TOXICOLOGY PATHOLOGY SERVICES

T.P.S. Inc., 10424 Middle Mt. Vernon Road, Mount Vernon, Indiana 47620 - Tel: 812/985-5900 - FAX 812/985-3403

IQB-9302: AN ACUTE SUBCUTANEOUS TOXICITY STUDY IN BEAGLE DOGS

FOR

LABORATORIOS INIBSA
CRTA DE SABADELL A GRANOLLERS, KM.14.5
08185 LLICA DE VALL (BARCELONA)
SPAIN

Study Director
L. J. Clare, D.V.M.

Performing Laboratory
T.P.S., Inc.
10424 Middle Mt. Vernon Road
Mt. Vernon, Indiana 47620

T.P.S. Study Number
616B-502-410-98

Sponsor I.D. No.:
030

CERTIFICATION OF GOOD LABORATORY PRACTICE

The enclosed report for T.P.S. Study No. 616B-502-410-98 accurately describes the methods and procedures used in the study and accurately reflects the raw data obtained. The study was conducted in compliance with the FDA Good Laboratory Practice for Nonclinical Laboratory Studies regulations as described in the Federal Register: 21 CFR Part 58. There were no differences discovered between practices used in conducting the study and those required by Good Laboratory Practice regulations.

3-17-99

L. J. Clare, D.V.M.
Study Director
T.P.S., Inc.

QUALITY ASSURANCE STATEMENT

Quality Assurance inspections of Study 616B-502-410-98 were made and the findings reported on the following dates:

11-09-98
11-18-98
12-09-98
01-19-99

This study was conducted in accordance with FDA Good Laboratory Practice for Nonclinical Laboratory Studies regulations (21 CFR 58). Data reported were compared to original raw data records and found to be accurate.

G L Ingram 12MAR99
G. L. Ingram, B.S.
Quality Assurance Auditor
T.P.S., Inc.

M J Bandoli 17 Mar 99
M. J. Bandoli, M.S.
Director of Quality Assurance
T.P.S., Inc.

T.P.S. Study No.: 616B-502-410-98
Sponsor Study No.: 030

T.P.S. Inc., 10424 Middle Mt. Vernon Road, Mount Vernon, Indiana 47620 - Tel: 812/985-5900 - FAX 812/985-3403

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FOR

LABORATORIOS INIBSA
CRTA DE SABADELL A GRANOLLERS, KM.14.5
08185 LLICA DE VALL (BARCELONA)
SPAIN

T.P.S. Study No.: 616B-502-410-98

Study Initiation:	10-28-98
Animal Phase Initiation:	11-09-98
Animal Phase Termination:	11-18-98

REPORTED BY:

L. J. Clare, D.V.M. 3-17-99
L. J. Clare, D.V.M.
Study Director
T.P.S., Inc.

APPROVED FOR RELEASE BY:

John B. Botta 3-17-99
J. B. Botta, B.S., B.A.
President
T.P.S., Inc.

TABLE OF CONTENTS

IQB-9302: AN ACUTE SUBCUTANEOUS TOXICITY STUDY IN BEAGLE DOGS

SUMMARY

One male and one female adult beagle dogs were dosed once subcutaneously with 2.5, 5.0, 10.0, 20.0, and 40.0 mg IQB-9302/kg body weight. Doses were administered every other day (Monday, Wednesday, Friday). The test article was dissolved in 0.9% Saline for Injection, USP at concentrations that allowed the appropriate dosage to be delivered in a volume of 5 mL/kg body weight. To avoid pressure necrosis at the injection sites, dose volumes were divided and administered at different sites on the back such that no more than 10 mL was injected at any one site. Subsequent doses were increased based on results of the previous dosage. Body weights obtained just prior to the first dose and weekly thereafter were used to determine the appropriate dosage to be administered. Each dog was observed for clinical effects hourly for up to 6 hours following dosing and a minimum of twice daily throughout the evaluation.

