks and blanks with internal standard must have an average response of no greater than 20.0% of the average response of the lowest acceptable standard.

- Sensitivity – The limit of quantitation for a run is the lowest acceptable standard that meets all acceptance criteria.

- Quality Control Samples - At least 67% (eight of twelve) of the QCs will have determined concentrations within 20.0 percent of the nominal concentration and at least 50% (two of the four) QCs at each concentration level will have

determined concentrations within 20.0 percent of the nominal concentration as measured by the %RE.

- Repeat Analysis of Study Samples – For samples that are repeated for technical reasons (e.g. failed run, bad injection) only one replicate must be run. For samples repeated for any other reason, at least duplicate aliquots must be analyzed, if sufficient sample is available. When multiple samples are analyzed, the average of all acceptable values will be reported, unless a value can be statistically excluded.

4.0 RESPONSE TO FAILURE TO MEET ACCEPTANCE CRITERIA

4.1 System Suitability

Verify that all calculations are correct. If the system suitability does not meet acceptance criteria and the standards and the QCs within the run meet acceptance criteria, a run set may be accepted with written justification and management approval. Otherwise, reinject the entire run set.

4.2 Drift

Verify that all calculations are correct. If the drift does not meet acceptance criteria and the standards and the QCs within the run meet acceptance criteria, a run set may be accepted with written justification and management approval. Otherwise, reinject the entire run set.

4.3 Accuracy of Standards and QCs

Verify that all calculations are correct . Verify that the proper instrument system (e.g., column, flow rates, etc.) was used for the analysis. If not, samples need to be re-injected using the correct instrument system.

If the correct instrument system was used, compare the current chromatograms to a past analysis. If the chromatography has changed substantially, determine and correct the problem with the instrument system and then re-inject the samples.

4.4 Linearity (Coefficient of Determination)

Verify that all concentrations, the regression model, integration, and calculations are correct.

If all the calculations are correct, repeat the analysis.

4.5 Selectivity/Carryover

If the results are greater than 20.0%, verify that the proper wash procedure was used, if applicable. If the proper wash conditions were utilized, assess the reason for carryover and the impact to the data. The LLOQ for the impacted set may be raised to the concentration level that meets the acceptable limits. The samples between the new LLOQ and the original LLOQ will be repeated with another extraction set.

5.0 RESULTS

Include printouts of the acquisition method, and any applicable spreadsheets in the data packet.

5.1 COMMENTS/CONCLUSIONS

_____
_____
_____
_____
_____
_____
_____
_____

6.0 DATA REVIEW

6.1 Technical Review

All data generated in the laboratory activities will be reviewed by the technical leader (or designee) for technical content, accuracy, and completeness of documentation.

The technical leader will assure the proper equipment was used, review experimental procedure, rejection of calibration standards, integration of chromatograms, chromatography data processing and acquisition parameters, calibration standard concentrations, regression model, and that this documentation was followed.

6.2 Data Accuracy Review

Review at least the following: completeness and correctness of data entry, formulas used to calculate all values, and accuracy of calculations.

7.0 SIGNATURES

Technical Review Signature/Date:

_____

_____

Signature of the technical reviewer will be considered documentation that all modifications and/or changes to this documentation form (documented during the course of conducting this task) are technically acceptable and have no adverse technical impact unless otherwise noted.

Data Accuracy Review Signature/Date:

_____

METHOD FOR THE ANALYSIS OF SYN546308 IN RAT WHOLEBLOOD:WATER (1:1) BY TANDEM LC-MS

1.0 PROCEDURE

1.1 General Instructions

USE TWO PAIR OF DISSIMILAR GLOVES DURING NEAT CHEMICAL HANDLING.

Calibrate all required balances according to the SOP on balance usage.

Make equivalent dilutions when the volume needed varies from the volume stated in the method.

Label all standard and reagent solutions as specified in the appropriate SOP.

Document all materials, equipment, and the chromatographic parameters. Initial and date on the top of each page of this document to signify that you have followed the instructions as written, all materials and reagents are current, and all equipment has been properly calibrated.

Initial and date the top of the page on the day that the work for that page was begun. Other entries made by the analyst on a later date or entries made by another person will be initialed and dated near the data entry.

The procedures are written in general chronological order. However, it is not essential that all sections be performed sequentially. The analyst may determine the order for conducting the task in the most efficient manner, unless the order for certain activities is specified.

Line through or "NA" any section that is not needed for a specific task. No formal explanation is required.

1.2 Samples

Reference the Chain of Custody or Sample Inventory Forms.

1.3 Materials

See Table 1 for all required chemicals, reagents, matrix, and solvents. Use Table 1 for documentation. Check the labels carefully to ensure that materials with expiration dates are current and are the proper grade/purity.

Table 1. Materials

Chemical	Supplier	Grade/Purity	Lot Number	Exp Date
SYN546308	See Analytical Standards Section 1.6			
Nifedipine	Sigma Aldrich	≥98%		
Water	Aqua Solutions Model 2121BL	ASTM Type 1	NA	NA
Acetonitrile	Sigma Aldrich	HPLC		
Methanol	Sigma Aldrich	HPLC		
Formic Acid	Fisher	HPLC		
Blank 1:1 Rat Wholeblood:Water[a]	Bioreclamation	Wistar Hannover (K3EDTA)		

a. Will be referred to as Blank blood throughout the document.

1.4 Equipment

All major pieces of equipment used for this method are listed in Table 2 and in the bulleted list below the table. Check the calibration of all equipment requiring calibration (e.g. balances and pipets) to ensure it is current. Document the actual piece of equipment, X or SN, and calibration due date in Table 2.

Table 2. Equipment

Equipment	Equipment ID	Calibration Due Date
Analytical Balance		
Weight Set		
Positive Displacement Pipet		
Positive Displacement Pipet		
Positive Displacement Pipet		
Positive Displacement Pipet		
Repeater Pipet		
Aqua Solutions Water Filtration	09011-21BL	NA
Refrigerator (2 to 8ºC)		NA
Freezer (~-70ºC)		NA

Balance Calibrated by: _____

The following lists the additional general equipment and supplies that are used throughout this method:

- Volumetric flasks, Class A
- Volumetric Pipets, Class A
- Sonicator
- Centrifuge
- Vortex
- Micro-centrifuge Tubes
- Polypropylene Centrifuge Tubes

1.5 Preparation of Solutions

1.5.1 Mobile Phase A, 0.1% Formic acid in Water

Add 1 mL of formic acid to 1 L of water. Cap and mix well. Store at room temperature. May be used for up to one month.

Date Prepared: _____

1.5.2 Mobile Phase B, 0.1% Formic acid in Methanol

Add 1 mL of formic acid to 1 L of methanol. Cap and mix well. Store at room temperature. May be used for up to one month.

Date Prepared: _____

1.6 Preparation of Analytical Standard Solutions

1.6.1 Analytical Standard Information

Document the analytical standard information in Table 3.

Table 3. Analytical Standard Information

Identification	**SYN546308**
Lot Number	13DEC13
Storage Location at Time of Use	
Supplier	Battelle
Grade or Purity	NA
Storage Conditions	RT
Expiration Date	12/13/18

1.6.2 Preparation of Stock Analytical Standard Solutions

Preparation of Stock SYN546308 Analytical Standard Solution

Weigh the amounts of SYN546308 analytical standard shown in Table 4 and transfer into a volumetric flask. Add 1 mL of acetonitrile. Cap and mix well. Sonicate for ~5 minutes if necessary. Dilute to volume with acetonitrile and mix thoroughly. Store refrigerated. May be used for up to three months.

Table 4. Preparation of Stock SYN546308 Analytical Standard Solution

ID	Target Conc. (µg/mL)	Target Weight (mg)	Actual Weight (mg)[a]	Final Volume (mL)
A	1000	2 ± 0.2		2

a. Weigh to at least the nearest 0.01 mg. Document weight to at least four significant figures.

Date Prepared: _____

1.6.3 Preparation of Solvent Standard Spiking Solution

Using an adjustable volume pipet, transfer the volumes of standard indicated in Table 5 into volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to three months or the expiration date of the source solution, whichever is the shortest.

Table 5. Preparation of Solvent Standard Spiking Solutions

ID	Target Conc. (ng/mL)	Source	Source Volume (mL) each	Final Volume (mL)
SS1	10000	A	0.10	10
SS2	5000	A	0.05	10
SS3	2000	SS1	1.00	5
SS4	500.0	SS2	0.50	5
SS5	200.0	SS3	0.50	5
SS6	50.00	SS4	0.50	5
SS7	20.00	SS5	0.50	5

Date Prepared: _____

1.7 Preparation of Blood Standards

1.7.1 Preparation of Blood Calibration Standards

Using an adjustable volume pipet transfer the volumes of spiking solution and blank blood indicated in Table 6 into the appropriate micro-centrifuge tubes. **Take care to avoid coagulated clumps.** Cap and mix vigorously by vortexing for approximately 1 minute. Prepare fresh each day of extraction. Process duplicate aliquots at each concentration level with each extraction set.

Table 6. Preparation of SYN546308 Blood Calibration Standards

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
CS1	500.0	SS1	0.05	0.95	1
CS2	250.0	SS2	0.05	0.95	1
CS3	100.0	SS3	0.05	0.95	1
CS4	25.00	SS4	0.05	0.95	1
CS5	10.00	SS5	0.05	0.95	1
CS6	2.500	SS6	0.05	0.95	1
CS7	1.000	SS7	0.05	0.95	1

1.8 Preparation of Blanks

On each day of extraction, process duplicate blood blanks and duplicate blanks + IS.

1.9 Preparation of Internal Standard Solutions

1.9.1 Preparation of Stock Internal Standard Solution

Weigh the amount of Nifedipine analytical standard shown in Table 7 and transfer into a volumetric flask. Dilute to volume with acetonitrile. Cap and mix well. Sonicate for ~5 minutes. If needed, add acetonitrile drop wise and sonicate until dissolved. Store refrigerated. May be used for up to six months.

Table 7. Preparation of Stock Internal Standard Solution

ID	Target Conc. (µg/mL)	Target Weight (mg)	Actual Weight (mg)[a]	Final Volume (mL)
Stock IS	1000	5 ± 0.5		5

b. Weigh to at least the nearest 0.01 mg. Document weight to at least four significant figures.

Date Prepared: _____

1.9.2 Preparation of Intermediate Internal Standard Solution

Using an adjustable volume pipet, transfer the volume of stock internal standard listed in Table 8 into a volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to six months or the expiration date of the stock internal standard solution, whichever is the shortest.

Table 8. Preparation of Intermediate Internal Standard Solution

ID	Target Conc. (µg/mL)	Source	Source Volume (mL)	Final Volume (mL)
Int IS	10	Stock IS	0.25	25

Date Prepared: _____

1.9.3 Preparation of Working Internal Standard (WIS) Solution

Using an adjustable volume pipet, transfer the volume of intermediate internal standard listed in Table 9 into a volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to six months or the expiration date of the stock internal standard solution, whichever is the shortest.

Table 9. Preparation of Working Internal Standard Solution

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Final Volume (mL)
WIS	250	Int IS	1.25	50

Date Prepared: _____

1.10 Preparation of Quality Control (QC) Samples

1.10.1 Preparation of Solvent Intermediate QC Spiking Solutions

Using an adjustable volume pipet, transfer the volumes of standard indicated in Table 10 into volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to one year or the expiration date of the source solution, whichever is the shortest.

Table 10. Preparation of Solvent Intermediate QC Spiking Solution

ID	Target Conc. (ng/mL)	Source	Source Volume (mL)	Final Volume (mL)
QC SS High	8000	A	0.08	10
QC SS Mid	4000	QC SS High	5.00	10
QC SS Low	60.00	QC SS Mid	0.15	10

Date Prepared: _____

1.10.2 Preparation of Blood QC Samples

Using an adjustable volume pipet, transfer the volumes of solutions indicated in Table 11 into individual micro-centrifuge tubes. **Take care to avoid coagulated clumps.** Cap and vortex to mix. Transfer ~0.5 mL aliquots to micro-centrifuge tubes for storage. Store frozen at ~ -70°C. May be used for up to one year. Process four replicates at each concentration for each extraction set

Table 11. Preparation of Blood QC Samples

ID	Target Conc. (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
QC High	400	QC SS High	0.1	1.9	2
QC Mid	200	QC SS Mid	0.1	1.9	2
QC Low	3	QC SS Low	0.1	1.9	2

Date Prepared: _____

1.11 Preparation of System Suitability (SYS)

Using a positive displacement pipet, transfer the volume of blank blood indicated in Table 12 into a micro-centrifuge tube. Using an adjustable volume pipet, transfer the volume of spiking solution indicated in Table 12 into the micro-centrifuge tube. Cap and vortex to mix. Prepare fresh on each day of extraction. Process four replicates with each extraction set and combine the extracts.

Table 12. Preparation of System Suitability Sample

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
SYS	12.5	CS2	0.05	0.95	1

1.12 Preparation of Blood Standards, Blanks, QCs, and Samples

- Allow blank blood, and samples to thaw at room temperature if previously frozen.
- Document any sample irregularities.
- A second analyst will vortex each study sample thoroughly and open each container one at a time while the primary extracting analyst removes the appropriate volume of sample from the container.

  Second Analyst Initials/Date: _____
- Using an adjustable volume pipet, transfer 20 µL of each calibration standard, blood blank, and study sample into individual micro-centrifuge tubes. Document any samples requiring dilution in Table 13. Use blank blood to bring any samples to 20 µL.

Table 13. Sample Dilution Table

Sample ID	Sample Volume (µL)	Sample ID	Sample Volume (µL)

Reference the sample list at the front of the data binder (if available) for any dilutions not recorded in Table 13.

- Add 100 µL of WIS to all samples, except the blanks without IS. For the blanks without IS, add 100 µL of Acetonitrile instead of WIS.
- Vortex for ~1 minute to mix.
- Centrifuge at a maximum speed for ~10 minutes.
- Transfer supernatant to auto-sampler vials.
- Submit for analysis by LC-MS.

- Sample extracts are stored at the temperature of the auto-sampler if not analyzed on the day of extraction. Storage location of the extracts until injection if not stored on the auto-sampler: _____

1.13 Analysis of Standards, Blanks, QC, and Samples

Use the LC-MS system conditions specified in Table 14. The conditions, which are designated, may be modified by the analyst to produce acceptable chromatography. Record the actual values inside the parentheses.

Set up the automated chromatography data software (CDS) system to collect the electronic output from each injection, acquiring the data.

If the instrument has not been previously conditioned, condition the instrument by injecting the SYS sample for approximately 1 hour prior to starting the system suitability injections. Conditioning run performed: _____ Yes _____ No

Make a minimum of three injections of the appropriate SYS sample prior to starting the sample run to ensure optimal operation of the system.

Make single injections of all calibration standards, blanks, QCs, and samples. Inject one calibration curve near the beginning of the run and one near the end of the run. The calibration standards near the beginning of the run will be injected from high to low concentration. The calibration standards near the end of the run will be injected from low to high concentration. The first replicate of the blank and blank with IS will be injected prior to the first curve. The second replicate of the blank and blank with IS will be injected immediately following the second calibration curve. Distribute the QCs throughout the run.

Make duplicate injections of a post-run SYS sample after the run to assess drift.

Table 14. LC-MS System

Data System	Analyst, Version 1.6.1
MRM	SYN546308 530 > 400 amu Nifedipine 347 > 254 amu
HPLC System	Pump[a]:_____ X/SN:_____ Autosampler[a]:_____ X/SN:_____
MS System	Sciex API _____(Toronto, Ontario Canada) SN:_____
Ionization Source	Heated Nebulizer, Positive Ion Mode
Analytical Column	Phenomenex Luna C18, 5 µm, 50 x 2 mm SN/Lot#:_____
Guard Column	Phenomenex C18 4 x 2 mm
Column Temperature	40ºC
Auto-Sampler Temperature	RT
Mobile Phase	A: 0.1% Formic Acid in Water B: 0.1% Formic Acid in Methanol
Mobile Phase Gradient[b]	Time (minutes) %B 0.0 35 0.2 90 2.2 90 2.3 35 4.5 35
Flow Rate (µL/min)[b]	500 (_____) µL/min
Injection Volume[b]	10 µL (_____) µL
Run Time[b]	4.5 minutes (_____) minutes

a. Agilent (Santa Clara, CA), Shimadzu (Kyoto, Japan), Leap CTC Analytics (Carrboro, NC).
b. Parameters which may be modified by analyst.

Run ID: _____

2.0 CALCULATIONS

Determine the optimal automatic integration parameters for the run. Examine the integration of the analyte and the internal standard by the chromatography system. Modify, if necessary, to obtain optimum consistent integration. Document the rationale of any manual or automated reintegration of peaks.

Use Microsoft Excel to perform the calculations as follows:

Calculate the individual and average percent area of each blank and blank with IS compared with the average area response of the Lower Limit of Quantitation (LLOQ) standard.

Calculate the individual percent area of the blank following the high standard compared with the area response of the nearest LLOQ standard.

Calculate the SYS average ratio and rsd. Calculate the Drift average area ratio. Compare the average ratio of the SYS to the average ratio of the Drift.

Calculate the exact concentrations of the plasma standards and plasma QCs. Enter the values into the Watson Bioanalytical LIMS.

Use the Watson Bioanalytical LIMS, version 7.4, to perform the calculations as follows:

Calculate the peak area response ratio (Analyte/IS) for each injection.

Determine optimal regression parameters and record the model and weighting in Table 15 for SYN546308 to calculate the regression equation.

Table 15. Regression Parameters for SYN546308

Model	
Weighting	
y-intercept	Calculate, Do not force through origin
y-values	Instrument Response
x-values	Nominal Concentration

Using the peak area response of the standards and the regression equation, calculate their determined concentrations.

Using the peak area response ratios of the QCs and samples, the regression equation, and any dilution factor, if applicable, calculate their individual determined concentrations.

Using the determined concentration, calculate the individual relative error (%RE) for each standard and QC concentration.

3.0 ACCEPTANCE CRITERIA

There are no specific acceptance criteria for a this assay. The following will be used to assess each individual run, however the task leader may technically decide to revise these criteria or accept criteria outside these limits.

- System Suitability – At least three consecutive injections of the SYS immediately preceding the run must have a RSD of ≤ 10.0% for the run to be valid using the area ratio.

- Drift – Average area ratio within 15.0% of the pre-run system suitability average area ratio.

- Carryover - The blank immediately following the second curve must have an area response of no greater than 20.0% of the area response of the nearest acceptable low standard.

- Linearity – The coefficient of determination will be ≥ 0.98.

- Calibration Standards – Standards will have determined concentrations within 15.0% of nominal as measured by %RE, except for the LLOQ, where it should be within 20.0% of nominal to be acceptable. If a standard is not acceptable, it will not be used to generate the calibration curve. The standards that are not acceptable will be removed one-by-one with the standard deviating furthest from nominal concentration as measured by %RE removed first; regression will be performed again after each removal. Up to six standards falling outside these limits can be discarded (deactivated), provided they do not change the established model. At least 75% (11 of 14) of the standards must be acceptable for the calibration curve to be acceptable. A minimum of six concentration levels must define the curve.

- Selectivity – The blanks and blanks with internal standard must have an average response of no greater than 20.0% of the average response of the lowest acceptable standard.

- Sensitivity – The limit of quantitation for a run is the lowest acceptable standard that meets all acceptance criteria.

- Quality Control Samples - At least 67% (eight of twelve) of the QCs will have determined concentrations within 20.0 percent of the nominal concentration and at least 50% (two of the four) QCs at each concentration level will have

determined concentrations within 20.0 percent of the nominal concentration as measured by the %RE.

- Repeat Analysis of Study Samples – For samples that are repeated for technical reasons (e.g. failed run, bad injection) only one replicate must be run. For samples repeated for any other reason, at least duplicate aliquots must be analyzed, if sufficient sample is available. When multiple samples are analyzed, the average of all acceptable values will be reported, unless a value can be statistically excluded.

4.0 RESPONSE TO FAILURE TO MEET ACCEPTANCE CRITERIA

4.1 System Suitability

Verify that all calculations are correct. If the system suitability does not meet acceptance criteria and the standards and the QCs within the run meet acceptance criteria, a run set may be accepted with written justification and management approval. Otherwise, reinject the entire run set.

4.2 Drift

Verify that all calculations are correct. If the drift does not meet acceptance criteria and the standards and the QCs within the run meet acceptance criteria, a run set may be accepted with written justification and management approval. Otherwise, reinject the entire run set.

4.3 Accuracy of Standards and QCs

Verify that all calculations are correct . Verify that the proper instrument system (e.g., column, flow rates, etc.) was used for the analysis. If not, samples need to be re-injected using the correct instrument system.

If the correct instrument system was used, compare the current chromatograms to a past analysis. If the chromatography has changed substantially, determine and correct the problem with the instrument system and then re-inject the samples.

4.4 Linearity (Coefficient of Determination)

Verify that all concentrations, the regression model, integration, and calculations are correct.

If all the calculations are correct, repeat the analysis.

4.5 Selectivity/Carryover

If the results are greater than 20.0%, verify that the proper wash procedure was used, if applicable. If the proper wash conditions were utilized, assess the reason for carryover and the impact to the data. The LLOQ for the impacted set may be raised to the concentration level that meets the acceptable limits. The samples between the new LLOQ and the original LLOQ will be repeated with another extraction set.

5.0 RESULTS

Include printouts of the acquisition method, and any applicable spreadsheets in the data packet.

5.1 COMMENTS/CONCLUSIONS

_____
_____
_____
_____
_____
_____
_____
_____

6.0 DATA REVIEW

6.1 Technical Review

All data generated in the laboratory activities will be reviewed by the technical leader (or designee) for technical content, accuracy, and completeness of documentation.

The technical leader will assure the proper equipment was used, review experimental procedure, rejection of calibration standards, integration of chromatograms, chromatography data processing and acquisition parameters, calibration standard concentrations, regression model, and that this documentation was followed.

6.2 Data Accuracy Review

Review at least the following: completeness and correctness of data entry, formulas used to calculate all values, and accuracy of calculations.

7.0 SIGNATURES

Technical Review Signature/Date:

_____

_____

Signature of the technical reviewer will be considered documentation that all modifications and/or changes to this documentation form (documented during the course of conducting this task) are technically acceptable and have no adverse technical impact unless otherwise noted.

Data Accuracy Review Signature/Date:

_____

METHOD FOR THE ANALYSIS OF SYN548097 IN RAT WHOLEBLOOD:WATER (1:1) BY TANDEM LC-MS

1.0 PROCEDURE

1.1 General Instructions

USE TWO PAIR OF DISSIMILAR GLOVES DURING NEAT CHEMICAL HANDLING.

Calibrate all required balances according to the SOP on balance usage.

Make equivalent dilutions when the volume needed varies from the volume stated in the method.

Label all standard and reagent solutions as specified in the appropriate SOP.

Document all materials, equipment, and the chromatographic parameters. Initial and date on the top of each page of this document to signify that you have followed the instructions as written, all materials and reagents are current, and all equipment has been properly calibrated.

Initial and date the top of the page on the day that the work for that page was begun. Other entries made by the analyst on a later date or entries made by another person will be initialed and dated near the data entry.

The procedures are written in general chronological order. However, it is not essential that all sections be performed sequentially. The analyst may determine the order for conducting the task in the most efficient manner, unless the order for certain activities is specified.

Line through or "NA" any section that is not needed for a specific task. No formal explanation is required.

1.2 Samples

Reference the Chain of Custody or Sample Inventory Forms.

1.3 Materials

See Table 1 for all required chemicals, reagents, matrix, and solvents. Use Table 1 for documentation. Check the labels carefully to ensure that materials with expiration dates are current and are the proper grade/purity.

Table 1. Materials

Chemical	Supplier	Grade/Purity	Lot Number	Exp Date
SYN548097	See Analytical Standards Section 1.6			
Nifedipine	Sigma Aldrich	≥98%		
Water	Aqua Solutions Model 2121BL	ASTM Type 1	NA	NA
Acetonitrile	Sigma Aldrich	HPLC		
Methanol	Sigma Aldrich	HPLC		
Formic Acid	Fisher	HPLC		
Blank 1:1 Rat Wholeblood:Water[a]	Bioreclamation	Wistar Hannover (K3EDTA)		

a. Will be referred to as Blank blood throughout the document.

1.4 Equipment

All major pieces of equipment used for this method are listed in Table 2 and in the bulleted list below the table. Check the calibration of all equipment requiring calibration (e.g. balances and pipets) to ensure it is current. Document the actual piece of equipment, X or SN, and calibration due date in Table 2.

Table 2. Equipment

Equipment	Equipment ID	Calibration Due Date
Analytical Balance		
Weight Set		
Positive Displacement Pipet		
Positive Displacement Pipet		
Positive Displacement Pipet		
Positive Displacement Pipet		
Repeater Pipet		
Aqua Solutions Water Filtration	09011-21BL	NA
Refrigerator (2 to 8ºC)		NA
Freezer (~-70ºC)		NA

Balance Calibrated by: _____

The following lists the additional general equipment and supplies that are used throughout this method:

- Volumetric flasks, Class A
- Volumetric Pipets, Class A
- Sonicator
- Centrifuge
- Vortex
- Micro-centrifuge Tubes
- Polypropylene Centrifuge Tubes

1.5 Preparation of Solutions

1.5.1 Mobile Phase A, 0.1% Formic acid in Water

Add 1 mL of formic acid to 1 L of water. Cap and mix well. Store at room temperature. May be used for up to one month.

Date Prepared: _____

1.5.2 Mobile Phase B, 0.1% Formic acid in Methanol

Add 1 mL of formic acid to 1 L of methanol. Cap and mix well. Store at room temperature. May be used for up to one month.

Date Prepared: _____

1.6 Preparation of Analytical Standard Solutions

1.6.1 Analytical Standard Information

Document the analytical standard information in Table 3.

Table 3. Analytical Standard Information (SYN548097)

Identification	SYN548097
Lot Number	12DEC13
Storage Location at Time of Use	
Supplier	Battelle
Grade or Purity	NA
Storage Conditions	RT
Expiration Date	12/12/18

1.6.2 Preparation of Stock Analytical Standard Solutions

Preparation of Stock Analytical Standard Solution

Weigh the amounts of analytical standard shown in Table 4 and transfer into a volumetric flask. Add 1 mL of acetonitrile. Cap and mix well. Sonicate for ~5 minutes if necessary. Dilute to volume with acetonitrile and mix thoroughly. Store refrigerated. May be used for up to one year.

Table 4. Preparation of Stock SYN548097 Analytical Standard Solution

ID	Target Conc. (µg/mL)	Target Weight (mg)	Actual Weight (mg)[a]	Final Volume (mL)
A	1000	2 ± 0.2		2

a. Weigh to at least the nearest 0.01 mg. Document weight to at least four significant figures.

Date Prepared: _____

1.6.3 Preparation of Solvent Standard Spiking Solution

Using an adjustable volume pipet, transfer the volumes of standard indicated in Table 5 into volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to one year or the expiration date of the source solution, whichever is the shortest.

Table 5. Preparation of Solvent Standard Spiking Solutions

ID	Target Conc. (ng/mL)	Source	Source Volume (mL) each	Final Volume (mL)
SS1	10000	A	0.10	10
SS2	5000	A	0.05	10
SS3	2000	SS1	1.00	5
SS4	500.0	SS2	0.50	5
SS5	200.0	SS3	0.50	5
SS6	50.00	SS4	0.50	5
SS7	20.00	SS5	0.50	5

Date Prepared: _____

1.7 Preparation of Blood Standards

1.7.1 Preparation of Blood Calibration Standards

Using an adjustable volume pipet transfer the volumes of spiking solution and blank blood indicated in Table 6 into the appropriate micro-centrifuge tubes. **Take care to avoid coagulated clumps.** Cap and mix vigorously by vortexing for approximately 1 minute. Prepare fresh each day of extraction. Process duplicate aliquots at each concentration level with each extraction set.

Table 6. Preparation of SYN548097 Blood Calibration Standards

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
CS1	500.0	SS1	0.05	0.95	1
CS2	250.0	SS2	0.05	0.95	1
CS3	100.0	SS3	0.05	0.95	1
CS4	25.00	SS4	0.05	0.95	1
CS5	10.00	SS5	0.05	0.95	1
CS6	2.500	SS6	0.05	0.95	1
CS7	1.000	SS7	0.05	0.95	1

1.8 Preparation of Blanks

On each day of extraction, process duplicate blood blanks and duplicate blanks + IS.

1.9 Preparation of Internal Standard Solutions

1.9.1 Preparation of Stock Internal Standard Solution

Weigh the amount of Nifedipine analytical standard shown in Table 7 and transfer into a volumetric flask. Dilute to volume with acetonitrile. Cap and mix well. Sonicate for ~5 minutes. If needed, add acetonitrile drop wise and sonicate until dissolved. Store refrigerated. May be used for up to six months.

Table 7. Preparation of Stock Internal Standard Solution

ID	Target Conc. (µg/mL)	Target Weight (mg)	Actual Weight (mg)[a]	Final Volume (mL)
Stock IS	1000	5 ± 0.5		5

b. Weigh to at least the nearest 0.01 mg. Document weight to at least four significant figures.

Date Prepared: _____

1.9.2 Preparation of Intermediate Internal Standard Solution

Using an adjustable volume pipet, transfer the volume of stock internal standard listed in Table 8 into a volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to six months or the expiration date of the stock internal standard solution, whichever is the shortest.

Table 8. Preparation of Intermediate Internal Standard Solution

ID	Target Conc. (µg/mL)	Source	Source Volume (mL)	Final Volume (mL)
Int IS	10	Stock IS	0.25	25

Date Prepared: _____

1.9.3 Preparation of Working Internal Standard (WIS) Solution

Using an adjustable volume pipet, transfer the volume of intermediate internal standard listed in Table 9 into a volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to six months or the expiration date of the stock internal standard solution, whichever is the shortest.

Table 9. Preparation of Working Internal Standard Solution

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Final Volume (mL)
WIS	250	Int IS	1.25	50

Date Prepared: _____

1.10 Preparation of Quality Control (QC) Samples

1.10.1 Preparation of QC Spiking Solutions

Using an adjustable volume pipet, transfer the volume of solutions indicated in Table 10 into volumetric flasks. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to one year or the expiration date of the stock internal standard solution, whichever is the shortest of the two.

Table 10. Preparation of QC Spiking Solution

ID	Target Conc. (ng/mL)	Source	Source Volume (mL)	Final Volume (mL)
QC SS High	8000	A	0.08	10
QC SS Mid	4000	QC SS High	5.00	10
QC SS Low	60.00	QC SS Mid	0.15	10

Date Prepared: _____

1.10.2 Preparation of Blood QC Samples

Using an adjustable volume pipet, transfer the volumes of solutions indicated in Table 11 into individual micro-centrifuge tubes. **Take care to avoid coagulated clumps.** Cap and vortex to mix. Transfer ~0.5 mL aliquots to micro-centrifuge tubes for storage. Store frozen at ~ -70°C. May be used for up to three months.. Process four replicates at each concentration for each extraction set

Table 11. Preparation of Blood QC Samples

ID	Target Conc. (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
QC High	400.0	QC SS High	0.1	1.9	2
QC Mid	200.0	QC SS Mid	0.1	1.9	2
QC Low	3.000	QC SS Low	0.1	1.9	2

Date Prepared: _____

1.11 Preparation of System Suitability (SYS)

Using a positive displacement pipet, transfer the volume of blank blood indicated in Table 12 into a micro-centrifuge tube. Using an adjustable volume pipet, transfer the volume of spiking solution indicated in Table 12 into the micro-centrifuge tube. Cap and vortex to mix. Prepare fresh on each day of extraction. Process four replicates with each extraction set and combine the extracts.

Table 12. Preparation of System Suitability Sample

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
SYS	12.5	CS2	0.05	0.95	1

1.12 Preparation of Blood Standards, Blanks, QCs, and Samples

- Allow blank blood, and samples to thaw at room temperature if previously frozen.

- Document any sample irregularities.

- A second analyst will vortex each study sample thoroughly and open each container one at a time while the primary extracting analyst removes the appropriate volume of sample from the container.

  Second Analyst Initials/Date: _____

- Using an adjustable volume pipet, transfer 20 µL of each calibration standard, blood blank, and study sample into individual micro-centrifuge tubes. Document any samples requiring dilution in Table 13. Use blank blood to bring any samples to 20 µL.

Table 13. Sample Dilution Table

Sample ID	Sample Volume (µL)	Sample ID	Sample Volume (µL)

Reference the sample list at the front of the data binder (if available) for any dilutions not recorded in Table 13.

- Add 100 µL of WIS to all samples, except the blanks without IS. For the blanks without IS, add 100 µL of Acetonitrile instead of WIS.

- Vortex for ~1 minute to mix.

- Centrifuge at a maximum speed for ~10 minutes.

- Transfer supernatant to auto-sampler vials.

- Submit for analysis by LC-MS.

- Sample extracts are stored at the temperature of the auto-sampler if not analyzed on the day of extraction. Storage location of the extracts until injection if not stored on the auto-sampler: _____

1.13 Analysis of Standards, Blanks, QC, and Samples

Use the LC-MS system conditions specified in Table 14. The conditions, which are designated, may be modified by the analyst to produce acceptable chromatography. Record the actual values inside the parentheses.

Set up the automated chromatography data software (CDS) system to collect the electronic output from each injection, acquiring the data.

If the instrument has not been previously conditioned, condition the instrument by injecting the SYS sample for approximately 1 hour prior to starting the system suitability injections. Conditioning run performed: _____ Yes _____ No

Make a minimum of three injections of the appropriate SYS sample prior to starting the sample run to ensure optimal operation of the system.

Make single injections of all calibration standards, blanks, QCs, and samples. Inject one calibration curve near the beginning of the run and one near the end of the run. The calibration standards near the beginning of the run will be injected from high to low concentration. The calibration standards near the end of the run will be injected from low to high concentration. The first replicate of the blank and blank with IS will be injected prior to the first curve. The second replicate of the blank and blank with IS will be injected immediately following the second calibration curve. Distribute the QCs throughout the run.

Make duplicate injections of a post-run SYS sample after the run to assess drift.

Table 14. LC-MS System

Data System	Analyst, Version 1.6.1
MRM	SYN548097 534 > 418 amu Nifedipine 347 > 254 amu
HPLC System	Pump[a]:_____ X/SN:_____ Autosampler[a]:_____ X/SN:_____
MS System	Sciex API _____(Toronto, Ontario Canada) SN:_____
Ionization Source	Heated Nebulizer, Positive Ion Mode
Analytical Column	Phenomenex Luna C18, 5 µm, 50 x 2 mm SN/Lot#:_____
Guard Column	Phenomenex C18 4 x 2 mm
Column Temperature	40ºC
Auto-Sampler Temperature	RT
Mobile Phase	A: 0.1% Formic Acid in Water B: 0.1% Formic Acid in Methanol
Mobile Phase Gradient[b]	Time (minutes) %B 0.0 35 0.2 90 2.2 90 2.3 35 4.5 35
Flow Rate (µL/min)[b]	500 (_____) µL/min
Injection Volume[b]	10 µL (_____) µL
Run Time[b]	4.5 minutes (_____) minutes

a. Agilent (Santa Clara, CA), Shimadzu (Kyoto, Japan), Leap CTC Analytics (Carrboro, NC).
b. Parameters which may be modified by analyst.

Run ID: _____

2.0 CALCULATIONS

Determine the optimal automatic integration parameters for the run. Examine the integration of the analyte and the internal standard by the chromatography system. Modify, if necessary, to obtain optimum consistent integration. Document the rationale of any manual or automated reintegration of peaks.

Use Microsoft Excel to perform the calculations as follows:

Calculate the individual and average percent area of each blank and blank with IS compared with the average area response of the Lower Limit of Quantitation (LLOQ) standard.

Calculate the individual percent area of the blank following the high standard compared with the area response of the nearest LLOQ standard.

Calculate the SYS average ratio and rsd. Calculate the Drift average area ratio. Compare the average ratio of the SYS to the average ratio of the Drift.

Calculate the exact concentrations of the plasma standards and plasma QCs. Enter the values into the Watson Bioanalytical LIMS.

Use the Watson Bioanalytical LIMS, version 7.4, to perform the calculations as follows:

Calculate the peak area response ratio (Analyte/IS) for each injection.

Determine optimal regression parameters and record the model and weighting in Table 15 to calculate the regression equation.

Table 15. Regression Parameters

Model	
Weighting	
y-intercept	Calculate, Do not force through origin
y-values	Instrument Response
x-values	Nominal Concentration

Using the peak area response of the standards and the regression equation, calculate their determined concentrations.

Using the peak area response ratios of the QCs and samples, the regression equation, and any dilution factor, if applicable, calculate their individual determined concentrations.

Using the determined concentration, calculate the individual relative error (%RE) for each standard and QC concentration.

3.0 ACCEPTANCE CRITERIA

There are no specific acceptance criteria for a this assay. The following will be used to assess each individual run, however the task leader may technically decide to revise these criteria or accept criteria outside these limits.

- System Suitability – At least three consecutive injections of the SYS immediately preceding the run must have a RSD of ≤ 10.0% for the run to be valid using the area ratio.

- Drift – Average area ratio within 15.0% of the pre-run system suitability average area ratio.

- Carryover - The blank immediately following the second curve must have an area response of no greater than 20.0% of the area response of the nearest acceptable low standard.

- Linearity – The coefficient of determination will be ≥ 0.98.

- Calibration Standards – Standards will have determined concentrations within 15.0% of nominal as measured by %RE, except for the LLOQ, where it should be within 20.0% of nominal to be acceptable. If a standard is not acceptable, it will not be used to generate the calibration curve. The standards that are not acceptable will be removed one-by-one with the standard deviating furthest from nominal concentration as measured by %RE removed first; regression will be performed again after each removal. Up to 3 standards falling outside these limits can be discarded (deactivated), provided they do not change the established model. At least 75% (11 of 14) of the standards must be acceptable for the calibration curve to be acceptable. A minimum of six concentration levels must define the curve.

- Selectivity – The blanks and blanks with internal standard must have an average response of no greater than 20.0% of the average response of the lowest acceptable standard.

- Sensitivity – The limit of quantitation for a run is the lowest acceptable standard that meets all acceptance criteria.

- Quality Control Samples - At least 67% (eight of twelve) of the QCs will have determined concentrations within 20.0 percent of the nominal concentration and at least 50% (two of the four) QCs at each concentration level will have

determined concentrations within 20.0 percent of the nominal concentration as measured by the %RE.

- Repeat Analysis of Study Samples – For samples that are repeated for technical reasons (e.g. failed run, bad injection) only one replicate must be run. For samples repeated for any other reason, at least duplicate aliquots must be analyzed, if sufficient sample is available. When multiple samples are analyzed, the average of all acceptable values will be reported, unless a value can be statistically excluded.

4.0 RESPONSE TO FAILURE TO MEET ACCEPTANCE CRITERIA

4.1 System Suitability

Verify that all calculations are correct. If the system suitability does not meet acceptance criteria and the standards and the QCs within the run meet acceptance criteria, a run set may be accepted with written justification and management approval. Otherwise, reinject the entire run set.

4.2 Drift

Verify that all calculations are correct. If the drift does not meet acceptance criteria and the standards and the QCs within the run meet acceptance criteria, a run set may be accepted with written justification and management approval. Otherwise, reinject the entire run set.

4.3 Accuracy of Standards and QCs

Verify that all calculations are correct . Verify that the proper instrument system (e.g., column, flow rates, etc.) was used for the analysis. If not, samples need to be re-injected using the correct instrument system.

If the correct instrument system was used, compare the current chromatograms to a past analysis. If the chromatography has changed substantially, determine and correct the problem with the instrument system and then re-inject the samples.

4.4 Linearity (Coefficient of Determination)

Verify that all concentrations, the regression model, integration, and calculations are correct.

If all the calculations are correct, repeat the analysis.

4.5 Selectivity/Carryover

If the results are greater than 20.0%, verify that the proper wash procedure was used, if applicable. If the proper wash conditions were utilized, assess the reason for carryover and the impact to the data. The LLOQ for the impacted set may be raised to the concentration level that meets the acceptable limits. The samples between the new LLOQ and the original LLOQ will be repeated with another extraction set.

5.0 RESULTS

Include printouts of the acquisition method, and any applicable spreadsheets in the data packet.

5.1 COMMENTS/CONCLUSIONS

_____
_____
_____
_____
_____
_____
_____
_____

6.0 DATA REVIEW

6.1 Technical Review

All data generated in the laboratory activities will be reviewed by the technical leader (or designee) for technical content, accuracy, and completeness of documentation.

The technical leader will assure the proper equipment was used, review experimental procedure, rejection of calibration standards, integration of chromatograms, chromatography data processing and acquisition parameters, calibration standard concentrations, regression model, and that this documentation was followed.

6.2 Data Accuracy Review

Review at least the following: completeness and correctness of data entry, formulas used to calculate all values, and accuracy of calculations.

7.0 SIGNATURES

Technical Review Signature/Date:

_____

_____

Signature of the technical reviewer will be considered documentation that all modifications and/or changes to this documentation form (documented during the course of conducting this task) are technically acceptable and have no adverse technical impact unless otherwise noted.

Data Accuracy Review Signature/Date:

_____

METHOD FOR THE ANALYSIS OF SYN548012 IN RAT WHOLEBLOOD:WATER (1:1) BY TANDEM LC-MS

1.0 PROCEDURE

1.1 General Instructions

USE TWO PAIR OF DISSIMILAR GLOVES DURING NEAT CHEMICAL HANDLING.

Calibrate all required balances according to the SOP on balance usage.

Make equivalent dilutions when the volume needed varies from the volume stated in the method.

Label all standard and reagent solutions as specified in the appropriate SOP.

Document all materials, equipment, and the chromatographic parameters. Initial and date on the top of each page of this document to signify that you have followed the instructions as written, all materials and reagents are current, and all equipment has been properly calibrated.

Initial and date the top of the page on the day that the work for that page was begun. Other entries made by the analyst on a later date or entries made by another person will be initialed and dated near the data entry.

The procedures are written in general chronological order. However, it is not essential that all sections be performed sequentially. The analyst may determine the order for conducting the task in the most efficient manner, unless the order for certain activities is specified.

Line through or "NA" any section that is not needed for a specific task. No formal explanation is required.

1.2 Samples

Reference the Chain of Custody or Sample Inventory Forms.

1.3 Materials

See Table 1 for all required chemicals, reagents, matrix, and solvents. Use Table 1 for documentation. Check the labels carefully to ensure that materials with expiration dates are current and are the proper grade/purity.

Table 1. Materials

Chemical	Supplier	Grade/Purity	Lot Number	Exp Date
SYN548012	See Analytical Standards Section 1.6			
Nifedipine	Sigma Aldrich	≥98%		
Water	Aqua Solutions Model 2121BL	ASTM Type 1	NA	NA
Acetonitrile	Sigma Aldrich	HPLC		
Methanol	Sigma Aldrich	HPLC		
Formic Acid	Fisher	HPLC		
Blank 1:1 Rat Wholeblood:Water[a]	Bioreclamation	Wistar Hannover (K3EDTA)		

a. Will be referred to as Blank blood throughout the document.

1.4 Equipment

All major pieces of equipment used for this method are listed in Table 2 and in the bulleted list below the table. Check the calibration of all equipment requiring calibration (e.g. balances and pipets) to ensure it is current. Document the actual piece of equipment, X or SN, and calibration due date in Table 2.

Table 2. Equipment

Equipment	Equipment ID	Calibration Due Date
Analytical Balance		
Weight Set		
Positive Displacement Pipet		
Positive Displacement Pipet		
Positive Displacement Pipet		
Positive Displacement Pipet		
Repeater Pipet		
Aqua Solutions Water Filtration	09011-21BL	NA
Refrigerator (2 to 8ºC)		NA
Freezer (~-70ºC)		NA

Balance Calibrated by: _____

The following lists the additional general equipment and supplies that are used throughout this method:

- Volumetric flasks, Class A
- Volumetric Pipets, Class A
- Sonicator
- Centrifuge
- Vortex
- Micro-centrifuge Tubes
- Polypropylene Centrifuge Tubes

1.5 Preparation of Solutions

1.5.1 Mobile Phase A, 0.1% Formic acid in Water

Add 1 mL of formic acid to 1 L of water. Cap and mix well. Store at room temperature. May be used for up to one month.

Date Prepared: _____

1.5.2 Mobile Phase B, 0.1% Formic acid in Methanol

Add 1 mL of formic acid to 1 L of methanol. Cap and mix well. Store at room temperature. May be used for up to one month.

Date Prepared: _____

1.6 Preparation of Analytical Standard Solutions

1.6.1 Analytical Standard Information

Document the analytical standard information in Table 3.

Table 3. Analytical Standard Information (SYN548012)

Identification	SYN548012
Lot Number	12DEC13
Storage Location at Time of Use	
Supplier	Battelle
Grade or Purity	NA
Storage Conditions	RT
Expiration Date	12/12/18

1.6.2 Preparation of Stock Analytical Standard Solutions

Preparation of Stock Analytical Standard Solution

Weigh the amounts of analytical standard shown in Table 4 and transfer into a volumetric flask. Add 1 mL of acetonitrile. Cap and mix well. Sonicate for ~5 minutes if necessary. Dilute to volume with acetonitrile and mix thoroughly. Store refrigerated. May be used for up to one year.

Table 4. Preparation of Stock SYN548012 Analytical Standard Solution

ID	Target Conc. (µg/mL)	Target Weight (mg)	Actual Weight (mg)[a]	Final Volume (mL)
A	1000	2 ± 0.2		2

a. Weigh to at least the nearest 0.01 mg. Document weight to at least four significant figures.

