AR226-0654

PFOS

Pharmacokinetic -3

Corning Hazleton Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd.
Madison, WI 53704
608.241.4471
608.241.7227 Fax

CORNING Hazleton

Sponsor:

3M
St. Paul, Minnesota

FINAL REPORT

Study Title:

Single-Dose Intravenous Pharmacokinetic Study
of T-6684 in Rabbits

Author:

F. Bud W. McDonald

Study Completion Date:

December 31, 1997

Performing Laboratory:

Corning Hazleton Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

CHW 6329-199

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Corning Hazleton Inc., in accordance with the Food and Drug Administration (FDA) Good Laboratory Practice Regulations, 21 CFR 58. The following inspections were conducted and findings reported to the Study Director and management.

Inspection Dates			Date Reported to	Date Reported to
From	To	Phase	Study Director	Management
12/24/96	12/24/96	Protocol Review	12/24/96	12/24/96
01/28/97	01/28/97	Necropsy	01/28/97	01/28/97
02/17/97	02/17/97	Protocol Amendment	02/17/97	02/17/97
03/27/97	03/31/97	Data/Report Review	03/31/97	03/31/97

Randy Leety
Representative, Quality Assurance Unit

12.31.97
Date

STUDY IDENTIFICATION

Single-Dose Intravenous Pharmacokinetic Study
of T-6684 in Rabbits

Test Material	T-6684
Sponsor	3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220
Sponsor's Representative	Roger G. Perkins, PhD, DABT 3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220 (612) 733-3222
Study Director	F. Bud W. McDonald Corning Hazleton Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 242-7901
Study Location	Corning Hazleton Inc. 3301 Kinsman Boulevard Madison, WI 53704
Study Timetable	
Study Initiation Date	January 3, 1997
Experimental (In-life) Start Date	January 14, 1997
In-life End Date	February 11, 1997
Experimental Termination Date	February 11, 1997
Study Completion Date	December 31, 1997

KEY PERSONNEL

Acute Studies

Steven M. Glaza
Manager

F. Bud W. McDonald
Study Director

Jeffrey B. Hicks
In-life Supervisor

Rose M. Bridge
Administrative Supervisor

Toxicology Support

Kathy Myers
Manager

Calvin L. Horton
Supervisor

Quality Assurance

Nancy M. Centanni
Manager

Laboratory Animal Medicine

Donna J. Clemons, DVM
Diplomate, ACLAM
Supervisor

Anatomical Pathology

Deborah L. Pirkel
Laurie J. Schuller
Supervisors
Necropsy

CONTENTS

SUMMARY

This study was done to assess the level of systemic exposure of T-6684 when administered by a single intravenous injection to rabbits.

The study was conducted using four male and four female acclimated rabbits of the Hra:(NZW)SPF strain for each treatment group as follows:

Group	Control/Test Material	Dose Level (mg/kg)[a]	Number of Animals[b] Male	Female
1 (Control)	DMSO	0.0	4	4
2	T-6684	1.0	4	4
3	T-6684	3.0	4	4
4	T-6684	10.0	4	4
5	T-6684	30.0	4	4

DMSO - Dimethyl Sulfoxide

a Administered at a dose volume of 0.5 mL/kg.

b Two animals/sex/dose level were sacrificed on Day 15. The remaining animals (two animals/sex/dose level) were sacrificed on Day 29.

The animals received a single intravenous injection of the control or test material at the indicated dose level into the marginal ear vein of the right ear. The test material was mixed with Dimethyl Sulfoxide (DMSO) to a specific concentration for each dose level in Groups 2-5. The respective control material and test mixtures were administered at a dose volume of 0.5 mL/kg of body weight. Two animals/sex/dose level were sacrificed on Day 15 and the remaining animals (two animals/sex/dose level) were sacrificed on Day 29.