No clinical signs or evidence of adverse local or systemic toxicity were noted in either dog at dosages of 2.5, 5.0, 10.0, and 20.0 mg IQB-9302/kg. Both dogs were found dead 1 hour following administration of the 40.0 mg/kg dosage. No clinical signs were noted prior to death. Gross necropsy examination of both dogs revealed white foam in the stomach, red tinged foam in and around the mouth, mottled light and dark areas on the spleen, a red thymus, and mottled light and dark areas on all lung lobes. One dog also had a white plague on the medial margin of the left diaphragmatic lung lobe. All injection sites from both dogs had red subcutaneous tissue with a gelatinous material present. In addition to the injection sites, the spleen, thymus and lungs of each dog were collected, saved in 10% neutral buffered formalin and processed for histopathologic evaluation by a board certified veterinary pathologist. Microscopic findings were consistent with acute death and included injection site hemorrhage, edema and leukocyte infiltrate; thymus congestion and hemorrhage; splenic depletion; and lung pneumonitis with or without hemorrhage for both dogs.

In summary, the test article, IQB-9302, produced no local or systemic indications of toxicity when given subcutaneously to adult beagle dogs at dosages of 2.5, 5.0, 10.0, and 20.0 mg/kg. Both dogs died within 1 hour of the subcutaneous administration of IQB-9302 at a dosage of 40.0 mg/kg. Based on the results of this study, the maximum tolerated dose of IQB-9302 when given subcutaneously to adult beagle dogs can be estimated to be more than 20.0 mg/kg but less than 40.0 mg/kg.

INTRODUCTION

The objective of this study was to determine the local tolerance and the maximum tolerated dose of IQB-9302 when administered subcutaneously to beagle dogs using an up and down procedure. The materials and methods used, the observations made, and the results obtained during the study are the subject of this report.

This study was conducted by T.P.S., Inc., 10424 Middle Mt. Vernon Road, Mt. Vernon, IN 47620 under the sponsorship of Laboratorios INIBSA to generate animal safety data which may be submitted to regulatory authorities. The laboratories of T.P.S., Inc. are licensed by the U.S.D.A. to conduct research in laboratory animals and all conditions of testing conformed to requirements of the Animal Welfare Act and its amendments. Maintenance of all records and performance of testing procedures were done in accordance with T.P.S. Standard Operating Procedures.

All work reported herein was done according to the requirements specified in the study protocol (Appendix III). The protocol was reviewed and approved by the sponsor.

The names, titles, and job functions of T.P.S., Inc. supervisory personnel involved in the conduct of the study are listed in Appendix IV.

All data reported herein were compared to original data and found to be valid and accurate. No known circumstances occurred during the study that may have adversely affected the quality or integrity of the data.

MATERIALS AND METHODS

TEST ARTICLE. The test article was received from LEBSA on 10/10/98 and identified as follows:

Name:	IQB-9302.HCl
Lot Number:	9454.001
Description:	White Powder
Storage Conditions:	Room temperature

All data relating to the identity, purity and stability of the test article are the responsibility of the sponsor. The Certificate of Analysis for the test article provided by the manufacturer is included in Appendix II.

VEHICLE. The vehicle was received from Henry Schein, Inc. and identified as follows:

Vehicle Name:	0.9% Saline for Injection, USP
Lot Number:	J8H672
Physical Description:	Clear liquid
Storage Condition:	Room temperature

DOSING SOLUTION PREPARATION. Dosing solutions were prepared under aseptic conditions just prior to administration by dissolving the appropriate amount of IQB-9302.HCl in 0.9% Saline for Injection, USP. The dissolved solution was filtered through a 0.2 µm Acrodisc® filter into a sterile glass vial. Dose 1 was prepared by weighing 67.5 mg (60 mg x 1.1249 [salt/base correction factor]) into a tared container and q.s.'d to 120 g with 0.9% Saline. Doses 2 through 5 were prepared using the same procedure by weighing 135 mg, 270 mg, 540 mg and 1080 mg, respectively, q.s.'d to 120 g with 0.9% Saline.

DOSE CONCENTRATIONS. The dose solutions were prepared such that all doses were administered in a dose volume of 5 mL/kg.

