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Sponsor:

3M
St. Paul, Minnesota

FINAL REPORT

Study Title:

Single-Dose Intravenous Pharmacokinetic Study of T-5904 in Rabbits

Author:

Steven M. Glaza

Study Completion Date:

October 14, 1994

Performing Laboratory:

Hazleton Wisconsin, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HWI 6329-117

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QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Hazleton Wisconsin, Inc., in accordance with the Food and Drug Administration (FDA) Good Laboratory Practice Regulations, 21 CFR 58.35 (b) (6) (7). The following inspections were conducted and findings reported to the Study Director and management. Written status reports of inspections and findings are issued to Hazleton management monthly according to standard operating procedures.

Inspection Dates			Date Reported to	Date
From	To	Phase	Study Director	to Management
03/01/94	03/01/94	Protocol Review	03/02/94	04/10/94
03/07/94	03/07/94	Body Weight	03/07/94	04/10/94
04/26/94	04/26/94	Data/Report Review	04/29/94	05/10/94
10/11/94	10/11/94	Report Re-Review	10/11/94	11/10/94

Tracy Diedrich for
Nancy Diedrich
Representative, Quality Assurance Unit

10.14.94
Date

STUDY IDENTIFICATION

Single-Dose Intravenous Pharmacokinetic
Study of T-5904 in Rabbits

Test Material	T-5904
Sponsor	3M Toxicology Services 220-2E-02 3M Center St. Paul, MN 55144
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Services 220-2E-02 3M Center St. Paul, MN 55144 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Study Timetable	
Experimental Start Date	March 7, 1994
Experimental Termination Date	March 9, 1994

KEY PERSONNEL

Acute Toxicology

Steven M. Glaza
Study Director
Manager

Steven R. Sorenson
Study Coordinator

Patricia Padgham
In-life Supervisor

Rose M. Bridge
Report Supervisor

Quality Assurance

Sherry R. W. Petsel
Manager

Laboratory Animal Medicine

Cindy J. Cary, DVM
Diplomate, ACLAM
Supervisor

Anatomical Pathology

Jack Serfort/
Deborah L. Pirkel
Supervisors
Necropsy

Anne Mosher
Supervisor
Pathology Data

CONTENTS

SUMMARY

This study was done to assess the level of systemic exposure of T-5904 when administered by intravenous injection to rabbits.

Female Hra:(NZW)SPF rabbits were assigned at random to five groups (one/group). On Day 0, the animals received a single intravenous injection of the vehicle [Dimethyl Sulfoxide (DMSO)] at a dose volume of 0.5 mL/kg or 1.0, 10.0, 50.0, or 100.0 mg of T-5904/kg of body weight.

Clinical observations were conducted at approximately 0.5, 2, 4, 24, and 48 hours after intravenous injection. Body weights were determined just before test material administration (Day 0). Blood samples were collected from the marginal ear vein of the animals at 2-, 4-, 6-, 12-, and 24-hours post-injection. In addition, at the time of experimental termination (48-hours post-injection) a large volume of blood was obtained from each surviving animal. All samples were centrifuged and separated into serum and cellular fractions. The animal found dead during the study was necropsied. Animals surviving to the end of the 48-hour post-injection period were anesthetized with sodium pentobarbital and exsanguinated without necropsy. The bile from each animal surviving to termination was collected and sent frozen to the Sponsor after termination of the in-life phase.

Administration of T-5904 resulted in a decrease in food consumption at all dose levels, hypoactivity in the animals treated at the 50.0 and 100.0 mg/kg dose levels, and death of the animal treated at the 100.0 mg/kg dose level.

OBJECTIVE

The objective of this study was to assess the level of systemic exposure to the test material, T-5904, when administered as a single intravenous injection to rabbits.

REGULATORY COMPLIANCE

This study was conducted in accordance with the U.S. Food and Drug Administration's Good Laboratory Practice Regulations for Nonclinical Laboratory Studies, 21 CFR 58. All procedures used in this study are in compliance with the Animals Welfare Act Regulations. In the opinion of the Sponsor and study director, the study did not unnecessarily duplicate any previous work. Hazleton Wisconsin (HWI) does not accept any responsibility for the analysis of the samples collected in this study nor are these results presented in this report.