Date Prepared: _____

1.6.3 Preparation of Solvent Standard Spiking Solution

Using an adjustable volume pipet, transfer the volumes of standard indicated in Table 5 into volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to one year or the expiration date of the source solution, whichever is the shortest.

Table 5. Preparation of Solvent Standard Spiking Solutions

ID	Target Conc. (ng/mL)	Source	Source Volume (mL) each	Final Volume (mL)
SS1	10000	A	0.10	10
SS2	5000	A	0.05	10
SS3	2000	SS1	1.00	5
SS4	500.0	SS2	0.50	5
SS5	200.0	SS3	0.50	5
SS6	50.00	SS4	0.50	5
SS7	20.00	SS5	0.50	5

Date Prepared: _____

1.7 Preparation of Blood Standards

1.7.1 Preparation of Blood Calibration Standards

Using an adjustable volume pipet transfer the volumes of spiking solution and blank blood indicated in Table 6 into the appropriate micro-centrifuge tubes. **Take care to avoid coagulated clumps.** Cap and mix vigorously by vortexing for approximately 1 minute. Prepare fresh each day of extraction. Process duplicate aliquots at each concentration level with each extraction set.

Table 6. Preparation of SYN548012 Blood Calibration Standards

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
CS1	500.0	SS1	0.05	0.95	1
CS2	250.0	SS2	0.05	0.95	1
CS3	100.0	SS3	0.05	0.95	1
CS4	25.00	SS4	0.05	0.95	1
CS5	10.00	SS5	0.05	0.95	1
CS6	2.500	SS6	0.05	0.95	1
CS7	1.000	SS7	0.05	0.95	1

1.8 Preparation of Blanks

On each day of extraction, process duplicate blood blanks and duplicate blanks + IS.

1.9 Preparation of Internal Standard Solutions

1.9.1 Preparation of Stock Internal Standard Solution

Weigh the amount of Nifedipine analytical standard shown in Table 7 and transfer into a volumetric flask. Dilute to volume with acetonitrile. Cap and mix well. Sonicate for ~5 minutes. If needed, add acetonitrile drop wise and sonicate until dissolved. Store refrigerated. May be used for up to six months.

Table 7. Preparation of Stock Internal Standard Solution

ID	Target Conc. (µg/mL)	Target Weight (mg)	Actual Weight (mg)[a]	Final Volume (mL)
Stock IS	1000	5 ± 0.5		5

b. Weigh to at least the nearest 0.01 mg. Document weight to at least four significant figures.

Date Prepared: _____

1.9.2 Preparation of Intermediate Internal Standard Solution

Using an adjustable volume pipet, transfer the volume of stock internal standard listed in Table 8 into a volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to six months or the expiration date of the stock internal standard solution, whichever is the shortest.

Table 8. Preparation of Intermediate Internal Standard Solution

ID	Target Conc. (µg/mL)	Source	Source Volume (mL)	Final Volume (mL)
Int IS	10	Stock IS	0.25	25

Date Prepared: _____

1.9.3 Preparation of Working Internal Standard (WIS) Solution

Using an adjustable volume pipet, transfer the volume of intermediate internal standard listed in Table 9 into a volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to six months or the expiration date of the stock internal standard solution, whichever is the shortest.

Table 9. Preparation of Working Internal Standard Solution

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Final Volume (mL)
WIS	250	Int IS	1.25	50

Date Prepared: _____

1.10 Preparation of Quality Control (QC) Samples

1.10.1 Preparation of QC Spiking Solutions

Using an adjustable volume pipet, transfer the volume of solutions indicated in Table 10 into volumetric flasks. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to one year or the expiration date of the stock internal standard solution, whichever is the shortest of the two.

Table 10. Preparation of QC Spiking Solution

ID	Target Conc. (ng/mL)	Source	Source Volume (mL)	Final Volume (mL)
QC SS High	8000	A	0.08	10
QC SS Mid	4000	QC SS High	5.00	10
QC SS Low	60.00	QC SS Mid	0.15	10

Date Prepared: _____

1.10.2 Preparation of Blood QC Samples

Using an adjustable volume pipet, transfer the volumes of solutions indicated in Table 11 into individual micro-centrifuge tubes. **Take care to avoid coagulated clumps.** Cap and vortex to mix. Transfer ~0.5 mL aliquots to micro-centrifuge tubes for storage. Store frozen at ~ -70°C. May be used for up to three months.. Process four replicates at each concentration for each extraction set

Table 11. Preparation of Blood QC Samples

ID	Target Conc. (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
QC High	400.0	QC SS High	0.1	1.9	2
QC Mid	200.0	QC SS Mid	0.1	1.9	2
QC Low	3.000	QC SS Low	0.1	1.9	2

Date Prepared: _____

1.11 Preparation of System Suitability (SYS)

Using a positive displacement pipet, transfer the volume of blank blood indicated in Table 12 into a micro-centrifuge tube. Using an adjustable volume pipet, transfer the volume of spiking solution indicated in Table 12 into the micro-centrifuge tube. Cap and vortex to mix. Prepare fresh on each day of extraction. Process four replicates with each extraction set and combine the extracts.

Table 12. Preparation of System Suitability Sample

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
SYS	12.5	CS2	0.05	0.95	1

1.12 Preparation of Blood Standards, Blanks, QCs, and Samples

- Allow blank blood, and samples to thaw at room temperature if previously frozen.
- Document any sample irregularities.
- A second analyst will vortex each study sample thoroughly and open each container one at a time while the primary extracting analyst removes the appropriate volume of sample from the container.

  Second Analyst Initials/Date: _____
- Using an adjustable volume pipet, transfer 20 µL of each calibration standard, blood blank, and study sample into individual micro-centrifuge tubes. Document any samples requiring dilution in Table 13. Use blank blood to bring any samples to 20 µL.

Table 13. Sample Dilution Table

Sample ID	Sample Volume (µL)	Sample ID	Sample Volume (µL)

Reference the sample list at the front of the data binder (if available) for any dilutions not recorded in Table 13.

- Add 100 µL of WIS to all samples, except the blanks without IS. For the blanks without IS, add 100 µL of Acetonitrile instead of WIS.
- Vortex for ~1 minute to mix.
- Centrifuge at a maximum speed for ~10 minutes.
- Transfer supernatant to auto-sampler vials.
- Submit for analysis by LC-MS.

- Sample extracts are stored at the temperature of the auto-sampler if not analyzed on the day of extraction. Storage location of the extracts until injection if not stored on the auto-sampler: _____

1.13 Analysis of Standards, Blanks, QC, and Samples

Use the LC-MS system conditions specified in Table 14. The conditions, which are designated, may be modified by the analyst to produce acceptable chromatography. Record the actual values inside the parentheses.

Set up the automated chromatography data software (CDS) system to collect the electronic output from each injection, acquiring the data.

If the instrument has not been previously conditioned, condition the instrument by injecting the SYS sample for approximately 1 hour prior to starting the system suitability injections. Conditioning run performed: _____ Yes _____ No

Make a minimum of three injections of the appropriate SYS sample prior to starting the sample run to ensure optimal operation of the system.

Make single injections of all calibration standards, blanks, QCs, and samples. Inject one calibration curve near the beginning of the run and one near the end of the run. The calibration standards near the beginning of the run will be injected from high to low concentration. The calibration standards near the end of the run will be injected from low to high concentration. The first replicate of the blank and blank with IS will be injected prior to the first curve. The second replicate of the blank and blank with IS will be injected immediately following the second calibration curve. Distribute the QCs throughout the run.

Make duplicate injections of a post-run SYS sample after the run to assess drift.

Table 14. LC-MS System

Data System	Analyst, Version 1.6.1
MRM	SYN548012 564 > 434 amu Nifedipine 347 > 254 amu
HPLC System	Pump[a]:_____ X/SN:_____ Autosampler[a]:_____ X/SN:_____
MS System	Sciex API _____(Toronto, Ontario Canada) SN:_____
Ionization Source	Heated Nebulizer, Positive Ion Mode
Analytical Column	Phenomenex Luna C18, 5 µm, 50 x 2 mm SN/Lot#:_____
Guard Column	Phenomenex C18 4 x 2 mm
Column Temperature	40ºC
Auto-Sampler Temperature	RT
Mobile Phase	A: 0.1% Formic Acid in Water B: 0.1% Formic Acid in Methanol
Mobile Phase Gradient[b]	Time (minutes) %B 0.0 35 0.2 90 2.2 90 2.3 35 4.5 35
Flow Rate (µL/min)[b]	500 (_____) µL/min
Injection Volume[b]	10 µL (_____) µL
Run Time[b]	4.5 minutes (_____) minutes

a. Agilent (Santa Clara, CA), Shimadzu (Kyoto, Japan), Leap CTC Analytics (Carrboro, NC).
b. Parameters which may be modified by analyst.

Run ID: _____

2.0 CALCULATIONS

Determine the optimal automatic integration parameters for the run. Examine the integration of the analyte and the internal standard by the chromatography system. Modify, if necessary, to obtain optimum consistent integration. Document the rationale of any manual or automated reintegration of peaks.

Use Microsoft Excel to perform the calculations as follows:

Calculate the individual and average percent area of each blank and blank with IS compared with the average area response of the Lower Limit of Quantitation (LLOQ) standard.

Calculate the individual percent area of the blank following the high standard compared with the area response of the nearest LLOQ standard.

Calculate the SYS average ratio and rsd. Calculate the Drift average area ratio. Compare the average ratio of the SYS to the average ratio of the Drift.

Calculate the exact concentrations of the plasma standards and plasma QCs. Enter the values into the Watson Bioanalytical LIMS.

Use the Watson Bioanalytical LIMS, version 7.4, to perform the calculations as follows:

Calculate the peak area response ratio (Analyte/IS) for each injection.

Determine optimal regression parameters and record the model and weighting in Table 15 to calculate the regression equation.

Table 15. Regression Parameters

Model	
Weighting	
y-intercept	Calculate, Do not force through origin
y-values	Instrument Response
x-values	Nominal Concentration

Using the peak area response of the standards and the regression equation, calculate their determined concentrations.

Using the peak area response ratios of the QCs and samples, the regression equation, and any dilution factor, if applicable, calculate their individual determined concentrations.

Using the determined concentration, calculate the individual relative error (%RE) for each standard and QC concentration.

3.0 ACCEPTANCE CRITERIA

There are no specific acceptance criteria for a this assay. The following will be used to assess each individual run, however the task leader may technically decide to revise these criteria or accept criteria outside these limits.

- System Suitability – At least three consecutive injections of the SYS immediately preceding the run must have a RSD of ≤ 10.0% for the run to be valid using the area ratio.

- Drift – Average area ratio within 15.0% of the pre-run system suitability average area ratio.

- Carryover - The blank immediately following the second curve must have an area response of no greater than 20.0% of the area response of the nearest acceptable low standard.

- Linearity – The coefficient of determination will be ≥ 0.98.

- Calibration Standards – Standards will have determined concentrations within 15.0% of nominal as measured by %RE, except for the LLOQ, where it should be within 20.0% of nominal to be acceptable. If a standard is not acceptable, it will not be used to generate the calibration curve. The standards that are not acceptable will be removed one-by-one with the standard deviating furthest from nominal concentration as measured by %RE removed first; regression will be performed again after each removal. Up to 3 standards falling outside these limits can be discarded (deactivated), provided they do not change the established model. At least 75% (11 of 14) of the standards must be acceptable for the calibration curve to be acceptable. A minimum of six concentration levels must define the curve.

- Selectivity – The blanks and blanks with internal standard must have an average response of no greater than 20.0% of the average response of the lowest acceptable standard.

- Sensitivity – The limit of quantitation for a run is the lowest acceptable standard that meets all acceptance criteria.

- Quality Control Samples - At least 67% (eight of twelve) of the QCs will have determined concentrations within 20.0 percent of the nominal concentration and at least 50% (two of the four) QCs at each concentration level will have

determined concentrations within 20.0 percent of the nominal concentration as measured by the %RE.

- Repeat Analysis of Study Samples – For samples that are repeated for technical reasons (e.g. failed run, bad injection) only one replicate must be run. For samples repeated for any other reason, at least duplicate aliquots must be analyzed, if sufficient sample is available. When multiple samples are analyzed, the average of all acceptable values will be reported, unless a value can be statistically excluded.

4.0 RESPONSE TO FAILURE TO MEET ACCEPTANCE CRITERIA

4.1 System Suitability

Verify that all calculations are correct. If the system suitability does not meet acceptance criteria and the standards and the QCs within the run meet acceptance criteria, a run set may be accepted with written justification and management approval. Otherwise, reinject the entire run set.

4.2 Drift

Verify that all calculations are correct. If the drift does not meet acceptance criteria and the standards and the QCs within the run meet acceptance criteria, a run set may be accepted with written justification and management approval. Otherwise, reinject the entire run set.

4.3 Accuracy of Standards and QCs

Verify that all calculations are correct . Verify that the proper instrument system (e.g., column, flow rates, etc.) was used for the analysis. If not, samples need to be re-injected using the correct instrument system.

If the correct instrument system was used, compare the current chromatograms to a past analysis. If the chromatography has changed substantially, determine and correct the problem with the instrument system and then re-inject the samples.

4.4 Linearity (Coefficient of Determination)

Verify that all concentrations, the regression model, integration, and calculations are correct.

If all the calculations are correct, repeat the analysis.

4.5 Selectivity/Carryover

If the results are greater than 20.0%, verify that the proper wash procedure was used, if applicable. If the proper wash conditions were utilized, assess the reason for carryover and the impact to the data. The LLOQ for the impacted set may be raised to the concentration level that meets the acceptable limits. The samples between the new LLOQ and the original LLOQ will be repeated with another extraction set.

5.0 RESULTS

Include printouts of the acquisition method, and any applicable spreadsheets in the data packet.

5.1 COMMENTS/CONCLUSIONS

_____
_____
_____
_____
_____
_____
_____
_____

6.0 DATA REVIEW

6.1 Technical Review

All data generated in the laboratory activities will be reviewed by the technical leader (or designee) for technical content, accuracy, and completeness of documentation.

The technical leader will assure the proper equipment was used, review experimental procedure, rejection of calibration standards, integration of chromatograms, chromatography data processing and acquisition parameters, calibration standard concentrations, regression model, and that this documentation was followed.

6.2 Data Accuracy Review

Review at least the following: completeness and correctness of data entry, formulas used to calculate all values, and accuracy of calculations.

7.0 SIGNATURES

Technical Review Signature/Date:

_____

_____

Signature of the technical reviewer will be considered documentation that all modifications and/or changes to this documentation form (documented during the course of conducting this task) are technically acceptable and have no adverse technical impact unless otherwise noted.

Data Accuracy Review Signature/Date:

_____

METHOD FOR THE ANALYSIS OF SYN548014 IN RAT WHOLEBLOOD:WATER (1:1) BY TANDEM LC-MS

1.0 PROCEDURE

1.1 General Instructions

USE TWO PAIR OF DISSIMILAR GLOVES DURING NEAT CHEMICAL HANDLING.

Calibrate all required balances according to the SOP on balance usage.

Make equivalent dilutions when the volume needed varies from the volume stated in the method.

Label all standard and reagent solutions as specified in the appropriate SOP.

Document all materials, equipment, and the chromatographic parameters. Initial and date on the top of each page of this document to signify that you have followed the instructions as written, all materials and reagents are current, and all equipment has been properly calibrated.

Initial and date the top of the page on the day that the work for that page was begun. Other entries made by the analyst on a later date or entries made by another person will be initialed and dated near the data entry.

The procedures are written in general chronological order. However, it is not essential that all sections be performed sequentially. The analyst may determine the order for conducting the task in the most efficient manner, unless the order for certain activities is specified.

Line through or "NA" any section that is not needed for a specific task. No formal explanation is required.

1.2 Samples

Reference the Chain of Custody or Sample Inventory Forms.

1.3 Materials

See Table 1 for all required chemicals, reagents, matrix, and solvents. Use Table 1 for documentation. Check the labels carefully to ensure that materials with expiration dates are current and are the proper grade/purity.

Table 1. Materials

Chemical	Supplier	Grade/Purity	Lot Number	Exp Date
SYN548014	See Analytical Standards Section 1.6			
Nifedipine	Sigma Aldrich	≥98%		
Water	Aqua Solutions Model 2121BL	ASTM Type 1	NA	NA
Acetonitrile	Sigma Aldrich	HPLC		
Methanol	Sigma Aldrich	HPLC		
Formic Acid	Fisher	HPLC		
Blank 1:1 Rat Wholeblood:Water[a]	Bioreclamation	Wistar Hannover (K3EDTA)		

a. Will be referred to as Blank blood throughout the document.

1.4 Equipment

All major pieces of equipment used for this method are listed in Table 2 and in the bulleted list below the table. Check the calibration of all equipment requiring calibration (e.g. balances and pipets) to ensure it is current. Document the actual piece of equipment, X or SN, and calibration due date in Table 2.

Table 2. Equipment

Equipment	Equipment ID	Calibration Due Date
Analytical Balance		
Weight Set		
Positive Displacement Pipet		
Positive Displacement Pipet		
Positive Displacement Pipet		
Positive Displacement Pipet		
Repeater Pipet		
Aqua Solutions Water Filtration	09011-21BL	NA
Refrigerator (2 to 8ºC)		NA
Freezer (~-70ºC)		NA

Balance Calibrated by: _____

The following lists the additional general equipment and supplies that are used throughout this method:

- Volumetric flasks, Class A
- Volumetric Pipets, Class A
- Sonicator
- Centrifuge
- Vortex
- Micro-centrifuge Tubes
- Polypropylene Centrifuge Tubes

1.5 Preparation of Solutions

1.5.1 Mobile Phase A, 0.1% Formic acid in Water

Add 1 mL of formic acid to 1 L of water. Cap and mix well. Store at room temperature. May be used for up to one month.

Date Prepared: _____

1.5.2 Mobile Phase B, 0.1% Formic acid in Methanol

Add 1 mL of formic acid to 1 L of methanol. Cap and mix well. Store at room temperature. May be used for up to one month.

Date Prepared: _____

1.6 Preparation of Analytical Standard Solutions

1.6.1 Analytical Standard Information

Document the analytical standard information in Table 3.

Table 3. Analytical Standard Information (SYN548014)

Identification	SYN548014
Lot Number	12DEC13
Storage Location at Time of Use	
Supplier	Battelle
Grade or Purity	NA
Storage Conditions	RT
Expiration Date	12/12/18

1.6.2 Preparation of Stock Analytical Standard Solutions

Preparation of Stock Analytical Standard Solution

Weigh the amounts of analytical standard shown in Table 4 and transfer into a volumetric flask. Add 1 mL of acetonitrile. Cap and mix well. Sonicate for ~5 minutes if necessary. Dilute to volume with acetonitrile and mix thoroughly. Store refrigerated. May be used for up to one year.

Table 4. Preparation of Stock SYN548014 Analytical Standard Solution

ID	Target Conc. (µg/mL)	Target Weight (mg)	Actual Weight (mg)[a]	Final Volume (mL)
A	1000	2 ± 0.2		2

a. Weigh to at least the nearest 0.01 mg. Document weight to at least four significant figures.

Date Prepared: _____

1.6.3 Preparation of Solvent Standard Spiking Solution

Using an adjustable volume pipet, transfer the volumes of standard indicated in Table 5 into volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to one year or the expiration date of the source solution, whichever is the shortest.

Table 5. Preparation of Solvent Standard Spiking Solutions

ID	Target Conc. (ng/mL)	Source	Source Volume (mL) each	Final Volume (mL)
SS1	10000	A	0.10	10
SS2	5000	A	0.05	10
SS3	2000	SS1	1.00	5
SS4	500.0	SS2	0.50	5
SS5	200.0	SS3	0.50	5
SS6	50.00	SS4	0.50	5
SS7	20.00	SS5	0.50	5

Date Prepared: _____

1.7 Preparation of Blood Standards

1.7.1 Preparation of Blood Calibration Standards

Using an adjustable volume pipet transfer the volumes of spiking solution and blank blood indicated in Table 6 into the appropriate micro-centrifuge tubes. **Take care to avoid coagulated clumps.** Cap and mix vigorously by vortexing for approximately 1 minute. Prepare fresh each day of extraction. Process duplicate aliquots at each concentration level with each extraction set.

Table 6. Preparation of SYN548014 Blood Calibration Standards

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
CS1	500.0	SS1	0.05	0.95	1
CS2	250.0	SS2	0.05	0.95	1
CS3	100.0	SS3	0.05	0.95	1
CS4	25.00	SS4	0.05	0.95	1
CS5	10.00	SS5	0.05	0.95	1
CS6	2.500	SS6	0.05	0.95	1
CS7	1.000	SS7	0.05	0.95	1

1.8 Preparation of Blanks

On each day of extraction, process duplicate blood blanks and duplicate blanks + IS.

1.9 Preparation of Internal Standard Solutions

1.9.1 Preparation of Stock Internal Standard Solution

Weigh the amount of Nifedipine analytical standard shown in Table 7 and transfer into a volumetric flask. Dilute to volume with acetonitrile. Cap and mix well. Sonicate for ~5 minutes. If needed, add acetonitrile drop wise and sonicate until dissolved. Store refrigerated. May be used for up to six months.

Table 7. Preparation of Stock Internal Standard Solution

ID	Target Conc. (µg/mL)	Target Weight (mg)	Actual Weight (mg)[a]	Final Volume (mL)
Stock IS	1000	5 ± 0.5		5

b. Weigh to at least the nearest 0.01 mg. Document weight to at least four significant figures.

Date Prepared: _____

1.9.2 Preparation of Intermediate Internal Standard Solution

Using an adjustable volume pipet, transfer the volume of stock internal standard listed in Table 8 into a volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to six months or the expiration date of the stock internal standard solution, whichever is the shortest.

Table 8. Preparation of Intermediate Internal Standard Solution

ID	Target Conc. (µg/mL)	Source	Source Volume (mL)	Final Volume (mL)
Int IS	10	Stock IS	0.25	25

Date Prepared: _____

1.9.3 Preparation of Working Internal Standard (WIS) Solution

Using an adjustable volume pipet, transfer the volume of intermediate internal standard listed in Table 9 into a volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to six months or the expiration date of the stock internal standard solution, whichever is the shortest.

Table 9. Preparation of Working Internal Standard Solution

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Final Volume (mL)
WIS	250	Int IS	1.25	50

Date Prepared: _____

1.10 Preparation of Quality Control (QC) Samples

1.10.1 Preparation of QC Spiking Solutions

Using an adjustable volume pipet, transfer the volume of solutions indicated in Table 10 into volumetric flasks. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to one year or the expiration date of the stock internal standard solution, whichever is the shortest of the two.

Table 10. Preparation of QC Spiking Solution

ID	Target Conc. (ng/mL)	Source	Source Volume (mL)	Final Volume (mL)
QC SS High	8000	A	0.08	10
QC SS Mid	4000	QC SS High	5.00	10
QC SS Low	60.00	QC SS Mid	0.15	10

Date Prepared: _____

1.10.2 Preparation of Blood QC Samples

Using an adjustable volume pipet, transfer the volumes of solutions indicated in Table 11 into individual micro-centrifuge tubes. **Take care to avoid coagulated clumps.** Cap and vortex to mix. Transfer ~0.5 mL aliquots to micro-centrifuge tubes for storage. Store frozen at ~ -70°C. May be used for up to three months.. Process four replicates at each concentration for each extraction set

Table 11. Preparation of Blood QC Samples

ID	Target Conc. (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
QC High	400.0	QC SS High	0.1	1.9	2
QC Mid	200.0	QC SS Mid	0.1	1.9	2
QC Low	3.000	QC SS Low	0.1	1.9	2

Date Prepared: _____

1.11 Preparation of System Suitability (SYS)

Using a positive displacement pipet, transfer the volume of blank blood indicated in Table 12 into a micro-centrifuge tube. Using an adjustable volume pipet, transfer the volume of spiking solution indicated in Table 12 into the micro-centrifuge tube. Cap and vortex to mix. Prepare fresh on each day of extraction. Process four replicates with each extraction set and combine the extracts.

Table 12. Preparation of System Suitability Sample

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
SYS	12.5	CS2	0.05	0.95	1

1.12 Preparation of Blood Standards, Blanks, QCs, and Samples

- Allow blank blood, and samples to thaw at room temperature if previously frozen.

- Document any sample irregularities.

- A second analyst will vortex each study sample thoroughly and open each container one at a time while the primary extracting analyst removes the appropriate volume of sample from the container.

  Second Analyst Initials/Date: _____

- Using an adjustable volume pipet, transfer 20 µL of each calibration standard, blood blank, and study sample into individual micro-centrifuge tubes. Document any samples requiring dilution in Table 13. Use blank blood to bring any samples to 20 µL.

Table 13. Sample Dilution Table

Sample ID	Sample Volume (µL)	Sample ID	Sample Volume (µL)

Reference the sample list at the front of the data binder (if available) for any dilutions not recorded in Table 13.

- Add 100 µL of WIS to all samples, except the blanks without IS. For the blanks without IS, add 100 µL of Acetonitrile instead of WIS.

- Vortex for ~1 minute to mix.

- Centrifuge at a maximum speed for ~10 minutes.

- Transfer supernatant to auto-sampler vials.

- Submit for analysis by LC-MS.

- Sample extracts are stored at the temperature of the auto-sampler if not analyzed on the day of extraction. Storage location of the extracts until injection if not stored on the auto-sampler: _____

1.13 Analysis of Standards, Blanks, QC, and Samples

Use the LC-MS system conditions specified in Table 14. The conditions, which are designated, may be modified by the analyst to produce acceptable chromatography. Record the actual values inside the parentheses.

Set up the automated chromatography data software (CDS) system to collect the electronic output from each injection, acquiring the data.

If the instrument has not been previously conditioned, condition the instrument by injecting the SYS sample for approximately 1 hour prior to starting the system suitability injections. Conditioning run performed: _____ Yes _____ No

Make a minimum of three injections of the appropriate SYS sample prior to starting the sample run to ensure optimal operation of the system.

Make single injections of all calibration standards, blanks, QCs, and samples. Inject one calibration curve near the beginning of the run and one near the end of the run. The calibration standards near the beginning of the run will be injected from high to low concentration. The calibration standards near the end of the run will be injected from low to high concentration. The first replicate of the blank and blank with IS will be injected prior to the first curve. The second replicate of the blank and blank with IS will be injected immediately following the second calibration curve. Distribute the QCs throughout the run.

Make duplicate injections of a post-run SYS sample after the run to assess drift.

Table 14. LC-MS System

Data System	Analyst, Version 1.6.1
MRM	SYN548014 568 > 438 amu Nifedipine 347 > 254 amu
HPLC System	Pump[a]:_____ X/SN:_____ Autosampler[a]:_____ X/SN:_____
MS System	Sciex API _____(Toronto, Ontario Canada) SN:_____
Ionization Source	Heated Nebulizer, Positive Ion Mode
Analytical Column	Phenomenex Luna C18, 5 µm, 50 x 2 mm SN/Lot#:_____
Guard Column	Phenomenex C18 4 x 2 mm
Column Temperature	40ºC
Auto-Sampler Temperature	RT
Mobile Phase	A: 0.1% Formic Acid in Water B: 0.1% Formic Acid in Methanol
Mobile Phase Gradient[b]	Time (minutes) %B 0.0 35 0.2 90 2.2 90 2.3 35 4.5 35
Flow Rate (µL/min)[b]	500 (_____) µL/min
Injection Volume[b]	10 µL (_____) µL
Run Time[b]	4.5 minutes (_____) minutes

a. Agilent (Santa Clara, CA), Shimadzu (Kyoto, Japan), Leap CTC Analytics (Carrboro, NC).
b. Parameters which may be modified by analyst.

Run ID: _____

2.0 CALCULATIONS

Determine the optimal automatic integration parameters for the run. Examine the integration of the analyte and the internal standard by the chromatography system. Modify, if necessary, to obtain optimum consistent integration. Document the rationale of any manual or automated reintegration of peaks.

Use Microsoft Excel to perform the calculations as follows:

Calculate the average response of the internal standard for the standards and QCs. Do not include any standards that were excluded from the calibration curve and QCs that were eliminated for technical reasons.

Calculate the variance from the average internal standard response for all study samples.

Calculate the individual and average percent area of each blank and blank with IS compared with the average area response of the Lower Limit of Quantitation (LLOQ) standard.

Calculate the individual percent area of the blank following the high standard compared with the area response of the nearest LLOQ standard.

Calculate the SYS average ratio and rsd. Calculate the Drift average area ratio. Compare the average ratio of the SYS to the average ratio of the Drift.

Calculate the exact concentrations of the plasma standards and plasma QCs. Enter the values into the Watson Bioanalytical LIMS.

Use the Watson Bioanalytical LIMS, version 7.4, to perform the calculations as follows:

Calculate the peak area response ratio (Analyte/IS) for each injection.

Determine optimal regression parameters and record the model and weighting in Table 15 to calculate the regression equation.

Table 15. Regression Parameters

Model	
Weighting	
y-intercept	Calculate, Do not force through origin
y-values	Instrument Response
x-values	Nominal Concentration

Using the peak area response of the standards and the regression equation, calculate their determined concentrations.

Using the peak area response ratios of the QCs and samples, the regression equation, and any dilution factor, if applicable, calculate their individual determined concentrations.

Using the determined concentration, calculate the individual relative error (%RE) for each standard and QC concentration.

3.0 ACCEPTANCE CRITERIA

There are no specific acceptance criteria for a this assay. The following will be used to assess each individual run, however the task leader may technically decide to revise these criteria or accept criteria outside these limits.

- System Suitability – At least three consecutive injections of the SYS immediately preceding the run must have a RSD of ≤ 10.0% for the run to be valid using the area ratio.

- Drift – Average area ratio within 15.0% of the pre-run system suitability average area ratio.

- Carryover - The blank immediately following the second curve must have an area response of no greater than 20.0% of the area response of the nearest acceptable low standard.

- Linearity – The coefficient of determination will be ≥ 0.98.

- Calibration Standards – Standards will have determined concentrations within 15.0% of nominal as measured by %RE, except for the LLOQ, where it should be within 20.0% of nominal to be acceptable. If a standard is not acceptable, it will not be used to generate the calibration curve. The standards that are not acceptable will be removed one-by-one with the standard deviating furthest from nominal concentration as measured by %RE removed first; regression will be performed again after each removal. Up to 3 standards falling outside these limits can be discarded (deactivated), provided they do not change the established model. At least 75% (11 of 14) of the standards must be acceptable for the calibration curve to be acceptable. A minimum of six concentration levels must define the curve.

- Selectivity – The blanks and blanks with internal standard must have an average response of no greater than 20.0% of the average response of the lowest acceptable standard.

- Sensitivity – The limit of quantitation for a run is the lowest acceptable standard that meets all acceptance criteria.

- Quality Control Samples - At least 67% (eight of twelve) of the QCs will have determined concentrations within 20.0 percent of the nominal concentration and at least 50% (two of the four) QCs at each concentration level will have

determined concentrations within 20.0 percent of the nominal concentration as measured by the %RE.

- Repeat Analysis of Study Samples – For samples that are repeated for technical reasons (e.g. failed run, bad injection) only one replicate must be run. For samples repeated for any other reason, at least duplicate aliquots must be analyzed, if sufficient sample is available. When multiple samples are analyzed, the average of all acceptable values will be reported, unless a value can be statistically excluded.

4.0 RESPONSE TO FAILURE TO MEET ACCEPTANCE CRITERIA

4.1 System Suitability

Verify that all calculations are correct. If the system suitability does not meet acceptance criteria and the standards and the QCs within the run meet acceptance criteria, a run set may be accepted with written justification and management approval. Otherwise, reinject the entire run set.

4.2 Drift

Verify that all calculations are correct. If the drift does not meet acceptance criteria and the standards and the QCs within the run meet acceptance criteria, a run set may be accepted with written justification and management approval. Otherwise, reinject the entire run set.

4.3 Accuracy of Standards and QCs

Verify that all calculations are correct . Verify that the proper instrument system (e.g., column, flow rates, etc.) was used for the analysis. If not, samples need to be re-injected using the correct instrument system.

If the correct instrument system was used, compare the current chromatograms to a past analysis. If the chromatography has changed substantially, determine and correct the problem with the instrument system and then re-inject the samples.

4.4 Linearity (Coefficient of Determination)

Verify that all concentrations, the regression model, integration, and calculations are correct.

If all the calculations are correct, repeat the analysis.

4.5 Selectivity/Carryover

If the results are greater than 20.0%, verify that the proper wash procedure was used, if applicable. If the proper wash conditions were utilized, assess the reason for carryover and the impact to the data. The LLOQ for the impacted set may be raised to the concentration level that meets the acceptable limits. The samples between the new LLOQ and the original LLOQ will be repeated with another extraction set.

5.0 RESULTS

Include printouts of the acquisition method, and any applicable spreadsheets in the data packet.

5.1 COMMENTS/CONCLUSIONS

_____
_____
_____
_____
_____
_____
_____
_____

6.0 DATA REVIEW

6.1 Technical Review

All data generated in the laboratory activities will be reviewed by the technical leader (or designee) for technical content, accuracy, and completeness of documentation.

The technical leader will assure the proper equipment was used, review experimental procedure, rejection of calibration standards, integration of chromatograms, chromatography data processing and acquisition parameters, calibration standard concentrations, regression model, and that this documentation was followed.

6.2 Data Accuracy Review

Review at least the following: completeness and correctness of data entry, formulas used to calculate all values, and accuracy of calculations.

7.0 SIGNATURES

Technical Review Signature/Date:

_____

_____

Signature of the technical reviewer will be considered documentation that all modifications and/or changes to this documentation form (documented during the course of conducting this task) are technically acceptable and have no adverse technical impact unless otherwise noted.

Data Accuracy Review Signature/Date:

_____

METHOD FOR THE ANALYSIS OF SYN548102 IN RAT WHOLEBLOOD:WATER (1:1) BY TANDEM LC-MS

1.0 PROCEDURE

1.1 General Instructions

USE TWO PAIR OF DISSIMILAR GLOVES DURING NEAT CHEMICAL HANDLING.

Calibrate all required balances according to the SOP on balance usage.

Make equivalent dilutions when the volume needed varies from the volume stated in the method.

Label all standard and reagent solutions as specified in the appropriate SOP.

Document all materials, equipment, and the chromatographic parameters. Initial and date on the top of each page of this document to signify that you have followed the instructions as written, all materials and reagents are current, and all equipment has been properly calibrated.

Initial and date the top of the page on the day that the work for that page was begun. Other entries made by the analyst on a later date or entries made by another person will be initialed and dated near the data entry.

The procedures are written in general chronological order. However, it is not essential that all sections be performed sequentially. The analyst may determine the order for conducting the task in the most efficient manner, unless the order for certain activities is specified.

Line through or "NA" any section that is not needed for a specific task. No formal explanation is required.

1.2 Samples

Reference the Chain of Custody or Sample Inventory Forms.

1.3 Materials

See Table 1 for all required chemicals, reagents, matrix, and solvents. Use Table 1 for documentation. Check the labels carefully to ensure that materials with expiration dates are current and are the proper grade/purity.

Table 1. Materials

Chemical	Supplier	Grade/Purity	Lot Number	Exp Date
SYN548102	See Analytical Standards Section 1.6			
Nifedipine	Sigma Aldrich	≥98%		
Water	Aqua Solutions Model 2121BL	ASTM Type 1	NA	NA
Acetonitrile	Sigma Aldrich	HPLC		
Methanol	Sigma Aldrich	HPLC		
Formic Acid	Fisher	HPLC		
Blank 1:1 Rat Wholeblood:Water[a]	Bioreclamation	Wistar Hannover (K3EDTA)		

a. Will be referred to as Blank blood throughout the document.

1.4 Equipment

All major pieces of equipment used for this method are listed in Table 2 and in the bulleted list below the table. Check the calibration of all equipment requiring calibration (e.g. balances and pipets) to ensure it is current. Document the actual piece of equipment, X or SN, and calibration due date in Table 2.

Table 2. Equipment

Equipment	Equipment ID	Calibration Due Date
Analytical Balance		
Weight Set		
Positive Displacement Pipet		
Positive Displacement Pipet		
Positive Displacement Pipet		
Positive Displacement Pipet		
Repeater Pipet		
Aqua Solutions Water Filtration	09011-21BL	NA
Refrigerator (2 to 8ºC)		NA
Freezer (~-70ºC)		NA

Balance Calibrated by: _____

The following lists the additional general equipment and supplies that are used throughout this method:

- Volumetric flasks, Class A
- Volumetric Pipets, Class A
- Sonicator
- Centrifuge
- Vortex
- Micro-centrifuge Tubes
- Polypropylene Centrifuge Tubes

1.5 Preparation of Solutions

1.5.1 Mobile Phase A, 0.1% Formic acid in Water

Add 1 mL of formic acid to 1 L of water. Cap and mix well. Store at room temperature. May be used for up to one month.

Date Prepared: _____

1.5.2 Mobile Phase B, 0.1% Formic acid in Methanol

Add 1 mL of formic acid to 1 L of methanol. Cap and mix well. Store at room temperature. May be used for up to one month.

Date Prepared: _____

1.6 Preparation of Analytical Standard Solutions

1.6.1 Analytical Standard Information

Document the analytical standard information in Table 3.

Table 3. Analytical Standard Information (SYN548102)

Identification	SYN548102
Lot Number	12DEC13
Storage Location at Time of Use	
Supplier	Battelle
Grade or Purity	NA
Storage Conditions	RT
Expiration Date	12/12/18

1.6.2 Preparation of Stock Analytical Standard Solutions

Preparation of Stock Analytical Standard Solution

Weigh the amounts of analytical standard shown in Table 4 and transfer into a volumetric flask. Add 1 mL of acetonitrile. Cap and mix well. Sonicate for ~5 minutes if necessary. Dilute to volume with acetonitrile and mix thoroughly. Store refrigerated. May be used for up to one year.

Table 4. Preparation of Stock SYN548102 Analytical Standard Solution

ID	Target Conc. (µg/mL)	Target Weight (mg)	Actual Weight (mg)[a]	Final Volume (mL)
A	1000	2 ± 0.2		2

a. Weigh to at least the nearest 0.01 mg. Document weight to at least four significant figures.

Date Prepared: _____

1.6.3 Preparation of Solvent Standard Spiking Solution

Using an adjustable volume pipet, transfer the volumes of standard indicated in Table 5 into volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to one year or the expiration date of the source solution, whichever is the shortest.

Table 5. Preparation of Solvent Standard Spiking Solutions

ID	Target Conc. (ng/mL)	Source	Source Volume (mL) each	Final Volume (mL)
SS1	10000	A	0.10	10
SS2	5000	A	0.05	10
SS3	2000	SS1	1.00	5
SS4	500.0	SS2	0.50	5
SS5	200.0	SS3	0.50	5
SS6	50.00	SS4	0.50	5
SS7	20.00	SS5	0.50	5

Date Prepared: _____

1.7 Preparation of Blood Standards

1.7.1 Preparation of Blood Calibration Standards

Using an adjustable volume pipet transfer the volumes of spiking solution and blank blood indicated in Table 6 into the appropriate micro-centrifuge tubes. **Take care to avoid coagulated clumps.** Cap and mix vigorously by vortexing for approximately 1 minute. Prepare fresh each day of extraction. Process duplicate aliquots at each concentration level with each extraction set.

Table 6. Preparation of SYN548102 Blood Calibration Standards

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
CS1	500.0	SS1	0.05	0.95	1
CS2	250.0	SS2	0.05	0.95	1
CS3	100.0	SS3	0.05	0.95	1
CS4	25.00	SS4	0.05	0.95	1
CS5	10.00	SS5	0.05	0.95	1
CS6	2.500	SS6	0.05	0.95	1
CS7	1.000	SS7	0.05	0.95	1

1.8 Preparation of Blanks

On each day of extraction, process duplicate blood blanks and duplicate blanks + IS.

1.9 Preparation of Internal Standard Solutions

1.9.1 Preparation of Stock Internal Standard Solution

Weigh the amount of Nifedipine analytical standard shown in Table 7 and transfer into a volumetric flask. Dilute to volume with acetonitrile. Cap and mix well. Sonicate for ~5 minutes. If needed, add acetonitrile drop wise and sonicate until dissolved. Store refrigerated. May be used for up to six months.

Table 7. Preparation of Stock Internal Standard Solution

ID	Target Conc. (µg/mL)	Target Weight (mg)	Actual Weight (mg)[a]	Final Volume (mL)
Stock IS	1000	5 ± 0.5		5

b. Weigh to at least the nearest 0.01 mg. Document weight to at least four significant figures.

Date Prepared: _____

1.9.2 Preparation of Intermediate Internal Standard Solution

Using an adjustable volume pipet, transfer the volume of stock internal standard listed in Table 8 into a volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to six months or the expiration date of the stock internal standard solution, whichever is the shortest.

Table 8. Preparation of Intermediate Internal Standard Solution

ID	Target Conc. (µg/mL)	Source	Source Volume (mL)	Final Volume (mL)
Int IS	10	Stock IS	0.25	25

Date Prepared: _____

1.9.3 Preparation of Working Internal Standard (WIS) Solution

Using an adjustable volume pipet, transfer the volume of intermediate internal standard listed in Table 9 into a volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to six months or the expiration date of the stock internal standard solution, whichever is the shortest.

Table 9. Preparation of Working Internal Standard Solution

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Final Volume (mL)
WIS	250	Int IS	1.25	50

Date Prepared: _____

1.10 Preparation of Quality Control (QC) Samples

1.10.1 Preparation of QC Spiking Solutions

Using an adjustable volume pipet, transfer the volume of solutions indicated in Table 10 into volumetric flasks. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to one year or the expiration date of the stock internal standard solution, whichever is the shortest of the two.

Table 10. Preparation of QC Spiking Solution

ID	Target Conc. (ng/mL)	Source	Source Volume (mL)	Final Volume (mL)
QC SS High	8000	A	0.08	10
QC SS Mid	4000	QC SS High	5.00	10
QC SS Low	60.00	QC SS Mid	0.15	10

Date Prepared: _____

1.10.2 Preparation of Blood QC Samples

Using an adjustable volume pipet, transfer the volumes of solutions indicated in Table 11 into individual micro-centrifuge tubes. **Take care to avoid coagulated clumps.** Cap and vortex to mix. Transfer ~0.5 mL aliquots to micro-centrifuge tubes for storage. Store frozen at ~ -70°C. May be used for up to three months.. Process four replicates at each concentration for each extraction set

Table 11. Preparation of Blood QC Samples

ID	Target Conc. (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
QC High	400.0	QC SS High	0.1	1.9	2
QC Mid	200.0	QC SS Mid	0.1	1.9	2
QC Low	3.000	QC SS Low	0.1	1.9	2

Date Prepared: _____

1.11 Preparation of System Suitability (SYS)

Using a positive displacement pipet, transfer the volume of blank blood indicated in Table 12 into a micro-centrifuge tube. Using an adjustable volume pipet, transfer the volume of spiking solution indicated in Table 12 into the micro-centrifuge tube. Cap and vortex to mix. Prepare fresh on each day of extraction. Process four replicates with each extraction set and combine the extracts.

Table 12. Preparation of System Suitability Sample

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
SYS	12.5	CS2	0.05	0.95	1

1.12 Preparation of Blood Standards, Blanks, QCs, and Samples

- Allow blank blood, and samples to thaw at room temperature if previously frozen.
- Document any sample irregularities.
- A second analyst will vortex each study sample thoroughly and open each container one at a time while the primary extracting analyst removes the appropriate volume of sample from the container.

  Second Analyst Initials/Date: _____
- Using an adjustable volume pipet, transfer 20 µL of each calibration standard, blood blank, and study sample into individual micro-centrifuge tubes. Document any samples requiring dilution in Table 13. Use blank blood to bring any samples to 20 µL.

Table 13. Sample Dilution Table

Sample ID	Sample Volume (µL)	Sample ID	Sample Volume (µL)

Reference the sample list at the front of the data binder (if available) for any dilutions not recorded in Table 13.

- Add 100 µL of WIS to all samples, except the blanks without IS. For the blanks without IS, add 100 µL of Acetonitrile instead of WIS.
- Vortex for ~1 minute to mix.
- Centrifuge at a maximum speed for ~10 minutes.
- Transfer supernatant to auto-sampler vials.
- Submit for analysis by LC-MS.

- Sample extracts are stored at the temperature of the auto-sampler if not analyzed on the day of extraction. Storage location of the extracts until injection if not stored on the auto-sampler: _____

1.13 Analysis of Standards, Blanks, QC, and Samples

Use the LC-MS system conditions specified in Table 14. The conditions, which are designated, may be modified by the analyst to produce acceptable chromatography. Record the actual values inside the parentheses.

Set up the automated chromatography data software (CDS) system to collect the electronic output from each injection, acquiring the data.

If the instrument has not been previously conditioned, condition the instrument by injecting the SYS sample for approximately 1 hour prior to starting the system suitability injections. Conditioning run performed: _____ Yes _____ No

Make a minimum of three injections of the appropriate SYS sample prior to starting the sample run to ensure optimal operation of the system.

Make single injections of all calibration standards, blanks, QCs, and samples. Inject one calibration curve near the beginning of the run and one near the end of the run. The calibration standards near the beginning of the run will be injected from high to low concentration. The calibration standards near the end of the run will be injected from low to high concentration. The first replicate of the blank and blank with IS will be injected prior to the first curve. The second replicate of the blank and blank with IS will be injected immediately following the second calibration curve. Distribute the QCs throughout the run.

Make duplicate injections of a post-run SYS sample after the run to assess drift.