Clinical observations were conducted predose and at approximately 0.5, 2.0, and 4.0 hours after intravenous injection. Additional clinical observations and twice a day

mortality checks were conducted daily thereafter until the scheduled sacrifice interval (a.m. mortality check only on days of scheduled sacrifice). Body weights were determined on Day -7 for randomization purposes, before test or control material administration (Day 1), and at the scheduled sacrifice interval (Day 15 or Day 29). A blood sample (approximately 4-mL) was collected from each animal on the day before control or test material administration. Blood samples (approximately 4-mL) were also collected from the animals at 4-, 8-, 12-, 24-, and 48-hours post-injection, and on Day 8, with the exception of one Group 1 male at the 8-hour collection interval from which only 1.7 mL of blood was collected. An approximate 4-mL blood sample was also collected on Days 15 and 22 from each animal scheduled for sacrifice on Day 29. In addition, at the time of the scheduled sacrifice (Day 15 or Day 29), approximately 20 to 40 mL of blood was obtained from each control animal and approximately 20 mL of blood was obtained from each Group 2-5 animal. All samples were centrifuged and separate samples of serum and cellular fractions were obtained and sent frozen on dry ice to the Sponsor. On Day 15 or 29, the animals were anesthetized with sodium pentobarbital, bled via the posterior vena cava, and exsanguinated. An abbreviated gross necropsy examination was not done, however, tissues were collected. The whole liver, bile, and both kidneys from each animal were collected, weighed (volume only determined for bile), and sent frozen to the Sponsor.

After being intravenously injected with T-6684 or the DMSO control, all animals appeared clinically normal and gained weight during the study, with the exception of one Group 3 female (3.0 mg/kg) which exhibited an insignificant loss in weight at its termination interval (Day 15).

OBJECTIVE

The objective of this study was to assess the level of systemic exposure to the test material, T-6684, 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, with the exception that analysis of the test mixtures for concentration, homogeneity/solubility, and stability was not conducted. All procedures used in this study were in compliance with the Animal Welfare Act Regulations. In the opinion of the Sponsor and study director, the study did not unnecessarily duplicate any previous work.

TEST AND CONTROL MATERIALS

Identification

The test material was identified as T-6684 and described as an off-white liquid. The control material was Dimethyl Sulfoxide, (Sigma Chemical Company, Lot No. 64H1007; Expiration March 19, 1997), and was described as a clear, colorless liquid.

Purity and Stability

The Sponsor assumes responsibility for test material purity and stability determinations (including under test conditions). Analysis of the test material mixtures for concentration, homogeneity/solubility, and stability was not conducted. The purity and stability of the control material were considered to be adequate for the purposes of this study.

Storage and Retention

The control and test materials were stored at room temperature. Reserve samples of the test and control materials were taken and stored in a freezer set to maintain a temperature of -20°C ±10°C. The control material reserve sample will be retained at CHW for 1 year. The test material reserve sample was sent to the Sponsor. Any unused test material will be returned to the Sponsor. Any remaining control material may be used for other testing and will not be discarded after issuance of the final report.

Safety Precautions

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

TEST SYSTEM

Test Animal

Adult albino rabbits of the Hra:(NZW)SPF strain were received from HRP, Inc., Kalamazoo, Michigan on December 11, 1996 and maintained at the CHW facility at 3301 Kinsman Boulevard, Madison, Wisconsin.

Housing

After receipt, the animals were acclimated for a period of at least 7 days. During acclimation and throughout the study, the animals were individually housed in suspended stainless steel cages in temperature- and humidity- controlled quarters. The only exception to this was one Group 1 male which was found outside its cage at the time of the a.m. mortality check on Day 2 of the study. Environmental controls for the animal room were set to maintain a temperature of 19° to 23°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark lighting cycle. The dark cycle was interrupted to conduct in-life procedures. In cases where variations from these conditions existed, they were documented and considered to have had no adverse effect on the study outcome.

