CALVERT
LABORATORIES, INC.

Study Protocol

Title: 5-Day Intraperitoneal Toxicity Study in Rats

Calvert Study No.: 0440RE27.001

Testing Facility:
Calvert Laboratories, Inc.
Scott Technology Park
100 Discovery Drive
Olyphant, PA 18447

Study Sponsor:
Emory University
1515 Dickey Drive
Atlanta, GA 30322

I. Table Of Contents

II. Introduction

A. Title

5-Day Intraperitoneal Toxicity Study in Rats

B. Objective

The purpose of this study is to evaluate the toxicity of a test article when administered intraperitoneally to Sprague Dawley rats once daily for a minimum of 5 consecutive days.

C. Regulatory Compliance

This is a non-regulated study. However, it will be run according to the Standard Operating Procedures (SOP's) of Calvert. There will be no formal involvement of the Quality Assurance Unit.

D. Calvert Study Number

0440RE27.001

E. Testing Facility

Calvert Laboratories, Inc. (Calvert)
Scott Technology Park
100 Discovery Drive
Olyphant, PA 18447

F. Sponsor

Emory University
1515 Dickey Drive
Atlanta, GA 30322

G. Study Director

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H. Sponsor's Representative

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I. Key Study Dates

Proposed Experimental Start Date:	12 Dec 2007
Proposed First Day of Dosing:	17 Dec 2007
Proposed Necropsy:	21 Dec 2007
Proposed Experimental Completion Date:	21 Dec 2007

III. Materials and Methods

A. Test Article

1. Test Article

Identification:	GB67B
Lot/Batch No.:	To be documented in study data
Physical Description:	White powder
Storage Conditions:	Room Temperature, protected from light

2. *Vehicle*

Identification:	DMSO:45% beta-cyclodextrin (10:90)
Lot/Batch No.:	To be documented in study data
Physical Description:	Semi-viscous solution
Storage Conditions:	Room temperature

3. *Dose Preparation*

Test article will be prepared according to instructions provided by the sponsor and Calvert SOPs on test article formulation.

4. *Accountability and Disposition*

Unused test article will be returned to the Sponsor or designee at the termination of this study or, if necessary, retained for use on related future studies. The Sponsor will be notified in advance of shipping and a transmittal letter will accompany the shipment. The material will be packed in a suitable container to maintain the conditions specified by the Sponsor during transit plus an adequate margin of safety to account for any possible transit delays.

B. Test System (Animals and Animal Care)

1. *Description*

Species:	Rat
Stock:	Sprague-Dawley - Hsd:SD
Total Number:	36 (18 males and 18 females)
Gender:	Male and female
Age Range:	5-7 weeks at start of dosing; records of dates of birth for animals used in this study will be retained in the Calvert archives.
Body Weight Range:	125-250 grams for males and females at the outset (Day 1) of the study.

Animal Source:	Harlan Sprague-Dawley
Experimental History:	Purpose-bred and experimentally naïve at the outset of the study.
Identification:	Eartag

2. *Rationale for Choice of Species and Number of Animals*

The conduct of a toxicity study in a rodent species is a requirement of worldwide regulatory agencies. The rat is a standard species used in toxicology studies based upon the substantial amounts of published historical data (1).

The number of animals used in this study is considered to be the minimum number necessary to evaluate the potential toxicity of the test article and to account for the inter-animal variability.

3. *Husbandry*

Housing:	Animals will be group-housed by sex upon receipt and individually housed upon assignment to study in compliance with National Research Council "Guide for the Care and Use of Laboratory Animals". The room in which the animals will be kept will be documented in the study records.
Lighting:	12 hours light/12 hours dark, except when room lights will be turned on during the dark cycle to accommodate blood sampling or other study procedures.
Room Temperature:	18 to 26°C
Relative Humidity:	30-70%
Food:	All animals will have access to Harlan Teklad Rodent Diet (certified) or equivalent, *ad libitum*, unless otherwise specified. The lot number(s) and specifications of each lot used are archived

at Calvert. No contaminants are known to be present in the certified diet at levels that would be expected to interfere with the results of this study. Analysis of the diet was limited to that performed by the manufacturer, records of which will be maintained in the Calvert archives.

Water: Water will be available *ad libitum,* to each animal via an automatic watering device. The water is routinely analyzed for contaminants as per Calvert SOP's. No contaminants are known to be present in the water at levels that would be expected to interfere with the results of this study. Results of the water analysis will be maintained in the Calvert archives.

Acclimation: Study animals will be acclimated to their housing for a minimum of 5 days prior to their first day of dosing.

4. *Prestudy Health Screen and Selection Criteria*

All animals received for this study will be assessed as to their general health by a member of the veterinary staff or other authorized personnel. During the acclimation period, each animal will be observed at least once daily for any abnormalities or for the development of infectious disease. Only animals that are determined by the veterinary staff and/or Study Director to be suitable for use will be assigned to this study. Any animals considered unacceptable for use in this study will be replaced with animals of similar age and weight from the same vendor.