Table 14. LC-MS System

Data System	Analyst, Version 1.6.1
MRM	SYN548102 608 > 478 amu Nifedipine 347 > 254 amu
HPLC System	Pump[a]:_____ X/SN:_____ Autosampler[a]:_____ X/SN:_____
MS System	Sciex API _____(Toronto, Ontario Canada) SN:_____
Ionization Source	Heated Nebulizer, Positive Ion Mode
Analytical Column	Phenomenex Luna C18, 5 µm, 50 x 2 mm SN/Lot#:_____
Guard Column	Phenomenex C18 4 x 2 mm
Column Temperature	40ºC
Auto-Sampler Temperature	RT
Mobile Phase	A: 0.1% Formic Acid in Water B: 0.1% Formic Acid in Methanol
Mobile Phase Gradient[b]	Time (minutes) %B 0.0 35 0.2 90 2.2 90 2.3 35 4.5 35
Flow Rate (µL/min)[b]	500 (_____) µL/min
Injection Volume[b]	10 µL (_____) µL
Run Time[b]	4.5 minutes (_____) minutes

a. Agilent (Santa Clara, CA), Shimadzu (Kyoto, Japan), Leap CTC Analytics (Carrboro, NC).
b. Parameters which may be modified by analyst.

Run ID: _____

2.0 CALCULATIONS

Determine the optimal automatic integration parameters for the run. Examine the integration of the analyte and the internal standard by the chromatography system. Modify, if necessary, to obtain optimum consistent integration. Document the rationale of any manual or automated reintegration of peaks.

Use Microsoft Excel to perform the calculations as follows:

Calculate the individual and average percent area of each blank and blank with IS compared with the average area response of the Lower Limit of Quantitation (LLOQ) standard.

Calculate the individual percent area of the blank following the high standard compared with the area response of the nearest LLOQ standard.

Calculate the SYS average ratio and rsd. Calculate the Drift average area ratio. Compare the average ratio of the SYS to the average ratio of the Drift.

Calculate the exact concentrations of the plasma standards and plasma QCs. Enter the values into the Watson Bioanalytical LIMS.

Use the Watson Bioanalytical LIMS, version 7.4, to perform the calculations as follows:

Calculate the peak area response ratio (Analyte/IS) for each injection.

Determine optimal regression parameters and record the model and weighting in Table 15 to calculate the regression equation.

Table 15. Regression Parameters

Model	
Weighting	
y-intercept	Calculate, Do not force through origin
y-values	Instrument Response
x-values	Nominal Concentration

Using the peak area response of the standards and the regression equation, calculate their determined concentrations.

Using the peak area response ratios of the QCs and samples, the regression equation, and any dilution factor, if applicable, calculate their individual determined concentrations.

Using the determined concentration, calculate the individual relative error (%RE) for each standard and QC concentration.

3.0 ACCEPTANCE CRITERIA

There are no specific acceptance criteria for a this assay. The following will be used to assess each individual run, however the task leader may technically decide to revise these criteria or accept criteria outside these limits.

- System Suitability – At least three consecutive injections of the SYS immediately preceding the run must have a RSD of ≤ 10.0% for the run to be valid using the area ratio.

- Drift – Average area ratio within 15.0% of the pre-run system suitability average area ratio.

- Carryover - The blank immediately following the second curve must have an area response of no greater than 20.0% of the area response of the nearest acceptable low standard.

- Linearity – The coefficient of determination will be ≥ 0.98.

- Calibration Standards – Standards will have determined concentrations within 15.0% of nominal as measured by %RE, except for the LLOQ, where it should be within 20.0% of nominal to be acceptable. If a standard is not acceptable, it will not be used to generate the calibration curve. The standards that are not acceptable will be removed one-by-one with the standard deviating furthest from nominal concentration as measured by %RE removed first; regression will be performed again after each removal. Up to 3 standards falling outside these limits can be discarded (deactivated), provided they do not change the established model. At least 75% (11 of 14) of the standards must be acceptable for the calibration curve to be acceptable. A minimum of six concentration levels must define the curve.

- Selectivity – The blanks and blanks with internal standard must have an average response of no greater than 20.0% of the average response of the lowest acceptable standard.

- Sensitivity – The limit of quantitation for a run is the lowest acceptable standard that meets all acceptance criteria.

- Quality Control Samples - At least 67% (eight of twelve) of the QCs will have determined concentrations within 20.0 percent of the nominal concentration and at least 50% (two of the four) QCs at each concentration level will have

determined concentrations within 20.0 percent of the nominal concentration as measured by the %RE.

- Repeat Analysis of Study Samples – For samples that are repeated for technical reasons (e.g. failed run, bad injection) only one replicate must be run. For samples repeated for any other reason, at least duplicate aliquots must be analyzed, if sufficient sample is available. When multiple samples are analyzed, the average of all acceptable values will be reported, unless a value can be statistically excluded.

4.0 RESPONSE TO FAILURE TO MEET ACCEPTANCE CRITERIA

4.1 System Suitability

Verify that all calculations are correct. If the system suitability does not meet acceptance criteria and the standards and the QCs within the run meet acceptance criteria, a run set may be accepted with written justification and management approval. Otherwise, reinject the entire run set.

4.2 Drift

Verify that all calculations are correct. If the drift does not meet acceptance criteria and the standards and the QCs within the run meet acceptance criteria, a run set may be accepted with written justification and management approval. Otherwise, reinject the entire run set.

4.3 Accuracy of Standards and QCs

Verify that all calculations are correct . Verify that the proper instrument system (e.g., column, flow rates, etc.) was used for the analysis. If not, samples need to be re-injected using the correct instrument system.

If the correct instrument system was used, compare the current chromatograms to a past analysis. If the chromatography has changed substantially, determine and correct the problem with the instrument system and then re-inject the samples.

4.4 Linearity (Coefficient of Determination)

Verify that all concentrations, the regression model, integration, and calculations are correct.

If all the calculations are correct, repeat the analysis.

4.5 Selectivity/Carryover

If the results are greater than 20.0%, verify that the proper wash procedure was used, if applicable. If the proper wash conditions were utilized, assess the reason for carryover and the impact to the data. The LLOQ for the impacted set may be raised to the concentration level that meets the acceptable limits. The samples between the new LLOQ and the original LLOQ will be repeated with another extraction set.

5.0 RESULTS

Include printouts of the acquisition method, and any applicable spreadsheets in the data packet.

5.1 COMMENTS/CONCLUSIONS

_____
_____
_____
_____
_____
_____
_____
_____

6.0 DATA REVIEW

6.1 Technical Review

All data generated in the laboratory activities will be reviewed by the technical leader (or designee) for technical content, accuracy, and completeness of documentation.

The technical leader will assure the proper equipment was used, review experimental procedure, rejection of calibration standards, integration of chromatograms, chromatography data processing and acquisition parameters, calibration standard concentrations, regression model, and that this documentation was followed.

6.2 Data Accuracy Review

Review at least the following: completeness and correctness of data entry, formulas used to calculate all values, and accuracy of calculations.

7.0 SIGNATURES

Technical Review Signature/Date:

_____

_____

Signature of the technical reviewer will be considered documentation that all modifications and/or changes to this documentation form (documented during the course of conducting this task) are technically acceptable and have no adverse technical impact unless otherwise noted.

Data Accuracy Review Signature/Date:

_____

METHOD FOR THE ANALYSIS OF SYN548117 IN RAT WHOLEBLOOD:WATER (1:1) BY TANDEM LC-MS

1.0 PROCEDURE

1.1 General Instructions

USE TWO PAIR OF DISSIMILAR GLOVES DURING NEAT CHEMICAL HANDLING.

Calibrate all required balances according to the SOP on balance usage.

Make equivalent dilutions when the volume needed varies from the volume stated in the method.

Label all standard and reagent solutions as specified in the appropriate SOP.

Document all materials, equipment, and the chromatographic parameters. Initial and date on the top of each page of this document to signify that you have followed the instructions as written, all materials and reagents are current, and all equipment has been properly calibrated.

Initial and date the top of the page on the day that the work for that page was begun. Other entries made by the analyst on a later date or entries made by another person will be initialed and dated near the data entry.

The procedures are written in general chronological order. However, it is not essential that all sections be performed sequentially. The analyst may determine the order for conducting the task in the most efficient manner, unless the order for certain activities is specified.

Line through or "NA" any section that is not needed for a specific task. No formal explanation is required.

1.2 Samples

Reference the Chain of Custody or Sample Inventory Forms.

1.3 Materials

See Table 1 for all required chemicals, reagents, matrix, and solvents. Use Table 1 for documentation. Check the labels carefully to ensure that materials with expiration dates are current and are the proper grade/purity.

Table 1. Materials

Chemical	Supplier	Grade/Purity	Lot Number	Exp Date
SYN548117	See Analytical Standards Section 1.6			
Nifedipine	Sigma Aldrich	≥98%		
Water	Aqua Solutions Model 2121BL	ASTM Type 1	NA	NA
Acetonitrile	Sigma Aldrich	HPLC		
Methanol	Sigma Aldrich	HPLC		
Formic Acid	Fisher	HPLC		
Blank 1:1 Rat Wholeblood:Water[a]	Bioreclamation	Wistar Hannover (K3EDTA)		

a. Will be referred to as Blank blood throughout the document.

1.4 Equipment

All major pieces of equipment used for this method are listed in Table 2 and in the bulleted list below the table. Check the calibration of all equipment requiring calibration (e.g. balances and pipets) to ensure it is current. Document the actual piece of equipment, X or SN, and calibration due date in Table 2.

Table 2. Equipment

Equipment	Equipment ID	Calibration Due Date
Analytical Balance		
Weight Set		
Positive Displacement Pipet		
Positive Displacement Pipet		
Positive Displacement Pipet		
Positive Displacement Pipet		
Repeater Pipet		
Aqua Solutions Water Filtration	09011-21BL	NA
Refrigerator (2 to 8ºC)		NA
Freezer (~-70ºC)		NA

Balance Calibrated by: _____

The following lists the additional general equipment and supplies that are used throughout this method:

- Volumetric flasks, Class A
- Volumetric Pipets, Class A
- Sonicator
- Centrifuge
- Vortex
- Micro-centrifuge Tubes
- Polypropylene Centrifuge Tubes

1.5 Preparation of Solutions

1.5.1 Mobile Phase A, 0.1% Formic acid in Water

Add 1 mL of formic acid to 1 L of water. Cap and mix well. Store at room temperature. May be used for up to one month.

Date Prepared: _____

1.5.2 Mobile Phase B, 0.1% Formic acid in Methanol

Add 1 mL of formic acid to 1 L of methanol. Cap and mix well. Store at room temperature. May be used for up to one month.

Date Prepared: _____

1.6 Preparation of Analytical Standard Solutions

1.6.1 Analytical Standard Information

Document the analytical standard information in Table 3.

Table 3. Analytical Standard Information (SYN548117)

Identification	SYN548117
Lot Number	12DEC13
Storage Location at Time of Use	
Supplier	Battelle
Grade or Purity	NA
Storage Conditions	RT
Expiration Date	12/12/18

1.6.2 Preparation of Stock Analytical Standard Solutions

Preparation of Stock Analytical Standard Solution

Weigh the amounts of analytical standard shown in Table 4 and transfer into a volumetric flask. Add 1 mL of acetonitrile. Cap and mix well. Sonicate for ~5 minutes if necessary. Dilute to volume with acetonitrile and mix thoroughly. Store refrigerated. May be used for up to one year.

Table 4. Preparation of Stock SYN548117 Analytical Standard Solution

ID	Target Conc. (µg/mL)	Target Weight (mg)	Actual Weight (mg)[a]	Final Volume (mL)
A	1000	2 ± 0.2		2

a. Weigh to at least the nearest 0.01 mg. Document weight to at least four significant figures.

Date Prepared: _____

1.6.3 Preparation of Solvent Standard Spiking Solution

Using an adjustable volume pipet, transfer the volumes of standard indicated in Table 5 into volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to one year or the expiration date of the source solution, whichever is the shortest.

Table 5. Preparation of Solvent Standard Spiking Solutions

ID	Target Conc. (ng/mL)	Source	Source Volume (mL) each	Final Volume (mL)
SS1	10000	A	0.10	10
SS2	5000	A	0.05	10
SS3	2000	SS1	1.00	5
SS4	500.0	SS2	0.50	5
SS5	200.0	SS3	0.50	5
SS6	50.00	SS4	0.50	5
SS7	20.00	SS5	0.50	5

Date Prepared: _____

1.7 Preparation of Blood Standards

1.7.1 Preparation of Blood Calibration Standards

Using an adjustable volume pipet transfer the volumes of spiking solution and blank blood indicated in Table 6 into the appropriate micro-centrifuge tubes. **Take care to avoid coagulated clumps.** Cap and mix vigorously by vortexing for approximately 1 minute. Prepare fresh each day of extraction. Process duplicate aliquots at each concentration level with each extraction set.

Table 6. Preparation of SYN548117 Blood Calibration Standards

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
CS1	500.0	SS1	0.05	0.95	1
CS2	250.0	SS2	0.05	0.95	1
CS3	100.0	SS3	0.05	0.95	1
CS4	25.00	SS4	0.05	0.95	1
CS5	10.00	SS5	0.05	0.95	1
CS6	2.500	SS6	0.05	0.95	1
CS7	1.000	SS7	0.05	0.95	1

1.8 Preparation of Blanks

On each day of extraction, process duplicate blood blanks and duplicate blanks + IS.

1.9 Preparation of Internal Standard Solutions

1.9.1 Preparation of Stock Internal Standard Solution

Weigh the amount of Nifedipine analytical standard shown in Table 7 and transfer into a volumetric flask. Dilute to volume with acetonitrile. Cap and mix well. Sonicate for ~5 minutes. If needed, add acetonitrile drop wise and sonicate until dissolved. Store refrigerated. May be used for up to six months.

Table 7. Preparation of Stock Internal Standard Solution

ID	Target Conc. (µg/mL)	Target Weight (mg)	Actual Weight (mg)[a]	Final Volume (mL)
Stock IS	1000	5 ± 0.5		5

b. Weigh to at least the nearest 0.01 mg. Document weight to at least four significant figures.

Date Prepared: _____

1.9.2 Preparation of Intermediate Internal Standard Solution

Using an adjustable volume pipet, transfer the volume of stock internal standard listed in Table 8 into a volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to six months or the expiration date of the stock internal standard solution, whichever is the shortest.

Table 8. Preparation of Intermediate Internal Standard Solution

ID	Target Conc. (µg/mL)	Source	Source Volume (mL)	Final Volume (mL)
Int IS	10	Stock IS	0.25	25

Date Prepared: _____

1.9.3 Preparation of Working Internal Standard (WIS) Solution

Using an adjustable volume pipet, transfer the volume of intermediate internal standard listed in Table 9 into a volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to six months or the expiration date of the stock internal standard solution, whichever is the shortest.

Table 9. Preparation of Working Internal Standard Solution

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Final Volume (mL)
WIS	250	Int IS	1.25	50

Date Prepared: _____

1.10 Preparation of Quality Control (QC) Samples

1.10.1 Preparation of QC Spiking Solutions

Using an adjustable volume pipet, transfer the volume of solutions indicated in Table 10 into volumetric flasks. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to one year or the expiration date of the stock internal standard solution, whichever is the shortest of the two.

Table 10. Preparation of QC Spiking Solution

ID	Target Conc. (ng/mL)	Source	Source Volume (mL)	Final Volume (mL)
QC SS High	8000	A	0.08	10
QC SS Mid	4000	QC SS High	5.00	10
QC SS Low	60.00	QC SS Mid	0.15	10

Date Prepared: _____

1.10.2 Preparation of Blood QC Samples

Using an adjustable volume pipet, transfer the volumes of solutions indicated in Table 11 into individual micro-centrifuge tubes. **Take care to avoid coagulated clumps.** Cap and vortex to mix. Transfer ~0.5 mL aliquots to micro-centrifuge tubes for storage. Store frozen at ~ -70°C. May be used for up to three months.. Process four replicates at each concentration for each extraction set

Table 11. Preparation of Blood QC Samples

ID	Target Conc. (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
QC High	400.0	QC SS High	0.1	1.9	2
QC Mid	200.0	QC SS Mid	0.1	1.9	2
QC Low	3.000	QC SS Low	0.1	1.9	2

Date Prepared: _____

1.11 Preparation of System Suitability (SYS)

Using a positive displacement pipet, transfer the volume of blank blood indicated in Table 12 into a micro-centrifuge tube. Using an adjustable volume pipet, transfer the volume of spiking solution indicated in Table 12 into the micro-centrifuge tube. Cap and vortex to mix. Prepare fresh on each day of extraction. Process four replicates with each extraction set and combine the extracts.

Table 12. Preparation of System Suitability Sample

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
SYS	12.5	CS2	0.05	0.95	1

1.12 Preparation of Blood Standards, Blanks, QCs, and Samples

- Allow blank blood, and samples to thaw at room temperature if previously frozen.

- Document any sample irregularities.

- A second analyst will vortex each study sample thoroughly and open each container one at a time while the primary extracting analyst removes the appropriate volume of sample from the container.

  Second Analyst Initials/Date: _____

- Using an adjustable volume pipet, transfer 20 µL of each calibration standard, blood blank, and study sample into individual micro-centrifuge tubes. Document any samples requiring dilution in Table 13. Use blank blood to bring any samples to 20 µL.

Table 13. Sample Dilution Table

Sample ID	Sample Volume (µL)	Sample ID	Sample Volume (µL)

Reference the sample list at the front of the data binder (if available) for any dilutions not recorded in Table 13.

- Add 100 µL of WIS to all samples, except the blanks without IS. For the blanks without IS, add 100 µL of Acetonitrile instead of WIS.

- Vortex for ~1 minute to mix.

- Centrifuge at a maximum speed for ~10 minutes.

- Transfer supernatant to auto-sampler vials.

- Submit for analysis by LC-MS.

- Sample extracts are stored at the temperature of the auto-sampler if not analyzed on the day of extraction. Storage location of the extracts until injection if not stored on the auto-sampler: _____

1.13 Analysis of Standards, Blanks, QC, and Samples

Use the LC-MS system conditions specified in Table 14. The conditions, which are designated, may be modified by the analyst to produce acceptable chromatography. Record the actual values inside the parentheses.

Set up the automated chromatography data software (CDS) system to collect the electronic output from each injection, acquiring the data.

If the instrument has not been previously conditioned, condition the instrument by injecting the SYS sample for approximately 1 hour prior to starting the system suitability injections. Conditioning run performed: _____ Yes _____ No

Make a minimum of three injections of the appropriate SYS sample prior to starting the sample run to ensure optimal operation of the system.

Make single injections of all calibration standards, blanks, QCs, and samples. Inject one calibration curve near the beginning of the run and one near the end of the run. The calibration standards near the beginning of the run will be injected from high to low concentration. The calibration standards near the end of the run will be injected from low to high concentration. The first replicate of the blank and blank with IS will be injected prior to the first curve. The second replicate of the blank and blank with IS will be injected immediately following the second calibration curve. Distribute the QCs throughout the run.

Make duplicate injections of a post-run SYS sample after the run to assess drift.

Table 14. LC-MS System

Data System	Analyst, Version 1.6.1
MRM	SYN548117 550 > 434 amu Nifedipine 347 > 254 amu
HPLC System	Pump[a]:_____ X/SN:_____ Autosampler[a]:_____ X/SN:_____
MS System	Sciex API _____(Toronto, Ontario Canada) SN:_____
Ionization Source	Heated Nebulizer, Positive Ion Mode
Analytical Column	Phenomenex Luna C18, 5 µm, 50 x 2 mm SN/Lot#:_____
Guard Column	Phenomenex C18 4 x 2 mm
Column Temperature	40ºC
Auto-Sampler Temperature	RT
Mobile Phase	A: 0.1% Formic Acid in Water B: 0.1% Formic Acid in Methanol
Mobile Phase Gradient[b]	Time (minutes) %B 0.0 35 0.2 90 2.2 90 2.3 35 4.5 35
Flow Rate (µL/min)[b]	500 (_____) µL/min
Injection Volume[b]	10 µL (_____) µL
Run Time[b]	4.5 minutes (_____) minutes

a. Agilent (Santa Clara, CA), Shimadzu (Kyoto, Japan), Leap CTC Analytics (Carrboro, NC).
b. Parameters which may be modified by analyst.

Run ID: _____

2.0 CALCULATIONS

Determine the optimal automatic integration parameters for the run. Examine the integration of the analyte and the internal standard by the chromatography system. Modify, if necessary, to obtain optimum consistent integration. Document the rationale of any manual or automated reintegration of peaks.

Use Microsoft Excel to perform the calculations as follows:

Calculate the average response of the internal standard for the standards and QCs. Do not include any standards that were excluded from the calibration curve and QCs that were eliminated for technical reasons.

Calculate the variance from the average internal standard response for all study samples.

Calculate the individual and average percent area of each blank and blank with IS compared with the average area response of the Lower Limit of Quantitation (LLOQ) standard.

Calculate the individual percent area of the blank following the high standard compared with the area response of the nearest LLOQ standard.

Calculate the SYS average ratio and rsd. Calculate the Drift average area ratio. Compare the average ratio of the SYS to the average ratio of the Drift.

Calculate the exact concentrations of the plasma standards and plasma QCs. Enter the values into the Watson Bioanalytical LIMS.

Use the Watson Bioanalytical LIMS, version 7.4, to perform the calculations as follows:

Calculate the peak area response ratio (Analyte/IS) for each injection.

Determine optimal regression parameters and record the model and weighting in Table 15 to calculate the regression equation.

Table 15. Regression Parameters

Model	
Weighting	
y-intercept	Calculate, Do not force through origin
y-values	Instrument Response
x-values	Nominal Concentration

Using the peak area response of the standards and the regression equation, calculate their determined concentrations.

Using the peak area response ratios of the QCs and samples, the regression equation, and any dilution factor, if applicable, calculate their individual determined concentrations.

Using the determined concentration, calculate the individual relative error (%RE) for each standard and QC concentration.

3.0 ACCEPTANCE CRITERIA

There are no specific acceptance criteria for a this assay. The following will be used to assess each individual run, however the task leader may technically decide to revise these criteria or accept criteria outside these limits.

- System Suitability – At least three consecutive injections of the SYS immediately preceding the run must have a RSD of ≤ 10.0% for the run to be valid using the area ratio.

- Drift – Average area ratio within 15.0% of the pre-run system suitability average area ratio.

- Carryover - The blank immediately following the second curve must have an area response of no greater than 20.0% of the area response of the nearest acceptable low standard.

- Linearity – The coefficient of determination will be ≥ 0.98.

- Calibration Standards – Standards will have determined concentrations within 15.0% of nominal as measured by %RE, except for the LLOQ, where it should be within 20.0% of nominal to be acceptable. If a standard is not acceptable, it will not be used to generate the calibration curve. The standards that are not acceptable will be removed one-by-one with the standard deviating furthest from nominal concentration as measured by %RE removed first; regression will be performed again after each removal. Up to 3 standards falling outside these limits can be discarded (deactivated), provided they do not change the established model. At least 75% (11 of 14) of the standards must be acceptable for the calibration curve to be acceptable. A minimum of six concentration levels must define the curve.

- Selectivity – The blanks and blanks with internal standard must have an average response of no greater than 20.0% of the average response of the lowest acceptable standard.

- Sensitivity – The limit of quantitation for a run is the lowest acceptable standard that meets all acceptance criteria.

- Quality Control Samples - At least 67% (eight of twelve) of the QCs will have determined concentrations within 20.0 percent of the nominal concentration and at least 50% (two of the four) QCs at each concentration level will have

determined concentrations within 20.0 percent of the nominal concentration as measured by the %RE.

- Repeat Analysis of Study Samples – For samples that are repeated for technical reasons (e.g. failed run, bad injection) only one replicate must be run. For samples repeated for any other reason, at least duplicate aliquots must be analyzed, if sufficient sample is available. When multiple samples are analyzed, the average of all acceptable values will be reported, unless a value can be statistically excluded.

4.0 RESPONSE TO FAILURE TO MEET ACCEPTANCE CRITERIA

4.1 System Suitability

Verify that all calculations are correct. If the system suitability does not meet acceptance criteria and the standards and the QCs within the run meet acceptance criteria, a run set may be accepted with written justification and management approval. Otherwise, reinject the entire run set.

4.2 Drift

Verify that all calculations are correct. If the drift does not meet acceptance criteria and the standards and the QCs within the run meet acceptance criteria, a run set may be accepted with written justification and management approval. Otherwise, reinject the entire run set.

4.3 Accuracy of Standards and QCs

Verify that all calculations are correct . Verify that the proper instrument system (e.g., column, flow rates, etc.) was used for the analysis. If not, samples need to be re-injected using the correct instrument system.

If the correct instrument system was used, compare the current chromatograms to a past analysis. If the chromatography has changed substantially, determine and correct the problem with the instrument system and then re-inject the samples.

4.4 Linearity (Coefficient of Determination)

Verify that all concentrations, the regression model, integration, and calculations are correct.

If all the calculations are correct, repeat the analysis.

4.5 Selectivity/Carryover

If the results are greater than 20.0%, verify that the proper wash procedure was used, if applicable. If the proper wash conditions were utilized, assess the reason for carryover and the impact to the data. The LLOQ for the impacted set may be raised to the concentration level that meets the acceptable limits. The samples between the new LLOQ and the original LLOQ will be repeated with another extraction set.

5.0 RESULTS

Include printouts of the acquisition method, and any applicable spreadsheets in the data packet.

5.1 COMMENTS/CONCLUSIONS

_____
_____
_____
_____
_____
_____
_____
_____

6.0 DATA REVIEW

6.1 Technical Review

All data generated in the laboratory activities will be reviewed by the technical leader (or designee) for technical content, accuracy, and completeness of documentation.

The technical leader will assure the proper equipment was used, review experimental procedure, rejection of calibration standards, integration of chromatograms, chromatography data processing and acquisition parameters, calibration standard concentrations, regression model, and that this documentation was followed.

6.2 Data Accuracy Review

Review at least the following: completeness and correctness of data entry, formulas used to calculate all values, and accuracy of calculations.

7.0 SIGNATURES

Technical Review Signature/Date:

_____

_____

Signature of the technical reviewer will be considered documentation that all modifications and/or changes to this documentation form (documented during the course of conducting this task) are technically acceptable and have no adverse technical impact unless otherwise noted.

Data Accuracy Review Signature/Date:

_____

METHOD FOR THE ANALYSIS OF SYN548098 IN RAT WHOLEBLOOD:WATER (1:1) BY TANDEM LC-MS

1.0 PROCEDURE

1.1 General Instructions

USE TWO PAIR OF DISSIMILAR GLOVES DURING NEAT CHEMICAL HANDLING.

Calibrate all required balances according to the SOP on balance usage.

Make equivalent dilutions when the volume needed varies from the volume stated in the method.

Label all standard and reagent solutions as specified in the appropriate SOP.

Document all materials, equipment, and the chromatographic parameters. Initial and date on the top of each page of this document to signify that you have followed the instructions as written, all materials and reagents are current, and all equipment has been properly calibrated.

Initial and date the top of the page on the day that the work for that page was begun. Other entries made by the analyst on a later date or entries made by another person will be initialed and dated near the data entry.

The procedures are written in general chronological order. However, it is not essential that all sections be performed sequentially. The analyst may determine the order for conducting the task in the most efficient manner, unless the order for certain activities is specified.

Line through or "NA" any section that is not needed for a specific task. No formal explanation is required.

1.2 Samples

Reference the Chain of Custody or Sample Inventory Forms.

1.3 Materials

See Table 1 for all required chemicals, reagents, matrix, and solvents. Use Table 1 for documentation. Check the labels carefully to ensure that materials with expiration dates are current and are the proper grade/purity.

Table 1. Materials

Chemical	Supplier	Grade/Purity	Lot Number	Exp Date
SYN548098	See Analytical Standards Section 1.6			
Nifedipine	Sigma Aldrich	≥98%		
Water	Aqua Solutions Model 2121BL	ASTM Type 1	NA	NA
Acetonitrile	Sigma Aldrich	HPLC		
Methanol	Sigma Aldrich	HPLC		
Formic Acid	Fisher	HPLC		
Blank 1:1 Rat Wholeblood:Water[a]	Bioreclamation	Wistar Hannover (K3EDTA)		

a. Will be referred to as Blank blood throughout the document.

1.4 Equipment

All major pieces of equipment used for this method are listed in Table 2 and in the bulleted list below the table. Check the calibration of all equipment requiring calibration (e.g. balances and pipets) to ensure it is current. Document the actual piece of equipment, X or SN, and calibration due date in Table 2.

Table 2. Equipment

Equipment	Equipment ID	Calibration Due Date
Analytical Balance		
Weight Set		
Positive Displacement Pipet		
Positive Displacement Pipet		
Positive Displacement Pipet		
Positive Displacement Pipet		
Repeater Pipet		
Aqua Solutions Water Filtration	09011-21BL	NA
Refrigerator (2 to 8ºC)		NA
Freezer (~-70ºC)		NA

Balance Calibrated by: _____

The following lists the additional general equipment and supplies that are used throughout this method:

- Volumetric flasks, Class A
- Volumetric Pipets, Class A
- Sonicator
- Centrifuge
- Vortex
- Micro-centrifuge Tubes
- Polypropylene Centrifuge Tubes

1.5 Preparation of Solutions

1.5.1 Mobile Phase A, 0.1% Formic acid in Water

Add 1 mL of formic acid to 1 L of water. Cap and mix well. Store at room temperature. May be used for up to one month.

Date Prepared: _____

1.5.2 Mobile Phase B, 0.1% Formic acid in Methanol

Add 1 mL of formic acid to 1 L of methanol. Cap and mix well. Store at room temperature. May be used for up to one month.

Date Prepared: _____

1.6 Preparation of Analytical Standard Solutions

1.6.1 Analytical Standard Information

Document the analytical standard information in Table 3.

Table 3. Analytical Standard Information (SYN548098)

Identification	SYN548098
Lot Number	12DEC13
Storage Location at Time of Use	
Supplier	Battelle
Grade or Purity	NA
Storage Conditions	RT
Expiration Date	12/12/18

1.6.2 Preparation of Stock Analytical Standard Solutions

Preparation of Stock Analytical Standard Solution

Weigh the amounts of analytical standard shown in Table 4 and transfer into a volumetric flask. Add 1 mL of acetonitrile. Cap and mix well. Sonicate for ~5 minutes if necessary. Dilute to volume with acetonitrile and mix thoroughly. Store refrigerated. May be used for up to one year.

Table 4. Preparation of Stock SYN548098 Analytical Standard Solution

ID	Target Conc. (µg/mL)	Target Weight (mg)	Actual Weight (mg)[a]	Final Volume (mL)
A	1000	2 ± 0.2		2

a. Weigh to at least the nearest 0.01 mg. Document weight to at least four significant figures.

Date Prepared: _____

1.6.3 Preparation of Solvent Standard Spiking Solution

Using an adjustable volume pipet, transfer the volumes of standard indicated in Table 5 into volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to one year or the expiration date of the source solution, whichever is the shortest.

Table 5. Preparation of Solvent Standard Spiking Solutions

ID	Target Conc. (ng/mL)	Source	Source Volume (mL) each	Final Volume (mL)
SS1	10000	A	0.10	10
SS2	5000	A	0.05	10
SS3	2000	SS1	1.00	5
SS4	500.0	SS2	0.50	5
SS5	200.0	SS3	0.50	5
SS6	50.00	SS4	0.50	5
SS7	20.00	SS5	0.50	5

Date Prepared: _____

1.7 Preparation of Blood Standards

1.7.1 Preparation of Blood Calibration Standards

Using an adjustable volume pipet transfer the volumes of spiking solution and blank blood indicated in Table 6 into the appropriate micro-centrifuge tubes. **Take care to avoid coagulated clumps.** Cap and mix vigorously by vortexing for approximately 1 minute. Prepare fresh each day of extraction. Process duplicate aliquots at each concentration level with each extraction set.

Table 6. Preparation of SYN548098 Blood Calibration Standards

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
CS1	500.0	SS1	0.05	0.95	1
CS2	250.0	SS2	0.05	0.95	1
CS3	100.0	SS3	0.05	0.95	1
CS4	25.00	SS4	0.05	0.95	1
CS5	10.00	SS5	0.05	0.95	1
CS6	2.500	SS6	0.05	0.95	1
CS7	1.000	SS7	0.05	0.95	1

1.8 Preparation of Blanks

On each day of extraction, process duplicate blood blanks and duplicate blanks + IS.

1.9 Preparation of Internal Standard Solutions

1.9.1 Preparation of Stock Internal Standard Solution

Weigh the amount of Nifedipine analytical standard shown in Table 7 and transfer into a volumetric flask. Dilute to volume with acetonitrile. Cap and mix well. Sonicate for ~5 minutes. If needed, add acetonitrile drop wise and sonicate until dissolved. Store refrigerated. May be used for up to six months.

Table 7. Preparation of Stock Internal Standard Solution

ID	Target Conc. (µg/mL)	Target Weight (mg)	Actual Weight (mg)[a]	Final Volume (mL)
Stock IS	1000	5 ± 0.5		5

b. Weigh to at least the nearest 0.01 mg. Document weight to at least four significant figures.

Date Prepared: _____

1.9.2 Preparation of Intermediate Internal Standard Solution

Using an adjustable volume pipet, transfer the volume of stock internal standard listed in Table 8 into a volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to six months or the expiration date of the stock internal standard solution, whichever is the shortest.

Table 8. Preparation of Intermediate Internal Standard Solution

ID	Target Conc. (µg/mL)	Source	Source Volume (mL)	Final Volume (mL)
Int IS	10	Stock IS	0.25	25

Date Prepared: _____

1.9.3 Preparation of Working Internal Standard (WIS) Solution

Using an adjustable volume pipet, transfer the volume of intermediate internal standard listed in Table 9 into a volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to six months or the expiration date of the stock internal standard solution, whichever is the shortest.

Table 9. Preparation of Working Internal Standard Solution

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Final Volume (mL)
WIS	250	Int IS	1.25	50

Date Prepared: _____

1.10 Preparation of Quality Control (QC) Samples

1.10.1 Preparation of QC Spiking Solutions

Using an adjustable volume pipet, transfer the volume of solutions indicated in Table 10 into volumetric flasks. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to one year or the expiration date of the stock internal standard solution, whichever is the shortest of the two.

Table 10. Preparation of QC Spiking Solution

ID	Target Conc. (ng/mL)	Source	Source Volume (mL)	Final Volume (mL)
QC SS High	8000	A	0.08	10
QC SS Mid	4000	QC SS High	5.00	10
QC SS Low	60.00	QC SS Mid	0.15	10

Date Prepared: _____

1.10.2 Preparation of Blood QC Samples

Using an adjustable volume pipet, transfer the volumes of solutions indicated in Table 11 into individual micro-centrifuge tubes. **Take care to avoid coagulated clumps.** Cap and vortex to mix. Transfer ~0.5 mL aliquots to micro-centrifuge tubes for storage. Store frozen at ~ -70°C. May be used for up to three months.. Process four replicates at each concentration for each extraction set

Table 11. Preparation of Blood QC Samples

ID	Target Conc. (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
QC High	400.0	QC SS High	0.1	1.9	2
QC Mid	200.0	QC SS Mid	0.1	1.9	2
QC Low	3.000	QC SS Low	0.1	1.9	2

Date Prepared: _____

1.11 Preparation of System Suitability (SYS)

Using a positive displacement pipet, transfer the volume of blank blood indicated in Table 12 into a micro-centrifuge tube. Using an adjustable volume pipet, transfer the volume of spiking solution indicated in Table 12 into the micro-centrifuge tube. Cap and vortex to mix. Prepare fresh on each day of extraction. Process four replicates with each extraction set and combine the extracts.

Table 12. Preparation of System Suitability Sample

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
SYS	12.5	CS2	0.05	0.95	1

1.12 Preparation of Blood Standards, Blanks, QCs, and Samples

- Allow blank blood, and samples to thaw at room temperature if previously frozen.
- Document any sample irregularities.
- A second analyst will vortex each study sample thoroughly and open each container one at a time while the primary extracting analyst removes the appropriate volume of sample from the container.

  Second Analyst Initials/Date: _____
- Using an adjustable volume pipet, transfer 20 µL of each calibration standard, blood blank, and study sample into individual micro-centrifuge tubes. Document any samples requiring dilution in Table 13. Use blank blood to bring any samples to 20 µL.

Table 13. Sample Dilution Table

Sample ID	Sample Volume (µL)	Sample ID	Sample Volume (µL)

Reference the sample list at the front of the data binder (if available) for any dilutions not recorded in Table 13.

- Add 100 µL of WIS to all samples, except the blanks without IS. For the blanks without IS, add 100 µL of Acetonitrile instead of WIS.
- Vortex for ~1 minute to mix.
- Centrifuge at a maximum speed for ~10 minutes.
- Transfer supernatant to auto-sampler vials.
- Submit for analysis by LC-MS.

- Sample extracts are stored at the temperature of the auto-sampler if not analyzed on the day of extraction. Storage location of the extracts until injection if not stored on the auto-sampler: _____

1.13 Analysis of Standards, Blanks, QC, and Samples

Use the LC-MS system conditions specified in Table 14. The conditions, which are designated, may be modified by the analyst to produce acceptable chromatography. Record the actual values inside the parentheses.

Set up the automated chromatography data software (CDS) system to collect the electronic output from each injection, acquiring the data.

If the instrument has not been previously conditioned, condition the instrument by injecting the SYS sample for approximately 1 hour prior to starting the system suitability injections. Conditioning run performed: _____ Yes _____ No

Make a minimum of three injections of the appropriate SYS sample prior to starting the sample run to ensure optimal operation of the system.

Make single injections of all calibration standards, blanks, QCs, and samples. Inject one calibration curve near the beginning of the run and one near the end of the run. The calibration standards near the beginning of the run will be injected from high to low concentration. The calibration standards near the end of the run will be injected from low to high concentration. The first replicate of the blank and blank with IS will be injected prior to the first curve. The second replicate of the blank and blank with IS will be injected immediately following the second calibration curve. Distribute the QCs throughout the run.

Make duplicate injections of a post-run SYS sample after the run to assess drift.

Table 14. LC-MS System

Data System	Analyst, Version 1.6.1
MRM	SYN548098 552 > 436 amu Nifedipine 347 > 254 amu
HPLC System	Pump[a]:_____ X/SN:_____ Autosampler[a]:_____ X/SN:_____
MS System	Sciex API _____(Toronto, Ontario Canada) SN:_____
Ionization Source	Heated Nebulizer, Positive Ion Mode
Analytical Column	Phenomenex Luna C18, 5 µm, 50 x 2 mm SN/Lot#:_____
Guard Column	Phenomenex C18 4 x 2 mm
Column Temperature	40ºC
Auto-Sampler Temperature	RT
Mobile Phase	A: 0.1% Formic Acid in Water B: 0.1% Formic Acid in Methanol
Mobile Phase Gradient[b]	Time (minutes) %B 0.0 35 0.2 90 2.2 90 2.3 35 4.5 35
Flow Rate (µL/min)[b]	500 (_____) µL/min
Injection Volume[b]	10 µL (_____) µL
Run Time[b]	4.5 minutes (_____) minutes

a. Agilent (Santa Clara, CA), Shimadzu (Kyoto, Japan), Leap CTC Analytics (Carrboro, NC).
b. Parameters which may be modified by analyst.

Run ID: _____

2.0 CALCULATIONS

Determine the optimal automatic integration parameters for the run. Examine the integration of the analyte and the internal standard by the chromatography system. Modify, if necessary, to obtain optimum consistent integration. Document the rationale of any manual or automated reintegration of peaks.

Use Microsoft Excel to perform the calculations as follows:

Calculate the individual and average percent area of each blank and blank with IS compared with the average area response of the Lower Limit of Quantitation (LLOQ) standard.

Calculate the individual percent area of the blank following the high standard compared with the area response of the nearest LLOQ standard.

Calculate the SYS average ratio and rsd. Calculate the Drift average area ratio. Compare the average ratio of the SYS to the average ratio of the Drift.

Calculate the exact concentrations of the plasma standards and plasma QCs. Enter the values into the Watson Bioanalytical LIMS.

Use the Watson Bioanalytical LIMS, version 7.4, to perform the calculations as follows:

Calculate the peak area response ratio (Analyte/IS) for each injection.

Determine optimal regression parameters and record the model and weighting in Table 15 to calculate the regression equation.

Table 15. Regression Parameters

Model	
Weighting	
y-intercept	Calculate, Do not force through origin
y-values	Instrument Response
x-values	Nominal Concentration

Using the peak area response of the standards and the regression equation, calculate their determined concentrations.

Using the peak area response ratios of the QCs and samples, the regression equation, and any dilution factor, if applicable, calculate their individual determined concentrations.

Using the determined concentration, calculate the individual relative error (%RE) for each standard and QC concentration.

3.0 ACCEPTANCE CRITERIA

There are no specific acceptance criteria for a this assay. The following will be used to assess each individual run, however the task leader may technically decide to revise these criteria or accept criteria outside these limits.

- System Suitability – At least three consecutive injections of the SYS immediately preceding the run must have a RSD of ≤ 10.0% for the run to be valid using the area ratio.

- Drift – Average area ratio within 15.0% of the pre-run system suitability average area ratio.

- Carryover - The blank immediately following the second curve must have an area response of no greater than 20.0% of the area response of the nearest acceptable low standard.

- Linearity – The coefficient of determination will be ≥ 0.98.

- Calibration Standards – Standards will have determined concentrations within 15.0% of nominal as measured by %RE, except for the LLOQ, where it should be within 20.0% of nominal to be acceptable. If a standard is not acceptable, it will not be used to generate the calibration curve. The standards that are not acceptable will be removed one-by-one with the standard deviating furthest from nominal concentration as measured by %RE removed first; regression will be performed again after each removal. Up to 3 standards falling outside these limits can be discarded (deactivated), provided they do not change the established model. At least 75% (11 of 14) of the standards must be acceptable for the calibration curve to be acceptable. A minimum of six concentration levels must define the curve.

- Selectivity – The blanks and blanks with internal standard must have an average response of no greater than 20.0% of the average response of the lowest acceptable standard.

- Sensitivity – The limit of quantitation for a run is the lowest acceptable standard that meets all acceptance criteria.

- Quality Control Samples - At least 67% (eight of twelve) of the QCs will have determined concentrations within 20.0 percent of the nominal concentration and at least 50% (two of the four) QCs at each concentration level will have

determined concentrations within 20.0 percent of the nominal concentration as measured by the %RE.

- Repeat Analysis of Study Samples – For samples that are repeated for technical reasons (e.g. failed run, bad injection) only one replicate must be run. For samples repeated for any other reason, at least duplicate aliquots must be analyzed, if sufficient sample is available. When multiple samples are analyzed, the average of all acceptable values will be reported, unless a value can be statistically excluded.

4.0 RESPONSE TO FAILURE TO MEET ACCEPTANCE CRITERIA

4.1 System Suitability

Verify that all calculations are correct. If the system suitability does not meet acceptance criteria and the standards and the QCs within the run meet acceptance criteria, a run set may be accepted with written justification and management approval. Otherwise, reinject the entire run set.

4.2 Drift

Verify that all calculations are correct. If the drift does not meet acceptance criteria and the standards and the QCs within the run meet acceptance criteria, a run set may be accepted with written justification and management approval. Otherwise, reinject the entire run set.

4.3 Accuracy of Standards and QCs

Verify that all calculations are correct . Verify that the proper instrument system (e.g., column, flow rates, etc.) was used for the analysis. If not, samples need to be re-injected using the correct instrument system.

If the correct instrument system was used, compare the current chromatograms to a past analysis. If the chromatography has changed substantially, determine and correct the problem with the instrument system and then re-inject the samples.

4.4 Linearity (Coefficient of Determination)

Verify that all concentrations, the regression model, integration, and calculations are correct.

If all the calculations are correct, repeat the analysis.

4.5 Selectivity/Carryover

If the results are greater than 20.0%, verify that the proper wash procedure was used, if applicable. If the proper wash conditions were utilized, assess the reason for carryover and the impact to the data. The LLOQ for the impacted set may be raised to the concentration level that meets the acceptable limits. The samples between the new LLOQ and the original LLOQ will be repeated with another extraction set.

5.0 RESULTS

Include printouts of the acquisition method, and any applicable spreadsheets in the data packet.

5.1 COMMENTS/CONCLUSIONS

_____
_____
_____
_____
_____
_____
_____
_____

6.0 DATA REVIEW

6.1 Technical Review

All data generated in the laboratory activities will be reviewed by the technical leader (or designee) for technical content, accuracy, and completeness of documentation.

The technical leader will assure the proper equipment was used, review experimental procedure, rejection of calibration standards, integration of chromatograms, chromatography data processing and acquisition parameters, calibration standard concentrations, regression model, and that this documentation was followed.

6.2 Data Accuracy Review

Review at least the following: completeness and correctness of data entry, formulas used to calculate all values, and accuracy of calculations.

7.0 SIGNATURES

Technical Review Signature/Date:

_____

_____

Signature of the technical reviewer will be considered documentation that all modifications and/or changes to this documentation form (documented during the course of conducting this task) are technically acceptable and have no adverse technical impact unless otherwise noted.

Data Accuracy Review Signature/Date:

_____

METHOD FOR THE ANALYSIS OF SYN548101 IN RAT WHOLEBLOOD:WATER (1:1) BY TANDEM LC-MS

1.0 PROCEDURE

1.1 General Instructions

USE TWO PAIR OF DISSIMILAR GLOVES DURING NEAT CHEMICAL HANDLING.

Calibrate all required balances according to the SOP on balance usage.

Make equivalent dilutions when the volume needed varies from the volume stated in the method.

Label all standard and reagent solutions as specified in the appropriate SOP.