Animal Diet

The animals were provided access to water *ad libitum* and a measured amount of Laboratory Rabbit Diet HF #5326, PMI Feeds, Inc. The feed is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed. There were no known contaminants in the feed or water at levels that would be expected to interfere with or affect the results of the study.

Selection of Test Animals

The animals were identified by animal number and corresponding ear tag and were placed into study groups based on a stratified body weight randomization program. The randomization body weights were determined on Day -7.

Study Design

Animals weighing from 2,372 to 2,800 g and approximately 17 weeks of age at initiation of treatment were placed into the following study groups:

Group	Control/Test Material	Dose Level (mg/kg)[a]	Number of Animals[b] Male	Female
1 (Control)	DMSO	0.0	4	4
2	T-6684	1.0	4	4
3	T-6684	3.0	4	4
4	T-6684	10.0	4	4
5	T-6684	30.0	4	4

a Administered at a dose volume of 0.5 mL/kg.

b Two animals/sex/dose level were sacrificed on Day 15. The remaining animals (two animals/sex/dose level) were sacrificed on Day 29.

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 in Groups 2 to 5. An individual dose of the control material or respective test mixture was calculated for each animal based on its body weight on the day of treatment (Day 1). The control material or respective test mixture was administered by intravenous injection into the marginal ear vein of the right ear over a period of 31 to 53 seconds. The prepared test mixtures were stored at room temperature until administered. After administration, any remaining test mixtures were discarded.

Reason for Route of Administration

Intravenous injection is an acceptable route to assess systemic exposure.

Observations of Animals

Clinical observations were conducted predose and at approximately 0.5, 2.0, and 4.0 hours after intravenous injection. Additional clinical observations and twice a day mortality checks were conducted daily thereafter until the scheduled sacrifice interval (a.m. mortality check only for animals sacrificed on Days 15 or 29).

Body weights were determined on Day -7 for randomization purposes, before test or control material administration (Day 1), and at the scheduled sacrifice interval (Day 15 or Day 29).

Blood Sample Collections/Shipment

A blood sample (approximately 4-mL) was collected from a marginal ear vein (left ear) of each animal on Day -4. Blood samples (approximately 4-mL each) were then collected from a marginal ear vein of each animal at 4-, 8-, 12-, 24-, and 48-hours post-injection, and on Day 8, with the exception of a Group 1 male from which a 1.7-mL blood sample was collected at the 8-hour interval. An approximate 4-mL blood sample was also collected on Days 15 and 22 from a marginal ear vein (either ear) of each animal

scheduled for sacrifice on Day 29. In addition, at the time of the scheduled sacrifice (Day 15 or Day 29) and via the posterior vena cava, approximately 20 to 40 mL of blood was obtained from each control animal and approximately 20 mL of blood was obtained from each animal in Groups 2-5. All samples were stored at room temperature until centrifuged. After centrifugation, separate samples of serum and cellular fractions were obtained. The serum and cellular fraction samples obtained pre-injection through Day 15 were stored in a freezer set to maintain a temperature of -20°C ±10°C until shipped frozen (on dry ice) to the Sponsor (James D. Johnson, 3M E.T. & S, Bldg. 2-3E-09, 935 Bush Avenue, St. Paul, MN, 55106) on Day 22. The serum and cellular fraction samples obtained on Days 22 and 29 were stored at CHW and shipped to the Sponsor the day after experimental (in-life) termination in the same manner as the samples obtained prior to Day 22.

Pathology

On Day 15, the first two animals/sex assigned to each dose level (based on the group assignment randomization) were anesthetized with sodium pentobarbital (via injection in the marginal ear vein), bled via the posterior vena cava, and exsanguinated. An abbreviated gross necropsy examination was not done, however, tissues were collected. The whole liver, bile, and both kidneys from each animal were collected, weighed (volume only determined for bile), and immediately placed in a freezer set to maintain a temperature of -20°C ±10°C. After tissue/bile collection, the animals were discarded. The remaining two animals/sex/dose level were anesthetized, bled, and exsanguinated on Day 29 in the same manner as the animals sacrificed on Day 15.