DESCRIPTION OF TEST SYSTEM. Adult beagle dogs were obtained from Ridglan Farms Inc., Mt. Horeb, Wisconsin. The dogs were housed individually, in concrete floored kennel runs within an isolated temperature and humidity controlled animal room (Room A, Bldg. 106) with filtered air supply (10-15 changes/hour) and cycled lighting (12 hours of light and 12 hours of darkness). Temperature (minimum, maximum and current) readings were recorded daily (69-73 °F) and humidity (35%) was recorded weekly. PMI® Laboratory Canine Diet #5006 (OCT 07 98 3) was provided *ad libitum*. Tested tap water (via an automatic water system) was provided *ad libitum*. Each dog was identified by a unique

permanent marking (USDA ear tattoo) in addition to an appropriately labelled cage card indicating the study number, animal number, tattoo number and sex.

ROUTE AND METHOD OF ADMINISTRATION. The test article solutions were administered subcutaneously. To avoid pressure necrosis, dose volumes were divided and administered at different sites such that no more than 10 mL was dosed at any one site. The number of sites and the location of the sites was documented in the raw data.

JUSTIFICATION OF ROUTE OF ADMINISTRATION. The route of administration was chosen in order to determine tolerance of the test article when administered subcutaneously.

FREQUENCY AND DURATION OF ADMINISTRATION. The study design was that of an Up and Down procedure. Each dog was dosed with the initial dose (2.5 mg/kg) and subsequent doses were increased based on the results obtained from the previous dose. Doses were administered every other day (Monday, Wednesday, Friday).

INITIAL DOSE LEVEL. Following an adequate acclimation period, 2 adult beagle dogs (1 male and 1 female) were selected for study and assigned to the following treatment group:

Group	Number of Dogs	Test Article	Initial Treatment**
BKF1	2	IQB-9302	2.5 mg/kg

**Subsequent doses were increased by a factor of 2 after a washout period of at least 1 day.

CLINICAL SIGNS. The dogs were observed hourly for 6 hours following dosing and once in the morning and once in the late afternoon every day throughout the study for signs of pharmacologic, toxicologic, or clinical effects including behavioral changes.

BODY WEIGHTS. Body weights were obtained prior to the first dose and weekly thereafter and used to determine the appropriate dosage to be administered.

GROSS NECROPSY. A gross necropsy was performed on all dogs that died during the study period. Findings are provided in Appendix I.

TISSUE PRESERVATION AND HISTOPATHOLOGY. The most recent injections sites and all gross lesions were removed and fixed in 10% neutral buffered formalin, slides prepared, stained with hematoxylin and eosin and examined by a veterinary pathologist employed by T.P.S., Inc.

CHRONOLOGICAL TABLE OF SIGNIFICANT EVENTS.

Animal Receipt:	09-25-98
Test Article Receipt	10-10-98
Study Initiation :	10-28-98
Animal Phase Initiation:	11-09-98
Dose 1 (2.5 mg/kg)	11-09-98
Dose 2 (5.0 mg/kg)	11-11-98
Dose 3 (10.0 mg/kg)	11-13-98
Dose 4 (20.0 mg/kg)	11-16-98
Dose 5 (40.0 mg/kg)	11-18-98
Animal Phase Termination:	11-18-98

PROTOCOL DEVIATIONS.

Protocol Section Body Weights (Page 6 of 8). Body weights were obtained prior to the first dose and weekly thereafter. The protocol specified body weights be recorded immediately prior to administration of the test article.

The deviation listed above did not adversely affect the integrity or evaluation of the data.

RESULTS

CLINICAL SIGNS. No clinical signs or indications of adverse local or systemic effects were noted in either dog at any time following administration of the 2.5, 5.0, 10.0, and 20.0 mg IQB-9302/kg dosages. Both dogs were found dead 1 hour following administration of the 40.0 mg/kg dosage.

BODY WEIGHT AND DOSE LEVELS.