Document all materials, equipment, and the chromatographic parameters. Initial and date on the top of each page of this document to signify that you have followed the instructions as written, all materials and reagents are current, and all equipment has been properly calibrated.

Initial and date the top of the page on the day that the work for that page was begun. Other entries made by the analyst on a later date or entries made by another person will be initialed and dated near the data entry.

The procedures are written in general chronological order. However, it is not essential that all sections be performed sequentially. The analyst may determine the order for conducting the task in the most efficient manner, unless the order for certain activities is specified.

Line through or "NA" any section that is not needed for a specific task. No formal explanation is required.

1.2 Samples

Reference the Chain of Custody or Sample Inventory Forms.

1.3 Materials

See Table 1 for all required chemicals, reagents, matrix, and solvents. Use Table 1 for documentation. Check the labels carefully to ensure that materials with expiration dates are current and are the proper grade/purity.

Table 1. Materials

Chemical	Supplier	Grade/Purity	Lot Number	Exp Date
SYN548101	See Analytical Standards Section 1.6			
Nifedipine	Sigma Aldrich	≥98%		
Water	Aqua Solutions Model 2121BL	ASTM Type 1	NA	NA
Acetonitrile	Sigma Aldrich	HPLC		
Methanol	Sigma Aldrich	HPLC		
Formic Acid	Fisher	HPLC		
Blank 1:1 Rat Wholeblood:Water[a]	Bioreclamation	Wistar Hannover (K3EDTA)		

a. Will be referred to as Blank blood throughout the document.

1.4 Equipment

All major pieces of equipment used for this method are listed in Table 2 and in the bulleted list below the table. Check the calibration of all equipment requiring calibration (e.g. balances and pipets) to ensure it is current. Document the actual piece of equipment, X or SN, and calibration due date in Table 2.

Table 2. Equipment

Equipment	Equipment ID	Calibration Due Date
Analytical Balance		
Weight Set		
Positive Displacement Pipet		
Positive Displacement Pipet		
Positive Displacement Pipet		
Positive Displacement Pipet		
Repeater Pipet		
Aqua Solutions Water Filtration	09011-21BL	NA
Refrigerator (2 to 8ºC)		NA
Freezer (~-70ºC)		NA

Balance Calibrated by: _____

The following lists the additional general equipment and supplies that are used throughout this method:

- Volumetric flasks, Class A
- Volumetric Pipets, Class A
- Sonicator
- Centrifuge
- Vortex
- Micro-centrifuge Tubes
- Polypropylene Centrifuge Tubes

1.5 Preparation of Solutions

1.5.1 Mobile Phase A, 0.1% Formic acid in Water

Add 1 mL of formic acid to 1 L of water. Cap and mix well. Store at room temperature. May be used for up to one month.

Date Prepared: _____

1.5.2 Mobile Phase B, 0.1% Formic acid in Methanol

Add 1 mL of formic acid to 1 L of methanol. Cap and mix well. Store at room temperature. May be used for up to one month.

Date Prepared: _____

1.6 Preparation of Analytical Standard Solutions

1.6.1 Analytical Standard Information

Document the analytical standard information in Table 3.

Table 3. Analytical Standard Information (SYN548101)

Identification	SYN548101
Lot Number	12DEC13
Storage Location at Time of Use	
Supplier	Battelle
Grade or Purity	NA
Storage Conditions	RT
Expiration Date	12/12/18

1.6.2 Preparation of Stock Analytical Standard Solutions

Preparation of Stock Analytical Standard Solution

Weigh the amounts of analytical standard shown in Table 4 and transfer into a volumetric flask. Add 1 mL of acetonitrile. Cap and mix well. Sonicate for ~5 minutes if necessary. Dilute to volume with acetonitrile and mix thoroughly. Store refrigerated. May be used for up to one year.

Table 4. Preparation of Stock SYN548101 Analytical Standard Solution

ID	Target Conc. (µg/mL)	Target Weight (mg)	Actual Weight (mg)[a]	Final Volume (mL)
A	1000	2 ± 0.2		2

a. Weigh to at least the nearest 0.01 mg. Document weight to at least four significant figures.

Date Prepared: _____

1.6.3 Preparation of Solvent Standard Spiking Solution

Using an adjustable volume pipet, transfer the volumes of standard indicated in Table 5 into volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to one year or the expiration date of the source solution, whichever is the shortest.

Table 5. Preparation of Solvent Standard Spiking Solutions

ID	Target Conc. (ng/mL)	Source	Source Volume (mL) each	Final Volume (mL)
SS1	10000	A	0.10	10
SS2	5000	A	0.05	10
SS3	2000	SS1	1.00	5
SS4	500.0	SS2	0.50	5
SS5	200.0	SS3	0.50	5
SS6	50.00	SS4	0.50	5
SS7	20.00	SS5	0.50	5

Date Prepared: _____

1.7 Preparation of Blood Standards

1.7.1 Preparation of Blood Calibration Standards

Using an adjustable volume pipet transfer the volumes of spiking solution and blank blood indicated in Table 6 into the appropriate micro-centrifuge tubes. **Take care to avoid coagulated clumps.** Cap and mix vigorously by vortexing for approximately 1 minute. Prepare fresh each day of extraction. Process duplicate aliquots at each concentration level with each extraction set.

Table 6. Preparation of SYN548101 Blood Calibration Standards

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
CS1	500.0	SS1	0.05	0.95	1
CS2	250.0	SS2	0.05	0.95	1
CS3	100.0	SS3	0.05	0.95	1
CS4	25.00	SS4	0.05	0.95	1
CS5	10.00	SS5	0.05	0.95	1
CS6	2.500	SS6	0.05	0.95	1
CS7	1.000	SS7	0.05	0.95	1

1.8 Preparation of Blanks

On each day of extraction, process duplicate blood blanks and duplicate blanks + IS.

1.9 Preparation of Internal Standard Solutions

1.9.1 Preparation of Stock Internal Standard Solution

Weigh the amount of Nifedipine analytical standard shown in Table 7 and transfer into a volumetric flask. Dilute to volume with acetonitrile. Cap and mix well. Sonicate for ~5 minutes. If needed, add acetonitrile drop wise and sonicate until dissolved. Store refrigerated. May be used for up to six months.

Table 7. Preparation of Stock Internal Standard Solution

ID	Target Conc. (µg/mL)	Target Weight (mg)	Actual Weight (mg)[a]	Final Volume (mL)
Stock IS	1000	5 ± 0.5		5

b. Weigh to at least the nearest 0.01 mg. Document weight to at least four significant figures.

Date Prepared: _____

1.9.2 Preparation of Intermediate Internal Standard Solution

Using an adjustable volume pipet, transfer the volume of stock internal standard listed in Table 8 into a volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to six months or the expiration date of the stock internal standard solution, whichever is the shortest.

Table 8. Preparation of Intermediate Internal Standard Solution

ID	Target Conc. (µg/mL)	Source	Source Volume (mL)	Final Volume (mL)
Int IS	10	Stock IS	0.25	25

Date Prepared: _____

1.9.3 Preparation of Working Internal Standard (WIS) Solution

Using an adjustable volume pipet, transfer the volume of intermediate internal standard listed in Table 9 into a volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to six months or the expiration date of the stock internal standard solution, whichever is the shortest.

Table 9. Preparation of Working Internal Standard Solution

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Final Volume (mL)
WIS	250	Int IS	1.25	50

Date Prepared: _____

1.10 Preparation of Quality Control (QC) Samples

1.10.1 Preparation of QC Spiking Solutions

Using an adjustable volume pipet, transfer the volume of solutions indicated in Table 10 into volumetric flasks. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to one year or the expiration date of the stock internal standard solution, whichever is the shortest of the two.

Table 10. Preparation of QC Spiking Solution

ID	Target Conc. (ng/mL)	Source	Source Volume (mL)	Final Volume (mL)
QC SS High	8000	A	0.08	10
QC SS Mid	4000	QC SS High	5.00	10
QC SS Low	60.00	QC SS Mid	0.15	10

Date Prepared: _____

1.10.2 Preparation of Blood QC Samples

Using an adjustable volume pipet, transfer the volumes of solutions indicated in Table 11 into individual micro-centrifuge tubes. **Take care to avoid coagulated clumps.** Cap and vortex to mix. Transfer ~0.5 mL aliquots to micro-centrifuge tubes for storage. Store frozen at ~ -70°C. May be used for up to three months.. Process four replicates at each concentration for each extraction set

Table 11. Preparation of Blood QC Samples

ID	Target Conc. (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
QC High	400.0	QC SS High	0.1	1.9	2
QC Mid	200.0	QC SS Mid	0.1	1.9	2
QC Low	3.000	QC SS Low	0.1	1.9	2

Date Prepared: _____

1.11 Preparation of System Suitability (SYS)

Using a positive displacement pipet, transfer the volume of blank blood indicated in Table 12 into a micro-centrifuge tube. Using an adjustable volume pipet, transfer the volume of spiking solution indicated in Table 12 into the micro-centrifuge tube. Cap and vortex to mix. Prepare fresh on each day of extraction. Process four replicates with each extraction set and combine the extracts.

Table 12. Preparation of System Suitability Sample

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
SYS	12.5	CS2	0.05	0.95	1

1.12 Preparation of Blood Standards, Blanks, QCs, and Samples

- Allow blank blood, and samples to thaw at room temperature if previously frozen.
- Document any sample irregularities.
- A second analyst will vortex each study sample thoroughly and open each container one at a time while the primary extracting analyst removes the appropriate volume of sample from the container.

  Second Analyst Initials/Date: _____
- Using an adjustable volume pipet, transfer 20 µL of each calibration standard, blood blank, and study sample into individual micro-centrifuge tubes. Document any samples requiring dilution in Table 13. Use blank blood to bring any samples to 20 µL.

Table 13. Sample Dilution Table

Sample ID	Sample Volume (µL)	Sample ID	Sample Volume (µL)

Reference the sample list at the front of the data binder (if available) for any dilutions not recorded in Table 13.

- Add 100 µL of WIS to all samples, except the blanks without IS. For the blanks without IS, add 100 µL of Acetonitrile instead of WIS.
- Vortex for ~1 minute to mix.
- Centrifuge at a maximum speed for ~10 minutes.
- Transfer supernatant to auto-sampler vials.
- Submit for analysis by LC-MS.

- Sample extracts are stored at the temperature of the auto-sampler if not analyzed on the day of extraction. Storage location of the extracts until injection if not stored on the auto-sampler: _____

1.13 Analysis of Standards, Blanks, QC, and Samples

Use the LC-MS system conditions specified in Table 14. The conditions, which are designated, may be modified by the analyst to produce acceptable chromatography. Record the actual values inside the parentheses.

Set up the automated chromatography data software (CDS) system to collect the electronic output from each injection, acquiring the data.

If the instrument has not been previously conditioned, condition the instrument by injecting the SYS sample for approximately 1 hour prior to starting the system suitability injections. Conditioning run performed: _____ Yes _____ No

Make a minimum of three injections of the appropriate SYS sample prior to starting the sample run to ensure optimal operation of the system.

Make single injections of all calibration standards, blanks, QCs, and samples. Inject one calibration curve near the beginning of the run and one near the end of the run. The calibration standards near the beginning of the run will be injected from high to low concentration. The calibration standards near the end of the run will be injected from low to high concentration. The first replicate of the blank and blank with IS will be injected prior to the first curve. The second replicate of the blank and blank with IS will be injected immediately following the second calibration curve. Distribute the QCs throughout the run.

Make duplicate injections of a post-run SYS sample after the run to assess drift.

Table 14. LC-MS System

Data System	Analyst, Version 1.6.1
MRM	SYN548101 564 > 434 amu Nifedipine 347 > 254 amu
HPLC System	Pump[a]:_____ X/SN:_____ Autosampler[a]:_____ X/SN:_____
MS System	Sciex API _____(Toronto, Ontario Canada) SN:_____
Ionization Source	Heated Nebulizer, Positive Ion Mode
Analytical Column	Phenomenex Luna C18, 5 µm, 50 x 2 mm SN/Lot#:_____
Guard Column	Phenomenex C18 4 x 2 mm
Column Temperature	40ºC
Auto-Sampler Temperature	RT
Mobile Phase	A: 0.1% Formic Acid in Water B: 0.1% Formic Acid in Methanol
Mobile Phase Gradient[b]	Time (minutes) %B 0.0 35 0.2 90 2.2 90 2.3 35 4.5 35
Flow Rate (µL/min)[b]	500 (_____) µL/min
Injection Volume[b]	10 µL (_____) µL
Run Time[b]	4.5 minutes (_____) minutes

a. Agilent (Santa Clara, CA), Shimadzu (Kyoto, Japan), Leap CTC Analytics (Carrboro, NC).
b. Parameters which may be modified by analyst.

2.0 CALCULATIONS

Determine the optimal automatic integration parameters for the run. Examine the integration of the analyte and the internal standard by the chromatography system. Modify, if necessary, to obtain optimum consistent integration. Document the rationale of any manual or automated reintegration of peaks.

Use Microsoft Excel to perform the calculations as follows:

Calculate the individual and average percent area of each blank and blank with IS compared with the average area response of the Lower Limit of Quantitation (LLOQ) standard.

Calculate the individual percent area of the blank following the high standard compared with the area response of the nearest LLOQ standard.

Calculate the SYS average ratio and rsd. Calculate the Drift average area ratio. Compare the average ratio of the SYS to the average ratio of the Drift.

Calculate the exact concentrations of the plasma standards and plasma QCs. Enter the values into the Watson Bioanalytical LIMS.

Use the Watson Bioanalytical LIMS, version 7.4, to perform the calculations as follows:

Calculate the peak area response ratio (Analyte/IS) for each injection.

Determine optimal regression parameters and record the model and weighting in Table 15 to calculate the regression equation.

Table 15. Regression Parameters

Model	
Weighting	
y-intercept	Calculate, Do not force through origin
y-values	Instrument Response
x-values	Nominal Concentration

Using the peak area response of the standards and the regression equation, calculate their determined concentrations.

Using the peak area response ratios of the QCs and samples, the regression equation, and any dilution factor, if applicable, calculate their individual determined concentrations.

Using the determined concentration, calculate the individual relative error (%RE) for each standard and QC concentration.

3.0 ACCEPTANCE CRITERIA

There are no specific acceptance criteria for a this assay. The following will be used to assess each individual run, however the task leader may technically decide to revise these criteria or accept criteria outside these limits.

- System Suitability – At least three consecutive injections of the SYS immediately preceding the run must have a RSD of ≤ 10.0% for the run to be valid using the area ratio.

- Drift – Average area ratio within 15.0% of the pre-run system suitability average area ratio.

- Carryover - The blank immediately following the second curve must have an area response of no greater than 20.0% of the area response of the nearest acceptable low standard.

- Linearity – The coefficient of determination will be ≥ 0.98.

- Calibration Standards – Standards will have determined concentrations within 15.0% of nominal as measured by %RE, except for the LLOQ, where it should be within 20.0% of nominal to be acceptable. If a standard is not acceptable, it will not be used to generate the calibration curve. The standards that are not acceptable will be removed one-by-one with the standard deviating furthest from nominal concentration as measured by %RE removed first; regression will be performed again after each removal. Up to 3 standards falling outside these limits can be discarded (deactivated), provided they do not change the established model. At least 75% (11 of 14) of the standards must be acceptable for the calibration curve to be acceptable. A minimum of six concentration levels must define the curve.

- Selectivity – The blanks and blanks with internal standard must have an average response of no greater than 20.0% of the average response of the lowest acceptable standard.

- Sensitivity – The limit of quantitation for a run is the lowest acceptable standard that meets all acceptance criteria.

- Quality Control Samples - At least 67% (eight of twelve) of the QCs will have determined concentrations within 20.0 percent of the nominal concentration and at least 50% (two of the four) QCs at each concentration level will have

determined concentrations within 20.0 percent of the nominal concentration as measured by the %RE.

- Repeat Analysis of Study Samples – For samples that are repeated for technical reasons (e.g. failed run, bad injection) only one replicate must be run. For samples repeated for any other reason, at least duplicate aliquots must be analyzed, if sufficient sample is available. When multiple samples are analyzed, the average of all acceptable values will be reported, unless a value can be statistically excluded.

4.0 RESPONSE TO FAILURE TO MEET ACCEPTANCE CRITERIA

4.1 System Suitability

Verify that all calculations are correct. If the system suitability does not meet acceptance criteria and the standards and the QCs within the run meet acceptance criteria, a run set may be accepted with written justification and management approval. Otherwise, reinject the entire run set.

4.2 Drift

Verify that all calculations are correct. If the drift does not meet acceptance criteria and the standards and the QCs within the run meet acceptance criteria, a run set may be accepted with written justification and management approval. Otherwise, reinject the entire run set.

4.3 Accuracy of Standards and QCs

Verify that all calculations are correct . Verify that the proper instrument system (e.g., column, flow rates, etc.) was used for the analysis. If not, samples need to be re-injected using the correct instrument system.

If the correct instrument system was used, compare the current chromatograms to a past analysis. If the chromatography has changed substantially, determine and correct the problem with the instrument system and then re-inject the samples.

4.4 Linearity (Coefficient of Determination)

Verify that all concentrations, the regression model, integration, and calculations are correct.

If all the calculations are correct, repeat the analysis.

4.5 Selectivity/Carryover

If the results are greater than 20.0%, verify that the proper wash procedure was used, if applicable. If the proper wash conditions were utilized, assess the reason for carryover and the impact to the data. The LLOQ for the impacted set may be raised to the concentration level that meets the acceptable limits. The samples between the new LLOQ and the original LLOQ will be repeated with another extraction set.

5.0 RESULTS

Include printouts of the acquisition method, and any applicable spreadsheets in the data packet.

5.1 COMMENTS/CONCLUSIONS

_____
_____
_____
_____
_____
_____
_____
_____

6.0 DATA REVIEW

6.1 Technical Review

All data generated in the laboratory activities will be reviewed by the technical leader (or designee) for technical content, accuracy, and completeness of documentation.

The technical leader will assure the proper equipment was used, review experimental procedure, rejection of calibration standards, integration of chromatograms, chromatography data processing and acquisition parameters, calibration standard concentrations, regression model, and that this documentation was followed.

6.2 Data Accuracy Review

Review at least the following: completeness and correctness of data entry, formulas used to calculate all values, and accuracy of calculations.

7.0 SIGNATURES

Technical Review Signature/Date:

_____

_____

Signature of the technical reviewer will be considered documentation that all modifications and/or changes to this documentation form (documented during the course of conducting this task) are technically acceptable and have no adverse technical impact unless otherwise noted.

Data Accuracy Review Signature/Date:

_____

METHOD FOR THE ANALYSIS OF SYN548013 IN RAT WHOLEBLOOD:WATER (1:1) BY TANDEM LC-MS

1.0 PROCEDURE

1.1 General Instructions

USE TWO PAIR OF DISSIMILAR GLOVES DURING NEAT CHEMICAL HANDLING.

Calibrate all required balances according to the SOP on balance usage.

Make equivalent dilutions when the volume needed varies from the volume stated in the method.

Label all standard and reagent solutions as specified in the appropriate SOP.

Document all materials, equipment, and the chromatographic parameters. Initial and date on the top of each page of this document to signify that you have followed the instructions as written, all materials and reagents are current, and all equipment has been properly calibrated.

Initial and date the top of the page on the day that the work for that page was begun. Other entries made by the analyst on a later date or entries made by another person will be initialed and dated near the data entry.

The procedures are written in general chronological order. However, it is not essential that all sections be performed sequentially. The analyst may determine the order for conducting the task in the most efficient manner, unless the order for certain activities is specified.

Line through or "NA" any section that is not needed for a specific task. No formal explanation is required.

1.2 Samples

Reference the Chain of Custody or Sample Inventory Forms.

1.3 Materials

See Table 1 for all required chemicals, reagents, matrix, and solvents. Use Table 1 for documentation. Check the labels carefully to ensure that materials with expiration dates are current and are the proper grade/purity.

Table 1. Materials

Chemical	Supplier	Grade/Purity	Lot Number	Exp Date
SYN548013	See Analytical Standards Section 1.6			
Nifedipine	Sigma Aldrich	≥98%		
Water	Aqua Solutions Model 2121BL	ASTM Type 1	NA	NA
Acetonitrile	Sigma Aldrich	HPLC		
Methanol	Sigma Aldrich	HPLC		
Formic Acid	Fisher	HPLC		
Blank 1:1 Rat Wholeblood:Water[a]	Bioreclamation	Wistar Hannover (K3EDTA)		

a. Will be referred to as Blank blood throughout the document.

1.4 Equipment

All major pieces of equipment used for this method are listed in Table 2 and in the bulleted list below the table. Check the calibration of all equipment requiring calibration (e.g. balances and pipets) to ensure it is current. Document the actual piece of equipment, X or SN, and calibration due date in Table 2.

Table 2. Equipment

Equipment	Equipment ID	Calibration Due Date
Analytical Balance		
Weight Set		
Positive Displacement Pipet		
Positive Displacement Pipet		
Positive Displacement Pipet		
Positive Displacement Pipet		
Repeater Pipet		
Aqua Solutions Water Filtration	09011-21BL	NA
Refrigerator (2 to 8ºC)		NA
Freezer (~-70ºC)		NA

Balance Calibrated by: _____

The following lists the additional general equipment and supplies that are used throughout this method:

- Volumetric flasks, Class A
- Volumetric Pipets, Class A
- Sonicator
- Centrifuge
- Vortex
- Micro-centrifuge Tubes
- Polypropylene Centrifuge Tubes

1.5 Preparation of Solutions

1.5.1 Mobile Phase A, 0.1% Formic acid in Water

Add 1 mL of formic acid to 1 L of water. Cap and mix well. Store at room temperature. May be used for up to one month.

Date Prepared: _____

1.5.2 Mobile Phase B, 0.1% Formic acid in Methanol

Add 1 mL of formic acid to 1 L of methanol. Cap and mix well. Store at room temperature. May be used for up to one month.

Date Prepared: _____

1.6 Preparation of Analytical Standard Solutions

1.6.1 Analytical Standard Information

Document the analytical standard information in Table 3.

Table 3. Analytical Standard Information (SYN548013)

Identification	SYN548013
Lot Number	12DEC13
Storage Location at Time of Use	
Supplier	Battelle
Grade or Purity	NA
Storage Conditions	RT
Expiration Date	12/12/18

1.6.2 Preparation of Stock Analytical Standard Solutions

Preparation of Stock Analytical Standard Solution

Weigh the amounts of analytical standard shown in Table 4 and transfer into a volumetric flask. Add 1 mL of acetonitrile. Cap and mix well. Sonicate for ~5 minutes if necessary. Dilute to volume with acetonitrile and mix thoroughly. Store refrigerated. May be used for up to one year.

Table 4. Preparation of Stock SYN548013 Analytical Standard Solution

ID	Target Conc. (µg/mL)	Target Weight (mg)	Actual Weight (mg)[a]	Final Volume (mL)
A	1000	2 ± 0.2		2

a. Weigh to at least the nearest 0.01 mg. Document weight to at least four significant figures.

Date Prepared: _____

1.6.3 Preparation of Solvent Standard Spiking Solution

Using an adjustable volume pipet, transfer the volumes of standard indicated in Table 5 into volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to one year or the expiration date of the source solution, whichever is the shortest.

Table 5. Preparation of Solvent Standard Spiking Solutions

ID	Target Conc. (ng/mL)	Source	Source Volume (mL) each	Final Volume (mL)
SS1	10000	A	0.10	10
SS2	5000	A	0.05	10
SS3	2000	SS1	1.00	5
SS4	500.0	SS2	0.50	5
SS5	200.0	SS3	0.50	5
SS6	50.00	SS4	0.50	5
SS7	20.00	SS5	0.50	5

Date Prepared: _____

1.7 Preparation of Blood Standards

1.7.1 Preparation of Blood Calibration Standards

Using an adjustable volume pipet transfer the volumes of spiking solution and blank blood indicated in Table 6 into the appropriate micro-centrifuge tubes. **Take care to avoid coagulated clumps.** Cap and mix vigorously by vortexing for approximately 1 minute. Prepare fresh each day of extraction. Process duplicate aliquots at each concentration level with each extraction set.

Table 6. Preparation of SYN548013 Blood Calibration Standards

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
CS1	500.0	SS1	0.05	0.95	1
CS2	250.0	SS2	0.05	0.95	1
CS3	100.0	SS3	0.05	0.95	1
CS4	25.00	SS4	0.05	0.95	1
CS5	10.00	SS5	0.05	0.95	1
CS6	2.500	SS6	0.05	0.95	1
CS7	1.000	SS7	0.05	0.95	1

1.8 Preparation of Blanks

On each day of extraction, process duplicate blood blanks and duplicate blanks + IS.

1.9 Preparation of Internal Standard Solutions

1.9.1 Preparation of Stock Internal Standard Solution

Weigh the amount of Nifedipine analytical standard shown in Table 7 and transfer into a volumetric flask. Dilute to volume with acetonitrile. Cap and mix well. Sonicate for ~5 minutes. If needed, add acetonitrile drop wise and sonicate until dissolved. Store refrigerated. May be used for up to six months.

Table 7. Preparation of Stock Internal Standard Solution

ID	Target Conc. (µg/mL)	Target Weight (mg)	Actual Weight (mg)[a]	Final Volume (mL)
Stock IS	1000	5 ± 0.5		5

b. Weigh to at least the nearest 0.01 mg. Document weight to at least four significant figures.

Date Prepared: _____

1.9.2 Preparation of Intermediate Internal Standard Solution

Using an adjustable volume pipet, transfer the volume of stock internal standard listed in Table 8 into a volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to six months or the expiration date of the stock internal standard solution, whichever is the shortest.

Table 8. Preparation of Intermediate Internal Standard Solution

ID	Target Conc. (µg/mL)	Source	Source Volume (mL)	Final Volume (mL)
Int IS	10	Stock IS	0.25	25

Date Prepared: _____

1.9.3 Preparation of Working Internal Standard (WIS) Solution

Using an adjustable volume pipet, transfer the volume of intermediate internal standard listed in Table 9 into a volumetric flask. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to six months or the expiration date of the stock internal standard solution, whichever is the shortest.

Table 9. Preparation of Working Internal Standard Solution

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Final Volume (mL)
WIS	250	Int IS	1.25	50

Date Prepared: _____

1.10 Preparation of Quality Control (QC) Samples

1.10.1 Preparation of QC Spiking Solutions

Using an adjustable volume pipet, transfer the volume of solutions indicated in Table 10 into volumetric flasks. Dilute to volume with acetonitrile. Cap and invert several times to mix. Store refrigerated. May be used for up to one year or the expiration date of the stock internal standard solution, whichever is the shortest of the two.

Table 10. Preparation of QC Spiking Solution

ID	Target Conc. (ng/mL)	Source	Source Volume (mL)	Final Volume (mL)
QC SS High	8000	A	0.08	10
QC SS Mid	4000	QC SS High	5.00	10
QC SS Low	60.00	QC SS Mid	0.15	10

Date Prepared: _____

1.10.2 Preparation of Blood QC Samples

Using an adjustable volume pipet, transfer the volumes of solutions indicated in Table 11 into individual micro-centrifuge tubes. **Take care to avoid coagulated clumps.** Cap and vortex to mix. Transfer ~0.5 mL aliquots to micro-centrifuge tubes for storage. Store frozen at ~ -70°C. May be used for up to three months.. Process four replicates at each concentration for each extraction set

Table 11. Preparation of Blood QC Samples

ID	Target Conc. (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
QC High	400.0	QC SS High	0.1	1.9	2
QC Mid	200.0	QC SS Mid	0.1	1.9	2
QC Low	3.000	QC SS Low	0.1	1.9	2

Date Prepared: _____

1.11 Preparation of System Suitability (SYS)

Using a positive displacement pipet, transfer the volume of blank blood indicated in Table 12 into a micro-centrifuge tube. Using an adjustable volume pipet, transfer the volume of spiking solution indicated in Table 12 into the micro-centrifuge tube. Cap and vortex to mix. Prepare fresh on each day of extraction. Process four replicates with each extraction set and combine the extracts.

Table 12. Preparation of System Suitability Sample

ID	Target Concentration (ng/mL)	Source	Source Volume (mL)	Blank Blood Volume (mL)	Final Volume (mL)
SYS	12.5	CS2	0.05	0.95	1

1.12 Preparation of Blood Standards, Blanks, QCs, and Samples

- Allow blank blood, and samples to thaw at room temperature if previously frozen.

- Document any sample irregularities.

- A second analyst will vortex each study sample thoroughly and open each container one at a time while the primary extracting analyst removes the appropriate volume of sample from the container.

  Second Analyst Initials/Date: _____

- Using an adjustable volume pipet, transfer 20 µL of each calibration standard, blood blank, and study sample into individual micro-centrifuge tubes. Document any samples requiring dilution in Table 13. Use blank blood to bring any samples to 20 µL.

Table 13. Sample Dilution Table

Sample ID	Sample Volume (µL)	Sample ID	Sample Volume (µL)

Reference the sample list at the front of the data binder (if available) for any dilutions not recorded in Table 13.

- Add 100 µL of WIS to all samples, except the blanks without IS. For the blanks without IS, add 100 µL of Acetonitrile instead of WIS.

- Vortex for ~1 minute to mix.

- Centrifuge at a maximum speed for ~10 minutes.

- Transfer supernatant to auto-sampler vials.

- Submit for analysis by LC-MS.

- Sample extracts are stored at the temperature of the auto-sampler if not analyzed on the day of extraction. Storage location of the extracts until injection if not stored on the auto-sampler: _____

1.13 Analysis of Standards, Blanks, QC, and Samples

Use the LC-MS system conditions specified in Table 14. The conditions, which are designated, may be modified by the analyst to produce acceptable chromatography. Record the actual values inside the parentheses.

Set up the automated chromatography data software (CDS) system to collect the electronic output from each injection, acquiring the data.

If the instrument has not been previously conditioned, condition the instrument by injecting the SYS sample for approximately 1 hour prior to starting the system suitability injections. Conditioning run performed: _____ Yes _____ No

Make a minimum of three injections of the appropriate SYS sample prior to starting the sample run to ensure optimal operation of the system.

Make single injections of all calibration standards, blanks, QCs, and samples. Inject one calibration curve near the beginning of the run and one near the end of the run. The calibration standards near the beginning of the run will be injected from high to low concentration. The calibration standards near the end of the run will be injected from low to high concentration. The first replicate of the blank and blank with IS will be injected prior to the first curve. The second replicate of the blank and blank with IS will be injected immediately following the second calibration curve. Distribute the QCs throughout the run.

Make duplicate injections of a post-run SYS sample after the run to assess drift.

Table 14. LC-MS System

Data System	Analyst, Version 1.6.1
MRM	SYN548013 586 > 456 amu Nifedipine 347 > 254 amu
HPLC System	Pump[a]:_____ X/SN:_____ Autosampler[a]:_____ X/SN:_____
MS System	Sciex API _____(Toronto, Ontario Canada) SN:_____
Ionization Source	Heated Nebulizer, Positive Ion Mode
Analytical Column	Phenomenex Luna C18, 5 µm, 50 x 2 mm SN/Lot#:_____
Guard Column	Phenomenex C18 4 x 2 mm
Column Temperature	40ºC
Auto-Sampler Temperature	RT
Mobile Phase	A: 0.1% Formic Acid in Water B: 0.1% Formic Acid in Methanol
Mobile Phase Gradient[b]	Time (minutes) %B 0.0 35 0.2 90 2.2 90 2.3 35 4.5 35
Flow Rate (µL/min)[b]	500 (_____) µL/min
Injection Volume[b]	10 µL (_____) µL
Run Time[b]	4.5 minutes (_____) minutes

a. Agilent (Santa Clara, CA), Shimadzu (Kyoto, Japan), Leap CTC Analytics (Carrboro, NC).
b. Parameters which may be modified by analyst.

Run ID: _____

2.0 CALCULATIONS

Determine the optimal automatic integration parameters for the run. Examine the integration of the analyte and the internal standard by the chromatography system. Modify, if necessary, to obtain optimum consistent integration. Document the rationale of any manual or automated reintegration of peaks.

Use Microsoft Excel to perform the calculations as follows:

Calculate the individual and average percent area of each blank and blank with IS compared with the average area response of the Lower Limit of Quantitation (LLOQ) standard.

Calculate the individual percent area of the blank following the high standard compared with the area response of the nearest LLOQ standard.

Calculate the SYS average ratio and rsd. Calculate the Drift average area ratio. Compare the average ratio of the SYS to the average ratio of the Drift.

Calculate the exact concentrations of the plasma standards and plasma QCs. Enter the values into the Watson Bioanalytical LIMS.

Use the Watson Bioanalytical LIMS, version 7.4, to perform the calculations as follows:

Calculate the peak area response ratio (Analyte/IS) for each injection.

Determine optimal regression parameters and record the model and weighting in Table 15 to calculate the regression equation.

Table 15. Regression Parameters

Model	
Weighting	
y-intercept	Calculate, Do not force through origin
y-values	Instrument Response
x-values	Nominal Concentration

Using the peak area response of the standards and the regression equation, calculate their determined concentrations.

Using the peak area response ratios of the QCs and samples, the regression equation, and any dilution factor, if applicable, calculate their individual determined concentrations.

Using the determined concentration, calculate the individual relative error (%RE) for each standard and QC concentration.

3.0 ACCEPTANCE CRITERIA

There are no specific acceptance criteria for a this assay. The following will be used to assess each individual run, however the task leader may technically decide to revise these criteria or accept criteria outside these limits.

- System Suitability – At least three consecutive injections of the SYS immediately preceding the run must have a RSD of ≤ 10.0% for the run to be valid using the area ratio.

- Drift – Average area ratio within 15.0% of the pre-run system suitability average area ratio.

- Carryover - The blank immediately following the second curve must have an area response of no greater than 20.0% of the area response of the nearest acceptable low standard.

- Linearity – The coefficient of determination will be ≥ 0.98.

- Calibration Standards – Standards will have determined concentrations within 15.0% of nominal as measured by %RE, except for the LLOQ, where it should be within 20.0% of nominal to be acceptable. If a standard is not acceptable, it will not be used to generate the calibration curve. The standards that are not acceptable will be removed one-by-one with the standard deviating furthest from nominal concentration as measured by %RE removed first; regression will be performed again after each removal. Up to 3 standards falling outside these limits can be discarded (deactivated), provided they do not change the established model. At least 75% (11 of 14) of the standards must be acceptable for the calibration curve to be acceptable. A minimum of six concentration levels must define the curve.

- Selectivity – The blanks and blanks with internal standard must have an average response of no greater than 20.0% of the average response of the lowest acceptable standard.

- Sensitivity – The limit of quantitation for a run is the lowest acceptable standard that meets all acceptance criteria.

- Quality Control Samples - At least 67% (eight of twelve) of the QCs will have determined concentrations within 20.0 percent of the nominal concentration and at least 50% (two of the four) QCs at each concentration level will have

determined concentrations within 20.0 percent of the nominal concentration as measured by the %RE.

- Repeat Analysis of Study Samples – For samples that are repeated for technical reasons (e.g. failed run, bad injection) only one replicate must be run. For samples repeated for any other reason, at least duplicate aliquots must be analyzed, if sufficient sample is available. When multiple samples are analyzed, the average of all acceptable values will be reported, unless a value can be statistically excluded.

4.0 RESPONSE TO FAILURE TO MEET ACCEPTANCE CRITERIA

4.1 System Suitability

Verify that all calculations are correct. If the system suitability does not meet acceptance criteria and the standards and the QCs within the run meet acceptance criteria, a run set may be accepted with written justification and management approval. Otherwise, reinject the entire run set.

4.2 Drift

Verify that all calculations are correct. If the drift does not meet acceptance criteria and the standards and the QCs within the run meet acceptance criteria, a run set may be accepted with written justification and management approval. Otherwise, reinject the entire run set.

4.3 Accuracy of Standards and QCs

Verify that all calculations are correct . Verify that the proper instrument system (e.g., column, flow rates, etc.) was used for the analysis. If not, samples need to be re-injected using the correct instrument system.

If the correct instrument system was used, compare the current chromatograms to a past analysis. If the chromatography has changed substantially, determine and correct the problem with the instrument system and then re-inject the samples.

4.4 Linearity (Coefficient of Determination)

Verify that all concentrations, the regression model, integration, and calculations are correct.

If all the calculations are correct, repeat the analysis.

4.5 Selectivity/Carryover

If the results are greater than 20.0%, verify that the proper wash procedure was used, if applicable. If the proper wash conditions were utilized, assess the reason for carryover and the impact to the data. The LLOQ for the impacted set may be raised to the concentration level that meets the acceptable limits. The samples between the new LLOQ and the original LLOQ will be repeated with another extraction set.

5.0 RESULTS

Include printouts of the acquisition method, and any applicable spreadsheets in the data packet.

5.1 COMMENTS/CONCLUSIONS

_____
_____
_____
_____
_____
_____
_____
_____

6.0 DATA REVIEW

6.1 Technical Review

All data generated in the laboratory activities will be reviewed by the technical leader (or designee) for technical content, accuracy, and completeness of documentation.

The technical leader will assure the proper equipment was used, review experimental procedure, rejection of calibration standards, integration of chromatograms, chromatography data processing and acquisition parameters, calibration standard concentrations, regression model, and that this documentation was followed.

6.2 Data Accuracy Review

Review at least the following: completeness and correctness of data entry, formulas used to calculate all values, and accuracy of calculations.

7.0 SIGNATURES

Technical Review Signature/Date:

_____

_____

Signature of the technical reviewer will be considered documentation that all modifications and/or changes to this documentation form (documented during the course of conducting this task) are technically acceptable and have no adverse technical impact unless otherwise noted.

Data Accuracy Review Signature/Date:

_____

TABLE 4-1 Individual Animal Blood Concentrations of SYN547407 Following Oral Administration

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
1	1	0.5	65.1	
2	1	1	331	
3	1	2	359	
4	1	4	477	
1	1	6	334	Collected 5 minutes late
2	1	6	337	Collected 3 minutes late
3	1	6	478	
4	1	6	435	Collected 1 minute late
5	1	6	331	
5	1	12	209	
1	1	24	117	
2	1	24	146	
3	1	24	103	
4	1	24	159	Collected 4 minutes late
5	1	24	65.0	
1	1	36	69.2	
2	1	48	21.4	Collected 1 minute late
1	1	72	7.76	
2	1	72	5.35	
3	1	72	3.13	
4	1	72	23.4	Collected 2 minutes late
5	1	72	BLOQ<2.02	
3	1	96	BLOQ<2.02	
4	1	120	2.80	Collected 1 minute late
5	1	144	BLOQ<2.02	
1	1	168	BLOQ<2.02	
2	1	168	BLOQ<2.02	
3	1	168	BLOQ<2.02	
4	1	168	BLOQ<2.02	
5	1	168	BLOQ<2.02	
6	2	0.5	407	
7	2	1	1110	
8	2	2	1020	
9	2	4	3710	
6	2	6	3160	
7	2	6	3500	
8	2	6	1760	

TABLE 4-1 Individual Animal Blood Concentrations of SYN547407 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
9	2	6	3130	
10	2	6	3260	
10	2	12	3930	
6	2	24	3560	
7	2	24	2410	
8	2	24	1360	
9	2	24	2310	Collected 1 minute early
10	2	24	3500	Collected 1 minute late
6	2	36	2240	
7	2	48	1330	Collected 5 minutes late
6	2	72	644	Collected 3 minutes late
7	2	72	844	
8	2	72	136	
9	2	72	612	Collected 2 minutes late
10	2	72	907	
8	2	96	33.3	
9	2	120	57.4	
10	2	144	36.1	
6	2	168	15.1	
7	2	168	17.7	
8	2	168	BLOQ<2.02	
9	2	168	2.25	
10	2	168	32.2	
11	3	0.5	701	
12	3	1	1150	
13	3	2	2950	
14	3	4	3780	
11	3	6	4140	Collected 1 minute late
12	3	6	2570	
13	3	6	3630	
14	3	6	4700	Collected 2 minutes late
15	3	6	2740	
15	3	12	4180	
11	3	24	3530	
12	3	24	8600	
13	3	24	4820	
14	3	24	3390	

TABLE 4-1 Individual Animal Blood Concentrations of SYN547407 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
15	3	24	3670	
11	3	36	5350	
12	3	48	1020	Collected 4 minutes late
11	3	72	3370	
12	3	72	304	
13	3	72	5960	
14	3	72	4370	
15	3	72	4620	
13	3	96	3690	
14	3	120	3700	
15	3	144	928	
11	3	168	97.9	
12	3	168	BLOQ<2.02	
13	3	168	318	
14	3	168	708	
15	3	168	419	
16	4	0.5	622	
17	4	1	1270	
18	4	2	3310	
19	4	4	4360	
16	4	6	5150	
17	4	6	3100	Collected 1 minute late
18	4	6	4160	
19	4	6	3920	Collected 1 minute early
20	4	6	3420	
20	4	12	5870	
16	4	24	5240	
17	4	24	3780	
18	4	24	5100	
19	4	24	5910	
20	4	24	5690	
16	4	36	5740	
17	4	48	5300	
16	4	72	5810	Collected 2 minutes late
17	4	72	6390	
18	4	72	5540	Collected 4 minutes late
19	4	72	8730	

TABLE 4-1 Individual Animal Blood Concentrations of SYN547407 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
20	4	72	6910	Collected 5 minutes late
18	4	96	6290	
19	4	120	5340	Collected 13 minutes late
20	4	144	2030	
16	4	168	1320	
17	4	168	4100	
18	4	168	8350	
19	4	168	3480	
20	4	168	1090	

TABLE 4-2 Individual Animal Blood Concentrations of SYN546308 Following Oral Administration

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
21	5	0.5	77.5	
22	5	1	138	
24	5	4	537	
21	5	6	633	
22	5	6	295	
23	5	6	277	
24	5	6	631	
25	5	6	665	
25	5	12	494	
21	5	24	253	
22	5	24	77.1	
23	5	24	147	Collected 1 minute late
24	5	24	219	
25	5	24	287	
21	5	36	138	Collected 1 minute late
22	5	48	10.6	
21	5	72	8.54	
22	5	72	BLOQ<2.06	
23	5	72	8.19	
25	5	72	9.31	
23	5	96	BLOQ<2.06	Collected 1 minute late
21	5	120	BLOQ<2.06	
25	5	144	BLOQ<2.06	
21	5	168	BLOQ<2.06	
22	5	168	BLOQ<2.06	
23	5	168	BLOQ<2.06	
25	5	168	BLOQ<2.06	
26	6	0.5	450	
27	6	1	623	
28	6	2	1600	
29	6	4	2530	
26	6	6	2850	
27	6	6	1280	Collected 1 minute late
28	6	6	2490	
29	6	6	2460	
30	6	6	2800.0	
30	6	12	3270	
26	6	24	2320	

TABLE 4-2 Individual Animal Blood Concentrations of SYN546308 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
27	6	24	1820	Collected 3 minutes late
28	6	24	2920	
30	6	24	3100	
26	6	36	1740	
27	6	48	991	Collected 1 minute late
26	6	72	730	
27	6	72	454	
28	6	72	494	
30	6	72	858	
28	6	96	183.0	Collected 1 minute late
26	6	120	57.9	Collected 1 minute late
30	6	144	37.6	
26	6	168	BLOQ<2.06	
27	6	168	4.96	
28	6	168	BLOQ<2.06	
30	6	168	6.55	
31	7	0.5	336.00	
32	7	1	944	
33	7	2	2280	Collected 1 minute late
34	7	4	2370	
31	7	6	1560	
32	7	6	1510	
33	7	6	2670	
34	7	6	2260	
35	7	6	3180	
35	7	12	2970	Collected 7 minutes late
31	7	24	2420	Collected 4 minutes late
32	7	24	1670	
33	7	24	4070	
34	7	24	2590	
31	7	36	1670	
32	7	48	582	Collected 1 minute late
31	7	72	755	
32	7	72	349	
33	7	72	3900	
34	7	72	2610	
35	7	72	3820	
33	7	96	2110	

TABLE 4-2 Individual Animal Blood Concentrations of SYN546308 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
34	7	120	613	
35	7	144	1580	
31	7	168	6.11	
32	7	168	BLOQ<2.06	
33	7	168	90.8	
34	7	168	46.2	
35	7	168	490	
36	8	0.5	396	
37	8	1	1610	
38	8	2	1720	
39	8	4	2930	
36	8	6	1760	
37	8	6	2600	
38	8	6	3000	
39	8	6	3390	
40	8	6	3850	Collected 1 minute early
40	8	12	4770	Collected 1 minute late
36	8	24	2780	
37	8	24	3960	
38	8	24	3080	
39	8	24	6250	
40	8	24	5720	
36	8	36	2750	Collected 6 minutes late
37	8	48	5810	
36	8	72	2630	
37	8	72	5690	Collected 7 minutes late
38	8	72	5690	Collected 2 minutes late
39	8	72	4820	Collected 1 minute late
38	8	96	4820	Collected 1 minute late
39	8	120	4950	Collected 2 minutes late
36	8	144	76.0	
36	8	168	4.00	
37	8	168	3730	
38	8	168	5070	
39	8	168	5490	

TABLE 4-3 Individual Animal Blood Concentrations of SYN548097 Following Oral Administration