TEST AND CONTROL MATERIAL

Identification

The test material was identified as T-5904 and described as a yellow solid. The control material was Dimethyl Sulfoxide (DMSO) (Sigma Chemical Company, St. Louis, Missouri; Lot No. 53H0634; Exp. March 4, 1995) and described as a clear, colorless liquid.

Purity and Stability

The Sponsor assumes responsibility for test material purity and stability determinations (including under test conditions). A sample of the test material/vehicle mixtures for concentration, solubility, homogeneity, and stability analyses was not taken before administration as this was not requested by the Sponsor. The certificate of analysis, obtained from the supplier, documents the purity of the control material as 99.9%. The stability of the control material was considered to be adequate for the purposes of this study.

Storage and Retention

The test and control material were stored at room temperature. Any unused test or control material will be discarded after issuance of the final report according to HWI Standard Operating Procedure (SOP).

Safety Precautions

The test and control material handling procedures were according to HWI SOPs and policies.

TEST SYSTEM

Test Animal

Adult albino rabbits of the Hra:(NZW)SPF strain were received from HRP, Inc., Kalamazoo, MI, on February 23, 1994 and maintained at the Hazleton Wisconsin facility at 3802 Packers Avenue, Madison, Wisconsin. Animal husbandry and housing at HWI comply with standards outlined in the "Guide for the Care and Use of Laboratory Animals".[1] The animals were individually housed in screen-bottom stainless steel cages in temperature- and humidity-controlled quarters, provided access to water *ad libitum* and a measured amount of Laboratory Rabbit Diet HF® #5326, PMI Feeds, Inc., and held for an acclimation period of at least 7 days. The feed is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of water are periodically analyzed by HWI. There were no known contaminants in the feed or water that would have interfered with or affected the results of the study.

All animals were identified by animal number and corresponding ear tag and maintained during the observation period as specified for the acclimation period. During the conduct of the study, the temperature of the animal room ranged from 20 to 23°C and the relative humidity ranged from 48 to 60%. The animals were housed under the required temperature and humidity conditions.

Selection of Test Animals

The animals were selected at random based on health and body weight requirements.

Study Design

Female animals weighing from 2,173 to 2,386 g at initiation of treatment were placed into the following study groups:

Group	Treatment	Dose Level (mg/kg)	Dose Volume (mL/kg)	Number of Animals
1 (Control)	DMSO	0.0	0.5	1
2	T-5904	1.0	0.5	1
3	T-5904	10.0	0.5	1
4	T-5904	50.0	0.5	1
5	T-5904	100.0	0.5	1

Justification for Species Selection

Historically, the New Zealand White albino rabbit has been the animal of choice because of the large amount of background information on this species.

PROCEDURES

Dose Preparation and Administration

The test material was diluted with DMSO to achieve a specific concentration for each dose level. An individual dose of each respective test solution or control was calculated for each animal based on its body weight on the day of treatment. The respective test solution was administered by intravenous injection into a marginal ear vein. The dose was given as a slow push (duration ranging from 35 to 63 seconds). The prepared test solutions were stored at room temperature until administered. After administration, any remaining test solutions were discarded.

Reason for Route of Administration

Intravenous injection is an acceptable route to assess systemic exposure.

Observations of Animals

Clinical observations were conducted at approximately 0.5, 2, 4, 24 and 48 hours after intravenous injection.

Body weights were determined just before test material administration (Day 0).

Sample Collections

Blood samples (approximately 2 mL) were collected from the marginal ear vein of all animals at 2, 4, and 6 hours post-injection. Subsequent collection of blood from surviving animals was conducted at 12 and 24 hours post-injection. At the time of necropsy (approximately 48-hours post-injection) a large volume of blood (approximately 45 to 60 mL) was obtained from each surviving animal. All samples were centrifuged and separated into serum and cellular fractions. The blood samples were then stored frozen (-20°C ±10°C) until shipped to the Sponsor.

Pathology

The animal found dead during the study was subjected to an abbreviated gross necropsy examination and any abnormalities were recorded. At termination of the experimental phase, surviving animals were anesthetized with sodium pentobarbital and exsanguinated without necropsy. The bile from each animal surviving to termination was collected and immediately placed on dry ice, then frozen by placing in a freezer set to maintain a temperature of -70°C ±10°C. After necropsy or bile collection, the animals were discarded.

Shipment of Blood and Bile

The blood samples and bile were sent frozen (on dry ice) to the Sponsor (James D. Johnson, 3M E.E. & P.C., Bldg. 2-3E-09, 935 Bush Avenue, St. Paul, MN, 55106) after completion of the in-life phase.