Shipment of Bile and Tissues

The tissues (whole livers and kidneys) and bile collected on Days 15 and 29, along with documentation of their corresponding weights or volumes, were sent frozen (on dry ice) to the Sponsor (James D. Johnson, 3M E.T. & S, Bldg. 2-3E-09, 935 Bush Avenue, St. Paul, MN, 55106) on Day 21 and six days after in-life termination, respectively. The Sponsor is responsible for the retention and disposition of the samples. CHW does not accept any responsibility for the analysis of the samples collected in this study nor are these results presented in this report.

Statistical Analyses

No statistical analyses were required by the protocol.

Location of Raw Data, Records, and Final Report

The raw data, records, and an original signed copy of the final report will be retained in the archives of CHW in accordance with CHW SOP.

RESULTS

Body Weights

Individual body weights are in Table 1. All animals gained weight during the study, with the exception of one Group 3 female (3.0 mg/kg) which exhibited an insignificant loss in weight (18 g) at its termination interval (Day 15).

Clinical Observations

Individual clinical signs are in Table 2. All animals appeared normal throughout the study.

Pathology

Individual animal tissue weights and bile volumes are in Table 3. The animals were not examined grossly, although tissues were saved.

DISCUSSION

The level of systemic exposure of T-6684 was evaluated in male and female albino rabbits when administered as a single intravenous injection at levels of 1.0, 3.0, 10.0, and 30.0 mg/kg. All animals appeared clinically normal and gained weight during the study, with the exception of one Group 3 female (3.0 mg/kg) which exhibited an insignificant loss in weight at its termination interval (Day 15).

SIGNATURE

F. Bud W. McDonald 12/31/97

F. Bud W. McDonald
Date

Study Director

Acute Studies

Table 1

Individual Body Weights (g)

Sex	Animal Number	Randomization (Day -7)	Initial (Day 1)	Terminal (Day 15)	Terminal (Day 29)
		Group 1 (Control) - DMSO (0.0 mg/kg)			
Male	F61522	2,604	2,625	2,749	*
	F61523	2,677	2,782	2,900	*
	F61524	2,458	2,492	-	2,941
	F61525	2,422	2,507	-	3,018
Female	F61542	2,293	2,372	2,481	*
	F61543	2,549	2,644	2,788	*
	F61544	2,449	2,525	-	2,991
	F61545	2,641	2,731	-	3,174
		Group 2 - T-6684 (1.0 mg/kg)			
Male	F61526	2,598	2,688	2,877	*
	F61527	2,437	2,556	2,664	*
	F61528	2,527	2,665	-	3,029
	F61529	2,410	2,551	-	2,875
Female	F61546	2,537	2,691	2,891	*
	F61547	2,544	2,676	2,829	*
	F61548	2,501	2,610	-	2,850
	F61549	2,592	2,719	-	3,036
		Group 3 - T-6684 (3.0 mg/kg)			
Male	F61530	2,585	2,691	2,694	*
	F61531	2,547	2,555	2,718	*
	F61532	2,599	2,713	-	3,181
	F61533	2,627	2,754	-	2,979
Female	F61550	2,472	2,425	2,669	*
	F61551	2,447	2,591	2,573	*
	F61552	2,416	2,487	-	2,832
	F61553	2,450	2,527	-	2,880

\- Not required.
* Animal sacrificed on Day 15.