Dog BKF1M01

Study Day	Body Weight	Dose Number	Dose Level	Dose Volume
Day 1	11.4 kg	Dose 1	2.5 mg/kg	5 mL/kg
Day 3	11.4 kg	Dose 2	5.0 mg/kg	5 mL/kg
Day 5	11.4 kg	Dose 3	10.0 mg/kg	5 mL/kg
Day 8	11.0 kg	Dose 4	20.0 mg/kg	5 mL/kg
Day 10	11.0 kg	Dose 5	40.0 mg/kg	5 mL/kg

Dog BKF1F01

Study Day	Body Weight	Dose Number	Dose Level	Dose Volume
Day 1	12.5 kg	Dose 1	2.5 mg/kg	5 mL/kg
Day 3	12.5 kg	Dose 2	5.0 mg/kg	5 mL/kg
Day 5	12.5 kg	Dose 3	10.0 mg/kg	5 mL/kg
Day 8	12.1 kg	Dose 4	20.0 mg/kg	5 mL/kg
Day 10	12.1 kg	Dose 5	40.0 mg/kg	5 mL/kg

HISTOPATHOLOGY.

Dog BKF1M01

GENERAL: Found dead within 1 hour after dosing at 40 mg/kg of IQB-9302. Red tinged foam present in and around mouth. Stomach contained white foam.

SPLEEN:
Gross: Mottled light and dark areas.
Microscopic: Slight depletion of the white pulp.

THYMUS:
Gross: Red in color.
Microscopic: Moderate diffuse congestion and moderate multifocal hemorrhage.

LUNGS:
Gross: White plaque area on medial margin of left diaphragmatic lobe. Mottled light and dark areas all lobes.
Microscopic: Slight pneumonitis.

INJECTION SITES 1 - 6:
Gross: Subcutaneous tissue red in color with gelatinous material present.
Microscopic:

- Site 1: Both sections had moderate hemorrhage, moderate edema and slight leukocyte infiltration.
- Site 2: Both sections had slight hemorrhage, slight edema and minimal leukocyte infiltration.
- Site 3: Both sections had slight hemorrhage, slight edema and minimal leukocyte infiltration.
- Site 4: Both sections had slight or moderate hemorrhage, slight or moderate edema and minimal or slight leukocyte infiltration.
- Site 5: Both sections had moderate hemorrhage, moderate edema and slight leukocyte infiltration.
- Site 6: Both sections had slight or moderate hemorrhage, slight or moderate edema and minimal or slight leukocyte infiltration.

HISTOPATHOLOGY cont'd.

Dog BKF1F01

GENERAL: Found dead within 1 hour after dosing at 40 mg/kg of IQB-9302. Red tinged foam present in and around mouth. Stomach contained white foam.

SPLEEN:
Gross: Mottled light and dark areas.
Microscopic: Slight depletion of the white pulp.

THYMUS:
Gross: Red in color.
Microscopic: Moderate diffuse congestion and moderate multifocal hemorrhage.

LUNGS:
Gross: Mottled light and dark areas all lobes.
Microscopic: Slight pneumonitis and slight multifocal hemorrhage.

INJECTION SITES 1 - 6:
Gross: Subcutaneous tissue red in color with gelatinous material present.
Microscopic:

Site 1: Both sections had slight hemorrhage, slight edema and minimal leukocyte infiltration.
Site 2: Both sections had slight hemorrhage, slight edema and minimal leukocyte infiltration.
Site 3: Both sections had moderate hemorrhage, moderate edema and slight leukocyte infiltration.
Site 4: Both sections had moderate hemorrhage, moderate edema and slight leukocyte infiltration.
Site 5: Both sections had moderate hemorrhage, moderate edema and slight leukocyte infiltration.
Site 6: Both sections had moderate hemorrhage, moderate edema and slight leukocyte infiltration.

HISTOPATHOLOGIC EVALUATION

Hematoxylin and eosin stained paraffin processed tissues sections were evaluated microscopically from 1 male and 1 female dog given IQB-9302 by subcutaneous injections. Both dogs died within 1 hour after dosing at 40 mg/kg. Microscopic findings were consistent with acute deaths and included injection site hemorrhage, edema and leukocyte infiltrate; thymus congestion and hemorrhage; splenic depletion; and lung pneumonitis with or without hemorrhage for both dogs.