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
41	9	0.5	52.2	
42	9	1	103	
43	9	2	134	
44	9	4	630	
41	9	6	514	
42	9	6	306	
43	9	6	640	
44	9	6	598	Collected 3 minutes late
45	9	6	526	
45	9	12	403	
41	9	24	108	
42	9	24	129	
43	9	24	177	
44	9	24	193	Collected 2 minutes late
45	9	24	197	
41	9	36	46.0	
42	9	48	32.2	
41	9	72	BLOQ<2.18	
42	9	72	4.95	
43	9	72	7.31	
45	9	72	8.30	
43	9	96	2.27	
41	9	120	BLOQ<2.18	
45	9	144	BLOQ<2.18	Collected 2 minutes late
41	9	168	BLOQ<2.18	
42	9	168	BLOQ<2.18	
43	9	168	BLOQ<2.18	Collected 1 minute late
45	9	168	BLOQ<2.18	
46	10	0.5	227	
47	10	1	893	Collected 1 minute late
48	10	2	1490	
49	10	4	2840	
46	10	6	3390	
47	10	6	3830	
48	10	6	3160	
49	10	6	3620	Collected 1 minute late
50	10	6	3150	
50	10	12	3840	

TABLE 4-3 Individual Animal Blood Concentrations of SYN548097 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
46	10	24	2430	
47	10	24	1860	
48	10	24	1620	
49	10	24	2360	
50	10	24	2060	
46	10	36	1150	Collected 2 minutes late
47	10	48	251	
46	10	72	221	
47	10	72	30.3	
48	10	72	52.3	
49	10	72	247	Collected 1 minute late
50	10	72	53.1	
48	10	96	13.9	
49	10	120	8.34	
50	10	144	BLOQ<2.18	Collected 1 minute late
46	10	168	2.86	
47	10	168	BLOQ<2.18	
48	10	168	BLOQ<2.18	
49	10	168	BLOQ<2.18	
50	10	168	BLOQ<2.18	
51	11	0.5	231	
52	11	1	753	
53	11	2	580	Collected 1 minute late
54	11	4	4100	
51	11	6	4700	
52	11	6	3570	
53	11	6	2890	
54	11	6	5720	
55	11	6	5430	
55	11	12	4510	
51	11	24	3720	
52	11	24	4170	
53	11	24	3360	
54	11	24	2830	Collected 1 minute late
55	11	24	3480	
51	11	36	4310	Collected 2 minutes late
52	11	48	3510	Collected 1 minute late
51	11	72	771	

TABLE 4-3 Individual Animal Blood Concentrations of SYN548097 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
52	11	72	646	Collected 1 minute late
53	11	72	7490	
54	11	72	2880	
55	11	72	82.7	
53	11	96	6480	Collected 2 minutes late
54	11	120	389	
55	11	144	2.22	
51	11	168	BLOQ<2.18	
52	11	168	224	
53	11	168	654	
54	11	168	17.6	Collected 1 minute late
55	11	168	BLOQ<2.18	
56	12	0.5	587	
57	12	1	1350	
58	12	2	2480	
59	12	4	BLOQ<2.18	
56	12	6	4880	
57	12	6	3930	
58	12	6	2790	Collected 5 minutes late
59	12	6	BLOQ<2.18	
60	12	6	5630	
60	12	12	6720	
56	12	24	6460	
57	12	24	5750	Collected 1 minute late
58	12	24	5880	
59	12	24	BLOQ<2.18	
60	12	24	7010	
57	12	48	9350	
56	12	72	10900	
57	12	72	10300	
58	12	72	9680	
59	12	72	2.36	
60	12	72	10300	
58	12	96	4840	Collected 1 minute late
59	12	120	BLOQ<2.18	Collected 12 minutes late
60	12	144	5200	
56	12	168	960	

TABLE 4-3 Individual Animal Blood Concentrations of SYN548097 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
57	12	168	65.3	
58	12	168	484	
60	12	168	1850	

TABLE 4-4 Individual Animal Blood Concentrations of SYN548012 Following Oral Administration

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
61	13	0.5	11.9	
62	13	1	88.2	
63	13	2	188	
64	13	4	308	
61	13	6	285	
62	13	6	288	
63	13	6	354	
64	13	6	279	Collected 1 minute late
65	13	6	326	
65	13	12	159	Collected 4 minutes late
61	13	24	132	
62	13	24	88.2	
63	13	24	139	
64	13	24	99.1	Collected 6 minutes late
65	13	24	128	
61	13	36	110	Collected 1 minute late
62	13	48	57.9	
61	13	72	54.5	Collected 1 minute late
62	13	72	16.2	
63	13	72	35.4	
64	13	72	13.4	
65	13	72	9.38	Collected 1 minute late
63	13	96	12.5	
64	13	120	2.55	
65	13	144	BLOQ<1.85	
61	13	168	5.44	
62	13	168	BLOQ<1.85	
63	13	168	BLOQ<1.85	Collected 1 minute late
64	13	168	BLOQ<1.85	Collected 1 minute late
65	13	168	BLOQ<1.85	
66	14	0.5	85.9	
67	14	1	313	
68	14	2	578	
69	14	4	1100	
66	14	6	1360	
67	14	6	1210	
68	14	6	1420	
69	14	6	1250	

TABLE 4-4 Individual Animal Blood Concentrations of SYN548012 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
70	14	6	1430	
70	14	12	1140	
66	14	24	833	
67	14	24	432	
68	14	24	609	
69	14	24	579	Collected 10 minutes late
70	14	24	1070	
66	14	36	444	Collected 2 minutes late
67	14	48	260	
66	14	72	203	
67	14	72	235	
68	14	72	291	
69	14	72	191	
70	14	72	487	
68	14	96	180	
69	14	120	31.3	Collected 1 minute late
70	14	144	67.3	
66	14	168	23.9	
67	14	168	32.0	
68	14	168	27.1	
69	14	168	10.9	
70	14	168	24.3	Collected 1 minute late
71	15	0.5	110	
72	15	1	195	Collected 2 minutes late
73	15	2	475	
74	15	4	1400	
71	15	6	1840	
72	15	6	1750	
73	15	6	1530	
74	15	6	1950	
75	15	6	1360	
75	15	12	1130	
71	15	24	943	
72	15	24	2570	
73	15	24	1520	
74	15	24	3230	
75	15	24	725	
71	15	36	642	

TABLE 4-4 Individual Animal Blood Concentrations of SYN548012 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
72	15	48	2020	Collected 4 minutes late
71	15	72	821	
72	15	72	1440	
73	15	72	457	
74	15	72	953	
75	15	72	446	
73	15	96	350	
74	15	120	502	
75	15	144	142	
71	15	168	80.1	
72	15	168	105	
73	15	168	46.4	
74	15	168	211	
75	15	168	100	
76	16	0.5	248	
77	16	1	518	
78	16	2	700	
79	16	4	1800	
76	16	6	2040	
77	16	6	2290	
78	16	6	1890	
79	16	6	2020	
80	16	6	1700	
80	16	12	2850	Collected 1 minute late
76	16	24	960	
77	16	24	1660	
78	16	24	1070	
79	16	24	1790	Collected 4 minutes late
80	16	24	2310	
76	16	36	838	
77	16	48	1060	
76	16	72	435	
77	16	72	603	
78	16	72	409	
79	16	72	663	
80	16	72	877	
78	16	96	344	

TABLE 4-4 Individual Animal Blood Concentrations of SYN548012 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
79	16	120	233	Collected 1 minute late
80	16	144	286	
76	16	168	117	
77	16	168	98.0	
78	16	168	40.9	
79	16	168	72.3	
80	16	168	128	

TABLE 4-5 Individual Animal Blood Concentrations of SYN548014 Following Oral Administration

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
81	17	0.5	27.9	
82	17	1	246	
83	17	2	458	
84	17	4	633	
81	17	6	231	
82	17	6	494	
83	17	6	837	
84	17	6	339	
85	17	6	481	
85	17	12	393	
81	17	24	59.3	
82	17	24	132	
83	17	24	98.3	
84	17	24	93.0	
85	17	24	176	
81	17	36	24.9	
82	17	48	30.3	Collected 1 minute late
81	17	72	BLOQ<2.14	Collected 1 minute late
82	17	72	5.38	
83	17	72	BLOQ<2.14	
84	17	72	BLOQ<2.14	
85	17	72	7.65	
83	17	96	BLOQ<2.14	
84	17	120	BLOQ<2.14	Collected 2 minutes late
85	17	144	BLOQ<2.14	
81	17	168	BLOQ<2.14	
82	17	168	BLOQ<2.14	
83	17	168	BLOQ<2.14	
84	17	168	BLOQ<2.14	
85	17	168	BLOQ<2.14	
86	18	0.5	307	
87	18	1	1650	
88	18	2	2140	
89	18	4	3770	
86	18	6	3020	
87	18	6	3770	
88	18	6	3950	
89	18	6	3760	

TABLE 4-5 Individual Animal Blood Concentrations of SYN548014 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
90	18	6	3440	
90	18	12	2350	
86	18	24	1900	
87	18	24	1490	
88	18	24	1620	
89	18	24	4200	
90	18	24	1530	
86	18	36	1090	
87	18	48	509	
86	18	72	145	Collected 1 minute late
87	18	72	54.0	
88	18	72	93.6	
89	18	72	416	
90	18	72	179	
88	18	96	21.3	
89	18	120	25.6	Collected 2 minutes late
90	18	144	7.31	
86	18	168	BLOQ<2.14	
87	18	168	BLOQ<2.14	
88	18	168	BLOQ<2.14	
89	18	168	BLOQ<2.14	
90	18	168	BLOQ<2.14	
91	19	0.5	541	
92	19	1	1470	
93	19	2	2000	
94	19	4	2600	
91	19	6	5400	
92	19	6	4860	
93	19	6	5490	Collected 5 minutes late
94	19	6	3190	
95	19	6	5200	
95	19	12	4780	
91	19	24	4890	
92	19	24	2530	
93	19	24	6170	
94	19	24	4050	
95	19	24	5830	
91	19	36	2150	

TABLE 4-5 Individual Animal Blood Concentrations of SYN548014 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
92	19	48	1810	
91	19	72	399	
92	19	72	466	
93	19	72	960	
94	19	72	207	
95	19	72	1390	
93	19	96	180	
94	19	120	8.13	Collected 3 minutes late
95	19	144	16.1	
91	19	168	BLOQ<2.14	Collected 2 minutes late
92	19	168	3.95	
93	19	168	BLOQ<2.14	
94	19	168	BLOQ<2.14	
95	19	168	2.31	
96	20	0.5	705	
97	20	1	1160	
98	20	2	3120	
99	20	4	3200	
96	20	6	7080	Collected 1 minute late
97	20	6	3810	
98	20	6	3750	
99	20	6	4610	
100	20	6	5860	
100	20	12	6030	
96	20	24	5360	
97	20	24	3780	
98	20	24	2860	
99	20	24	6930	
100	20	24	7160	
96	20	36	6880	
97	20	48	6080	
96	20	72	5140	
97	20	72	5060	
98	20	72	252	
99	20	72	6870	
100	20	72	5950	
98	20	96	69.4	
99	20	120	638	

TABLE 4-5 Individual Animal Blood Concentrations of SYN548014 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
100	20	144	595	
96	20	168	66.5	
97	20	168	165	
98	20	168	BLOQ<2.14	
99	20	168	12.4	
100	20	168	66.7	

TABLE 4-6 Individual Animal Blood Concentrations of SYN548102 Following Oral Administration

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
101	21	0.5	157	
102	21	1	318	
103	21	2	432	
104	21	4	798	
101	21	6	569	Collected 10 minutes late
102	21	6	935	
103	21	6	695	
104	21	6	943	Collected 1 minute late
105	21	6	271	
105	21	12	168	Collected 1 minute late
101	21	24	181	
102	21	24	178	
103	21	24	299	
104	21	24	298	
105	21	24	70.6	
101	21	36	133	
102	21	48	94.5	
101	21	72	18.1	
102	21	72	39.9	
103	21	72	51.0	
104	21	72	55.7	Collected 1 minute late
105	21	72	3.94	
103	21	96	27.0	
104	21	120	10.2	Collected 1 minute late
105	21	144	BLOQ<2.02	
101	21	168	BLOQ<2.02	
102	21	168	BLOQ<2.02	
103	21	168	BLOQ<2.02	
104	21	168	2.11	Collected 8 minutes late
105	21	168	BLOQ<2.02	
106	22	0.5	101	
107	22	1	826	
108	22	2	1840	Collected 1 minute late
109	22	4	2500	
106	22	6	2320	
107	22	6	3630	
108	22	6	2880	
109	22	6	2180	

TABLE 4-6 Individual Animal Blood Concentrations of SYN548102 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
110	22	6	3800	Collected 1 minute late
110	22	12	3840	
106	22	24	3230	
107	22	24	2160	
108	22	24	1420	
109	22	24	3770	
110	22	24	2790	Collected 8 minutes late
106	22	36	3690	
107	22	48	1520	
106	22	72	1130	
107	22	72	741	
108	22	72	543	
109	22	72	767	Collected 4 minutes late
110	22	72	1430	
108	22	96	266	
109	22	120	64.8	Collected 3 minutes late
110	22	144	126	
106	22	168	52.3	
107	22	168	29.2	
108	22	168	19.4	
109	22	168	3.32	
110	22	168	51.0	
111	23	0.5	274	Collected 1 minute late
112	23	1	1050	Collected 1 minute late
113	23	2	1210	
114	23	4	1340	
111	23	6	1650	
112	23	6	1600	
113	23	6	1870	
114	23	6	1810	
115	23	6	2150	
115	23	12	2180	
111	23	24	3540	
112	23	24	2290	
113	23	24	3010	
114	23	24	3020	
115	23	24	3170	
111	23	36	3970	

TABLE 4-6 Individual Animal Blood Concentrations of SYN548102 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
112	23	48	1310	Collected 1 minute late
111	23	72	2600	
112	23	72	1230	
113	23	72	3730	
114	23	72	2010	Collected 4 minutes late
115	23	72	2210	
113	23	96	2300	
114	23	120	850	Collected 2 minutes late
115	23	144	499	
111	23	168	784	
112	23	168	148	
113	23	168	1510	
114	23	168	139	Collected 1 minute late
115	23	168	292	
116	24	0.5	445	
117	24	1	1360	Collected 1 minute late
118	24	2	1330	
119	24	4	1270	
116	24	6	3180	
117	24	6	2240	
118	24	6	1840	
119	24	6	1430	Collected 1 minute late
120	24	6	2160	
120	24	12	3500	
116	24	24	3680	
117	24	24	2910	
118	24	24	3160	
119	24	24	2150	
120	24	24	3230	
116	24	36	5720	
117	24	48	4750	
116	24	72	5580	
117	24	72	3550	Collected 10 minutes late
118	24	72	3600	Collected 2 minutes late
119	24	72	3150	
120	24	72	3580	Collected 4 minutes late
118	24	96	6690	

TABLE 4-6 Individual Animal Blood Concentrations of SYN548102 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
119	24	120	3720	Collected 9 minutes late
116	24	144	3280	
116	24	168	2050	
117	24	168	2080	

TABLE 4-7 Individual Animal Blood Concentrations of SYN548117 Following Oral Administration

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
121	25	0.5	16.8	Collected 2 minutes late
121	25	6	581	
121	25	24	326	Collected 4 minutes late
121	25	36	331	
121	25	72	74.0	
121	25	168	3.96	Collected 2 minutes late
122	25	1	167	
122	25	6	680	
122	25	24	293	
122	25	48	152	Collected 2 minutes late
122	25	72	75.2	
122	25	168	2.50	
123	25	2	440	
123	25	6	493	
123	25	24	321	
123	25	72	88.5	
123	25	96	46.9	
123	25	168	5.70	
124	25	4	666	
124	25	6	602	Collected 1 minute late
124	25	24	311	
124	25	72	30.3	
124	25	120	BLOQ<2.02	Collected 2 minutes late
124	25	168	BLOQ<2.02	Collected 3 minutes late
125	25	6	881	
125	25	12	645	
125	25	24	353	
125	25	72	64.9	
125	25	144	2.31	
125	25	168	BLOQ<2.02	
126	26	0.5	272	Collected 2 minutes late
126	26	6	2010	
126	26	24	2660	
126	26	36	3040	
126	26	72	1390	
126	26	168	119	
127	26	1	430	
127	26	6	1390	

TABLE 4-7 Individual Animal Blood Concentrations of SYN548117 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
127	26	24	2330	
127	26	48	4380	
127	26	72	671.0	
127	26	168	23.5	
128	26	2	1860	
128	26	6	2340	
128	26	24	2410	
128	26	72	2680	
128	26	96	1670	
128	26	168	257	
129	26	4	2050	
129	26	6	2020	Collected 4 minutes late
129	26	24	2510	Collected 11 minutes late
129	26	72	1360	
129	26	120	269	Collected 1 minute late
129	26	168	168	Collected 4 minutes late
130	26	6	1630	
130	26	12	2790	
130	26	24	2640	
130	26	72	2090	
130	26	144	363	Collected 2 minutes late
130	26	168	188	Collected 2 minutes late
131	27	0.5	494	
131	27	6	2220	
131	27	24	2790	
131	27	36	2760	Collected 16 minutes late
131	27	72	3120	
131	27	168	871	
132	27	1	468	
132	27	6	2580	
132	27	24	3150	
132	27	48	4790	
132	27	72	5410	
132	27	168	1830	Collected 15 minutes late
133	27	2	1550	
133	27	6	2280	
133	27	24	3290	
133	27	72	1620	

TABLE 4-7 Individual Animal Blood Concentrations of SYN548117 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
133	27	96	1410	Collected 1 minute late
133	27	168	150	Collected 2 minutes late
134	27	4	2020	
134	27	6	1910	
134	27	24	3210	
134	27	72	3530	
134	27	120	3080	Collected 4 minutes late
134	27	168	1050	
135	27	6	1640	
135	27	12	3280	
135	27	24	3000	
135	27	72	1800	
135	27	144	265	Collected 2 minutes early
135	27	168	109	
136	28	0.5	689	
136	28	6	2770	
136	28	24	3020	
136	28	36	3840	
136	28	72	3630	Collected 10 minutes late
137	28	1	532	Collected 2 minutes late
137	28	6	1720	
137	28	24	3220	
137	28	48	1380	
137	28	72	3590	
138	28	2	2620	
138	28	6	3200	
138	28	24	2840	
138	28	72	3270	Collected 16 minutes late
138	28	96	3870	
138	28	168	6110	Collected 11 minutes late
139	28	4	1580	
139	28	6	2580	
139	28	24	2500	
139	28	72	3950	
139	28	120	3890	Collected 5 minutes late
139	28	168	6680	Collected 5 minutes late
140	28	6	2930	
140	28	12	3490	

TABLE 4-8 Individual Animal Blood Concentrations of SYN548098 Following Oral Administration

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
140	28	24	3940	
140	28	144	6730	
140	28	168	6030	Collected 11 minutes late
141	29	0.5	25.2	
142	29	1	76.7	
143	29	2	124	
144	29	4	302	
141	29	6	346	
142	29	6	389	
143	29	6	366	
144	29	6	423	
145	29	6	299	
145	29	12	272	
141	29	24	162	
142	29	24	167	
143	29	24	196	
144	29	24	361	
145	29	24	154	
141	29	36	171	
142	29	48	67.9	
141	29	72	46.1	
142	29	72	32.4	
143	29	72	51.6	
144	29	72	86.0	Collected 1 minute late
145	29	72	38.0	
143	29	96	24.0	
144	29	120	22.1	Collected 4 minutes late
145	29	144	3.58	
141	29	168	BLOQ<1.94	
142	29	168	BLOQ<1.94	
143	29	168	BLOQ<1.94	
144	29	168	5.83	
145	29	168	BLOQ<1.94	
146	30	0.5	9.05	Collected 1 minute late
147	30	1	222	Collected 1 minute late
148	30	2	648.0	Collected 1 minute late
149	30	4	882	
146	30	6	875	

TABLE 4-8 Individual Animal Blood Concentrations of SYN548098 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
147	30	6	1120	
148	30	6	1520	
149	30	6	1070	
150	30	6	1040	
150	30	12	969	
146	30	24	783	
147	30	24	615	
148	30	24	915	
149	30	24	911	
150	30	24	763	
146	30	36	514	
147	30	48	460	Collected 1 minute late
146	30	72	203	
147	30	72	216	
148	30	72	2370	
149	30	72	418	
150	30	72	465	
148	30	96	1310	
149	30	120	135	Collected 4 minutes late
150	30	144	50.3	
146	30	168	24.5	
147	30	168	26.5	
148	30	168	268	
149	30	168	38.7	
150	30	168	20.7	
151	31	0.5	106	
152	31	1	516	Collected 1 minute late
153	31	2	799	
154	31	4	1440	
151	31	6	1910	
152	31	6	1930	Collected 2 minutes late
153	31	6	2010	
154	31	6	2170	
155	31	6	1240	
155	31	12	1610	
151	31	24	2760	
152	31	24	953	

TABLE 4-8 Individual Animal Blood Concentrations of SYN548098 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
153	31	24	1420	Collected 1 minute late
154	31	24	1600	
155	31	24	1010	
151	31	36	3530	
152	31	48	574	Collected 2 minutes late
151	31	72	1110	
152	31	72	511	Collected 2 minutes late
153	31	72	1050	
154	31	72	665	
155	31	72	412	Collected 2 minutes late
153	31	96	946	
154	31	120	230	Collected 3 minutes late
155	31	144	195	
151	31	168	494	
152	31	168	583	
153	31	168	156	
154	31	168	533	
155	31	168	96.0	
156	32	0.5	479	
157	32	1	1220	
158	32	2	2410	
159	32	4	1820	
156	32	6	4630	
157	32	6	10800	Collected 2 minutes early
158	32	6	3510	
159	32	6	2710	Collected 2 minutes late
160	32	6	3450	
160	32	12	3770	
156	32	24	3660	
157	32	24	3220	
158	32	24	4550	
159	32	24	3920	Collected 5 minutes late
160	32	24	4820	
156	32	36	4070	
157	32	48	2950	Collected 8 minutes late
156	32	72	4200	
157	32	72	2510	
158	32	72	2240	

TABLE 4-8 Individual Animal Blood Concentrations of SYN548098 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
159	32	72	5140	
160	32	72	4490	
158	32	96	1010	Collected 6 minutes late
159	32	120	4910	Collected 2 minutes late
160	32	144	2880	
156	32	168	2620	
157	32	168	726	
158	32	168	354	
159	32	168	2470	Collected 1 minute late
160	32	168	4070	

TABLE 4-9 Individual Animal Blood Concentrations of SYN548101 Following Oral Administration

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
161	33	0.5	102	
162	33	1	266	Collected 1 minute late
163	33	2	221	
164	33	4	314	Collected 3 minutes late
161	33	6	389	
162	33	6	260	
163	33	6	427	
164	33	6	279	
165	33	6	67.4	
165	33	12	54.8	
161	33	24	122	
162	33	24	74.5	
163	33	24	76.8	
164	33	24	99.1	Collected 2 minutes early
165	33	24	21.9	
161	33	36	74.5	
162	33	48	25.4	Collected 2 minutes late
161	33	72	7.63	
162	33	72	8.36	
163	33	72	6.98	
164	33	72	2.35	
165	33	72	2.68	
163	33	96	BLOQ<1.83	
164	33	120	BLOQ<1.83	Collected 1 minute late
165	33	144	BLOQ<1.83	
161	33	168	BLOQ<1.83	
162	33	168	BLOQ<1.83	
163	33	168	BLOQ<1.83	
164	33	168	BLOQ<1.83	
165	33	168	BLOQ<1.83	
166	34	0.5	307	
167	34	1	859	
168	34	2	1440	
169	34	4	981	Collected 3 minutes late
166	34	6	2090	
167	34	6	2230	
168	34	6	1390	Collected 12 minutes late
169	34	6	1500	

TABLE 4-9 Individual Animal Blood Concentrations of SYN548101 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
170	34	6	1930	
170	34	12	1860	
167	34	24	2560	
168	34	24	1860	
169	34	24	2080	
170	34	24	1880	
169	34	36	1470	Collected 3 minutes late
167	34	48	1840	
167	34	72	1050	
168	34	72	1160	
169	34	72	536	Collected 6 minutes late
170	34	72	975	
168	34	96	800	
169	34	120	313	Collected 2 minutes late
170	34	144	33.9	
167	34	168	49.0	
168	34	168	58.1	
169	34	168	50.0	
170	34	168	7.09	
171	35	0.5	438	
172	35	1	997	
173	35	2	1630	
174	35	4	1620	Collected 1 minute late
171	35	6	1500	
172	35	6	1490	
173	35	6	1670	
174	35	6	2060	
175	35	6	1970	
175	35	12	3290	
171	35	24	3650	
172	35	24	2520	
173	35	24	2920	Collected 2 minutes late
174	35	24	2710	
175	35	24	2830	
171	35	36	4850	
172	35	48	3020	
171	35	72	3380	
172	35	72	2190	

TABLE 4-9 Individual Animal Blood Concentrations of SYN548101 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
173	35	72	3010	Collected 1 minute late
174	35	72	4110	
175	35	72	4410	Collected 1 minute late
173	35	96	2780	Collected 2 minutes late
174	35	120	5590	Collected 1 minute late
175	35	144	3380	
171	35	168	880	
172	35	168	2220	
173	35	168	2480	
175	35	168	2420	
176	36	0.5	407	
177	36	1	1780	
178	36	2	1780	
179	36	4	2190	Collected 1 minute late
176	36	6	1570	
177	36	6	1630	
178	36	6	1850	
179	36	6	2020	
180	36	6	2080	
180	36	12	3440	
176	36	24	3010	
177	36	24	2760	
178	36	24	3140	
179	36	24	2280	
180	36	24	3230	
176	36	36	3380	Collected 1 minute late
177	36	48	3810	Collected 1 minute late
176	36	72	4220	
177	36	72	3530	
178	36	72	2860	Collected 13 minutes late
179	36	72	3570	
180	36	72	3790	Collected 2 minutes late
178	36	96	3830	Collected 8 minutes late
180	36	120	4390	
180	36	144	3090	
178	36	168	5620	
180	36	168	1520	

TABLE 4-10 Individual Animal Blood Concentrations of SYN548013 Following Oral Administration

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
181	37	0.5	60.4	
182	37	1	275	Collected 8 minutes late
183	37	2	497	
184	37	4	627	
181	37	6	756	
182	37	6	723	
183	37	6	731	
184	37	6	584	
185	37	6	662	
185	37	12	425	
181	37	24	300	
182	37	24	406	
183	37	24	330	Collected 1 minute late
184	37	24	192	
185	37	24	283	
181	37	36	214	
182	37	48	134	
181	37	72	44.4	
182	37	72	57.7	
183	37	72	77.5	Collected 4 minutes late
184	37	72	16.1	
185	37	72	44.1	
183	37	96	37.7	Collected 3 minutes late
184	37	120	2.27	Collected 1 minute early
185	37	144	2.95	
181	37	168	BLOQ<2.18	
182	37	168	2.71	
183	37	168	8.80	Collected 2 minutes late
184	37	168	BLOQ<2.18	
185	37	168	BLOQ<2.18	
186	38	0.5	38.6	Collected 2 minutes late
187	38	1	540	Collected 1 minute late
188	38	2	2140	
189	38	4	3140	
186	38	6	1990	
187	38	6	3730	
188	38	6	3320	
189	38	6	3580	

TABLE 4-10 Individual Animal Blood Concentrations of SYN548013 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
190	38	6	2900	
190	38	12	3020	
186	38	24	2310	
187	38	24	1660	
188	38	24	1960	
189	38	24	1770	Collected 1 minute late
190	38	24	2510	
186	38	36	1900	
187	38	48	1600	
186	38	72	887	
187	38	72	758	Collected 2 minutes late
188	38	72	522	
189	38	72	684	
190	38	72	1080	
188	38	96	222	Collected 4 minutes late
189	38	120	218	Collected 1 minute early
190	38	144	117	
186	38	168	66.9	
187	38	168	29.5	
188	38	168	14.6	
189	38	168	43.4	
190	38	168	42.2	
191	39	0.5	313	
192	39	1	779	Collected 1 minute late
193	39	2	2250	
194	39	4	2810	
191	39	6	4610	
192	39	6	2440	
193	39	6	4660	
194	39	6	3160	
195	39	6	4380	
195	39	12	3600	
191	39	24	3900	
192	39	24	2900	
193	39	24	7620	
194	39	24	3260	
195	39	24	3200	
191	39	36	4100	

TABLE 4-10 Individual Animal Blood Concentrations of SYN548013 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
192	39	48	2210	
191	39	72	2550	
192	39	72	1320	
193	39	72	5060	
194	39	72	2590	
195	39	72	2150	
193	39	96	3440	
194	39	120	1430	Collected 1 minute late
195	39	144	478	
191	39	168	261	
192	39	168	103	
193	39	168	1240	
194	39	168	523	
195	39	168	280	Collected 4 minutes late
196	40	0.5	466	
197	40	1	1500	
198	40	2	2100	
199	40	4	4400	Collected 1 minute late
196	40	6	4510	
197	40	6	6520	
198	40	6	3860	
199	40	6	4530	
200	40	6	5980	
200	40	12	5490	
196	40	24	7310	
197	40	24	5800	
198	40	24	6510	
199	40	24	5110	
200	40	24	11100	Collected 1 minute late
196	40	36	6930	
197	40	48	4180	Collected 3 minutes late
196	40	72	5450	
197	40	72	2450	
198	40	72	3650	Collected 2 minutes late
199	40	72	4260	
200	40	72	6000	Collected 1 minute late
198	40	96	2430	Collected 6 minutes late
199	40	120	1270	

TABLE 4-10 Individual Animal Blood Concentrations of SYN548013 Following Oral Administration (Continued)

Subject	Group	Time Point (hr)	Concentration (ng/mL)	Comment
200	40	144	2570	Collected 1 minute late
196	40	168	1610	
197	40	168	181	
198	40	168	1100	Collected 1 minute late
199	40	168	432	
200	40	168	1860	Collected 3 minutes late

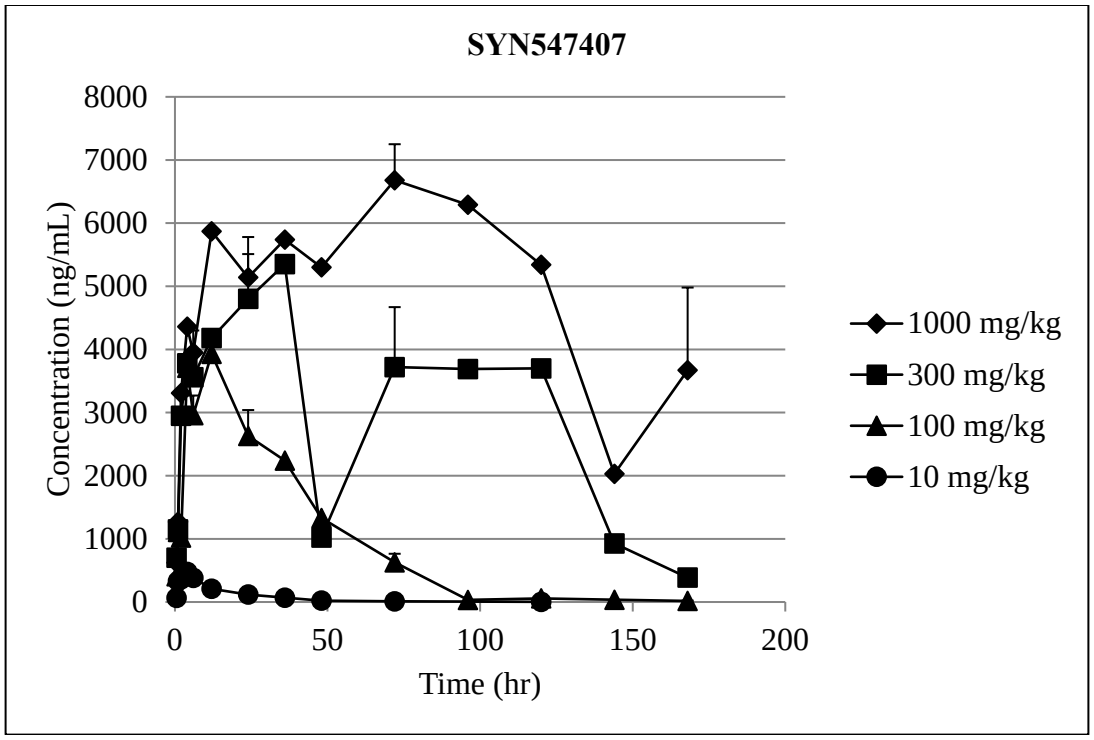


FIGURE 4-1 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN547407

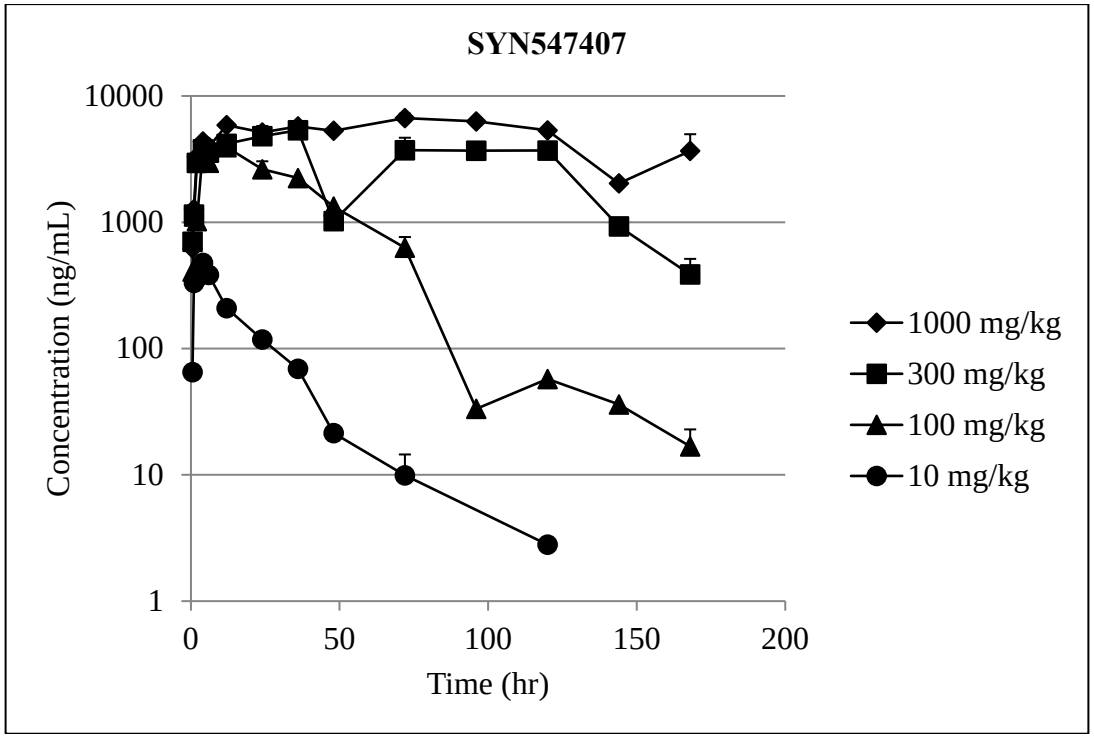


FIGURE 4-2 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN547407 – Logarithmic Scale

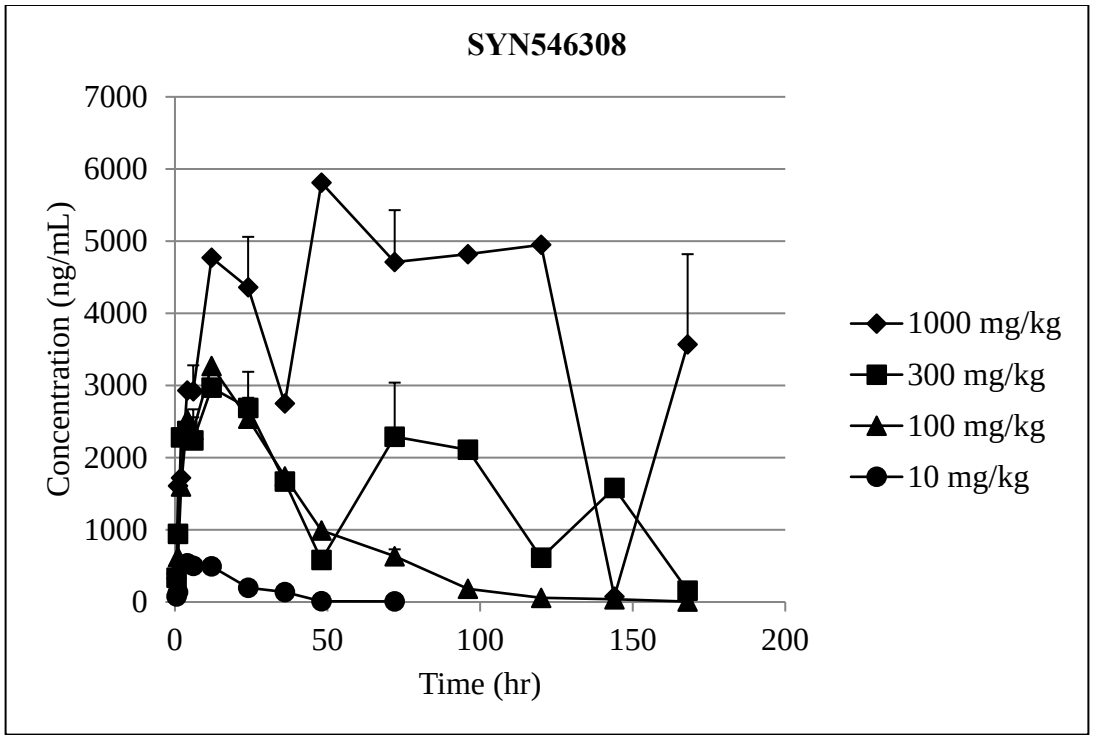


FIGURE 4-3 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN546308

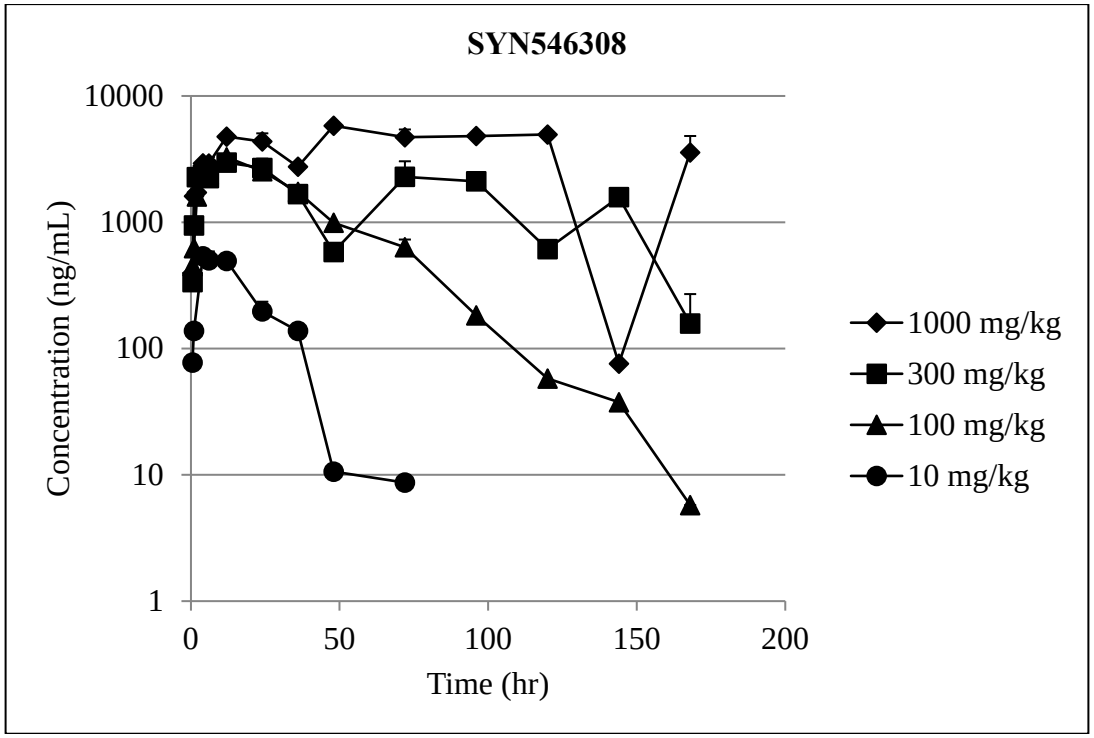


FIGURE 4-4 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN546308 – Logarithmic Scale

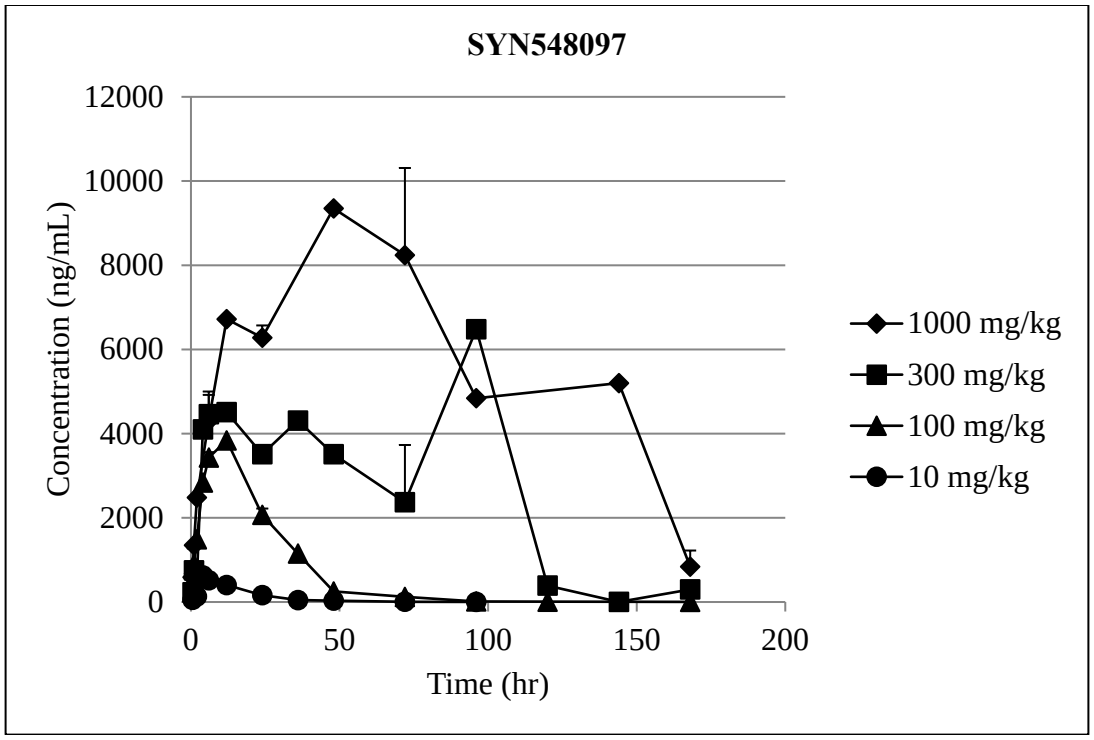


FIGURE 4-5 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548097

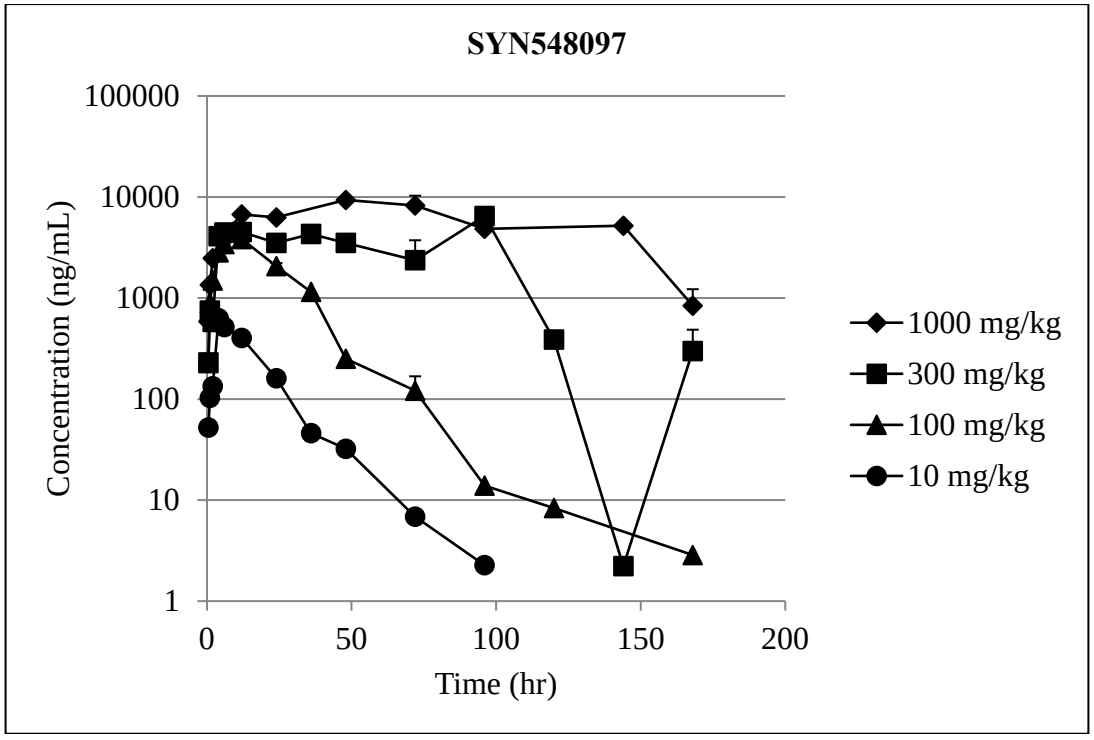


FIGURE 4-6 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548097 – Logarithmic Scale