Table 1 (Continued)

Individual Body Weights (g)

Sex	Animal Number	Randomization (Day -7)	Initial (Day 1)	Terminal (Day 15)	Terminal (Day 29)
		Group 4 - T-6684 (10.0 mg/kg)			
Male	F61534	2,593	2,800	2,953	*
	F61535	2,419	2,576	2,708	*
	F61536	2,413	2,487	-	2,818
	F61537	2,394	2,493	-	2,811
Female	F61554	2,381	2,486	2,574	*
	F61555	2,555	2,612	2,803	*
	F61556	2,493	2,544	-	2,871
	F61557	2,481	2,539	-	3,002
		Group 5 - T-6684 (30.0 mg/kg)			
Male	F61538	2,436	2,569	2,746	*
	F61539	2,409	2,500	2,620	*
	F61540	2,646	2,610	-	2,969
	F61541	2,594	2,619	-	2,860
Female	F61558	2,637	2,637	2,771	*
	F61559	2,427	2,492	2,643	*
	F61560	2,498	2,656	-	3,093
	F61561	2,571	2,610	-	2,924

\- Not required.
* Animal sacrificed on Day 15.

Table 2

Individual Clinical Signs

Sex	Animal Number	Observation	Hour (Day 1)[a] 0.5	2.0	4.0	Days 2-8	9-15	16-29
		Group 1 (Control) - DMSO (0.0 mg/kg)						
Male	F61522	Appeared Normal	✓	✓	✓	✓	✓	*
	F61523	Appeared Normal	✓	✓	✓	✓	✓	*
	F61524	Appeared Normal	✓	✓	✓	✓	✓	✓
	F61525	Appeared Normal	✓	✓	✓	✓	✓	✓
Female	F61542	Appeared Normal	✓	✓	✓	✓	✓	*
	F61543	Appeared Normal	✓	✓	✓	✓	✓	*
	F61544	Appeared Normal	✓	✓	✓	✓	✓	✓
	F61545	Appeared Normal	✓	✓	✓	✓	✓	✓
		Group 2 - T-6684 (1.0 mg/kg)						
Male	F61526	Appeared Normal	✓	✓	✓	✓	✓	*
	F61527	Appeared Normal	✓	✓	✓	✓	✓	*
	F61528	Appeared Normal	✓	✓	✓	✓	✓	✓
	F61529	Appeared Normal	✓	✓	✓	✓	✓	✓
Female	F61546	Appeared Normal	✓	✓	✓	✓	✓	*
	F61547	Appeared Normal	✓	✓	✓	✓	✓	*
	F61548	Appeared Normal	✓	✓	✓	✓	✓	✓
	F61549	Appeared Normal	✓	✓	✓	✓	✓	✓
		Group 3 - T-6684 (3.0 mg/kg)						
Male	F61530	Appeared Normal	✓	✓	✓	✓	✓	*
	F61531	Appeared Normal	✓	✓	✓	✓	✓	*
	F61532	Appeared Normal	✓	✓	✓	✓	✓	✓
	F61533	Appeared Normal	✓	✓	✓	✓	✓	✓
Female	F61550	Appeared Normal	✓	✓	✓	✓	✓	*
	F61551	Appeared Normal	✓	✓	✓	✓	✓	*
	F61552	Appeared Normal	✓	✓	✓	✓	✓	✓
	F61553	Appeared Normal	✓	✓	✓	✓	✓	✓

a Each animal appeared normal prior to control or test material administration.
* Animal sacrificed on Day 15.
✓ Condition existed.

Table 2 (Continued)