Histopathologic Evaluation Submitted by:

3-4-99

Dean Barnett, DVM, Ph.D., (ACVP)
Veterinary Pathologist

T.P.S. Study No.: 616B-502-410-98
Sponsor Study No.: 030

DISCUSSION AND CONCLUSIONS

The subcutaneous administration of IQB-9302 at dosages of 2.5, 5.0, 10.0 and 20.0 mg/kg body weight in a dose volume of 5 mL/kg caused no clinical signs or evidence of adverse local or systemic toxicity. Both study dogs died within 1 hour of administration of 40.0 mg/kg. No clinical signs were observed prior to death. Gross necropsy examination of both dogs revealed white foam in the stomach, red tinged foam in and around the mouth, mottled light and dark areas on the spleen, a red thymus, and mottled light and dark areas on all lung lobes. One dog (Dog BKF1M01) also had an area of white plaque on the medial margin of the left diaphragmatic lobe. The injection sites for both dogs had red subcutaneous tissue with a gelatinous material present. In addition to the injection sites, the spleen, thymus and lungs of each dog were collected and saved in 10% neutral buffered formalin. Microscopic findings were consistent with acute death and included injection site hemorrhage, edema and leukocyte infiltrate; thymus congestion and hemorrhage; splenic depletion; and lung pneumonitis with or without hemorrhage for both dogs.

In conclusion, the test article, IQB-9302, produced no local or systemic clinical signs of toxicity when given subcutaneously to adult beagle dogs at dosages of 2.5, 5.0, 10.0, and 20.0 mg/kg. Both study dogs died within 1 hour of the subcutaneous administration of IQB-9302 at a dosage of 40.0 mg/kg. Based on the results of this study, the maximum tolerated dose of IQB-9302 when given subcutaneously to adult beagle dogs can be estimated to be more than 20.0 mg/kg but less than 40.0 mg/kg.

ARCHIVES

RECORDS. Original data entries made in laboratory notebooks or other data input forms and one copy of the final report with original signatures will be maintained in the T.P.S., Inc. archives for a period of at least 5 years. Laboratorios INIBSA will be notified to approve the destruction of these records, transfer to their facility or agree to additional archiving charges.

TEST MATERIAL. The unused test material will be returned to the sponsor, Laboratorios INIBSA, following completion of related studies. Data relating to the identity, purity, and stability of the test article will be maintained by the sponsor.

T.P.S. RAW DATA REFERENCES

Form 101	Body Weight and Food Consumption: Nos. 12686, 12687
Form 108	Room Log: No. 8535
Form 120	Test Data Sheet: Nos. 37001-37005
Form 121	Test Material/Control Article Storage Record: Nos. 1986, 2008
Form 122	Animal Receipt Record: No. 766
Form 128	Study Maintenance Log: No. 3466
Form 133	Clinical Observation Record: Nos. 11167-11171
Form 133A	Observation Record: No. 460
Form 135A	Weekly Dosing Record: Nos. 30747, 30813
Form 143	Test/Control Article Usage Log Sheet: Nos. 8695, 8696
Form 164	Study Director Notification: No. 713

APPENDIX I

NECROPSY REPORT

NECROPSY REPORT

Following a subcutaneous dose of 40.0 mg IQB-9302/kg body weight, both study dogs were found dead at 1 hour postdosing and were subjected to a complete gross necropsy.