5. *Assignment to Study Groups*

Animals will be assigned to study groups by a computerized randomization program (LABCAT In Life module version 8.0, developed by Innovative Program Associates, Inc. 303 Wall Street, Princeton, NJ 08540-1515) designed to achieve similar group mean body weights.

6. *Humane Care of Animals*

Treatment of animals will be in accordance with the study protocol and also in accordance with Calvert SOP's which adhere to the regulations outlined in the USDA Animal Welfare Act (9 CFR Parts 1, 2 and 3) and the conditions specified in the Guide for the Care and Use of Laboratory Animals (ILAR publication, 1996, National Academy Press). The Calvert Institutional Animal Care and Use Committee (IACUC) will approve the study protocol prior to finalization to insure compliance with acceptable standard animal welfare and humane care.

No alternative test systems exist which have been adequately validated to permit replacement of the use of live animals in this study. Every effort has been made to obtain the maximum amount of information while reducing to a minimum the number of animals required for this study. The assessment of pain and distress in study animals and the use or non-use of pain alleviating medications will be in accordance with Standard Operating Procedure VET-19, Criteria for Assessing Pain and Distress in Laboratory Animals. The study will be terminated in part or whole for humane reasons if unnecessary pain occurs. To the best of our knowledge, this study is not unnecessary or duplicative.

C. Test Article Administration

1. *Group Assignments and Dose Levels*

Group	Dose Level (mg/kg)	Concentration (mg/ml)	Dose Volume (ml/kg)	Number of Animals	
				Male	Female
1. Vehicle	0	0	10	3	3
2. Low dose	100	10	10	3	3
3. Low-mid dose	200	20	10	3	3
4. Mid dose	500	50	10	3	3
5. Mid-high dose	1000	100	10	3	3
6. High dose	2000	200	10	3	3

2. *Dosing*

Route: Intraperitoneally

Frequency: Once daily for a minimum of 5 consecutive days.

Procedure: Doses will be administered once daily for 5 consecutive days. Each animal will receive a specific mg/kg dose based upon its most recent body weight.

3. *Justification for Route, Dose Levels and Dosing Schedule*

The intraperitoneal route was chosen to avoid complications from administering all doses of test article.

Dose levels were selected by the Sponsor based on the lack of toxicity in preliminary toxicology studies in mice.

D. In-Life Observations and Measurements

1. *Mortality/Morbidity*

Frequency: Twice daily (a.m. and p.m.) on Days 1-5 and once prior to sacrifice on Day 6.

Each animal observed for evidence of death or impending death (as per Calvert SOP VET-14).

2. *Clinical Observations*

Frequency: Prior to dose administration and a minimum of once post-dose (approximately 2 hours post-dose) on Days 1-5.

Animals will be observed once prior to terminal sacrifice on Day 6.

3. *Body Weight*

Frequency: Body weight will be recorded for all animals at the time of randomization/selection, prior to dose administration on Day 1 and following final dose administration on Day 5.

A fasted body weight will be recorded prior to sacrifice on Day 6.

4. *Food Consumption*

Frequency: Full feeder weights and/or feeder weigh backs will be recorded on Day 1 and Day 5 for determination of food consumption.

E. Clinical Pathology Evaluation

1. *Sample Collection*

Blood samples for evaluation of hematology and serum chemistry parameters will be collected from all animals prior to sacrifice on Day 6. Animals will be anesthetized by CO_2 inhalation prior to blood collection. Animals will be fasted overnight (approximately 12-24 hours) prior to blood collection for clinical pathology evaluation.

2. *Collection Procedures, Processing and Analysis*

a) Hematology

Method of Collection: Cardiocentesis

Anticoagulant: EDTA

Parameters Analyzed:

Hematology Parameters	
Red Blood Cell Count (RBC) and Morphology	Platelet count (PLT)
White Blood Cell Count (WBC)*	Hematocrit (HCT)
Mean Corpuscular Hemoglobin (MCH)	Hemoglobin (HGB)
Mean Corpuscular Hemoglobin Concentration (MCHC)	Reticulocyte Count (Retic)
Mean Corpuscular Volume (MCV)	

*Total and differential white blood cell counts, including neutrophils, basophils, eosinophils, monocytes, lymphocytes and large unstained cells

b) Serum Clinical Chemistry

Method of Collection: Cardiocentesis

Anticoagulant: None

Parameters Analyzed:

Clinical Chemistry Parameters	
Alanine Aminotransferase (ALT)	Globulin (calculated)(GLOB)
Albumin (ALB)	Glucose (GLU)
Albumin/Globulin ratio (calculated)(A/G)	Phosphorus (PHOS)
Alkaline Phosphatase (ALP)	Potassium (K)
Aspartate Aminotransferase (AST)	Sodium (NA)
Calcium (CA)	Total Bilirubin (T-BIL)
Chloride (CL)	Total Protein (TP)
Cholesterol (CHOL)	Triglycerides (TRIG)
Creatinine (CREAT)	Urea Nitrogen (BUN)

F. Terminal Procedures and Anatomic Pathology

1. Termination

a) Scheduled Sacrifice

All surviving animals will be euthanized by CO_2 asphyxiation on Day 6.

b) Final Body Weight

A fasted terminal body weight will be recorded prior to sacrifice on Day 6. This body weight will be used to calculate organ to body weight ratios.