Individual Clinical Signs

Sex	Animal Number	Observation	Hour (Day 1)[a] 0.5	2.0	4.0	Days 2-8	9-15	16-29
		Group 4 - T-6684 (10.0 mg/kg)						
Male	F61534	Appeared Normal	✓	✓	✓	✓	✓	*
	F61535	Appeared Normal	✓	✓	✓	✓	✓	*
	F61536	Appeared Normal	✓	✓	✓	✓	✓	✓
	F61537	Appeared Normal	✓	✓	✓	✓	✓	✓
Female	F61554	Appeared Normal	✓	✓	✓	✓	✓	*
	F61555	Appeared Normal	✓	✓	✓	✓	✓	*
	F61556	Appeared Normal	✓	✓	✓	✓	✓	✓
	F61557	Appeared Normal	✓	✓	✓	✓	✓	✓
		Group 5 - T-6684 (30.0 mg/kg)						
Male	F61538	Appeared Normal	✓	✓	✓	✓	✓	*
	F61539	Appeared Normal	✓	✓	✓	✓	✓	*
	F61540	Appeared Normal	✓	✓	✓	✓	✓	✓
	F61541	Appeared Normal	✓	✓	✓	✓	✓	✓
Female	F61558	Appeared Normal	✓	✓	✓	✓	✓	*
	F61559	Appeared Normal	✓	✓	✓	✓	✓	*
	F61560	Appeared Normal	✓	✓	✓	✓	✓	✓
	F61561	Appeared Normal	✓	✓	✓	✓	✓	✓

a Each animal appeared normal prior to test material administration.
* Animal sacrificed on Day 15.
✓ Condition existed.

TABLE 3

Individual Animal Tissue Weights and Bile Volumes

Corning Hazleton Inc.
Madison, Wisconsin USA

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=ALL;SUBSET=ALL

Sex	Dose Group	Animal Number	Kidney (g)	Liver (g)	Bile (mL)	Sex	Dose Group	Animal Number	Kidney (g)	Liver (g)	Bile (mL)
				Group 1 (Control) - Dimethyl Sulfoxide (0.0 mg/kg)							
M	1	F61522[a]	15.4076	85.4694	0.1000	F	1	F61542[a]	14.5255	70.3337	1.1000
M	1	F61523[a]	14.8791	97.1462	1.3000	F	1	F61543[a]	14.4908	83.8694	1.3000
M	1	F61524[b]	15.2507	95.4341	0.3000	F	1	F61544[b]	13.4878	80.7886	0.5000
M	1	F61525[b]	16.3977	92.9237	0.4000	F	1	F61545[b]	15.1213	91.5821	0.9000
				Group 2 - T6684 (1.0 mg/kg)							
M	2	F61526[a]	17.2412	92.1133	0.5000	F	2	F61546[a]	12.0996	67.3471	1.3000
M	2	F61527[a]	13.9506	97.4926	1.3000	F	2	F61547[a]	13.6341	80.3894	0.8000
M	2	F61528[b]	14.6632	81.6418	1.4000	F	2	F61548[b]	14.2870	70.6582	1.0000
M	2	F61529[b]	13.4739	78.2645	0.5000	F	2	F61549[b]	14.2304	83.7193	0.8000
				Group 3 - T6684 (3.0 mg/kg)							
M	3	F61530[a]	12.9111	70.4048	0.7000	F	3	F61550[a]	13.2410	90.1444	1.2000
M	3	F61531[a]	15.9940	71.5495	0.6000	F	3	F61551[a]	14.1205	65.4078	1.1000
M	3	F61532[b]	15.4256	79.8862	0.4000	F	3	F61552[b]	14.5109	64.3275	0.9000
M	3	F61533[b]	13.7796	65.6058	1.7000	F	3	F61553[b]	15.2481	70.2567	0.5000
				Group 4 - T6684 (10.0 mg/kg)							
M	4	F61534[a]	16.0135	77.8536	0.3000	F	4	F61554[a]	13.3187	74.8795	1.0000
M	4	F61535[a]	19.3318	109.4233	1.8000	F	4	F61555[a]	13.9257	79.1617	0.9000
M	4	F61536[b]	14.5952	71.0696	0.6000	F	4	F61556[b]	14.1232	59.5147	0.4000
M	4	F61537[b]	16.5857	81.7135	0.5000	F	4	F61557[b]	11.7310	76.3246	0.8000
				Group 5 - T6684 (30.0 mg/kg)							
M	5	F61538[a]	16.1940	91.2777	0.9000	F	5	F61558[a]	16.4529	72.4410	0.8000
M	5	F61539[a]	16.5633	77.5408	0.2000	F	5	F61559[a]	13.8245	78.6216	0.6000
M	5	F61540[b]	15.9951	87.0437	0.9000	F	5	F61560[b]	13.2574	93.3025	0.5000
M	5	F61541[b]	17.9059	80.6534	0.8000	F	5	F61561[b]	13.8642	62.2193	1.3000

a Sacrificed on Day 15.
b Sacrificed on Day 29.