IQB-9302: 40 mg/kg Group BKF1

Animal No.	Necropsy Finding
BKF1M01	Red tinged foam in and around the mouth Stomach – contained white foam Spleen – mottled light and dark areas Thymus – red in color Lungs – white plaque area on medial margin of left diaphragmatic lobe, mottled light and dark areas all lobes Injection sites – subcutaneous tissue red in color with gelatinous material present
BKF1F01	Red tinged foam in and around the mouth Stomach – contained white foam Spleen – mottled light and dark areas Thymus – red in color Lungs – mottled light and dark areas all lobes Injection sites – subcutaneous tissue red in color with gelatinous material present

APPENDIX II

TEST ARTICLE CERTIFICATE OF ANALYSIS

LEBSA

LABORATORIOS ESPINOS Y BOFILL, S.A.
Investigación y síntesis de productos químicos
Ctra. de l'Hospitalet, 30
08940 Cornellá (Barcelona)
Apartado 14.012 de Barcelona
Teléfono 93 377 00 51 Fax 93 377 51 58
E-mail: lebsa@sefes.es
Telex. 93051 LEB-E

CERTIFICATE OF ANALYSIS

PRODUCT: CIPROCAINE HYDROCHLORIDE CONTROL #: 9810034

LOT #: 9454.001 DATE: 8th Oct.1998

ANALYTICAL DATA	SPECIFICATIONS	RESULT
Appearance	White powder	Conforms
Identification		
I.R. Spectrum	Similar to standard	Conforms
Chlorides	To pass test	Conforms
Appearance of solution	Clear and colourless	Conforms
Acidity or alkalinity	To pass test	Conforms
Related substances	Not more than 0.5%	Conforms
2,6-Dimethylaniline	Not more than 100ppm	Conforms
Heavy metals	Not more than 10 ppm	Conforms
Loss on drying	Not more than 1.0%	0.35%
Sulphates ash	Not more than 0.1%	0.04%
Assay	98.5 - 101.0%	101.0%
Residual Isopropanol	Not more than 0.5%	0.23%

Analyst
Silvia Dieguez

Analytical Department Manager
Anna Pons

R.M. Barcelona, Insc. 1ª, Sec. 2ª, L. 1.025, T. 1.594, F. 171, H. 13.690 - CIF/VAT - ESA/ 08150450

APPENDIX III

PROTOCOL

IQB-9302: AN ACUTE SUBCUTANEOUS TOXICITY STUDY IN BEAGLE DOGS	
FACILITY NAME & ADDRESS: T.P.S., Inc. 10424 Middle Mt. Vernon Road Mt. Vernon, IN 47620 Telephone: (812) 985-5900 Facsimile: (812) 985-3403	**SPONSOR NAME & ADDRESS:** Laboratorios INIBSA Crta de Sabadell a Granollers, KM.14.5 08185 Llica de Vall (Barcelona) Spain
T.P.S. STUDY NO.: 616B-502-410-98	**SPONSOR STUDY NO.:** 030
APPROVED BY: 10-28-98 L. J. Clare, D.V.M. Date	**APPROVED BY:** 29-10-98 Alvaro Galiano Ramos Instituto Quimico y Biologico Date
REVIEWED BY: 10/28/98 R. J. Wheeler, B.S. Date Vice President of Marketing	**REVIEWED BY:** Date

IQB-9302: AN ACUTE SUBCUTANEOUS TOXICITY STUDY IN BEAGLE DOGS

GENERAL INFORMATION

Identification:

T.P.S. Study No.: 616B-502-410-98
Sponsor I.D. No.: 030

Sponsor:

Laboratorios INIBSA
Crta de Sabadell a Granollers, KM.14.5
08185 Lliça de Vall (Barcelona)
Spain

Objective: To determine the local tolerance and the maximum tolerated dose of IQB-9302 when given subcutaneously to dogs using an up and down procedure.

Location of Study and Conditions of Testing:

It is the intention of Laboratorios INIBSA in sponsoring this study to generate animal safety data which may be submitted to regulatory authorities. The laboratories of T.P.S., Inc., 10424 Middle Mt. Vernon Road, Mt. Vernon, Indiana 47620 are licensed by the United States Department of Agriculture to conduct research in laboratory animals, and all the conditions of testing will conform to the Animal Welfare Act and its amendments. T.P.S., Inc. will follow all requirements specified in this approved protocol and all applicable governmental regulations regarding Good Laboratory Practices as well as T.P.S., Inc. Standard Operating Procedures. Changes in the protocol may be made by consultation with and approval from Laboratorios INIBSA, followed by written verification of the change. Laboratorios INIBSA, reserves the right to inspect facilities and procedures used in this study by means of announced or unannounced site visits.