2. Gross Necropsy

A complete gross necropsy will be performed by Calvert personnel on all animals that are sacrificed or found dead during the study. The necropsy will include examination of:

- the external body surface
- all orifices
- the cranial, thoracic and abdominal cavities and their contents.

All abnormalities will be described completely and recorded. If a necropsy cannot be performed immediately on any animal found dead, it will be refrigerated until necropsy can be performed. All animals necropsied will have tissues collected and preserved as designated

below in Section 4.

3. *Organ Weights*

At scheduled sacrifice on Day 6, the following organs (when present) will be weighed before fixation, after dissection of excess fat and other excess tissues. Paired organs will be weighed together unless gross abnormalities are present, in which case they will be weighed separately. The thyroids/parathyroids will be weighed fixed at Calvert. Organs will not be weighed for animals found dead or sacrificed moribund.

Organs Weighed	
Adrenals	Ovaries
Brain	Spleen
Heart	Testes
Kidneys	Thyroids/parathyroids
Liver	

Organ to body weight ratios will be calculated (using the final body weight obtained prior to necropsy), as well as organ to brain weight ratios.

4. *Tissue Collection and Preservation*

For all animals necropsied, the tissues listed in the table below will be preserved in 10% neutral buffered formalin (except for the testes that will be preserved in Bouin's fixative).

Tissues Collected	
Cardiovascular	**Urogenital**
Aorta	Kidneys
Heart	Urinary Bladder
Digestive	Ovaries
Salivary gland(s)	Uterus
Tongue	Cervix
Esophagus	Vagina
Stomach	Testes
Small Intestine	Epididymides
Duodenum	Prostate
Jejunum	**Endocrine**

Ileum	Adrenals
Large Intestine	Pituitary
Cecum	Thyroid/Parathyroid
Colon	**Skin/Musculoskeletal**
Rectum	Skin
Pancreas	Mammary Gland
Liver	Skeletal Muscle (thigh)
Respiratory	Femur with articular surface
Trachea	**Nervous/Special Sense**
Larynx	Eye with optic nerve
Lung with mainstem bronchus	Sciatic Nerve
Lymphoid/Hematopoietic	Brain
Sternum with bone marrow	Spinal Cord – cervical
Thymus	Spinal Cord – midthoracic
Spleen	Spinal Cord – lumbar
Lymph Nodes	Lacrimal Glands
Mandibular	**Other**
Mesenteric	Unique Animal Identifier (not for evaluation)
	Gross Findings

5. *Histopathology*

All tissues will be saved for possible future histopathological evaluation.

IV. Records and Reports

A. Statistical Analysis

Data will be collected and/or evaluated using LABCAT In Life module version 8.0, LABCAT Clinical Pathology module version 8.0 and LABCAT Organ Weights/Necropsy module version 3.28. All LABCAT modules were developed by Innovative Program Associates, Inc. (303 Wall Street, Princeton, NJ 08540-1515).

Body weight, food consumption, hematology, clinical chemistry and organ weight data will be evaluated. If the data is homogeneous, the evaluation of the equality of means will be made by a one-way analysis of variance using

the F distribution to assess statistical significance. If statistically significant differences between the means are found, Dunnett's test will then be used to determine the degree of significance from the control means ($p < 0.05$ and $p< 0.01$). If the data is non-homogeneous, the Kuskal-Wallis test will be used to assess statistical significance. If statistically significant differences are found, Dunnett's test will then be used to determine the degree of significance from the control means ($p < 0.05$ and $p< 0.01$).

B. Storage of Records

Test article preparation, test article tracking, in-life data, necropsy data, clinical pathology data, protocol, protocol amendments (if applicable) and draft report(s) that have been submitted to a regulatory agency, and the original final report generated as a result of this study will be archived at Calvert, 105 Edella Road, Suite 100, Clarks Summit, PA 18411. After 2 years, the Sponsor will be contacted to determine final disposition of all study materials.

Six months following the submission of the audited draft report, if there are no client comments generated by the Sponsor and/or Study Monitor, the Sponsor/Study Monitor will be notified and the report will be finalized and archived according to the terms stated in the protocol.

V. Miscellaneous

A. Confidentiality Statement

The information contained herein is for the personal use of the intended recipient(s).

B. References

1. Gad, Shayne C. (ed.). (1995). *Safety Assessment for Pharmaceuticals.* Van Nostrand Reinhold, New York, pp. 111-128.

2. Speid, L.H., Lumley, C.E. and Walker, S.R. (1990), Harmonization of Guidelines for Toxicity Testing of Pharmaceuticals by 1992. *Regulatory Toxicology and Pharmacology*, 12:179-211.

VI. Protocol Approval Signatures

Jill L. Fogleman, Ph.D.
Study Director
Calvert Laboratories, Inc.

11 Dec 2007
Date

Scientific Management
Calvert Laboratories, Inc.

9 Dec 07
Date

IACUC
Calvert Laboratories, Inc.

7 DEC 2007
Date

Gregory Bluemling
Sponsor Representative
Emory University

12-6-2007
Date