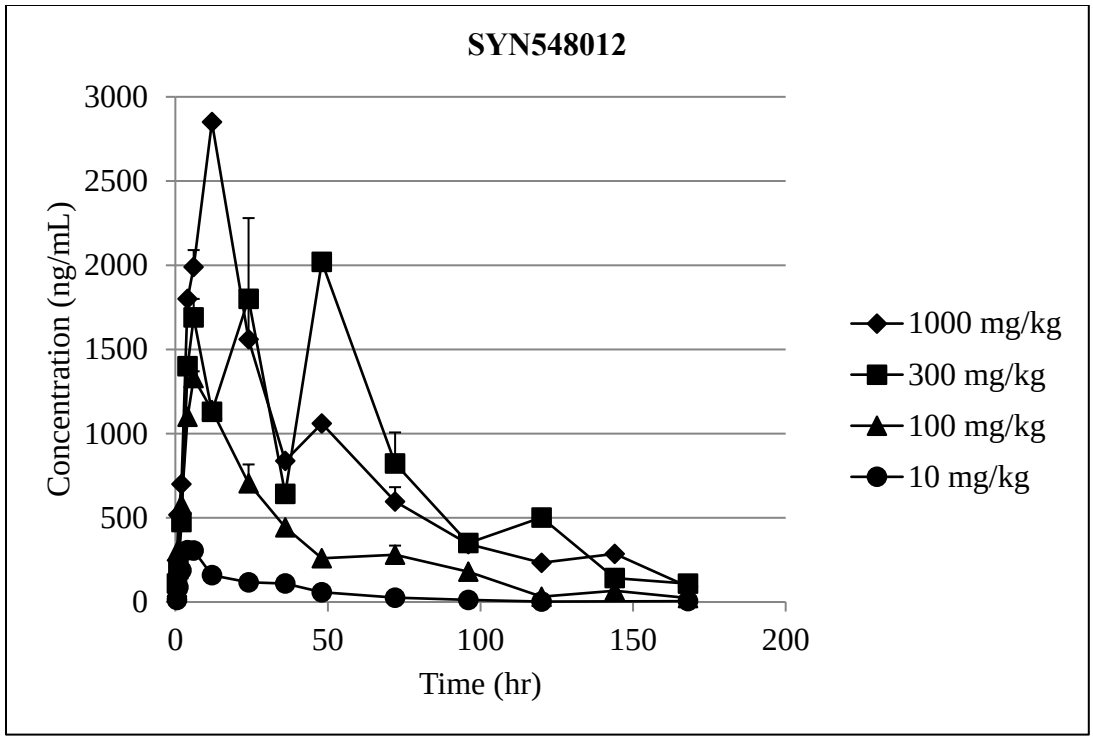


FIGURE 4-7 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548012

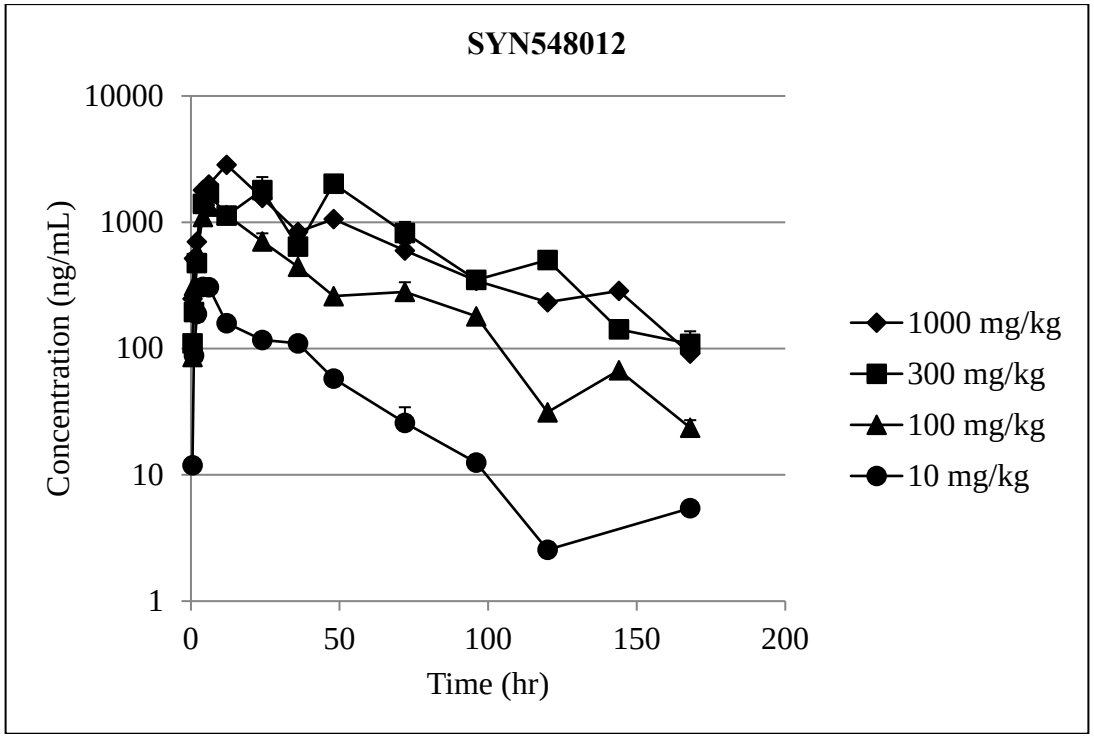


FIGURE 4-8 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548012 – Logarithmic Scale

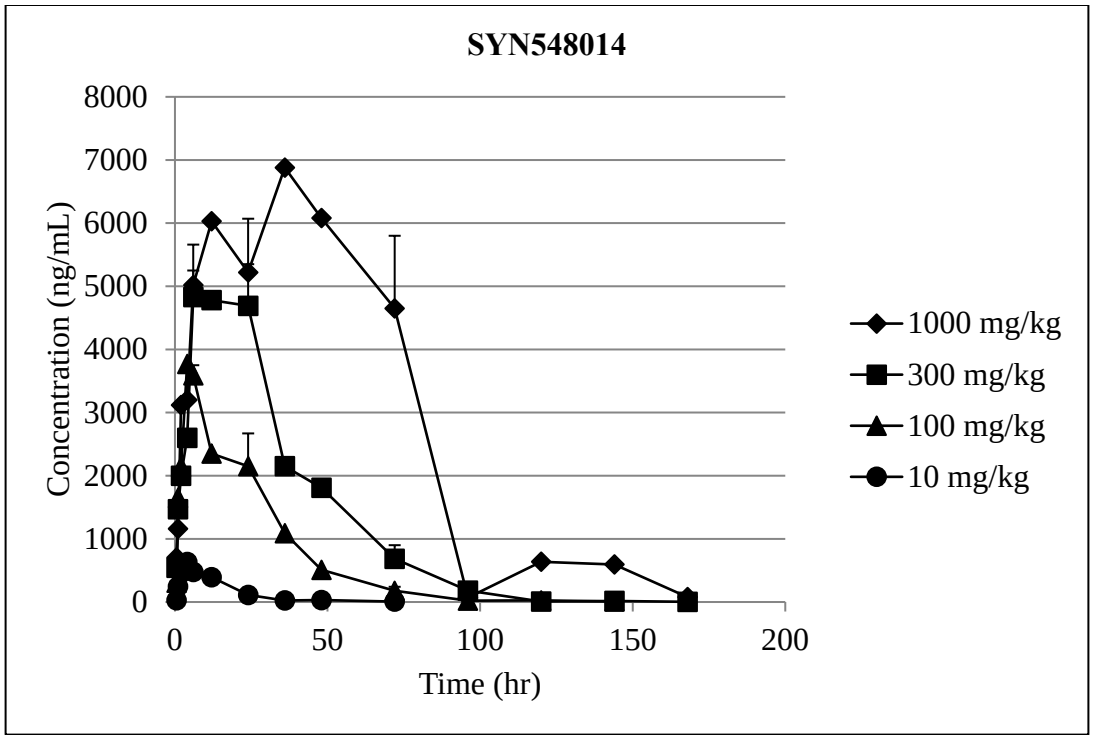


FIGURE 4-9 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548014

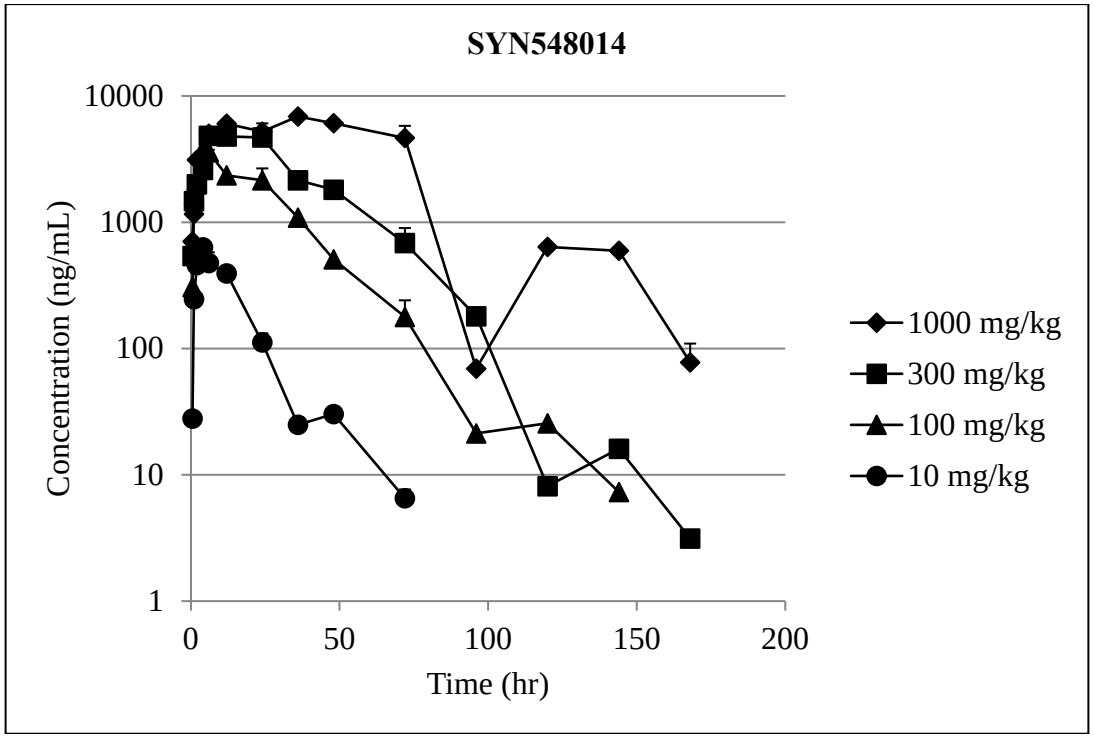


FIGURE 4-10 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548014 – Logarithmic Scale

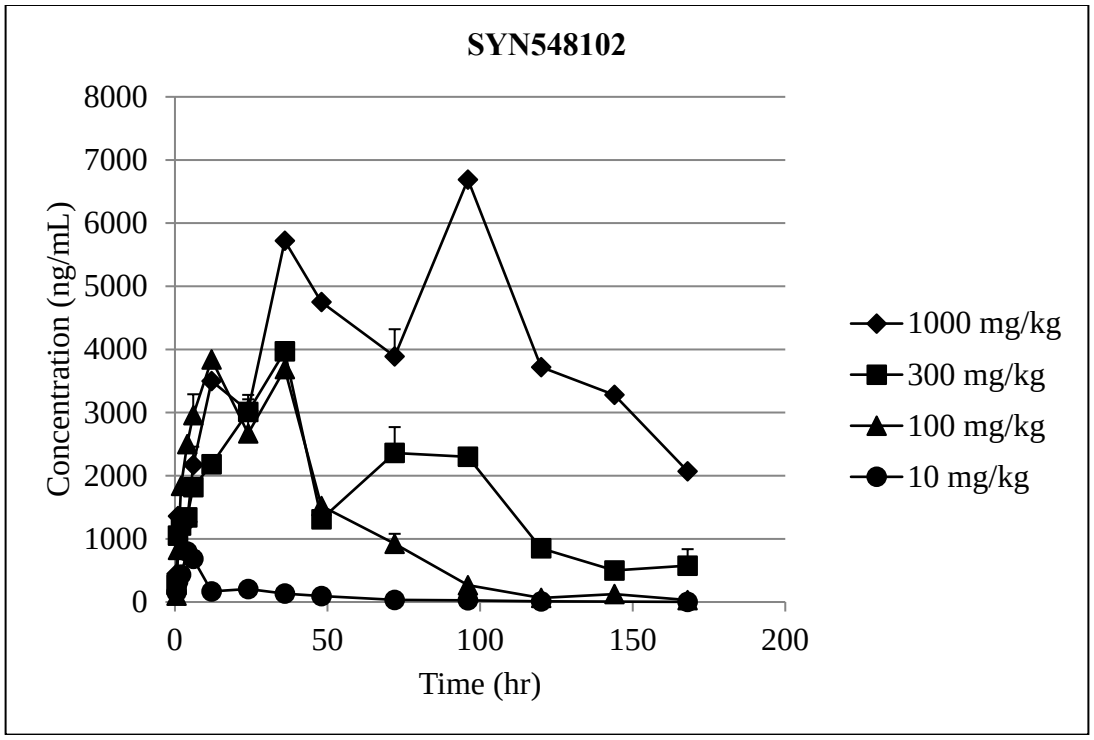


FIGURE 4-11 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548102

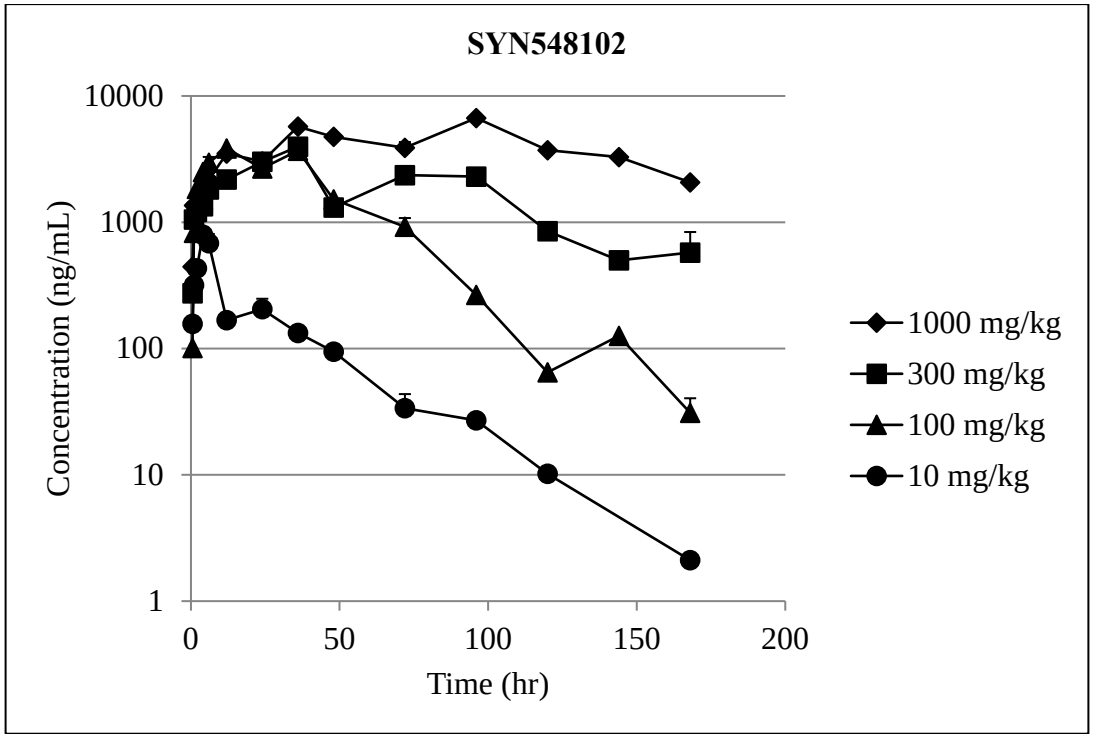


FIGURE 4-12 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548102 – Logarithmic Scale

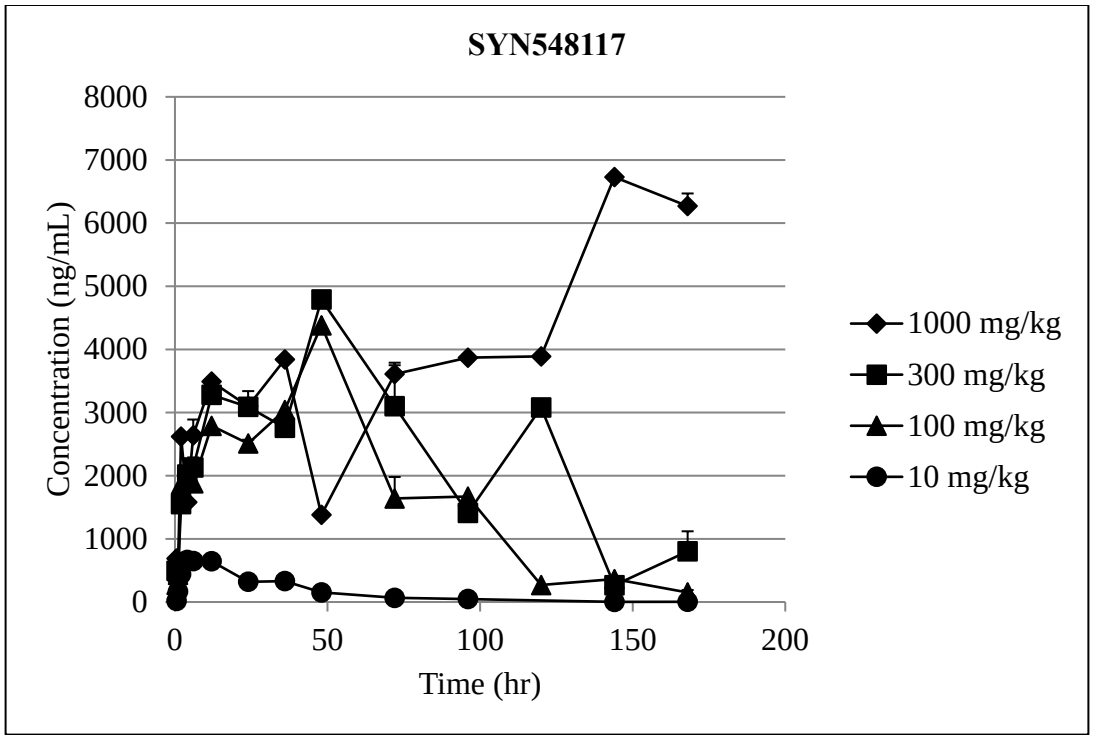


FIGURE 4-13 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548117

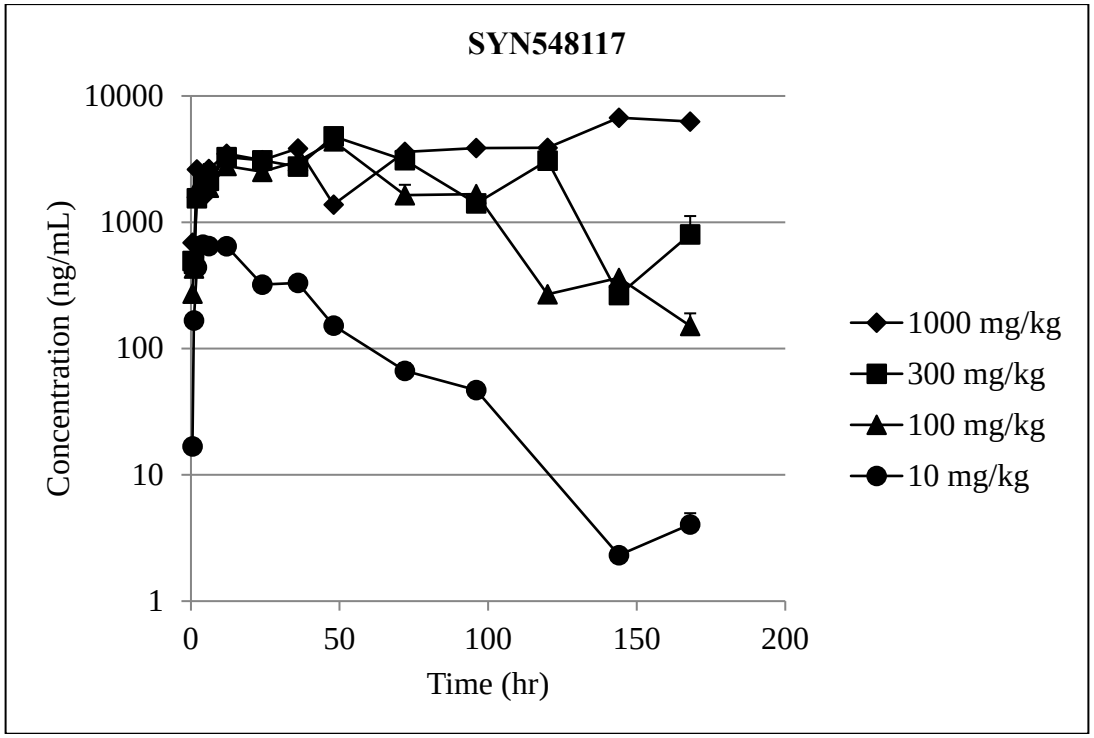


FIGURE 4-14 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548117 – Logarithmic Scale

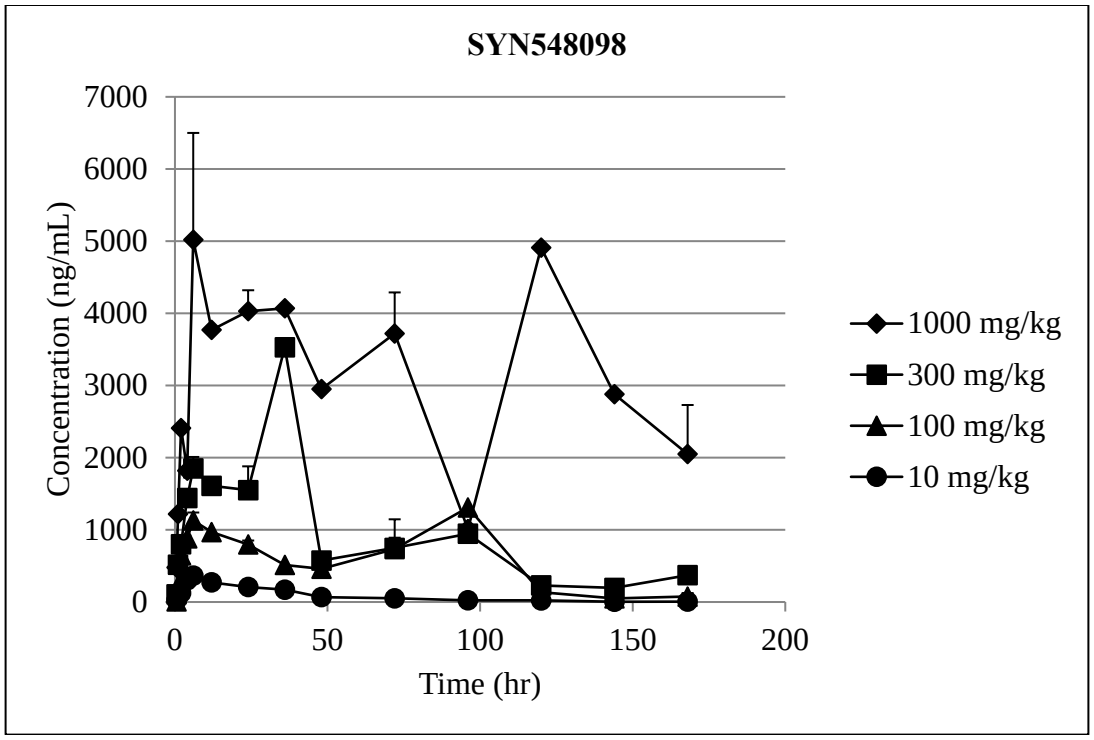


FIGURE 4-15 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548098

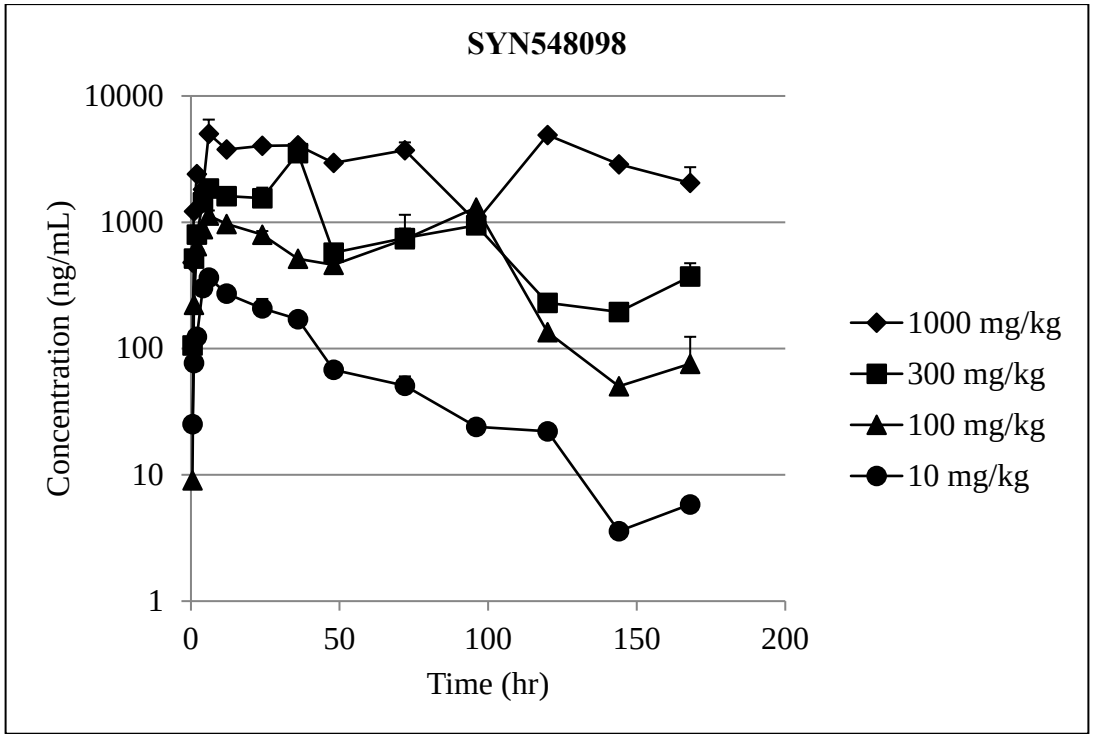


FIGURE 4-16 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548098 – Logarithmic Scale

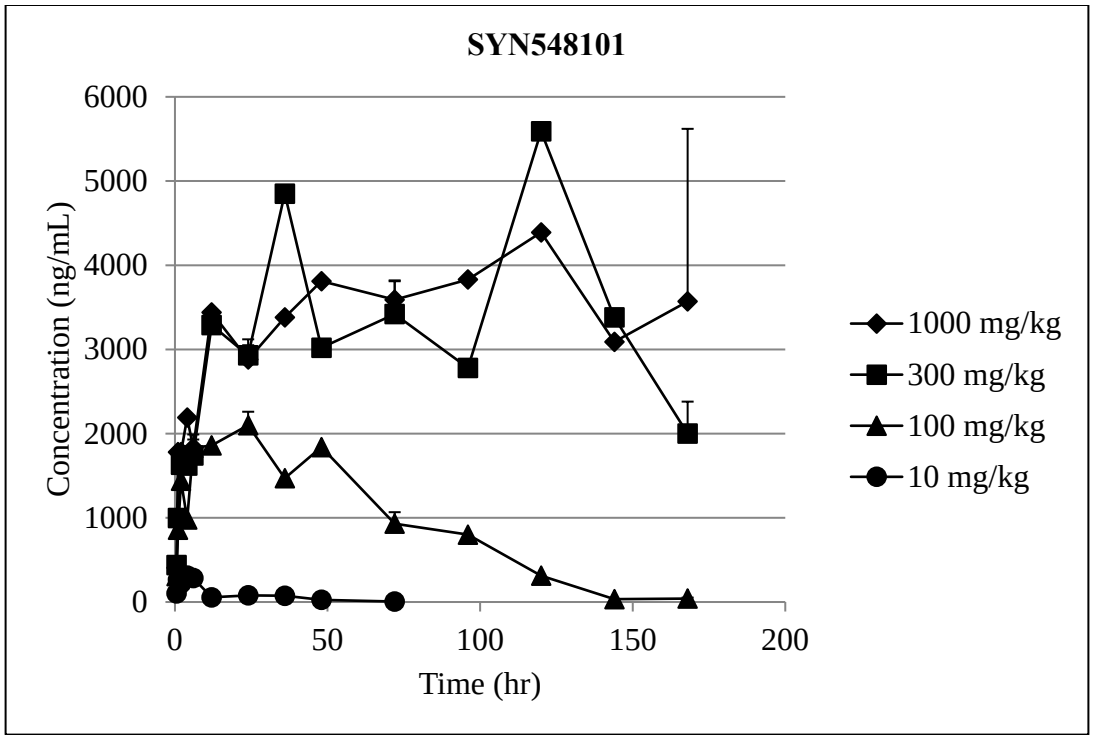


FIGURE 4-17 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548101

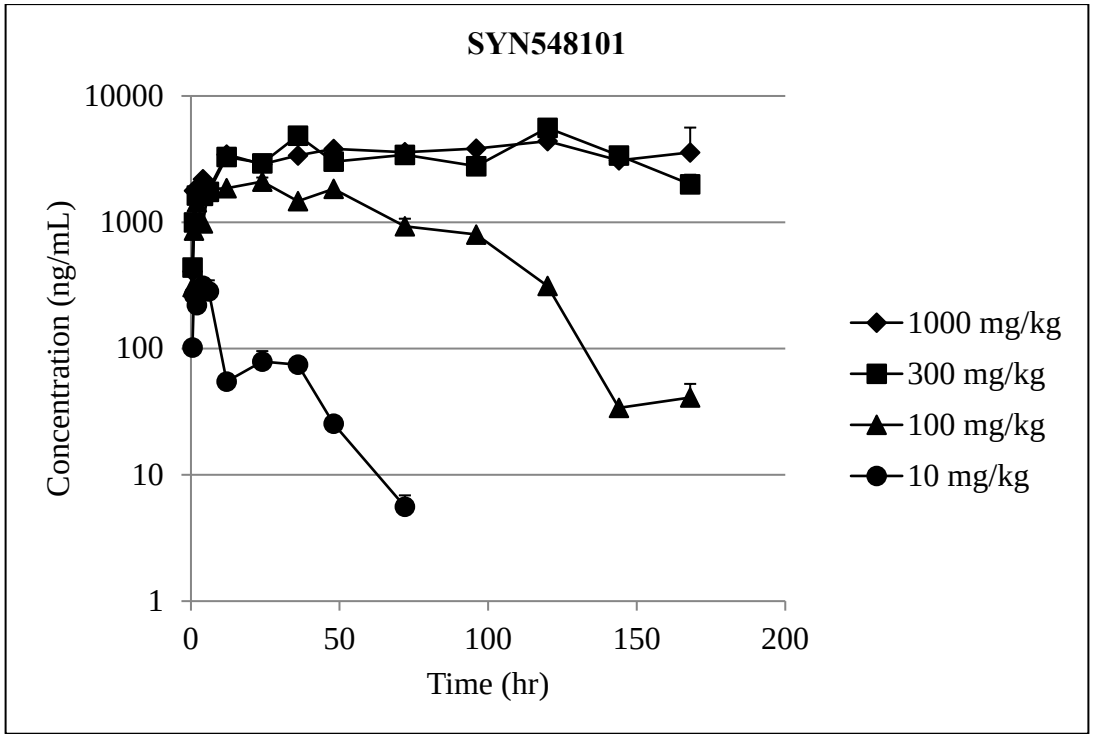


FIGURE 4-18 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548101 – Logarithmic Scale

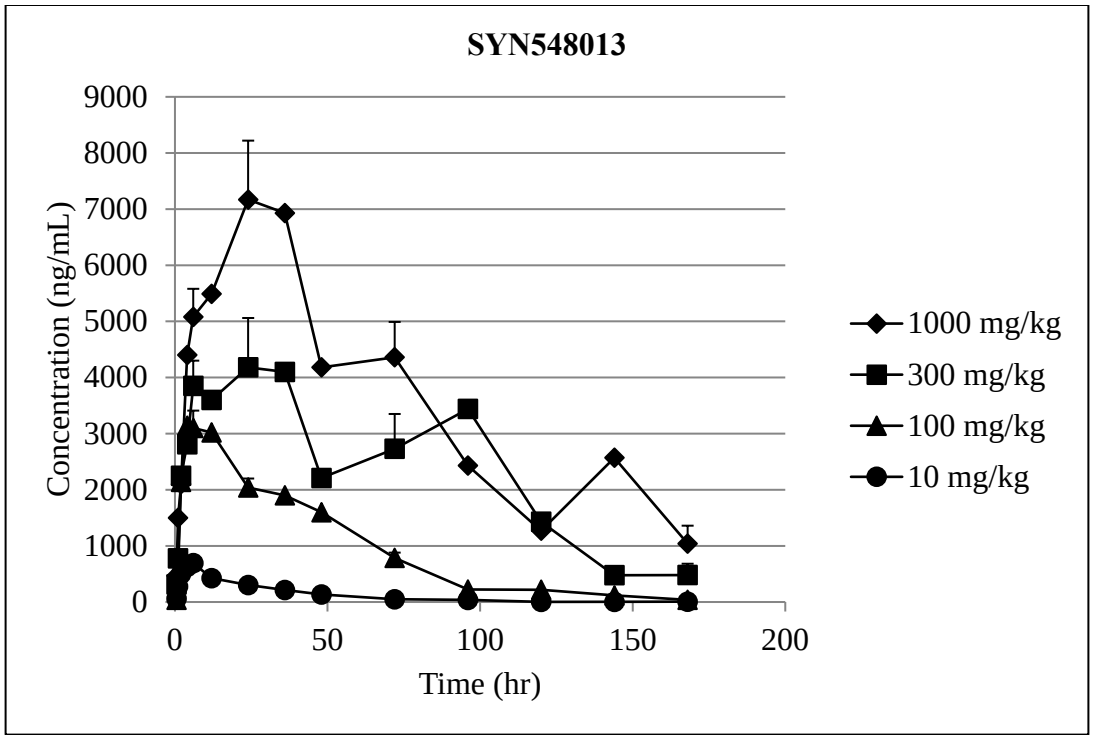


FIGURE 4-19 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548013

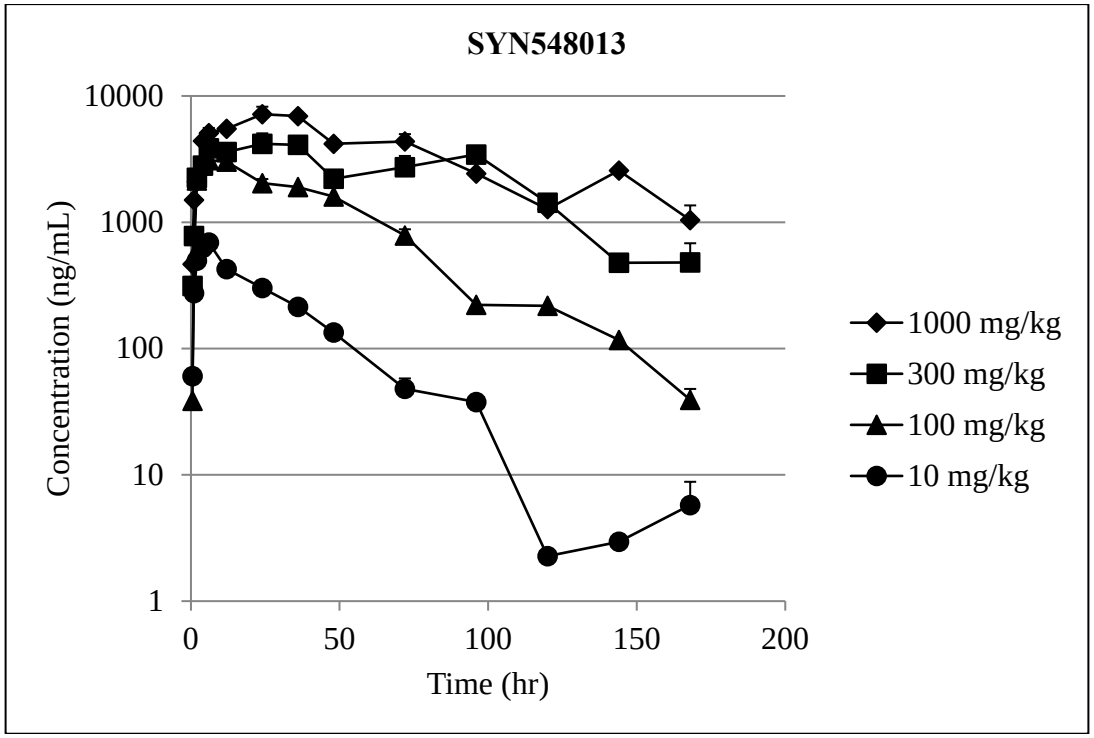


FIGURE 4-20 Group Mean Concentration-Time Profile for Rats Administered 10, 100, 300, or 1000 mg/kg SYN548013 – Logarithmic Scale

APPENDIX 5 Clinical Pathology Narrative

CLINICAL PATHOLOGY NARRATIVE

SYN547407, SYN546308, SYN548097, SYN548012, SYN548014, SYN548102, SYN548117, SYN548098, SYN548101, AND SYN548013 - SEVEN DAY ACUTE ORAL GAVAGE TOXICITY STUDY IN FEMALE WISTAR HAN RATS

Battelle Study No. 39292

May 27, 2016

Prepared By:

Sam Harbo 05/27/16

Sam J. Harbo D. V. M., P.M.P.
Diplomate, A.C.V.P.
Study Clinical Pathologist

Date

Approved By:

A. Skowronek 5/27/2016

Anthony J. Skowronek, D.V.M., Ph.D.
Diplomate, A.C.V.P.
Technical Review

Date

BATTELLE
Toxicology West Jefferson
1425 State Route 142
(Plain City-Georgesvilles Road)
West Jefferson, OH 43162

TABLE OF CONTENTS

LIST OF TABLES

1.0 CLINICAL PATHOLOGY STUDY DESIGN

This narrative contains comparison of the results from the individual test substances to the appropriate control group (see Table 1). A comparison between the different test substances will not be conducted.

The following test article groups will be compared to the control Vehicle Group 41: SYN547407, SYN546308, SYN548097, SYN548012, and SYN548014. The following test article groups will be compared to Vehicle Group 42: SYN548102, SYN548117, SYN548098, SYN548101, and SYN548013.

Data in the tables are labeled Days 1, 2, 4, and 8 that correspond to approximate study timepoints and targets of 6 hours, 24 hours, 72 hours, and 168 hours, respectively.

2.0 SERUM TRIGLYCERIDES

6 hours: There were statistically significant decreases in the 100 and 1000 mg/kg SYN548102 group means when compared to the mean Vehicle Control Group 42.

24 hours: There were no statistically significant differences.

72 hours: There were statistically significant decreases in the 1000 mg/kg SYN546308 and SYN548097 group means when compared to the mean Vehicle Control Group 41.

168 hours: There were no statistically significant differences.

These statistically significant decreases were considered test article related.

Table 1. Study Design

Group	Test Substance[b]	Target Dosage (mg/kg)	Formulation Concentration (mg/mL)[a]	Number of Rats (Females)	Animal IDs
1	SYN547407	10	1	5	1-5
2		100	10	5	6-10
3		300	30	5	11-15
4		1000	100	5	16-20
5	SYN546308	10	1	5	21-25
6		100	10	5	26-30
7		300	30	5	31-35
8		1000	100	5	36-40

Group	Test Substance[b]	Target Dosage (mg/kg)	Formulation Concentration (mg/mL)[a]	Number of Rats (Females)	Animal IDs
9	SYN548097	10	1	5	41-45
10		100	10	5	46-50
11		300	30	5	51-55
12		1000	100	5	56-60
13	SYN548012	10	1	5	61-65
14		100	10	5	66-70
15		300	30	5	71-75
16		1000	100	5	76-80
17	SYN548014	10	1	5	81-85
18		100	10	5	86-90
19		300	30	5	91-95
20		1000	100	5	96-100
21	SYN548102	10	1	5	101-105
22		100	10	5	106-110
23		300	30	5	111-115
24		1000	100	5	116-120
25	SYN548117	10	1	5	121-125
26		100	10	5	126-130
27		300	30	5	131-135
28		1000	100	5	136-140
29	SYN548098	10	1	5	141-145
30		100	10	5	146-150
31		300	30	5	151-155
32		1000	100	5	156-160
33	SYN548101	10	1	5	161-165
34		100	10	5	166-170
35		300	30	5	171-175
36		1000	100	5	176-180
37	SYN548013	10	1	5	181-185
38		100	10	5	186-190
39		300	30	5	191-195
40		1000	100	5	196-200
41	Vehicle Control (0.5% CMC)	0	0	5	201-205
42	Vehicle Control (0.5% CMC)	0	0	5	206-210

a. Dose volume is 10 mL/kg.
b. Dose administration will occur over two days (five test substances per day). Five vehicle control rats will be administered 0.5% CMC on each day of dose administration.