Personnel:

Sponsor:	Laboratorios INIBSA
Study Monitor:	Alvaro Galiano Ramos Instituto Quimico y Biologico 28230 Las Rozas (Madrid) Spain
Telephone:	+ 34 91 631 60 26
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T.P.S., Inc.:
10424 Middle Mt. Vernon Road
Mt. Vernon, IN 47620

Telephone: (812) 985-5900
Facsimile: (812) 985-3403
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Study Director:	L. J. Clare, D.V.M.
Colony Manager:	M. A. Kempf, LAT
Study Manager:	M. A. Kempf, LAT
Director of QAU:	M. J. Bandoli, M.S.
Veterinarian:	L. J. Clare, D.V.M.

Proposed Schedule:

Animal Phase Initiation:	November 1998
Animal Phase Termination:	December 1998
Report Date:	January 1998

TEST ARTICLE AND DOSING

TEST ARTICLE

Name: IBQ-9302

Lot Number: To be included in the raw data and final report.

Source and Manufacturer: LEBSA

Purity: To be given in the Certificate of Analysis to be provided by the manufacturer and included in the raw data and final report.

Bulk Drug Storage: The test article is to be stored in the original containers at room temperature.

Stability: The sponsor has data indicating the bulk drug is stable at room temperature for at least 4 years. The sponsor is conducting stability studies according to FDA and EMEA guidelines.

VEHICLE:

Name: 0.9% Saline for Injection, USP

Lot Number: To be included in the raw data and final report.

Source and Manufacturer: To be included in the raw data and final report.

Storage: Room temperature in the original containers.

DOSING SOLUTION PREPARATION

Preparation of Dose Formulation: The test article will be prepared as a solution under aseptic conditions using 0.9% Saline for Injection, USP. The method of preparation and description of the dose formulation will be documented in the raw data and final report

Dose Concentrations: The dose solutions will be prepared such that all doses are administered in a dose volume of 5 ml/kg.

Frequency of Preparation: Once prior to dosing.

DESCRIPTION OF TEST SYSTEM

Species/Breed: Canine/Beagle

Source: T.P.S., Inc. stock colony obtained from a USDA licensed supplier.

Sex and Number: Two males and/or females.

Age and Body Weight: Adult dogs weighing 8-15 kg at study initiation.

Acclimation: Dogs will be acclimated at least one week prior to test initiation.

Identification: Animal runs will be marked with an identification card inscribed with the study number and animal number. Dogs will be identified by ear tattoo (USDA number).

Justification: The beagle is a standard non-rodent test animal used in drug safety evaluations.

Control of Bias: Since the number of animals is small (two) and both will be receiving the same treatment, control of bias is not necessary.

Environment: Animals will be in held an isolated animal room with filtered air supply (10-15 fresh changes per hour), temperature (64-84°F) and humidity (30-70%) control, and fluorescent lighting (12 hours on and 12 hours off). Temperature will be recorded daily and humidity will be recorded weekly.

Caging: Animals will be housed in individual wire-mesh runs with concrete floors and resting boards. Fresh dry wood shavings will be supplied daily; all shavings will be removed and the runs washed down biweekly.

Diet: PMI® Laboratory Canine Diet (#5006) will be provided ***ad libitum***.

Water: Tested tap water derived from a deep well will be provided ***ad libitum*** via an automatic watering system.

Contaminants: The Study Director is not aware of any dietary contaminants which would interfere with the conduct or purpose of this evaluation.

EXPERIMENTAL DESIGN

Route and Method of Administration: The test article solution will be administered subcutaneously. The dose site(s) will be documented in the raw data. To avoid pressure necrosis, dose volumes greater than 10 mL will be divided and administered at different sites such that no more than 10 mL is dosed at any one site. The number of sites and the location of the sites will be documented in the raw data.

Justification of Route of Administration: To determine tolerance of the test article when administered subcutaneously.