Statistical Analyses

No statistical analyses were required by the protocol.

Location of Raw Data, Records, and Final Report

The raw data, records, and a copy of the final report will be retained in the archives of HWI in accordance with HWI SOP.

RESULTS

Body Weights

Individual and mean body weights are in Table 1.

Clinical Observations

Individual clinical signs are in Table 2. The animals treated at 0.0, 1.0, 10.0, and 50.0 mg/kg appeared normal at 0.5 and 2 hours post-injection. The animal treated at 0.0 mg/kg exhibited a decrease in food consumption at 4, 24, and 48 hours. The animals treated at 1.0 and 10.0 mg/kg exhibited a decrease in food consumption at 4 and 24 hours, then returned to a normal appearance at the 48-hour observation. The animal treated at 50.0 mg/kg exhibited hypoactivity at 4 and 24 hours and a decrease in food consumption at 4, 24, and 48 hours. The animal treated at 100.0 mg/kg was found dead approximately 7.5 hours post-injection following signs of hypoactivity (at 2 and 4 hours) and decreased food consumption (at 4 hours).

Pathology

The animals treated at 0.0, 1.0, 10.0, and 50.0 mg/kg survived to termination of the experimental phase and were not necropsied. The animal treated at 100 mg/kg died on the day of treatment and was necropsied. There were no visible lesions in this animal.

DISCUSSION

The level of systemic exposure of T-5904 was evaluated in female albino rabbits when administered as a single intravenous injection at levels of 0.0, 1.0, 10.0, 50.0, and 100.0 mg/kg using DMSO as the vehicle. Administration of this material resulted in a decrease in food consumption at all dose levels, hypoactivity at the 50.0 and 100.0 mg/kg dose levels, and death of the animal treated at the 100.0 mg/kg dose level.

SIGNATURE

Steven M. Glaza
Study Director
Acute Toxicology

10-14-94
Date

REFERENCE

1. NIH Publication No. 86-23 (revised 1985).

Table 1
Individual Body Weights (g)

Group	Dose Level (mg/kg)	Sex	Animal Number	Day 0
1	0.0	Female	F50169	2,231
2	1.0	Female	F50170	2,386
3	10.0	Female	F50171	2,176
4	50.0	Female	F50097	2,173
5	100.0	Female	F50098	2,300

Table 2
Individual Clinical Signs

Group	Dose Level (mg/kg)	Sex	Animal Number	Observation	Hour 0.5	2	4	24	48
1	0.0	Female	F50169	Appeared normal	✓	✓	-	-	-
				Decreased food consumption	-	-	✓	✓	✓
2	1.0	Female	F50170	Appeared normal	✓	✓	-	-	✓
				Decreased food consumption	-	-	✓	✓	-
3	10.0	Female	F50171	Appeared normal	✓	✓	-	-	✓
				Decreased food consumption	-	-	✓	✓	-
4	50.0	Female	F50097	Appeared normal	✓	✓	-	-	-
				Hypoactivity	-	-	✓	✓	-
				Decreased food consumption	-	-	✓	✓	✓
5	100.0	Female	F50098	Appeared normal	✓	-	-		
				Hypoactivity	-	✓	✓		
				Decreased food consumption	-	-	✓		
				Found dead (≈7.5 hours post-injection)	-	-	✓		

✓ Indicates condition exists.
- Not applicable.

APPENDIX A

Protocol Deviations
Protocol TP8084.PK

Protocol Deviations

Protocol	Actual Procedure
Page 6, 7. Experimental Design, C. Dosing Procedures, (1) Dosing Route. Intravenous injection into a marginal ear vein over approximately 30 seconds.	The rate of injection ranged from 35 to 63 seconds.
Page 7, 7. Experimental Design, D. Observation of Animals, (3) Sample Collections, (c) Method of Collection. Blood samples (2 mL) will be collected from the marginal ear vein. In addition, at the time of necropsy approximately 50 - 70 mL of blood will be obtained from each animal.	A separate 2-mL sample of blood was not taken at the time of the schedule sacrifice (48 hours post-injection) since as much blood volume as possible (approximately 45 to 60 mL) was obtained from each animal.
Page 8, 7. Experimental Design, E. Termination, (2) Schedule Sacrifice, (a) Sample Collection. The bile from each animal surviving to termination will be collected and immediately placed on dry ice then frozen by placing in a freezer set to maintain a temperature of -20°C ±10°C.	The bile samples were placed in a freezer set to maintain a temperature of -70°C ±10°C.