Table 2. Group Mean Triglycerides Data

Day: 1 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN547407 10 mg/kg	Mean	68
	SD	16
	N	5
SYN547407 100 mg/kg	Mean	41
	SD	14
	N	5
SYN547407 300 mg/kg	Mean	37
	SD	18
	N	5
SYN547407 1000 mg/kg	Mean	36
	SD	6
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 1 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN546308 10 mg/kg	Mean	59
	SD	31
	N	5
SYN546308 100 mg/kg	Mean	31
	SD	8
	N	5
SYN546308 300 mg/kg	Mean	38
	SD	7
	N	5
SYN546308 1000 mg/kg	Mean	42
	SD	7
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 1 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548097 10 mg/kg	Mean	64
	SD	23
	N	5
SYN548097 100 mg/kg	Mean	51
	SD	4
	N	5
SYN548097 300 mg/kg	Mean	54
	SD	17
	N	5
SYN548097 1000 mg/kg	Mean	42
	SD	12
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 1 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548012 10 mg/kg	Mean	52
	SD	23
	N	5
SYN54012 100 mg/kg	Mean	81
	SD	27
	N	5
SYN548012 300 mg/kg	Mean	48
	SD	12
	N	5
SYN548012 1000 mg/kg	Mean	50
	SD	18
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 1 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548014 10 mg/kg	Mean	58
	SD	17
	N	5
SYN548014 100 mg/kg	Mean	50
	SD	11
	N	5
SYN548014 300 mg/kg	Mean	43
	SD	13
	N	5
SYN548014 1000 mg/kg	Mean	39
	SD	6
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 1 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
Control	Mean	71 R,k[1]
0 mg/kg	SD	43
	N	5

1 [R,k - Auto Transformation: Rank, Group Factor Test: Kruskal-Wallis $p < 0.05$]

Table 2. Group Mean Triglycerides Data (Continued)

Day: 2 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN547407 10 mg/kg	Mean	54
	SD	15
	N	5
SYN547407 100 mg/kg	Mean	43
	SD	16
	N	5
SYN547407 300 mg/kg	Mean	35
	SD	13
	N	5
SYN547407 1000 mg/kg	Mean	39
	SD	12
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 2 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN546308 10 mg/kg	Mean	51
	SD	18
	N	5
SYN546308 100 mg/kg	Mean	30
	SD	8
	N	4
SYN546308 300 mg/kg	Mean	29
	SD	2
	N	4
SYN546308 1000 mg/kg	Mean	40
	SD	12
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 2 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548097 10 mg/kg	Mean	44
	SD	10
	N	5
SYN548097 100 mg/kg	Mean	40
	SD	5
	N	5
SYN548097 300 mg/kg	Mean	49
	SD	17
	N	5
SYN548097 1000 mg/kg	Mean	42
	SD	18
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 2 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548012	Mean	49
10 mg/kg	SD	11
	N	5
SYN54012	Mean	57
100 mg/kg	SD	21
	N	5
SYN548012	Mean	44
300 mg/kg	SD	18
	N	5
SYN548012	Mean	49
1000 mg/kg	SD	12
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 2 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548014 10 mg/kg	Mean	65
	SD	17
	N	5
SYN548014 100 mg/kg	Mean	44
	SD	10
	N	5
SYN548014 300 mg/kg	Mean	36
	SD	10
	N	5
SYN548014 1000 mg/kg	Mean	36
	SD	6
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 2 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
Control	Mean	63 R,k[1]
0 mg/kg	SD	27
	N	5

1 [R,k - Auto Transformation: Rank, Group Factor Test: Kruskal-Wallis $p < 0.05$]

Table 2. Group Mean Triglycerides Data (Continued)

Day: 4 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN547407 10 mg/kg	Mean	84
	SD	56
	N	5
SYN547407 100 mg/kg	Mean	102
	SD	23
	N	5
SYN547407 300 mg/kg	Mean	48
	SD	6
	N	5
SYN547407 1000 mg/kg	Mean	39
	SD	8
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 4 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry Triglycerides (mg/dL)	
SYN546308	Mean	71	
10 mg/kg	SD	14	
	N	4	
SYN546308	Mean	54	
100 mg/kg	SD	25	
	N	4	
SYN546308	Mean	37	
300 mg/kg	SD	17	
	N	5	
SYN546308	Mean	29	d[1]
1000 mg/kg	SD	4	
	N	4	

1 [d - Test: Dunnett 2 Sided p < 0.05]

Table 2. Group Mean Triglycerides Data (Continued)

Day: 4 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry Triglycerides (mg/dL)	
SYN548097	Mean	49	
10 mg/kg	SD	16	
	N	4	
SYN548097	Mean	75	
100 mg/kg	SD	21	
	N	5	
SYN548097	Mean	60	
300 mg/kg	SD	18	
	N	5	
SYN548097	Mean	33	d[1]
1000 mg/kg	SD	10	
	N	5	

1 [d - Test: Dunnett 2 Sided p < 0.05]

Table 2. Group Mean Triglycerides Data (Continued)

Day: 4 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548012 10 mg/kg	Mean	70
	SD	27
	N	5
SYN54012 100 mg/kg	Mean	116
	SD	58
	N	5
SYN548012 300 mg/kg	Mean	83
	SD	44
	N	5
SYN548012 1000 mg/kg	Mean	92
	SD	26
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 4 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548014 10 mg/kg	Mean	87
	SD	21
	N	5
SYN548014 100 mg/kg	Mean	80
	SD	18
	N	5
SYN548014 300 mg/kg	Mean	72
	SD	15
	N	5
SYN548014 1000 mg/kg	Mean	55
	SD	32
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 4 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
Control 0 mg/kg	Mean	70 L,a[1]
	SD	35
	N	5

1 [L,a - Auto Transformation: Log, Group Factor Test: Analysis of Variance $p < 0.05$]

Table 2. Group Mean Triglycerides Data (Continued)

Day: 8 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN547407 10 mg/kg	Mean	76
	SD	34
	N	5
SYN547407 100 mg/kg	Mean	101
	SD	19
	N	5
SYN547407 300 mg/kg	Mean	92
	SD	51
	N	5
SYN547407 1000 mg/kg	Mean	77
	SD	46
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 8 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN546308 10 mg/kg	Mean	104
	SD	74
	N	4
SYN546308 100 mg/kg	Mean	84
	SD	25
	N	4
SYN546308 300 mg/kg	Mean	72
	SD	6
	N	5
SYN546308 1000 mg/kg	Mean	48
	SD	29
	N	4

Table 2. Group Mean Triglycerides Data (Continued)

Day: 8 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548097 10 mg/kg	Mean	54
	SD	12
	N	4
SYN548097 100 mg/kg	Mean	79
	SD	17
	N	5
SYN548097 300 mg/kg	Mean	68
	SD	26
	N	5
SYN548097 1000 mg/kg	Mean	88
	SD	12
	N	4

Table 2. Group Mean Triglycerides Data (Continued)

Day: 8 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548012 10 mg/kg	Mean	68
	SD	35
	N	5
SYN54012 100 mg/kg	Mean	91
	SD	50
	N	5
SYN548012 300 mg/kg	Mean	70
	SD	20
	N	5
SYN548012 1000 mg/kg	Mean	92
	SD	30
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 8 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548014 10 mg/kg	Mean	109
	SD	31
	N	5
SYN548014 100 mg/kg	Mean	74
	SD	16
	N	5
SYN548014 300 mg/kg	Mean	73
	SD	15
	N	5
SYN548014 1000 mg/kg	Mean	79
	SD	12
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 8 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry	
		Triglycerides (mg/dL)	
Control	Mean	71	R[1]
0 mg/kg	SD	31	
	N	5	

1 [R - Auto Transformation: Rank]

Table 2. Group Mean Triglycerides Data (Continued)

Day: 1 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry Triglycerides (mg/dL)	
SYN548102	Mean	64	
10 mg/kg	SD	20	
	N	5	
SYN548102	Mean	39	d [1]
100 mg/kg	SD	9	
	N	5	
SYN548102	Mean	43	
300 mg/kg	SD	11	
	N	5	
SYN548102	Mean	37	d [1]
1000 mg/kg	SD	8	
	N	5	

1 [d - Test: Dunnett 2 Sided p < 0.05]

Table 2. Group Mean Triglycerides Data (Continued)

Day: 1 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry	
		Triglycerides (mg/dL)	
SYN548117	Mean	74	
10 mg/kg	SD	35	
	N	5	
SYN548117	Mean	38	d[1]
100 mg/kg	SD	11	
	N	5	
SYN548117	Mean	41	
300 mg/kg	SD	12	
	N	5	
SYN548117	Mean	33	d[1]
1000 mg/kg	SD	4	
	N	5	

1 [d - Test: Dunnett 2 Sided p < 0.05]

Table 2. Group Mean Triglycerides Data (Continued)

Day: 1 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548098 10 mg/kg	Mean	59
	SD	20
	N	5
SYN548098 100 mg/kg	Mean	54
	SD	5
	N	5
SYN548098 300 mg/kg	Mean	46
	SD	10
	N	5
SYN548098 1000 mg/kg	Mean	49
	SD	11
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 1 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry Triglycerides (mg/dL)	
SYN548101	Mean	51	
10 mg/kg	SD	11	
	N	5	
SYN548101	Mean	41	
100 mg/kg	SD	4	
	N	5	
SYN548101	Mean	45	
300 mg/kg	SD	10	
	N	5	
SYN548101	Mean	36	d[1]
1000 mg/kg	SD	12	
	N	5	

1 [d - Test: Dunnett 2 Sided p < 0.05]

Table 2. Group Mean Triglycerides Data (Continued)

Day: 1 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548013 10 mg/kg	Mean	67
	SD	17
	N	5
SYN548013 100 mg/kg	Mean	52
	SD	14
	N	5
SYN548013 300 mg/kg	Mean	64
	SD	12
	N	5
SYN548013 1000 mg/kg	Mean	47
	SD	13
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 1 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
Control 0 mg/kg	Mean	64 L,a[1]
	SD	10
	N	5

1 [L,a - Auto Transformation: Log, Group Factor Test: Analysis of Variance $p < 0.05$]

Table 2. Group Mean Triglycerides Data (Continued)

Day: 2 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548102 10 mg/kg	Mean	75
	SD	15
	N	5
SYN548102 100 mg/kg	Mean	42
	SD	17
	N	5
SYN548102 300 mg/kg	Mean	33
	SD	7
	N	5
SYN548102 1000 mg/kg	Mean	32
	SD	5
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 2 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548117 10 mg/kg	Mean	85
	SD	50
	N	5
SYN548117 100 mg/kg	Mean	31
	SD	9
	N	5
SYN548117 300 mg/kg	Mean	47
	SD	11
	N	5
SYN548117 1000 mg/kg	Mean	35
	SD	9
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 2 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548098 10 mg/kg	Mean	96
	SD	91
	N	5
SYN548098 100 mg/kg	Mean	62
	SD	33
	N	5
SYN548098 300 mg/kg	Mean	61
	SD	24
	N	5
SYN548098 1000 mg/kg	Mean	34
	SD	10
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 2 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548101 10 mg/kg	Mean	52
	SD	17
	N	5
SYN548101 100 mg/kg	Mean	38
	SD	17
	N	5
SYN548101 300 mg/kg	Mean	31
	SD	3
	N	5
SYN548101 1000 mg/kg	Mean	31
	SD	6
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 2 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548013 10 mg/kg	Mean	62
	SD	11
	N	5
SYN548013 100 mg/kg	Mean	50
	SD	14
	N	5
SYN548013 300 mg/kg	Mean	41
	SD	8
	N	5
SYN548013 1000 mg/kg	Mean	43
	SD	22
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 2 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
Control	Mean	93 R,k[1]
0 mg/kg	SD	40
	N	5

1 [R,k - Auto Transformation: Rank, Group Factor Test: Kruskal-Wallis $p < 0.05$]

Table 2. Group Mean Triglycerides Data (Continued)

Day: 4 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548102	Mean	54
10 mg/kg	SD	8
	N	5
SYN548102	Mean	86
100 mg/kg	SD	23
	N	5
SYN548102	Mean	89
300 mg/kg	SD	28
	N	5
SYN548102	Mean	45
1000 mg/kg	SD	13
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 4 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548117 10 mg/kg	Mean	100
	SD	48
	N	5
SYN548117 100 mg/kg	Mean	52
	SD	7
	N	5
SYN548117 300 mg/kg	Mean	50
	SD	13
	N	5
SYN548117 1000 mg/kg	Mean	49
	SD	9
	N	4

Table 2. Group Mean Triglycerides Data (Continued)

Day: 4 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548098 10 mg/kg	Mean	117
	SD	79
	N	5
SYN548098 100 mg/kg	Mean	74
	SD	32
	N	5
SYN548098 300 mg/kg	Mean	66
	SD	8
	N	4
SYN548098 1000 mg/kg	Mean	53
	SD	22
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 4 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548101 10 mg/kg	Mean	63
	SD	10
	N	5
SYN548101 100 mg/kg	Mean	93
	SD	62
	N	4
SYN548101 300 mg/kg	Mean	36
	SD	12
	N	5
SYN548101 1000 mg/kg	Mean	39
	SD	9
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 4 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548013 10 mg/kg	Mean	56
	SD	29
	N	5
SYN548013 100 mg/kg	Mean	69
	SD	23
	N	5
SYN548013 300 mg/kg	Mean	70
	SD	20
	N	5
SYN548013 1000 mg/kg	Mean	39
	SD	9
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 4 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
Control 0 mg/kg	Mean	103 R,k[1]
	SD	25
	N	5

1 [R,k - Auto Transformation: Rank, Group Factor Test: Kruskal-Wallis $p < 0.05$]

Table 2. Group Mean Triglycerides Data (Continued)

Day: 8 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548102 10 mg/kg	Mean	72
	SD	27
	N	5
SYN548102 100 mg/kg	Mean	84
	SD	32
	N	5
SYN548102 300 mg/kg	Mean	94
	SD	29
	N	5
SYN548102 1000 mg/kg	Mean	53
	SD	16
	N	2

Table 2. Group Mean Triglycerides Data (Continued)

Day: 8 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548117 10 mg/kg	Mean	87
	SD	26
	N	5
SYN548117 100 mg/kg	Mean	81
	SD	34
	N	5
SYN548117 300 mg/kg	Mean	68
	SD	27
	N	5
SYN548117 1000 mg/kg	Mean	40
	SD	9
	N	3

Table 2. Group Mean Triglycerides Data (Continued)

Day: 8 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548098 10 mg/kg	Mean	84
	SD	46
	N	5
SYN548098 100 mg/kg	Mean	69
	SD	13
	N	5
SYN548098 300 mg/kg	Mean	67
	SD	25
	N	5
SYN548098 1000 mg/kg	Mean	66
	SD	24
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 8 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548101 10 mg/kg	Mean	60
	SD	25
	N	5
SYN548101 100 mg/kg	Mean	85
	SD	27
	N	4
SYN548101 300 mg/kg	Mean	65
	SD	12
	N	4
SYN548101 1000 mg/kg	Mean	80
	SD	-
	N	1

Table 2. Group Mean Triglycerides Data (Continued)

Day: 8 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
SYN548013 10 mg/kg	Mean	52
	SD	23
	N	5
SYN548013 100 mg/kg	Mean	61
	SD	18
	N	5
SYN548013 300 mg/kg	Mean	78
	SD	14
	N	5
SYN548013 1000 mg/kg	Mean	61
	SD	24
	N	5

Table 2. Group Mean Triglycerides Data (Continued)

Day: 8 Relative to Start Date (TSG)

Sex: Female		Cobas Serum Chemistry
		Triglycerides (mg/dL)
Control	Mean	110 [1]
0 mg/kg	SD	21
	N	5

1 [I - Auto Transformation: Identity]

APPENDIX 6 Anatomical Pathology Narrative

ANATOMIC PATHOLOGY NARRATIVE

SYN547407, SYN546308, SYN548097, SYN548012, SYN548014, SYN548102, SYN548117, SYN548098, SYN548101, AND SYN548013 – SEVEN DAY ACUTE ORAL GAVAGE TOXICITY STUDY IN FEMALE WISTAR HAN RATS

Battelle Study No. 39292

September 14, 2016

Prepared By:

A. Skowronek 9-14-16

Anthony J. Skowronek, D.V.M., Ph.D.
Diplomate, A.C.V.P.
Study Pathologist

Date

Approved By:

Katherine M. Gideon 9-14-16

Katherine M. Gideon, M.E.T.M.
Manager, Pathology
Technical Review

Date

BATTELLE
Columbus Operations
505 King Avenue
Columbus, Ohio 43201

TABLE OF CONTENTS

LIST OF TABLES

1.0 INTRODUCTION

The objective of this study was to determine the acute toxicity of ten test substances (SYN547407, SYN546308, SYN548097, SYN548012, SYN548014, SYN548102, SYN548117, SYN548098, SYN548101, and SYN548013) following a single oral (gavage) administration in female Wistar Han rats.

Twenty rats were assigned to one of ten treatment groups. For each treatment group, subgroups of five rats received an escalating dose of test substance (10, 100, 300, or 1000 mg/kg); for a total of 20 rats per treatment group. An additional two groups of rats (5 rats/group) received a single oral dose of 0.5 percent carboxymethylcellulose; serving as vehicle control groups. Dose administration occurred over two days; with five test substance groups and one vehicle control group administered per day.

Gross necropsies were performed on all rats regardless of whether death was scheduled or unscheduled. Organ weights (adrenal glands and liver) were collected from rats terminated at the scheduled necropsy but also from two moribund sacrificed rats (119 and 137, see Deviation Report 272-WJ).

Protocol-designated tissues were collected at necropsy and fixed per facility SOPs. All designated tissues (to include gross lesions) were trimmed, processed, stained with hematoxylin and eosin, and examined microscopically from vehicle control groups (41 and 42) and test substance groups (1-40).

2.0 PATHOLOGY

2.1 Terminal Body Weights

Mean terminal body weight data are presented in Table 3 along with percent organ-to-body-weight ratios. Individual animal terminal body weights are presented in Table 6 with the applicable individual animal organ-to-body weight ratio data.

When compared to the respective control group, there were no statistically significant changes in mean terminal body weight for test substances at all respective doses.

2.2 Organ Weights

Mean absolute and mean organ-to-body weight ratios are presented in Tables 2 and 3. Individual animal absolute organ weights are presented in Tables 5 and 6.

When compared to respective control, a few statistically significant organ weight changes were noted to include:

- SYN548102 (1000 mg/kg): increased mean adrenal absolute gland weight

- SYN547407 (1000 mg/kg): increased mean adrenal gland-to-body weight ratio
- SYN546308 (1000 mg/kg): increased mean adrenal gland-to-body weight ratio.

These increased mean adrenal gland weights (absolute weight and adrenal gland-body weight ratio) were deemed attributable to cytoplasm vacuolation; predominantly to involve the zona fasiculata region.

2.3 Necropsy

Gross necropsies were performed on all rats regardless of whether death was scheduled or unscheduled. A few gross findings (Table 1) were noted across test substance groups. These were interpreted to be spontaneous in occurrence and unrelated to test substance administration.

A proximate cause for early death (or moribund sacrificed) Rats 24, 29, 40, 44, 59, 118, 120, 136, 166, 174, 176, 177, and 179 was not ascertained (in the limited tissues examined). Microscopic examination of protocol specified tissues from moribund sacrificed Rats 119 (Group 24, SYN548102, 1000 mg/kg) and 137 (Group 28, SYN548117, 1000 mg/kg) did not reveal a proximate cause of moribundity.

Table 1. Summary of Microscopic Observations

Early Death/Moribund Termination		
Animal No.	**Microscopic Findings**	**Microscopic Correlate**
29	SYN546308, 100 mg/kg, eye, bilateral, discoloration, opaque,	Not examined
40	SYN546308, 1000 mg/kg, eye, bilateral, discoloration, opaque,	Not examined
119	SYN548102, 1000 mg/kg, brain, meninges, discoloration, dark,	Peracute meningeal hemorrhage
137	SYN548117, 1000 mg/kg, brain, meninges, discoloration, dark	Peracute meningeal hemorrhage
137	SYN548117, 1000 mg/kg, liver, discoloration, dark	Congestion, acute
Scheduled Sacrifice		
149	SYN548098, 100 mg/kg, liver, left, deformity (G1 = agenesis)	No microscopic correlate (lobe = missing)
	SYN548098, 100 mg/kg, liver, median, small, 0.5X	No microscopic correlate (lobe = normal)
	SYN548098, 100 mg/kg, liver, right posterior, enlarged, 2X	Congestion, acute
	SYN548098, 100 mg/kg, liver, right anterior, enlarged, 2X	Congestion, acute
	SYN548098, 100 mg/kg, liver, caudate, enlarged, 2X	Congestion, acute

Protocol-required tissues (to include gross lesions and tissues specified in Deviation Report 272-WJ) were collected, fixed in 10 percent neutral buffered formalin, trimmed, and processed routinely to slides.

2.4 Histopathology

Prepared slides were stained with hematoxylin and eosin and examined by a board-certified veterinary pathologist. Diagnoses were entered into Provantis data-management system and tables prepared. Summary incidences of microscopic observations are presented in Table 4.

A variety of non-neoplastic lesions were noted in various tissues. These were semiquantitatively graded across a 4-point scale, where Grade 1 (minimal) referred to a minor change of negligible significance, and which affected less than 10 percent of the presented tissue area; Grade 2 (mild) referred to a slight change which affected 10 to 19 percent of the tissue area; Grade 3 (moderate) referred to a change of biologic relevance, or which affected at least 20 percent of the tissue area; and Grade 4 (marked) was reserved for lesions considered to be of maximal morphologic change.

The results from test substances (each compared to the respective control) are discussed below.

2.4.1 SYN547407

Treatment-related changes, limited to the highest dose group (1000 mg/kg), were observed in the adrenal glands, duodenum, and jejunum. In the adrenal gland, fine (microvesicular) cytoplasm vacuolization primarily involved cells within the zona fasiculata region. Due to the subtlety of this microscopic finding, cytoplasm vacuolization was uniformly coded as “minimal” in the tables. Similar cytoplasm vacuolization was evident in the mucosal epithelium (enterocytes) of the duodenum and jejunum; primarily involving enterocytes lining the mid and distal portion of villi. Affected villi often appeared elongate; giving the mucosa a hypercellular appearance.

2.4.2 SYN546308

Treatment-related changes, limited to the highest dose group (1000 mg/kg), were observed in the adrenal glands, duodenum, and jejunum. In the adrenal gland, fine (microvesicular) cytoplasm vacuolization primarily involved cells within the zona fasiculata region. Similar cytoplasm vacuolization was evident in the mucosal epithelium (enterocytes) of the duodenum and jejunum; primarily involving enterocytes lining the mid and distal portion of villi. Affected villi often appeared elongate; giving the mucosa a hypercellular appearance.

2.4.3 SYN548097

There were no findings associated with SYN548097 administration.

2.4.4 SYN548012

There were no findings associated with SYN548012 administration.

2.4.5 SYN548014

There were no findings associated with SYN548014 administration.

2.4.6 SYN548102

A treatment-related change, limited to the highest dose group (1000 mg/kg), was observed in the adrenal glands to consist of fine (microvesicular) cytoplasm vacuolization primarily involved cells within the zona fasiculata region.

2.4.7 SYN548117

Treatment-related changes, limited to the highest dose group (1000 mg/kg), were observed in the duodenum and jejunum to consist of cytoplasm vacuolization of mucosal epithelium (enterocytes); primarily involving enterocytes lining the mid and distal portion of villi. Affected villi often appeared elongate; giving the mucosa a hypercellular appearance.

2.4.8 SYN548098

There were no findings associated with SYN548098 administration.

2.4.9 SYN548101

A treatment-related change, limited to the highest dose group (1000 mg/kg), was observed in the adrenal glands to consist of fine (microvesicular) cytoplasm vacuolization primarily involved cells within the zona fasiculata region.

2.4.10 SYN548013

There were no findings associated with SYN548013 administration.

2.4.11 Overall Salient Discussion

In this study, the adrenal glands and/or duodenum/jejunum appeared as target tissues in five out of ten test substances examined; only at the 1000/mg/kg target dosage. A few additional microscopic findings were rendered in the summary and individual animal data tables. These findings were deemed incidental and spontaneous changes; of neither biologic nor toxicologic significance.

3.0 CONCLUSIONS

Exposure of female Wistar Han rats to SYN547407, SYN546308, SYN548012, SYN548117, and SYN548101 at concentrations of 1000 mg/kg resulted in treatment related findings in the adrenal glands and/or duodenum/jejunum to involve cytoplasm vacuolization of adrenal zona fasiculata and intestinal villous enterocytes, respectively. Increased adrenal gland weights (absolute or percent of body weight) in groups SYN548102 (1000 mg/kg), SYN547407 (1000 mg/kg), and SYN546308 (1000 mg/kg) were deemed attributable to cytoplasm vacuolation; predominantly to involve the zona fasiculata region.

Table 2. Group Mean Absolute Organ Weights

Sex: Female		Adrenal Glands (g)	Liver (g)
SYN547407	Mean	0.072	7.240
10 mg/kg	SD	0.008	0.800
	N	5	5
SYN547407	Mean	0.074	7.533
100 mg/kg	SD	0.011	0.967
	N	5	5
SYN547407	Mean	0.085	7.698
300 mg/kg	SD	0.016	0.534
	N	5	5
SYN547407	Mean	0.101	7.105
1000 mg/kg	SD	0.010	0.895
	N	5	5

Table 2. Group Mean Organ Weights (Continued)

Sex: Female		Adrenal Glands (g)	Liver (g)
SYN546308	Mean	0.074	7.939
10 mg/kg	SD	0.005	0.200
	N	4	4
SYN546308	Mean	0.077	7.952
100 mg/kg	SD	0.007	0.450
	N	4	4
SYN546308	Mean	0.079	8.524
300 mg/kg	SD	0.014	0.932
	N	5	5
SYN546308	Mean	0.093	7.225
1000 mg/kg	SD	0.008	2.259
	N	4	4

Table 2. Group Mean Absolute Organ Weights (Continued)

Sex: Female		Adrenal Glands (g)	Liver (g)
SYN548097	Mean	0.071	7.678
10 mg/kg	SD	0.007	0.521
	N	4	4
SYN548097	Mean	0.072	8.195
100 mg/kg	SD	0.005	0.583
	N	5	5
SYN548097	Mean	0.077	7.755
300 mg/kg	SD	0.012	0.610
	N	5	5
SYN548097	Mean	0.083	7.655
1000 mg/kg	SD	0.012	0.892
	N	4	4

Table 2. Group Mean Absolute Organ Weights (Continued)

Sex: Female		Adrenal Glands (g)	Liver (g)
SYN548012	Mean	0.073	7.728
10 mg/kg	SD	0.006	0.639
	N	5	5
SYN54012	Mean	0.071	7.969
100 mg/kg	SD	0.014	0.766
	N	5	5
SYN548012	Mean	0.074	7.698
300 mg/kg	SD	0.004	0.895
	N	5	5
SYN548012	Mean	0.075	7.967
1000 mg/kg	SD	0.003	0.515
	N	5	5

Table 2. Group Mean Absolute Organ Weights (Continued)

Sex: Female		Adrenal Glands (g)	Liver (g)
SYN548014	Mean	0.066	7.496
10 mg/kg	SD	0.007	0.400
	N	5	5
SYN548014	Mean	0.075	7.951
100 mg/kg	SD	0.010	0.350
	N	5	5
SYN548014	Mean	0.073	7.739
300 mg/kg	SD	0.009	0.755
	N	5	5
SYN548014	Mean	0.071	7.962
1000 mg/kg	SD	0.008	0.735
	N	5	5

Table 2. Group Mean Absolute Organ Weights (Continued)

Sex: Female		Adrenal Glands (g)	Liver (g)
Control	Mean	0.070 R,k[1]	6.946 R[2]
0 mg/kg	SD	0.012	0.421
	N	5	5

1 [R,k - Auto Transformation: Rank, Group Factor Test: Kruskal-Wallis $p < 0.05$]

2 [R - Auto Transformation: Rank]

Table 2. Group Mean Absolute Organ Weights (Continued)

Sex: Female		Adrenal Glands (g)	Liver (g)
SYN548102	Mean	0.070	8.289
10 mg/kg	SD	0.009	0.991
	N	5	5
SYN548102	Mean	0.069	7.774
100 mg/kg	SD	0.010	0.398
	N	5	5
SYN548102	Mean	0.081	8.525
300 mg/kg	SD	0.010	1.369
	N	5	5
SYN548102	Mean	0.103 d[1]	6.559
1000 mg/kg	SD	0.018	1.447
	N	3	3

1 [d - Test: Dunnett 2 Sided p < 0.05]

Table 2. Group Mean Absolute Organ Weights (Continued)

Sex: Female		Adrenal Glands (g)	Liver (g)
SYN548117	Mean	0.067	8.304
10 mg/kg	SD	0.008	0.329
	N	5	5
SYN548117	Mean	0.072	8.211
100 mg/kg	SD	0.007	0.805
	N	5	5
SYN548117	Mean	0.078	7.611
300 mg/kg	SD	0.007	1.413
	N	5	5
SYN548117	Mean	0.078	6.526
1000 mg/kg	SD	0.017	0.579
	N	4	4

Table 2. Group Mean Absolute Organ Weights (Continued)

Sex: Female		Adrenal Glands (g)	Liver (g)
SYN548098	Mean	0.067	8.483
10 mg/kg	SD	0.013	0.967
	N	5	5
SYN548098	Mean	0.078	8.022
100 mg/kg	SD	0.010	0.765
	N	5	5
SYN548098	Mean	0.067	7.220
300 mg/kg	SD	0.004	0.584
	N	5	5
SYN548098	Mean	0.077	7.548
1000 mg/kg	SD	0.021	1.244
	N	5	5

Table 2. Group Mean Absolute Organ Weights (Continued)

Sex: Female		Adrenal Glands (g)	Liver (g)
SYN548101	Mean	0.069	8.087
10 mg/kg	SD	0.003	0.577
	N	5	5
SYN548101	Mean	0.073	7.482
100 mg/kg	SD	0.008	0.639
	N	4	4
SYN548101	Mean	0.087	7.995
300 mg/kg	SD	0.008	0.726
	N	4	4
SYN548101	Mean	0.090	6.475
1000 mg/kg	SD	0.008	1.385
	N	2	2

Table 2. Group Mean Absolute Organ Weights (Continued)

Sex: Female		Adrenal Glands (g)	Liver (g)
SYN548013	Mean	0.073	7.629
10 mg/kg	SD	0.010	1.325
	N	5	5
SYN548013	Mean	0.071	7.468
100 mg/kg	SD	0.012	0.467
	N	5	5
SYN548013	Mean	0.068	7.982
300 mg/kg	SD	0.011	0.573
	N	5	5
SYN548013	Mean	0.077	7.968
1000 mg/kg	SD	0.003	1.039
	N	5	5

Table 2. Group Mean Absolute Organ Weights (Continued)

Sex: Female		Adrenal Glands (g)	Liver (g)
Control	Mean	0.0711,a[1]	7.956 [2]
0 mg/kg	SD	0.008	0.713
	N	5	5

1 [l,a - Auto Transformation: Identity, Group Factor Test: Analysis of Variance p < 0.05]

2 [l - Auto Transformation: Identity]

Table 3. Group Mean Terminal Body Weights and Organ-to-Body Weights Ratios

Sex: Female		Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
SYN547407	Mean	168.600	0.043	4.287
10 mg/kg	SD	6.630	0.004	0.349
	N	5	5	5
SYN547407	Mean	172.092	0.043	4.367
100 mg/kg	SD	8.806	0.008	0.388
	N	5	5	5
SYN547407	Mean	165.612	0.051	4.652
300 mg/kg	SD	4.429	0.009	0.361
	N	5	5	5
SYN547407	Mean	149.058	0.069 d[1]	4.774
1000 mg/kg	SD	14.058	0.013	0.481
	N	5	5	5

1 [d - Test: Dunnett 2 Sided p < 0.05]

Table 3. Group Mean Terminal Body Weights and Organ-to-Body Weights Ratios (Continued)

Sex: Female		Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
SYN546308	Mean	169.498	0.043	4.686
10 mg/kg	SD	4.513	0.002	0.156
	N	4	4	4
SYN546308	Mean	164.306	0.045	4.673
100 mg/kg	SD	16.734	0.006	0.097
	N	5	4	4
SYN546308	Mean	174.074	0.045	4.889
300 mg/kg	SD	12.100	0.006	0.246
	N	5	5	5
SYN546308	Mean	146.260	0.064 d[1]	4.744
1000 mg/kg	SD	23.997	0.016	0.691
	N	5	4	4

1 [d - Test: Dunnett 2 Sided p < 0.05]

Table 3. Group Mean Terminal Body Weights and Organ-to-Body Weights Ratios (Continued)

Sex: Female		Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
SYN548097	Mean	167.724	0.042	4.468
10 mg/kg	SD	12.307	0.006	0.083
	N	5	4	4
SYN548097	Mean	172.588	0.042	4.752
100 mg/kg	SD	8.160	0.003	0.341
	N	5	5	5
SYN548097	Mean	168.928	0.046	4.596
300 mg/kg	SD	13.243	0.009	0.226
	N	5	5	5
SYN548097	Mean	159.854	0.049	4.500
1000 mg/kg	SD	24.950	0.008	0.350
	N	5	4	4

Table 3. Group Mean Terminal Body Weights and Organ-to-Body Weights Ratios (Continued)

Sex: Female		Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
SYN548012	Mean	171.902	0.043	4.497
10 mg/kg	SD	5.304	0.003	0.372
	N	5	5	5
SYN54012	Mean	169.600	0.042	4.710
100 mg/kg	SD	6.331	0.008	0.563
	N	5	5	5
SYN548012	Mean	172.584	0.043	4.454
300 mg/kg	SD	9.997	0.004	0.378
	N	5	5	5
SYN548012	Mean	171.936	0.044	4.635
1000 mg/kg	SD	4.354	0.002	0.302
	N	5	5	5

Table 3. Group Mean Terminal Body Weights and Organ-to-Body Weights Ratios (Continued)

Sex: Female		Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
SYN548014	Mean	170.500	0.039	4.399
10 mg/kg	SD	5.579	0.005	0.260
	N	5	5	5
SYN548014	Mean	171.744	0.043	4.635
100 mg/kg	SD	6.781	0.004	0.266
	N	5	5	5
SYN548014	Mean	171.580	0.042	4.509
300 mg/kg	SD	6.718	0.004	0.377
	N	5	5	5
SYN548014	Mean	166.536	0.043	4.775
1000 mg/kg	SD	8.752	0.007	0.262
	N	5	5	5

Table 3. Group Mean Terminal Body Weights and Organ-to-Body Weights Ratios (Continued)

Sex: Female		Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
Control	Mean	161.862 R[1]	0.043 L,a[2]	4.292 L[3]
0 mg/kg	SD	8.641	0.007	0.136
	N	5	5	5

1 [R - Auto Transformation: Rank]

2 [L,a - Auto Transformation: Log, Group Factor Test: Analysis of Variance $p < 0.05$]

Table 3. Group Mean Terminal Body Weights and Organ-to-Body Weights Ratios (Continued)

Sex: Female		Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
SYN548102	Mean	174.718	0.040	4.742
10 mg/kg	SD	9.971	0.005	0.470
	N	5	5	5
SYN548102	Mean	167.502	0.041	4.647
100 mg/kg	SD	6.368	0.006	0.292
	N	5	5	5
SYN548102	Mean	173.230	0.047	4.903
300 mg/kg	SD	7.683	0.006	0.554
	N	5	5	5
SYN548102	Mean	133.773	0.077	4.721
1000 mg/kg	SD	20.059	0.027	0.325
	N	4	3	3

Table 3. Group Mean Terminal Body Weights and Organ-to-Body Weights Ratios (Continued)

Sex: Female		Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
SYN548117	Mean	176.746	0.038	4.699
10 mg/kg	SD	4.063	0.004	0.163
	N	5	5	5
SYN548117	Mean	175.868	0.041	4.670
100 mg/kg	SD	4.681	0.005	0.445
	N	5	5	5
SYN548117	Mean	161.718	0.049	4.685
300 mg/kg	SD	15.867	0.008	0.506
	N	5	5	5
SYN548117	Mean	134.490	0.056	4.693
1000 mg/kg	SD	15.805	0.012	0.531
	N	5	4	4

Table 3. Group Mean Terminal Body Weights and Organ-to-Body Weights Ratios (Continued)

Sex: Female		Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
SYN548098	Mean	175.078	0.038	4.833
10 mg/kg	SD	11.102	0.006	0.278
	N	5	5	5
SYN548098	Mean	173.074	0.045	4.628
100 mg/kg	SD	7.826	0.006	0.270
	N	5	5	5
SYN548098	Mean	170.866	0.039	4.224
300 mg/kg	SD	7.127	0.001	0.267
	N	5	5	5
SYN548098	Mean	166.286	0.046	4.523
1000 mg/kg	SD	11.274	0.010	0.510
	N	5	5	5

Table 3. Group Mean Terminal Body Weights and Organ-to-Body Weights Ratios (Continued)

Sex: Female		Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
SYN548101	Mean	172.730	0.040	4.679
10 mg/kg	SD	6.142	0.002	0.240
	N	5	5	5
SYN548101	Mean	168.683	0.044	4.434
100 mg/kg	SD	11.484	0.007	0.181
	N	4	4	4
SYN548101	Mean	154.034	0.054	4.951
300 mg/kg	SD	18.375	0.008	0.202
	N	5	4	4
SYN548101	Mean	140.550	0.064	4.584
1000 mg/kg	SD	21.227	0.004	0.293
	N	2	2	2

Table 3. Group Mean Terminal Body Weights and Organ-to-Body Weights Ratios (Continued)

Sex: Female		Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
SYN548013	Mean	170.682	0.043	4.454
10 mg/kg	SD	7.208	0.004	0.625
	N	5	5	5
SYN548013	Mean	172.568	0.041	4.331
100 mg/kg	SD	7.299	0.005	0.275
	N	5	5	5
SYN548013	Mean	175.434	0.039	4.548
300 mg/kg	SD	5.813	0.006	0.255
	N	5	5	5
SYN548013	Mean	171.660	0.045	4.629
1000 mg/kg	SD	7.568	0.002	0.388
	N	5	5	5

Table 3. Group Mean Terminal Body Weights and Organ-to-Body Weights Ratios (Continued)

Sex: Female		Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
Control	Mean	174.640 R,k[1]	0.041 R,k[1]	4.555 I[2]
0 mg/kg	SD	8.996	0.003	0.314
	N	5	5	5

1 [R,k - Auto Transformation: Rank, Group Factor Test: Kruskal-Wallis $p < 0.05$]

2 [I - Auto Transformation: Identity]

Table 4. Incidence Summary of Microscopic Observations

Removal Reason: ALL	Female				
	SYN547407 10 mg/kg	SYN547407 100 mg/kg	SYN547407 300 mg/kg	SYN547407 1000 mg/kg	
Number of Animals:	5	5	5	5	
ADRENAL GLANDS					
Examined	5	5	5	5	
No Visible Lesions	5	5	5	1	
ANGIECTASIS	0	0	0	0	
HYPERPLASIA	0	0	0	0	
VACUOLIZATION CYTOPLASM	0	0	0	4	
BRAIN					
Examined	0	0	0	0	
HEMORRHAGE	.	.	.	.	
MENINGES; HEMORRHAGE	.	.	.	.	
DUODENUM					
Examined	5	5	5	5	
No Visible Lesions	5	5	5	3	
VACUOLIZATION CYTOPLASM	0	0	0	2	
JEJUNUM					
Examined	5	5	5	5	
No Visible Lesions	5	5	5	2	
VACUOLIZATION CYTOPLASM	0	0	0	3	
LIVER					
Examined	5	5	5	4	
No Visible Lesions	5	5	4	3	
CLEAR CELL FOCUS	0	0	0	0	
CONGESTION	0	0	0	0	
INFILTRATE, CELLULAR; LYMPHOCYTIC	0	0	1	0	
HEPATOCYTE; DIFFUSE; VACUOLIZATION CYTOPLASM	0	0	0	1	
NO CORRELATE	0	0	0	0	
NO CORRELATE					
Examined	0	0	0	0	
NO CORRELATE	.	.	.	.	

Table 4. Incidence Summary of Microscopic Observations (Continued)

Removal Reason: ALL	Female				
	SYN546308 10 mg/kg	SYN546308 100 mg/kg	SYN546308 300 mg/kg	SYN546308 1000 mg/kg	
Number of Animals:	5	5	5	5	
ADRENAL GLANDS					
Examined	4	4	5	4	
No Visible Lesions	4	4	4	2	
ANGIECTASIS	0	0	0	0	
HYPERPLASIA	0	0	1	0	
VACUOLIZATION CYTOPLASM	0	0	0	2	
BRAIN					
Examined	0	0	0	0	
HEMORRHAGE	.	.	.	.	
MENINGES; HEMORRHAGE	.	.	.	.	
DUODENUM					
Examined	4	4	5	4	
No Visible Lesions	4	4	4	1	
VACUOLIZATION CYTOPLASM	0	0	1	3	
JEJUNUM					
Examined	4	4	5	4	
No Visible Lesions	4	4	5	1	
VACUOLIZATION CYTOPLASM	0	0	0	3	
LIVER					
Examined	4	4	5	4	
No Visible Lesions	4	4	4	4	
CLEAR CELL FOCUS	0	0	0	0	
CONGESTION	0	0	0	0	
INFILTRATE, CELLULAR; LYMPHOCYTIC	0	0	1	0	
HEPATOCYTE; DIFFUSE; VACUOLIZATION CYTOPLASM	0	0	0	0	
NO CORRELATE	0	0	0	0	
NO CORRELATE					
Examined	0	0	0	0	
NO CORRELATE	.	.	.	.	

Table 4. Incidence Summary of Microscopic Observations (Continued)

Removal Reason: ALL	Female				
	SYN54809 7 10 mg/kg	SYN54809 7 100 mg/kg	SYN54809 7 300 mg/kg	SYN54809 7 1000 mg/kg	
Number of Animals:	5	5	5	5	
ADRENAL GLANDS					
Examined	4	5	5	4	
No Visible Lesions	4	5	5	4	
ANGIECTASIS	0	0	0	0	
HYPERPLASIA	0	0	0	0	
VACUOLIZATION CYTOPLASM	0	0	0	0	
BRAIN					
Examined	0	0	0	0	
HEMORRHAGE	.	.	.	.	
MENINGES; HEMORRHAGE	.	.	.	.	
DUODENUM					
Examined	4	5	5	4	
No Visible Lesions	4	5	5	4	
VACUOLIZATION CYTOPLASM	0	0	0	0	
JEJUNUM					
Examined	4	5	5	4	
No Visible Lesions	4	5	5	4	
VACUOLIZATION CYTOPLASM	0	0	0	0	
LIVER					
Examined	4	5	5	4	
No Visible Lesions	4	4	5	4	
CLEAR CELL FOCUS	0	1	0	0	
CONGESTION	0	0	0	0	
INFILTRATE, CELLULAR; LYMPHOCYTIC	0	1	0	0	
HEPATOCYTE; DIFFUSE; VACUOLIZATION CYTOPLASM	0	0	0	0	
NO CORRELATE	0	0	0	0	
NO CORRELATE					
Examined	0	0	0	0	
NO CORRELATE	.	.	.	.	

Table 4. Incidence Summary of Microscopic Observations (Continued)

Removal Reason: ALL	Female				
	SYN54801 2 10 mg/kg	SYN54012 100 mg/kg	SYN54801 2 300 mg/kg	SYN54801 2 1000 mg/kg	
Number of Animals:	5	5	5	5	
ADRENAL GLANDS					
Examined	5	5	5	5	
No Visible Lesions	5	5	5	5	
ANGIECTASIS	0	0	0	0	
HYPERPLASIA	0	0	0	0	
VACUOLIZATION CYTOPLASM	0	0	0	0	
BRAIN					
Examined	0	0	0	0	
HEMORRHAGE	.	.	.	.	
MENINGES; HEMORRHAGE	.	.	.	.	
DUODENUM					
Examined	5	5	5	5	
No Visible Lesions	5	5	5	5	
VACUOLIZATION CYTOPLASM	0	0	0	0	
JEJUNUM					
Examined	5	5	5	5	
No Visible Lesions	5	5	5	5	
VACUOLIZATION CYTOPLASM	0	0	0	0	
LIVER					
Examined	5	5	5	5	
No Visible Lesions	5	5	4	4	
CLEAR CELL FOCUS	0	0	0	0	
CONGESTION	0	0	0	0	
INFILTRATE, CELLULAR; LYMPHOCYTIC	0	0	1	1	
HEPATOCYTE; DIFFUSE; VACUOLIZATION CYTOPLASM	0	0	0	0	
NO CORRELATE	0	0	0	0	
NO CORRELATE					
Examined	0	0	0	0	
NO CORRELATE	.	.	.	.	

Table 4. Incidence Summary of Microscopic Observations (Continued)

Removal Reason: ALL	Female				
	SYN548014 10 mg/kg	SYN548014 100 mg/kg	SYN548014 300 mg/kg	SYN548014 1000 mg/kg	
Number of Animals:	5	5	5	5	
ADRENAL GLANDS					
Examined	5	5	5	5	
No Visible Lesions	5	5	5	5	
ANGIECTASIS	0	0	0	0	
HYPERPLASIA	0	0	0	0	
VACUOLIZATION CYTOPLASM	0	0	0	0	
BRAIN					
Examined	0	0	0	0	
HEMORRHAGE	.	.	.	.	
MENINGES; HEMORRHAGE	.	.	.	.	
DUODENUM					
Examined	5	5	5	5	
No Visible Lesions	5	5	5	5	
VACUOLIZATION CYTOPLASM	0	0	0	0	
JEJUNUM					
Examined	5	5	5	5	
No Visible Lesions	5	5	5	5	
VACUOLIZATION CYTOPLASM	0	0	0	0	
LIVER					
Examined	5	5	5	5	
No Visible Lesions	5	4	5	5	
CLEAR CELL FOCUS	0	0	0	0	
CONGESTION	0	0	0	0	
INFILTRATE, CELLULAR; LYMPHOCYTIC	0	1	0	0	
HEPATOCYTE; DIFFUSE; VACUOLIZATION CYTOPLASM	0	0	0	0	
NO CORRELATE	0	0	0	0	
NO CORRELATE					
Examined	0	0	0	0	
NO CORRELATE	.	.	.	.	

Table 4. Incidence Summary of Microscopic Observations (Continued)

Removal Reason: ALL	Female				
	SYN548102 10 mg/kg	SYN548102 100 mg/kg	SYN548102 300 mg/kg	SYN548102 1000 mg/kg	
Number of Animals:	5	5	5	5	
ADRENAL GLANDS					
Examined	5	5	5	3	
No Visible Lesions	5	5	5	0	
ANGIECTASIS	0	0	0	0	
HYPERPLASIA	0	0	0	0	
VACUOLIZATION CYTOPLASM	0	0	0	3	
BRAIN					
Examined	0	0	0	1	
HEMORRHAGE	.	.	.	1	
MENINGES; HEMORRHAGE	.	.	.	0	
DUODENUM					
Examined	5	5	5	3	
No Visible Lesions	5	5	5	3	
VACUOLIZATION CYTOPLASM	0	0	0	0	
JEJUNUM					
Examined	5	5	5	3	
No Visible Lesions	5	5	5	3	
VACUOLIZATION CYTOPLASM	0	0	0	0	
LIVER					
Examined	5	5	5	3	
No Visible Lesions	5	5	5	3	
CLEAR CELL FOCUS	0	0	0	0	
CONGESTION	0	0	0	0	
INFILTRATE, CELLULAR; LYMPHOCYTIC	0	0	0	0	
HEPATOCYTE; DIFFUSE; VACUOLIZATION CYTOPLASM	0	0	0	0	
NO CORRELATE	0	0	0	0	
NO CORRELATE					
Examined	0	0	0	0	
NO CORRELATE	.	.	.	.	

Table 4. Incidence Summary of Microscopic Observations (Continued)

Removal Reason: ALL	Female				
	SYN54811 7 10 mg/kg	SYN54811 7 100 mg/kg	SYN54811 7 300 mg/kg	SYN54811 7 1000 mg/kg	
Number of Animals:	5	5	5	5	
ADRENAL GLANDS					
Examined	5	5	5	4	
No Visible Lesions	5	5	5	4	
ANGIECTASIS	0	0	0	0	
HYPERPLASIA	0	0	0	0	
VACUOLIZATION CYTOPLASM	0	0	0	0	
BRAIN					
Examined	0	0	0	1	
HEMORRHAGE	.	.	.	0	
MENINGES; HEMORRHAGE	.	.	.	1	
DUODENUM					
Examined	5	5	5	4	
No Visible Lesions	5	5	5	2	
VACUOLIZATION CYTOPLASM	0	0	0	2	
JEJUNUM					
Examined	5	5	5	4	
No Visible Lesions	5	5	5	3	
VACUOLIZATION CYTOPLASM	0	0	0	1	
LIVER					
Examined	5	5	5	4	
No Visible Lesions	5	5	4	3	
CLEAR CELL FOCUS	0	0	0	0	
CONGESTION	0	0	0	1	
INFILTRATE, CELLULAR; LYMPHOCYTIC	0	0	1	0	
HEPATOCYTE; DIFFUSE; VACUOLIZATION CYTOPLASM	0	0	0	0	
NO CORRELATE	0	0	0	0	
NO CORRELATE					
Examined	0	0	0	0	
NO CORRELATE	.	.	.	.	