Frequency and Duration of Administration: The study design is that of an Up and Down procedure. Each dog will be dosed with the initial dose (2.5 mg/kg) and subsequent doses will be increased or decreased depending on results obtained. Doses will be administered every other day (Monday, Wednesday, Friday) until clinical signs indicate that a maximum tolerated non-lethal dose has been reached.

Initial Dose Level:

Group	Initial Treatment mg/kg*	No. of Dogs
BKF1	2.5	2

* Additional doses will be increased or decreased depending on results until a maximum tolerated non-lethal dose has been determined.

Clinical Signs: The animals will be observed hourly for 6 hours following each dose and once in the morning and once in the late afternoon every day during the wash out period including weekends and holidays for signs of pharmacologic, toxicologic or clinical effects including behavioral changes.

Clinically Affected Dogs: Clinically affected dogs may be examined more frequently as determined necessary by the attending veterinarian. The date of onset, degree, progression and duration of any clinical sign will be recorded in the raw data.

Body Weights: Body weights will be recorded immediately prior to administration of test article. Body weights are the basis for determining the appropriate dosage to be administered.

Moribund and Dead Dogs: Moribund dogs or dogs not expected to survive until the next observation period will be humanely sacrificed (see protocol p. 7 Method of Euthanasia) to prevent further suffering.

Gross Necropsy: Necropsies will be performed on all moribund animals, any animals found dead during the study and on all animals the day after the last dose.

Tissue Preservation and Histopathology: The most recent injection site and any gross lesions will be removed and fixed in 10% neutral buffered formalin, slides prepared, stained with hematoxylin and eosin and examined by a veterinary pathologist employed by T.P.S., Inc.

Method of Euthanasia: Each dog will be euthanized with an intravenous injection of sodium pentobarbital for euthanasia administered at a minimum dose of 1 mL/4.5 kg followed by exsanguination.

RECORDS

All records generated during the course of the study will be retained in T.P.S., Inc. archives for a period of at least five years after which Laboratorios INIBSA will be notified and must approve destruction of these records, transfer to their facilities, or agree to additional archiving charges. Remaining test article and any specimens generated during the study will be returned to the sponsor.

REPORT

A comprehensive final report will be issued within 30 days of study termination and will include all observations made during the evaluation, test article identification, description of administration and remarkable signs exhibited by the animals.

STATISTICS

Statistical evaluations are not applicable due to the small number of animals on the study.

SAFETY

All safety precautions described in the T.P.S., Inc. standard operating procedures and material MSDS are to be strictly followed.

ANIMAL WELFARE COMPLIANCE

This study will comply with all applicable sections of the final rules of the Animal Welfare Act regulations (9 CFR) and the "Guide for the Care and Use of Laboratory Animals" (National Academy Press, 1996). Wherever possible, procedures used in this study are designed to avoid or minimize discomfort, distress and pain to animals. All procedures are described in this study protocol or in written laboratory procedures. These procedures are based on the most currently available technologies concerning proper laboratory animal use and management.

In the event that any aspect of this study causes undue pain or distress to the animals, the Study Director shall determine if the administration of appropriate sedatives, analgesics or anesthetics would be contradicted by the objectives of the study and document the resultant course of actions. Animals that experience severe or chronic pain or distress that cannot be relieved will be painlessly euthanized. Methods of euthanasia used during this study are in conformance with the above referenced regulations.

QUALITY ASSURANCE AND GOOD LABORATORY PRACTICES

This is a GLP study designed to conform to all applicable Good Laboratory Practice regulations. The entire study will be subjected to inspections, and the final report will be reviewed by the T.P.S. Quality Assurance Unit in accordance with T.P.S. Standard Operating Procedures.

APPENDIX IV

T.P.S. SUPERVISORY PERSONNEL

T.P.S., INC. SUPERVISORY PERSONNEL

Name/Title	Job Function
L. J. Clare, D.V.M. Toxicologist/Attending Veterinarian	Study Director
J. P. Devine, Jr., B.S., LAT Toxicologist I	Study Manager
M. J. Bandoli, M.S. Director of Quality Assurance	Quality Assurance