These deviations are not considered to have had an adverse effect on the outcome of the study.

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a CORNING Laboratory Services Company

Sponsor:

3M
St. Paul, Minnesota

PROTOCOL TP8084.PK

Study Title:

Single-Dose Intravenous Pharmacokinetic Study
of T-5904 in Rabbits

Date:

March 4, 1994

Performing Laboratory:

Hazleton Wisconsin, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HWI 6329-117

STUDY IDENTIFICATION

Single-Dose Intravenous Pharmacokinetic Study
of T-5904 in Rabbits

HWI No.	6329-117
Test Material	T-5904
Sponsor	3M Toxicology Services 220-2E-02 3M Center St. Paul, MN 55144
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Services 220-2E-02 3M Center St. Paul, MN 55144 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Proposed Study Timetable	
Experimental Start Date	Week of March 7, 1994
Experimental Termination Date	Week of March 7, 1994
Draft Report Date	Week of April 11, 1994

1. Study
Single-Dose Intravenous Pharmacokinetic Study in Rabbits

2. Purpose
To assess the level of systemic exposure to the test material when it is administered as a single intravenous injection to rabbits

3. Regulatory Compliance
This study will be conducted in accordance with the following Good Laboratory Practice Regulations/Standards/Guidelines:

[ ] Conduct as a Nonregulated Study
[X] 21 CFR 58 (FDA)
[ ] 40 CFR 160 (EPA-FIFRA)
[ ] 40 CFR 792 (EPA-TSCA)
[ ] C(81)30 (Final) (OECD)
[ ] Notification No. 3850, August 10, 1984 (Japanese MAFF)
[ ] Notification No. 313, March 31, 1982, and as amended by Notification No. 870, October 5, 1988 (Japanese MOHW)

All procedures in this protocol are in compliance with the Animal Welfare Act Regulations. In the opinion of the Sponsor and study director, the study does not unnecessarily duplicate any previous work. Hazleton does not accept any responsibility for the analysis of the samples or tissues collected in this study nor will these results be presented in the report of this study.

4. Quality Assurance
The protocol, study conduct, and the final report will be audited by the Quality Assurance Unit in accordance with Hazleton Wisconsin (HWI) Standard Operating Procedures (SOPs) and policies.

5. Test Material

A. Identification
T-5904

B. Physical Description
Yellow solid

C. Purity and Stability
The Sponsor assumes responsibility for purity and stability determinations (including under test conditions). Samples of test material/vehicle mixture(s) (if applicable) for concentration, solubility, homogeneity, and stability analyses will be taken before administration if requested by the Sponsor. These samples (if taken) will be sent to the Sponsor after experimental termination for possible analysis.

D. Storage
Room temperature

E. Reserve Samples
Reserve samples will not be required for this study.

F. Retention
Any unused test material will be discarded after issuance of the final report, unless directed otherwise by the Sponsor.

G. Safety Precautions
As required by HWI SOPs and policies

6. Vehicle

A. Identification
Dimethyl Sulfoxide (DMSO); Lot number, source, and expiration date to be recorded in the data file and included in the final report.

B. Physical Description
(To be documented in the raw data)

C. Purity and Stability
To be documented by HWI (information from the supplier).

D. Storage
Room temperature

E. Reserve Samples
See Section, 5. E. Reserve Samples

F. Retention
Any remaining vehicle will be discarded after issuance of the final report.

G. Safety Precautions
As required by HWI SOPs and policies

7. Experimental Design

A. Animals

(1) Species
Rabbit

(2) Strain/Source
Hra:(NZW)SPF/HRP, Inc.

(3) Age at Initiation
Adult

(4) Weight at Initiation
2.0 to 3.0 kg

(5) Number and Sex
5 females

(6) Identification
Individual numbered ear tag

(7) Husbandry

(a) Housing
Individually, in screen-bottom stainless steel cages (heavy gauge)

(b) Food
A measured amount of Laboratory Rabbit Diet HF #5326 (PMI Feeds, Inc.). Animals will not be fasted before dose administration. The food is routinely analyzed by the manufacturer for nutritional components and environmental contaminants.