Table 4. Incidence Summary of Microscopic Observations (Continued)

Removal Reason: ALL	Female				
	SYN548098 10 mg/kg	SYN548098 100 mg/kg	SYN548098 300 mg/kg	SYN548098 1000 mg/kg	
Number of Animals:	5	5	5	5	
ADRENAL GLANDS					
Examined	5	5	5	5	
No Visible Lesions	5	5	5	5	
ANGIECTASIS	0	0	0	0	
HYPERPLASIA	0	0	0	0	
VACUOLIZATION CYTOPLASM	0	0	0	0	
BRAIN					
Examined	0	0	0	0	
HEMORRHAGE	.	.	.	.	
MENINGES; HEMORRHAGE	.	.	.	.	
DUODENUM					
Examined	5	5	5	5	
No Visible Lesions	5	5	5	5	
VACUOLIZATION CYTOPLASM	0	0	0	0	
JEJUNUM					
Examined	5	5	5	5	
No Visible Lesions	5	5	5	5	
VACUOLIZATION CYTOPLASM	0	0	0	0	
LIVER					
Examined	5	5	5	5	
No Visible Lesions	5	4	5	5	
CLEAR CELL FOCUS	0	0	0	0	
CONGESTION	0	1	0	0	
INFILTRATE, CELLULAR; LYMPHOCYTIC	0	0	0	0	
HEPATOCYTE; DIFFUSE; VACUOLIZATION CYTOPLASM	0	0	0	0	
NO CORRELATE	0	1	0	0	
NO CORRELATE					
Examined	0	1	0	0	
NO CORRELATE	.	1	.	.	

Table 4. Incidence Summary of Microscopic Observations (Continued)

Removal Reason: ALL	Female				
	SYN548101 10 mg/kg	SYN548101 100 mg/kg	SYN548101 300 mg/kg	SYN548101 1000 mg/kg	
Number of Animals:	5	5	5	5	
ADRENAL GLANDS					
Examined	5	4	4	2	
No Visible Lesions	5	4	4	0	
ANGIECTASIS	0	0	0	0	
HYPERPLASIA	0	0	0	0	
VACUOLIZATION CYTOPLASM	0	0	0	2	
BRAIN					
Examined	0	0	0	0	
HEMORRHAGE	.	.	.	.	
MENINGES; HEMORRHAGE	.	.	.	.	
DUODENUM					
Examined	5	4	4	2	
No Visible Lesions	5	4	4	2	
VACUOLIZATION CYTOPLASM	0	0	0	0	
JEJUNUM					
Examined	5	4	4	2	
No Visible Lesions	5	4	4	2	
VACUOLIZATION CYTOPLASM	0	0	0	0	
LIVER					
Examined	5	4	4	2	
No Visible Lesions	3	4	4	2	
CLEAR CELL FOCUS	0	0	0	0	
CONGESTION	0	0	0	0	
INFILTRATE, CELLULAR; LYMPHOCYTIC	2	0	0	0	
HEPATOCYTE; DIFFUSE; VACUOLIZATION CYTOPLASM	0	0	0	0	
NO CORRELATE	0	0	0	0	
NO CORRELATE					
Examined	0	0	0	0	
NO CORRELATE	.	.	.	.	

Table 4. Incidence Summary of Microscopic Observations (Continued)

Removal Reason: ALL	Female				
	SYN54801 3 10 mg/kg	SYN54801 3 100 mg/kg	SYN54801 3 300 mg/kg	SYN54801 3 1000 mg/kg	
Number of Animals:	5	5	5	5	
ADRENAL GLANDS					
Examined	5	5	5	5	
No Visible Lesions	5	5	5	5	
ANGIECTASIS	0	0	0	0	
HYPERPLASIA	0	0	0	0	
VACUOLIZATION CYTOPLASM	0	0	0	0	
BRAIN					
Examined	0	0	0	0	
HEMORRHAGE	.	.	.	.	
MENINGES; HEMORRHAGE	.	.	.	.	
DUODENUM					
Examined	5	5	5	5	
No Visible Lesions	5	5	5	5	
VACUOLIZATION CYTOPLASM	0	0	0	0	
JEJUNUM					
Examined	5	5	5	5	
No Visible Lesions	5	5	5	5	
VACUOLIZATION CYTOPLASM	0	0	0	0	
LIVER					
Examined	5	5	5	5	
No Visible Lesions	4	4	3	5	
CLEAR CELL FOCUS	0	0	0	0	
CONGESTION	0	0	0	0	
INFILTRATE, CELLULAR; LYMPHOCYTIC	1	1	2	0	
HEPATOCYTE; DIFFUSE; VACUOLIZATION CYTOPLASM	0	0	0	0	
NO CORRELATE	0	0	0	0	
NO CORRELATE					
Examined	0	0	0	0	
NO CORRELATE	.	.	.	.	

Table 4. Incidence Summary of Microscopic Observations (Continued)

Removal Reason: ALL	Female		
	Control 0 mg/kg	Control 0 mg/kg	
Number of Animals:	5	5	
ADRENAL GLANDS			
Examined	5	5	
No Visible Lesions	4	5	
ANGIECTASIS	1	0	
HYPERPLASIA	0	0	
VACUOLIZATION CYTOPLASM	0	0	
BRAIN			
Examined	0	0	
HEMORRHAGE	.	.	
MENINGES; HEMORRHAGE	.	.	
DUODENUM			
Examined	5	5	
No Visible Lesions	5	5	
VACUOLIZATION CYTOPLASM	0	0	
JEJUNUM			
Examined	5	5	
No Visible Lesions	5	5	
VACUOLIZATION CYTOPLASM	0	0	
LIVER			
Examined	5	5	
No Visible Lesions	3	5	
CLEAR CELL FOCUS	0	0	
CONGESTION	0	0	
INFILTRATE, CELLULAR; LYMPHOCYTIC	2	0	
HEPATOCYTE; DIFFUSE; VACUOLIZATION CYTOPLASM	0	0	
NO CORRELATE	0	0	
NO CORRELATE			
Examined	0	0	
NO CORRELATE	.	.	

Table 5. Individual Animal Absolute Organ Weights

SYN547407 10 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
1	8	0.069	6.270
2	8	0.063	7.052
3	8	0.082	8.245
4	8	0.079	6.786
5	8	0.068	7.847

SYN547407 100 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
6	8	0.075	6.652
7	8	0.061	6.571
8	8	0.089	7.411
9	8	0.079	8.737
10	8	0.064	8.293

SYN547407 300 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
11	8	0.080	7.223
12	8	0.072	7.066
13	8	0.094	8.018
14	8	0.108	7.868
15	8	0.072	8.317

Table 5. Individual Animal Absolute Organ Weights (Continued)

SYN547407 1000 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
16	8	0.094	7.509
17	8	0.113	5.999
18	8	0.110	6.349
19	8	0.097	8.137
20	8	0.092	7.532

SYN546308 10 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
21	8	0.068	7.868
22	8	0.071	8.140
23	8	0.079	8.054
24	2	-	-
25	8	0.077	7.692

SYN546308 100 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
26	8	0.067	8.086
27	8	0.077	8.485
28	8	0.082	7.821
29	2	-	-
30	8	0.080	7.415

Table 5. Individual Animal Absolute Organ Weights (Continued)

SYN546308 300 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
31	8	0.064	8.422
32	8	0.074	7.822
33	8	0.071	7.921
34	8	0.093	8.329
35	8	0.094	10.127

SYN546308 1000 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
36	8	0.089	10.418
37	8	0.085	6.039
38	8	0.103	5.301
39	8	0.093	7.143
40	2	-	-

Table 5. Individual Animal Absolute Organ Weights (Continued)

SYN548097 10 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
41	8	0.074	8.014
42	8	0.078	6.901
43	8	0.070	7.925
44	4	-	-
45	8	0.062	7.870

SYN548097 100 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
46	8	0.076	8.350
47	8	0.063	9.094
48	8	0.076	8.065
49	8	0.071	7.528
50	8	0.074	7.936

Table 5. Individual Animal Absolute Organ Weights (Continued)

SYN548097 300 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
51	8	0.072	8.477
52	8	0.079	8.265
53	8	0.087	7.003
54	8	0.059	7.629
55	8	0.087	7.399

SYN548097 1000 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
56	8	0.076	8.732
57	8	0.087	7.299
58	8	0.098	7.943
59	8	-	-
60	8	0.070	6.646

Table 5. Individual Animal Absolute Organ Weights (Continued)

SYN548012 10 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
61	8	0.084	8.631
62	8	0.067	7.092
63	8	0.072	7.485
64	8	0.074	7.295
65	8	0.070	8.138

SYN54012 100 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
66	8	0.080	9.098
67	8	0.058	7.492
68	8	0.057	7.486
69	8	0.069	7.339
70	8	0.090	8.432

SYN548012 300 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
71	8	0.072	8.629
72	8	0.071	7.834
73	8	0.075	6.260
74	8	0.081	8.185
75	8	0.070	7.581

Table 5. Individual Animal Absolute Organ Weights (Continued)

SYN548012 1000 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
76	8	0.070	7.681
77	8	0.074	8.253
78	8	0.077	7.782
79	8	0.078	8.709
80	8	0.075	7.408

Table 5. Individual Animal Absolute Organ Weights (Continued)

SYN548014 10 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
81	8	0.057	7.250
82	8	0.069	7.768
83	8	0.071	6.921
84	8	0.060	7.885
85	8	0.073	7.654

SYN548014 100 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
86	8	0.079	8.220
87	8	0.065	7.984
88	8	0.089	8.227
89	8	0.068	7.957
90	8	0.072	7.368

SYN548014 300 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
91	8	0.064	8.549
92	8	0.074	7.289
93	8	0.064	6.974
94	8	0.081	8.556
95	8	0.081	7.328

Table 5. Individual Animal Absolute Organ Weights (Continued)

SYN548014 1000 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
96	8	0.070	8.288
97	8	0.069	8.518
98	8	0.059	8.643
99	8	0.080	7.010
100	8	0.077	7.350

Table 5. Individual Animal Absolute Organ Weights (Continued)

SYN548102 10 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
101	8	0.063	7.571
102	8	0.080	9.120
103	8	0.060	7.322
104	8	0.079	7.872
105	8	0.067	9.560

SYN548102 100 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
106	8	0.077	8.001
107	8	0.061	7.393
108	8	0.073	7.437
109	8	0.079	7.704
110	8	0.057	8.337

SYN548102 300 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
111	8	0.083	10.926
112	8	0.067	7.929
113	8	0.087	7.768
114	8	0.076	7.646
115	8	0.094	8.358

Table 5. Individual Animal Absolute Organ Weights (Continued)

SYN548102 1000 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
116	8	0.100	7.780
117	8	0.086	6.936
118	7	-	-
119	8	0.122	4.960
120	7	-	-

Table 5. Individual Animal Absolute Organ Weights (Continued)

SYN548117 10 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
121	8	0.068	8.056
122	8	0.067	8.579
123	8	0.054	7.854
124	8	0.071	8.486
125	8	0.076	8.547

SYN548117 100 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
126	8	0.061	6.967
127	8	0.078	8.229
128	8	0.075	8.019
129	8	0.076	8.899
130	8	0.070	8.943

SYN548117 300 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
131	8	0.080	6.916
132	8	0.081	6.131
133	8	0.068	7.211
134	8	0.086	7.940
135	8	0.075	9.858

Table 5. Individual Animal Absolute Organ Weights (Continued)

SYN548117 1000 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
136	7	-	-
137	8	0.056	5.959
138	8	0.086	7.283
139	8	0.073	6.217
140	8	0.096	6.645

Table 5. Individual Animal Absolute Organ Weights (Continued)

SYN548098 10 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
141	8	0.083	9.418
142	8	0.066	8.617
143	8	0.077	8.834
144	8	0.052	6.847
145	8	0.056	8.699

SYN548098 100 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
146	8	0.091	8.298
147	8	0.068	7.150
148	8	0.067	8.417
149	8	0.082	8.938
150	8	0.081	7.308

Table 5. Individual Animal Absolute Organ Weights (Continued)

SYN548098 300 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
151	8	0.068	7.755
152	8	0.062	6.625
153	8	0.072	7.367
154	8	0.069	7.767
155	8	0.065	6.584

SYN548098 1000 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
156	8	0.049	6.314
157	8	0.108	9.364
158	8	0.073	6.516
159	8	0.078	8.095
160	8	0.076	7.452

Table 5. Individual Animal Absolute Organ Weights (Continued)

SYN548101 10 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
161	8	0.063	8.503
162	8	0.071	8.212
163	8	0.068	8.519
164	8	0.071	8.091
165	8	0.070	7.110

SYN548101 100 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
166	2	-	-
167	8	0.063	6.974
168	8	0.070	8.417
169	8	0.078	7.256
170	8	0.082	7.282

SYN548101 300 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
171	8	0.084	8.510
172	8	0.078	8.708
173	8	0.095	7.219
174	7	-	-
175	8	0.092	7.541

Table 5. Individual Animal Absolute Organ Weights (Continued)

SYN548101 1000 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
176	6	-	-
177	5	-	-
178	8	0.084	5.495
179	6	-	-
180	8	0.096	7.454

Table 5. Individual Animal Absolute Organ Weights (Continued)

SYN548013 10 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
181	8	0.087	8.822
182	8	0.068	8.962
183	8	0.066	5.958
184	8	0.079	7.780
185	8	0.064	6.625

SYN548013 100 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
186	8	0.064	8.055
187	8	0.085	7.578
188	8	0.065	7.135
189	8	0.060	6.875
190	8	0.083	7.699

SYN548013 300 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
191	8	0.062	8.223
192	8	0.077	8.023
193	8	0.079	7.409
194	8	0.068	8.792
195	8	0.052	7.465

Table 5. Individual Animal Absolute Organ Weights (Continued)

SYN548013 1000 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
196	8	0.079	7.436
197	8	0.075	7.682
198	8	0.072	7.302
199	8	0.080	7.614
200	8	0.079	9.808

Table 5. Individual Animal Absolute Organ Weights (Continued)

Control 0 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
201	8	0.068	7.611
202	8	0.061	6.745
203	8	0.056	6.497
204	8	0.085	6.826
205	8	0.078	7.052

Control 0 mg/kg	Day of Death	Adrenal Glands (g)	Liver (g)
206	8	0.065	7.169
207	8	0.071	7.929
208	8	0.064	7.380
209	8	0.072	8.413
210	8	0.083	8.891

Table 6. Individual Animal Terminal Body Weights and Organ-to-Body Weight Ratios

SYN547407 10 mg/kg	Day of Death	Terminal BW (g)		Adrenal Glands:TBW (%)	Liver:TBW (%)
1	8	157.290		0.044	3.986
2	8	168.530	>	0.037	4.184
3	8	172.760	>	0.047	4.773
4	8	170.700	>	0.046	3.975
5	8	173.720	>	0.039	4.517

SYN547407 100 mg/kg	Day of Death	Terminal BW (g)		Adrenal Glands:TBW (%)	Liver:TBW (%)
6	8	162.330	>	0.046	4.098
7	8	168.230	>	0.036	3.906
8	8	167.760	>	0.053	4.418
9	8	177.910	>	0.044	4.911
10	8	184.230	>	0.035	4.501

SYN547407 300 mg/kg	Day of Death	Terminal BW (g)		Adrenal Glands:TBW (%)	Liver:TBW (%)
11	8	167.600	>	0.048	4.310
12	8	163.840	>	0.044	4.313
13	8	172.330	>	0.055	4.653
14	8	163.190	>	0.066	4.821
15	8	161.100	>	0.045	5.163

Table 6. Individual Animal Terminal Body Weights and Organ-to-Body Weight Ratios (Continued)

SYN547407 1000 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
16	8	152.930	0.061	4.910
17	8	124.440	0.091	4.821
18	8	157.780	0.070	4.024
19	8	151.820	0.064	5.360
20	8	158.320	0.058	4.757

Table 6. Individual Animal Terminal Body Weights and Organ-to-Body Weight Ratios (Continued)

SYN546308 10 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
21	8	164.330 >	0.041	4.788
22	8	168.070 >	0.042	4.843
23	8	175.100 >	0.045	4.600
24	2	-	-	-
25	8	170.490 >	0.045	4.512

SYN546308 100 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
26	8	177.760 >	0.038	4.549
27	8	180.640 >	0.043	4.697
28	8	167.770 >	0.049	4.662
29	2	140.350	-	-
30	8	155.010 >	0.052	4.784

SYN546308 300 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
31	8	174.560 >	0.037	4.825
32	8	167.540 >	0.044	4.669
33	8	159.400 >	0.045	4.969
34	8	176.930 >	0.053	4.708
35	8	191.940 >	0.049	5.276

Table 6. Individual Animal Terminal Body Weights and Organ-to-Body Weight Ratios (Continued)

SYN546308 1000 mg/kg	Day of Death	Terminal BW (g)		Adrenal Glands:TBW (%)	Liver:TBW (%)
36	8	180.280	>	0.049	5.779
37	8	139.090		0.061	4.342
38	8	120.430	<	0.086	4.402
39	8	160.360	>	0.058	4.454
40	2	131.140		-	-

Table 6. Individual Animal Terminal Body Weights and Organ-to-Body Weight Ratios (Continued)

SYN548097 10 mg/kg	Day of Death	Terminal BW (g)		Adrenal Glands:TBW (%)	Liver:TBW (%)
41	8	179.580	>	0.041	4.463
42	8	157.950		0.049	4.369
43	8	177.320	>	0.039	4.469
44	4	151.650		-	-
45	8	172.120	>	0.036	4.572

SYN548097 100 mg/kg	Day of Death	Terminal BW (g)		Adrenal Glands:TBW (%)	Liver:TBW (%)
46	8	181.740	>	0.042	4.594
47	8	170.250	>	0.037	5.342
48	8	173.040	>	0.044	4.661
49	8	160.280	>	0.044	4.697
50	8	177.630	>	0.042	4.468

SYN548097 300 mg/kg	Day of Death	Terminal BW (g)		Adrenal Glands:TBW (%)	Liver:TBW (%)
51	8	181.600	>	0.040	4.668
52	8	173.720	>	0.045	4.758
53	8	147.380		0.059	4.752
54	8	166.210	>	0.035	4.590
55	8	175.730	>	0.050	4.210

Table 6. Individual Animal Terminal Body Weights and Organ-to-Body Weight Ratios (Continued)

SYN548097 1000 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
56	8	182.420 >	0.042	4.787
57	8	155.010 >	0.056	4.709
58	8	176.640 >	0.055	4.497
59	8	119.360 <	-	-
60	8	165.840 >	0.042	4.007

Table 6. Individual Animal Terminal Body Weights and Organ-to-Body Weight Ratios (Continued)

SYN548012 10 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
61	8	177.970 >	0.047	4.850
62	8	171.630 >	0.039	4.132
63	8	175.880 >	0.041	4.256
64	8	169.470 >	0.044	4.305
65	8	164.560 >	0.043	4.945

SYN54012 100 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
66	8	160.640 >	0.050	5.664
67	8	169.860 >	0.034	4.411
68	8	167.080	0.034	4.480
69	8	172.900 >	0.040	4.245
70	8	177.520 >	0.051	4.750

SYN548012 300 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
71	8	179.640 >	0.040	4.803
72	8	168.880 >	0.042	4.639
73	8	157.810 >	0.048	3.967
74	8	173.160 >	0.047	4.727
75	8	183.430 >	0.038	4.133

Table 6. Individual Animal Terminal Body Weights and Organ-to-Body Weight Ratios (Continued)

SYN548012 1000 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
76	8	174.720 >	0.040	4.396
77	8	165.530 >	0.045	4.986
78	8	171.180 >	0.045	4.546
79	8	177.030 >	0.044	4.920
80	8	171.220 >	0.044	4.327

Table 6. Individual Animal Terminal Body Weights and Organ-to-Body Weight Ratios (Continued)

SYN548014 10 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
81	8	169.320 >	0.034	4.282
82	8	162.260 >	0.043	4.787
83	8	169.670 >	0.042	4.079
84	8	176.620 >	0.034	4.464
85	8	174.630 >	0.042	4.383

SYN548014 100 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
86	8	174.020 >	0.045	4.724
87	8	162.250 >	0.040	4.921
88	8	179.690 >	0.050	4.578
89	8	167.830 >	0.041	4.741
90	8	174.930 >	0.041	4.212

SYN548014 300 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
91	8	169.090 >	0.038	5.056
92	8	175.750 >	0.042	4.147
93	8	164.500 >	0.039	4.240
94	8	181.070 >	0.045	4.725
95	8	167.490 >	0.048	4.375

Table 6. Individual Animal Terminal Body Weights and Organ-to-Body Weight Ratios (Continued)

SYN548014 1000 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
96	8	170.160 >	0.041	4.871
97	8	165.810 >	0.042	5.137
98	8	179.210 >	0.033	4.823
99	8	156.420	0.051	4.482
100	8	161.080	0.048	4.563

Table 6. Individual Animal Terminal Body Weights and Organ-to-Body Weight Ratios (Continued)

SYN548102 10 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
101	8	162.530 >	0.039	4.658
102	8	188.790 >	0.042	4.831
103	8	177.810 >	0.034	4.118
104	8	168.410 >	0.047	4.674
105	8	176.050 >	0.038	5.430

SYN548102 100 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
106	8	163.760 >	0.047	4.886
107	8	168.660 >	0.036	4.383
108	8	160.680 >	0.045	4.628
109	8	177.500 >	0.045	4.340
110	8	166.910 >	0.034	4.995

SYN548102 300 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
111	8	186.090 >	0.045	5.871
112	8	169.930 >	0.039	4.666
113	8	165.980 >	0.052	4.680
114	8	170.560 >	0.045	4.483
115	8	173.590 >	0.054	4.815

Table 6. Individual Animal Terminal Body Weights and Organ-to-Body Weight Ratios (Continued)

SYN548102 1000 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
116	8	153.890	0.065	5.056
117	8	147.580	0.058	4.700
118	7	-	-	-
119	8	112.550 <	0.108	4.407
120	7	121.070 <	-	-

Table 6. Individual Animal Terminal Body Weights and Organ-to-Body Weight Ratios (Continued)

SYN548117 10 mg/kg	Day of Death	Terminal BW (g)		Adrenal Glands:TBW (%)	Liver:TBW (%)
121	8	179.440	>	0.038	4.490
122	8	180.310	>	0.037	4.758
123	8	171.490	>	0.031	4.580
124	8	173.250	>	0.041	4.898
125	8	179.240	>	0.042	4.768

SYN548117 100 mg/kg	Day of Death	Terminal BW (g)		Adrenal Glands:TBW (%)	Liver:TBW (%)
126	8	176.720	>	0.035	3.942
127	8	170.270	>	0.046	4.833
128	8	174.710	>	0.043	4.590
129	8	174.530	>	0.044	5.099
130	8	183.110	>	0.038	4.884

SYN548117 300 mg/kg	Day of Death	Terminal BW (g)		Adrenal Glands:TBW (%)	Liver:TBW (%)
131	8	157.340	>	0.051	4.396
132	8	136.700	<	0.059	4.485
133	8	169.460	>	0.040	4.255
134	8	166.730	>	0.052	4.762
135	8	178.360	>	0.042	5.527

Table 6. Individual Animal Terminal Body Weights and Organ-to-Body Weight Ratios (Continued)

SYN548117 1000 mg/kg	Day of Death	Terminal BW (g)		Adrenal Glands:TBW (%)	Liver:TBW (%)
136	7	113.460	<	-	-
137	8	125.600		0.045	4.744
138	8	137.400	<	0.063	5.301
139	8	155.230		0.047	4.005
140	8	140.760		0.068	4.721

Table 6. Individual Animal Terminal Body Weights and Organ-to-Body Weight Ratios (Continued)

SYN548098 10 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
141	8	185.150 >	0.045	5.087
142	8	175.320 >	0.038	4.915
143	8	176.840 >	0.044	4.995
144	8	156.480 >	0.033	4.376
145	8	181.600 >	0.031	4.790

SYN548098 100 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
146	8	174.750 >	0.052	4.748
147	8	168.350 >	0.040	4.247
148	8	172.790 >	0.039	4.871
149	8	185.090 >	0.044	4.829
150	8	164.390 >	0.049	4.446

SYN548098 300 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
151	8	168.390 >	0.040	4.605
152	8	161.190 >	0.038	4.110
153	8	177.130 >	0.041	4.159
154	8	178.650 >	0.039	4.348
155	8	168.970 >	0.038	3.897

Table 6. Individual Animal Terminal Body Weights and Organ-to-Body Weight Ratios (Continued)

SYN548098 1000 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
156	8	161.110 >	0.030	3.919
157	8	186.260 >	0.058	5.027
158	8	159.090 >	0.046	4.096
159	8	161.500 >	0.048	5.012
160	8	163.470 >	0.046	4.559

Table 6. Individual Animal Terminal Body Weights and Organ-to-Body Weight Ratios (Continued)

SYN548101 10 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
161	8	171.650 >	0.037	4.954
162	8	178.160 >	0.040	4.609
163	8	179.630 >	0.038	4.743
164	8	169.270 >	0.042	4.780
165	8	164.940 >	0.042	4.311

SYN548101 100 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
166	2	-	-	-
167	8	166.450 >	0.038	4.190
168	8	184.990 >	0.038	4.550
169	8	158.060 >	0.049	4.591
170	8	165.230 >	0.050	4.407

SYN548101 300 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
171	8	167.800 >	0.050	5.072
172	8	171.420	0.046	5.080
173	8	155.150	0.061	4.653
174	7	124.920	-	-
175	8	150.880	0.061	4.998

Table 6. Individual Animal Terminal Body Weights and Organ-to-Body Weight Ratios (Continued)

SYN548101 1000 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
176	6	-	-	-
177	5	-	-	-
178	8	125.540	0.067	4.377
179	6	-	-	-
180	8	155.560	0.062	4.792

Table 6. Individual Animal Terminal Body Weights and Organ-to-Body Weight Ratios (Continued)

SYN548013 10 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
181	8	179.600 >	0.048	4.912
182	8	174.670 >	0.039	5.131
183	8	166.780 >	0.040	3.572
184	8	171.500 >	0.046	4.536
185	8	160.860 >	0.040	4.118

SYN548013 100 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
186	8	167.550 >	0.038	4.808
187	8	181.700 >	0.047	4.171
188	8	171.540 >	0.038	4.159
189	8	164.000 >	0.037	4.192
190	8	178.050 >	0.047	4.324

SYN548013 300 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
191	8	183.100 >	0.034	4.491
192	8	172.270	0.045	4.657
193	8	174.910 >	0.045	4.236
194	8	178.840 >	0.038	4.916
195	8	168.050 >	0.031	4.442

Table 6. Individual Animal Terminal Body Weights and Organ-to-Body Weight Ratios (Continued)

SYN548013 1000 mg/kg	Day of Death	Terminal BW (g)	Adrenal Glands:TBW (%)	Liver:TBW (%)
196	8	165.190 >	0.048	4.501
197	8	169.840 >	0.044	4.523
198	8	169.000 >	0.043	4.321
199	8	169.490 >	0.047	4.492
200	8	184.780 >	0.043	5.308

Table 6. Individual Animal Terminal Body Weights and Organ-to-Body Weight Ratios (Continued)

Control 0 mg/kg	Day of Death	Terminal BW (g)		Adrenal Glands:TBW (%)	Liver:TBW (%)
201	8	176.300	>	0.039	4.317
202	8	157.610		0.039	4.280
203	8	155.510	>	0.036	4.178
204	8	163.440		0.052	4.176
205	8	156.450		0.050	4.508

Control 0 mg/kg	Day of Death	Terminal BW (g)		Adrenal Glands:TBW (%)	Liver:TBW (%)
206	8	178.240	>	0.036	4.022
207	8	168.620	>	0.042	4.702
208	8	163.230	>	0.039	4.521
209	8	176.620	>	0.041	4.763
210	8	186.490	>	0.045	4.768

Table 7. Individual Gross and Microscopic Observations

Animal:	1	Group:	1 - Group 1	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54740710 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	2	Group:	1 - Group 1	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54740710 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	3	Group:	1 - Group 1	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54740710 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	4	Group:	1 - Group 1	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54740710 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	5	Group:	1 - Group 1	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54740710 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	6	Group:	2 - Group 2	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN547407100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	7	Group:	2 - Group 2	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN547407100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	8	Group:	2 - Group 2	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN547407100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	9	Group:	2 - Group 2	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN547407100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	10	Group:	2 - Group 2	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN547407100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	11	Group:	3 - Group 3	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN547407300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	12	Group:	3 - Group 3	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN547407300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	13	Group:	3 - Group 3	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN547407300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	14	Group:	3 - Group 3	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN547407300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

LIVER : INFILTRATE, CELLULAR; LYMPHOCYTIC, MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	15	Group:	3 - Group 3	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN547407300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	16	Group:	4 - Group 4	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5474071000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

ADRENAL GLANDS : VACUOLIZATION CYTOPLASM; MINIMAL
LIVER : HEPATOCYTE; DIFFUSE; VACUOLIZATION CYTOPLASM; MILD

Histo Pathology - The following Tissues were Within Normal Limits:

DUODENUM; JEJUNUM

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	17	Group:	4 - Group 4	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5474071000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

DUODENUM : VACUOLIZATION CYTOPLASM; MILD
JEJUNUM : VACUOLIZATION CYTOPLASM; MILD

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	18	Group:	4 - Group 4	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5474071000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

ADRENAL GLANDS : VACUOLIZATION CYTOPLASM; MINIMAL
DUODENUM : VACUOLIZATION CYTOPLASM; MINIMAL
JEJUNUM : VACUOLIZATION CYTOPLASM; MILD

Histo Pathology - The following Tissues were Within Normal Limits:

LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	19	Group:	4 - Group 4	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5474071000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

ADRENAL GLANDS : VACUOLIZATION CYTOPLASM; MINIMAL
JEJUNUM : VACUOLIZATION CYTOPLASM; MILD

Histo Pathology - The following Tissues were Within Normal Limits:

DUODENUM

Histo Pathology - The following Protocol Required Tissues were Not Processed:

LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	20	Group:	4 - Group 4	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5474071000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

ADRENAL GLANDS : VACUOLIZATION CYTOPLASM; MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	21	Group:	5 - Group 5	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54630810 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	22	Group:	5 - Group 5	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54630810 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	23	Group:	5 - Group 5	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54630810 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	24	Group:	5 - Group 5	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54630810 mg/kg		
Death Date:	1/14/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	2 (1)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LACRIMAL GLAND; LIVER; SPINAL CORD

Histo Pathology - The following Protocol Required Tissues were Not Processed:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	25	Group:	5 - Group 5	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54630810 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	26	Group:	6 - Group 6	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN546308100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	27	Group:	6 - Group 6	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN546308100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	28	Group:	6 - Group 6	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN546308100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	29	Group:	6 - Group 6	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN546308100 mg/kg		
Death Date:	1/14/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	2 (1)		

Gross Pathology Observations [Correlation]:

EYES : BILATERAL; DISCOLORATION(S) : opaque

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology - The following Protocol Required Tissues were Not Processed:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	30	Group:	6 - Group 6	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN546308100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	31	Group:	7 - Group 7	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN546308300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	32	Group:	7 - Group 7	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN546308300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

ADRENAL GLANDS : HYPERPLASIA
LIVER : INFILTRATE, CELLULAR; LYMPHOCYTIC, MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

DUODENUM; JEJUNUM

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	33	Group:	7 - Group 7	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN546308300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	34	Group:	7 - Group 7	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN546308300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	35	Group:	7 - Group 7	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN546308300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

DUODENUM : VACUOLIZATION CYTOPLASM; MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	36	Group:	8 - Group 8	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5463081000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

ADRENAL GLANDS : VACUOLIZATION CYTOPLASM; MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	37	Group:	8 - Group 8	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5463081000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

DUODENUM : VACUOLIZATION CYTOPLASM; MILD
JEJUNUM : VACUOLIZATION CYTOPLASM; MILD

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	38	Group:	8 - Group 8	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5463081000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

DUODENUM : VACUOLIZATION CYTOPLASM; MILD
JEJUNUM : VACUOLIZATION CYTOPLASM; MILD

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	39	Group:	8 - Group 8	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5463081000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

ADRENAL GLANDS : VACUOLIZATION CYTOPLASM; MINIMAL
DUODENUM : VACUOLIZATION CYTOPLASM; MILD
JEJUNUM : VACUOLIZATION CYTOPLASM; MILD

Histo Pathology - The following Tissues were Within Normal Limits:

LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	40	Group:	8 - Group 8	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5463081000 mg/kg		
Death Date:	1/14/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	2 (1)		

Gross Pathology Observations [Correlation]:

EYES : BILATERAL; DISCOLORATION(S) : opaque

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology - The following Protocol Required Tissues were Not Processed:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	41	Group:	9 - Group 9	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54809710 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	42	Group:	9 - Group 9	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54809710 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	43	Group:	9 - Group 9	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54809710 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	44	Group:	9 - Group 9	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54809710 mg/kg		
Death Date:	1/16/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	4 (1)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology - The following Protocol Required Tissues were Not Processed:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	45	Group:	9 - Group 9	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54809710 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	46	Group:	10 - Group 10	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548097100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	47	Group:	10 - Group 10	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548097100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	48	Group:	10 - Group 10	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548097100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	49	Group:	10 - Group 10	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548097100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

LIVER : CLEAR CELL FOCUS
LIVER : INFILTRATE, CELLULAR; LYMPHOCYTIC, MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	50	Group:	10 - Group 10	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548097100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	51	Group:	11 - Group 11	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548097300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	52	Group:	11 - Group 11	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548097300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	53	Group:	11 - Group 11	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548097300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	54	Group:	11 - Group 11	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548097300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	55	Group:	11 - Group 11	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548097300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	56	Group:	12 - Group 12	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480971000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	57	Group:	12 - Group 12	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480971000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	58	Group:	12 - Group 12	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480971000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	59	Group:	12 - Group 12	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480971000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Found Dead		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology - The following Protocol Required Tissues were Not Processed:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	60	Group:	12 - Group 12	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480971000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	61	Group:	13 - Group 13	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54801210 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	62	Group:	13 - Group 13	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54801210 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	63	Group:	13 - Group 13	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54801210 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	64	Group:	13 - Group 13	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54801210 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	65	Group:	13 - Group 13	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54801210 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	66	Group:	14 - Group 14	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54012100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	67	Group:	14 - Group 14	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54012100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	68	Group:	14 - Group 14	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54012100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	69	Group:	14 - Group 14	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54012100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	70	Group:	14 - Group 14	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54012100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	71	Group:	15 - Group 15	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548012300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

LIVER : INFILTRATE, CELLULAR; LYMPHOCYTIC, MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	72	Group:	15 - Group 15	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548012300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	73	Group:	15 - Group 15	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548012300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	74	Group:	15 - Group 15	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548012300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	75	Group:	15 - Group 15	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548012300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	76	Group:	16 - Group 16	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480121000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	77	Group:	16 - Group 16	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480121000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

LIVER : INFILTRATE, CELLULAR; LYMPHOCYTIC, MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	78	Group:	16 - Group 16	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480121000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	79	Group:	16 - Group 16	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480121000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	80	Group:	16 - Group 16	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480121000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	81	Group:	17 - Group 17	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54801410 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	82	Group:	17 - Group 17	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54801410 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	83	Group:	17 - Group 17	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54801410 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	84	Group:	17 - Group 17	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54801410 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	85	Group:	17 - Group 17	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54801410 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	86	Group:	18 - Group 18	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548014100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	87	Group:	18 - Group 18	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548014100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	88	Group:	18 - Group 18	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548014100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

LIVER : INFILTRATE, CELLULAR; LYMPHOCYTIC, MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	89	Group:	18 - Group 18	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548014100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	90	Group:	18 - Group 18	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548014100 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	91	Group:	19 - Group 19	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548014300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	92	Group:	19 - Group 19	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548014300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	93	Group:	19 - Group 19	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548014300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	94	Group:	19 - Group 19	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548014300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	95	Group:	19 - Group 19	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548014300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	96	Group:	20 - Group 20	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480141000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	97	Group:	20 - Group 20	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480141000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	98	Group:	20 - Group 20	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480141000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	99	Group:	20 - Group 20	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480141000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	100	Group:	20 - Group 20	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480141000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	101	Group:	21 - Group 21	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54810210 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	102	Group:	21 - Group 21	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54810210 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	103	Group:	21 - Group 21	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54810210 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	104	Group:	21 - Group 21	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54810210 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	105	Group:	21 - Group 21	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54810210 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	106	Group:	22 - Group 22	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548102100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	107	Group:	22 - Group 22	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548102100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	108	Group:	22 - Group 22	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548102100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	109	Group:	22 - Group 22	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548102100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	110	Group:	22 - Group 22	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548102100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	111	Group:	23 - Group 23	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548102300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	112	Group:	23 - Group 23	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548102300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	113	Group:	23 - Group 23	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548102300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	114	Group:	23 - Group 23	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548102300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	115	Group:	23 - Group 23	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548102300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	116	Group:	24 - Group 24	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5481021000 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

ADRENAL GLANDS : VACUOLIZATION CYTOPLASM; MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	117	Group:	24 - Group 24	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5481021000 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

ADRENAL GLANDS : VACUOLIZATION CYTOPLASM; MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	118	Group:	24 - Group 24	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5481021000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	7 (1)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology - The following Protocol Required Tissues were Not Processed:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	119	Group:	24 - Group 24	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5481021000 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

BRAIN : MENINGES; DISCOLORATION(S); DARK

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

ADRENAL GLANDS : VACUOLIZATION CYTOPLASM; MINIMAL
BRAIN : Hemorrhage appears peracute without a cellular reaction; considered to be an agonal finding.
BRAIN : HEMORRHAGE; MILD

Histo Pathology - The following Tissues were Within Normal Limits:

DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	120	Group:	24 - Group 24	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5481021000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Found Dead		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	7 (1)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology - The following Protocol Required Tissues were Not Processed:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	121	Group:	25 - Group 25	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54811710 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	122	Group:	25 - Group 25	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54811710 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	123	Group:	25 - Group 25	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54811710 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	124	Group:	25 - Group 25	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54811710 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	125	Group:	25 - Group 25	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54811710 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	126	Group:	26 - Group 26	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548117100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	127	Group:	26 - Group 26	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548117100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	128	Group:	26 - Group 26	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548117100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	129	Group:	26 - Group 26	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548117100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	130	Group:	26 - Group 26	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548117100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	131	Group:	27 - Group 27	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548117300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	132	Group:	27 - Group 27	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548117300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

LIVER : INFILTRATE, CELLULAR; LYMPHOCYTIC, MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	133	Group:	27 - Group 27	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548117300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	134	Group:	27 - Group 27	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548117300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	135	Group:	27 - Group 27	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548117300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	136	Group:	28 - Group 28	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5481171000 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Found Dead		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	7 (1)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology - The following Protocol Required Tissues were Not Processed:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	137	Group:	28 - Group 28	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5481171000 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

BRAIN : MENINGES; DISCOLORATION(S); DARK
LIVER : DISCOLORATION(S); DARK

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; DUODENUM; JEJUNUM; SPINAL CORD

Histo Pathology Observations [Correlation]:

BRAIN : Hemorrhage is peracute without cellular reaction and appears to be agonal.
BRAIN : MENINGES; HEMORRHAGE; MINIMAL
LIVER : CONGESTION; MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	138	Group:	28 - Group 28	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5481171000 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

DUODENUM : VACUOLIZATION CYTOPLASM; MILD

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	139	Group:	28 - Group 28	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5481171000 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	140	Group:	28 - Group 28	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5481171000 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

DUODENUM : VACUOLIZATION CYTOPLASM; MILD
JEJUNUM : VACUOLIZATION CYTOPLASM; MILD

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	141	Group:	29 - Group 29	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54809810 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	142	Group:	29 - Group 29	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54809810 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	143	Group:	29 - Group 29	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54809810 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	144	Group:	29 - Group 29	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54809810 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	145	Group:	29 - Group 29	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54809810 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	146	Group:	30 - Group 30	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548098100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	147	Group:	30 - Group 30	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548098100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	148	Group:	30 - Group 30	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548098100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	149	Group:	30 - Group 30	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548098100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

LIVER : LEFT LATERAL LOBE; DEFORMITY : Deformity (G1, left lobe) = agenesis
LIVER : CAUDATE LOBE; ENLARGED : caudate lobe enlarged = 2x
LIVER : RIGHT LATERAL LOBE; ANTERIOR; ENLARGED : right anterior enlarged = 2x
LIVER : RIGHT LATERAL LOBE; POSTERIOR; ENLARGED : right posterior lobe = 2x enlarged
LIVER : MEDIAN LOBE; SMALL : small = 0.5x

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; SPINAL CORD

Histo Pathology Observations [Correlation]:

LIVER : CONGESTION; MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	150	Group:	30 - Group 30	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548098100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	151	Group:	31 - Group 31	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548098300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	152	Group:	31 - Group 31	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548098300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	153	Group:	31 - Group 31	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548098300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	154	Group:	31 - Group 31	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548098300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	155	Group:	31 - Group 31	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548098300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	156	Group:	32 - Group 32	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480981000 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	157	Group:	32 - Group 32	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480981000 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	158	Group:	32 - Group 32	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480981000 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	159	Group:	32 - Group 32	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480981000 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	160	Group:	32 - Group 32	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480981000 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	161	Group:	33 - Group 33	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54810110 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	162	Group:	33 - Group 33	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54810110 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	163	Group:	33 - Group 33	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54810110 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

LIVER : INFILTRATE, CELLULAR; LYMPHOCYTIC, MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	164	Group:	33 - Group 33	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54810110 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

LIVER : INFILTRATE, CELLULAR; LYMPHOCYTIC, MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	165	Group:	33 - Group 33	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54810110 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	166	Group:	34 - Group 34	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548101100 mg/kg		
Death Date:	1/15/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	2 (1)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology - The following Protocol Required Tissues were Not Processed:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	167	Group:	34 - Group 34	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548101100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	168	Group:	34 - Group 34	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548101100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	169	Group:	34 - Group 34	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548101100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	170	Group:	34 - Group 34	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548101100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	171	Group:	35 - Group 35	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548101300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	172	Group:	35 - Group 35	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548101300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	173	Group:	35 - Group 35	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548101300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	174	Group:	35 - Group 35	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548101300 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Found Dead		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	7 (1)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology - The following Protocol Required Tissues were Not Processed:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	175	Group:	35 - Group 35	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548101300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	176	Group:	36 - Group 36	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5481011000 mg/kg		
Death Date:	1/19/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	6 (1)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology - The following Protocol Required Tissues were Not Processed:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	177	Group:	36 - Group 36	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5481011000 mg/kg		
Death Date:	1/18/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	5 (1)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology - The following Protocol Required Tissues were Not Processed:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	178	Group:	36 - Group 36	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5481011000 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

ADRENAL GLANDS : VACUOLIZATION CYTOPLASM; MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	179	Group:	36 - Group 36	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5481011000 mg/kg		
Death Date:	1/19/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	6 (1)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology - The following Protocol Required Tissues were Not Processed:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	180	Group:	36 - Group 36	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5481011000 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

ADRENAL GLANDS : VACUOLIZATION CYTOPLASM; MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	181	Group:	37 - Group 37	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54801310 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	182	Group:	37 - Group 37	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54801310 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

LIVER : INFILTRATE, CELLULAR; LYMPHOCYTIC, MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	183	Group:	37 - Group 37	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54801310 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	184	Group:	37 - Group 37	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54801310 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	185	Group:	37 - Group 37	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN54801310 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	186	Group:	38 - Group 38	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548013100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

LIVER : INFILTRATE, CELLULAR; LYMPHOCYTIC, MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	187	Group:	38 - Group 38	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548013100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	188	Group:	38 - Group 38	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548013100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	189	Group:	38 - Group 38	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548013100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	190	Group:	38 - Group 38	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548013100 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	191	Group:	39 - Group 39	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548013300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

LIVER : INFILTRATE, CELLULAR; LYMPHOCYTIC, MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	192	Group:	39 - Group 39	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548013300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

LIVER : INFILTRATE, CELLULAR; LYMPHOCYTIC, MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	193	Group:	39 - Group 39	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548013300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	194	Group:	39 - Group 39	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548013300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	195	Group:	39 - Group 39	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN548013300 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	196	Group:	40 - Group 40	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480131000 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	197	Group:	40 - Group 40	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480131000 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	198	Group:	40 - Group 40	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480131000 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	199	Group:	40 - Group 40	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480131000 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	200	Group:	40 - Group 40	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	SYN5480131000 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	201	Group:	41 - Group 41	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	Control0 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

LIVER : INFILTRATE, CELLULAR; LYMPHOCYTIC, MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	202	Group:	41 - Group 41	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	Control0 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

ADRENAL GLANDS : ANGIECTASIS; MINIMAL
LIVER : INFILTRATE, CELLULAR; LYMPHOCYTIC, MINIMAL

Histo Pathology - The following Tissues were Within Normal Limits:

DUODENUM; JEJUNUM

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	203	Group:	41 - Group 41	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	Control0 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	204	Group:	41 - Group 41	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	Control0 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	205	Group:	41 - Group 41	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	Control0 mg/kg		
Death Date:	1/20/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/20/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	206	Group:	42 - Group 42	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	Control0 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	207	Group:	42 - Group 42	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	Control0 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	208	Group:	42 - Group 42	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	Control0 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	209	Group:	42 - Group 42	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	Control0 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER

Table 7. Individual Gross and Microscopic Observations (Continued)

Animal:	210	Group:	42 - Group 42	Sex:	Female
Species:	Rat	Strain:	Wistar		
Study Type:	Acute (1-14 days) - Oral	Route:	Oral Gavage		
Material:	Test Article 1	Dose:	Control0 mg/kg		
Death Date:	1/21/2014	Removal Reason:	Core Necropsy		
Necropsy Date:	1/21/2014	Study Day (Week) of Death:	8 (2)		

Gross Pathology Observations [Correlation]:

Gross Pathology - The following Tissues had No Visible Lesions:

ADRENAL GLANDS; BRAIN; DUODENUM; JEJUNUM; LIVER; SPINAL CORD

Histo Pathology Observations [Correlation]:

Histo Pathology - The following Tissues were Within Normal Limits:

ADRENAL GLANDS; DUODENUM; JEJUNUM; LIVER