(c) Water
Ad libitum from an automatic system. Samples of the water are analyzed by HWI for total dissolved solids, hardness, and specified microbiological content and for selected elements, heavy metals, organophosphates, and chlorinated hydrocarbons.

(d) Contaminants
There are no known contaminants in the food or water that would interfere with this study.

(e) Environment
Environmental controls for the animal room will be set to maintain a temperature of 19 to 23°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark cycle.

(f) Acclimation
At least 7 days

(8) Selection of Test Animals
Based on health and body weight according to HWI SOPs. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test.

(9) Justification for Species Selection
Historically, the New Zealand White albino rabbit has been the animal of choice because of the large amount of background information on this species.

B. Dose Administration

(1) Test Groups

Group	Dose Level (mg/kg)[a]	Number of Females
1	0 (Control)	1
2	1.0	1
3	10.0	1
4	50.0	1
5	100.0	1

a The dose volume will be 0.5 mL/kg of body weight

C. Dosing Procedures

(1) Dosing Route
Intravenous injection into a marginal ear vein over approximately 30 seconds.

(2) Reason for Dosing Route
Intravenous injection is an acceptable route to assess systemic exposure.

(3) Dosing Duration
Single dose

(4) Dose Preparation
The test material will be diluted with dimethyl sulfoxide (DMSO) to achieve a specific concentration for each dose level. Individual doses will be calculated based on the animal's body weight taken just before test material administration. The dose volume will be equal between all groups. The prepared test mixtures will be stored at room temperature until administration. After administration, any remaining test mixtures will be discarded.

D. Observation of Animals

(1) Clinical Observations
The animals will be observed for clinical signs of toxicity at approximately 0.5, 2.0, 4.0, 24, and 48 hours after treatment.

(2) Body Weights
Just before test material administration.

(3) Sample Collections

(a) Frequency
2, 4, 6, 8, 12, 24, and 48 hours post-injection

(b) Number of Animals
All

(c) Method of Collection
Blood samples (2 mL) will be collected from the marginal ear vein. In addition, at the time of necropsy, approximately 50 - 70 mL of blood will be obtained from each animal. The samples will be stored at room temperature and then centrifuged, and the separate serum and cellular fractions stored at -10°C to -30°C. The separated serum and cellular fractions will be sent frozen to the Sponsor after experimental termination for possible future analysis.

Samples will be shipped to:

James D. Johnson
3M E.E. & P.C.
Bldg. 2-3E-09
935 Bush Avenue
St. Paul, MN 55106

James D. Johnson will be notified by telephone at (612) 778-5294 prior to the shipment of the samples.

E. Termination

(1) Unscheduled Sacrifices and Deaths
An abbreviated gross necropsy will be done. Animals in a moribund condition will be anesthetized with sodium pentobarbital and exsanguinated without necropsy.

(2) Scheduled Sacrifice
At approximately 48 hours post-injection, animals will be anesthetized with sodium pentobarbital and exsanguinated without necropsy.

(a) Sample Collection
The bile from each animal surviving to termination will be collected and immediately placed on dry ice then frozen by placing in a freezer set to maintain a temperature of -20°C ±10°C.

The bile (frozen) will be sent on dry ice to the Sponsor after experimental termination for possible future analysis. The samples will be shipped to the person listed in Section 7.D.(3).(c).

F. Statistical Analyses
No statistical analyses are required.

8. Report
A final report including those items listed below will be submitted.

Description of the test material
Description of the test system
Procedures
Dates of experimental initiation and termination
Description of any toxic effects

9. Location of Raw Data, Records, and Final Report
Original data, or copies thereof, will be available at HWI to facilitate auditing the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including those item listed below will be retained in the archives of HWI according to HWI SOP.

Protocol and protocol amendments
Dose preparation records
In-life records
  Body weights
  Dose administration
  Observations
Sample collection records
Study correspondence
Final report (original signed copy)

The following supporting records will be retained at HWI but will not be archived with the study data.

Animal receipt/acclimation records
Water analysis records
Animal room temperature and humidity records
Refrigerator and freezer temperature records
Instrument calibration and maintenance records

PROTOCOL APPROVAL

John L. Butenhoff, PhD
Sponsor's Representative
3M

March 9, 1994
Date

Steven M. Glaza
Study Director
Acute Toxicology
Hazleton Wisconsin, Inc.

3-4-94
Date

Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

3/4/94
Date

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