APPENDIX

Protocol Deviations
Protocol TP6797
Protocol Amendment No. 1

Protocol Deviations

Protocol	Actual Procedure
Page 5, 7. Experimental Design, A. Animals, (7) Husbandry, (a) Housing. Individually, in suspended stainless steel cages.	One Group 1 male was found outside its cage at the time of the a.m. check on Day 2.
Page 8, 7. Experimental Design, D. Observation of Animals, (3) Blood Sample Collections, (b) Method of Collection/Number of Animals, first paragraph, first sentence. Blood samples (approximately 4 mL)... and from the marginal ear vein (left ear) at 4-hours postdose through Day 8.	Blood samples were collected at the following intervals (postdose) from the **right** marginal ear vein of the following animals (animal number/group/sex): 12- and 24-hour: F61524 (1M), F61557 (4F). 48-hour and Day 8: F61524 (1M), F61549 (2F), F61555 (4F), F61557 (4F).
Page 9, 7. Experimental Design, E. Termination (3) Sample Collection. The whole liver, bile, and both kidneys (collected as one sample) from each animal will be collected, weighed (volume only determined for bile), and immediately placed into a freezer set to maintain a temperature of -20°C ±10°C.	Only one kidney for Animal No. F61531 (Group 3M) was weighed at the time of tissue collection. The other kidney was weighed the next day.

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

Corning Hazleton Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd.
Madison, WI 53704
608.241.4471
608.241.7227 Fax

Sponsor:

CORNING Hazleton

3M
St. Paul, Minnesota

PROTOCOL TP6797

Study Title:

Single-Dose Intravenous Pharmacokinetic Study
of T-6684 in Rabbits

Date:

January 3, 1997

Performing Laboratory:

Corning Hazleton Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

CHW 6329-199

STUDY IDENTIFICATION

Single-Dose Intravenous Pharmacokinetic Study
of T-6684 in Rabbits

CHW No.	6329-199
Test Material	T-6684
Sponsor	3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220
Sponsor's Representative	Roger G. Perkins, PhD, DABT 3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220 (612) 733-3222
Study Director	F. Bud W. McDonald Corning Hazleton Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 242-7901
Study Location	Corning Hazleton Inc. 3301 Kinsman Boulevard Madison, WI 53704
Proposed Study Timetable	
Experimental Start Date	Week of January 13, 1997
Experimental Termination Date	Week of February 10, 1997
Draft Report Date	Week of March 24, 1997

1. **Study**
   Single-Dose Intravenous Pharmacokinetic Study in Rabbits

2. **Purpose**
   To assess the level of systemic exposure when the test material 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 with the exception that analysis of the test material mixtures for concentration, solubility, homogeneity, and stability will not be conducted.

   [ ] 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)
   [ ] 59 NohSan No. 3850 (Japanese MAFF)
   [ ] Notification Nos. 313 and 870 (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.

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

5. **Test Material**

   A. **Identification**
      T-6684

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

   C. **Purity and Stability**
      The Sponsor assumes responsibility for purity and stability determinations (including under test conditions). Samples of test material/vehicle mixture(s)

for concentration, homogeneity/solubility, and stability analyses will not be taken before administration unless requested otherwise by the Sponsor. These samples (if taken) will be sent to the Sponsor after experimental termination.

D. Storage
Room temperature

E. Reserve Samples
Reserve sample(s) of each batch/lot of test material will be taken for this study.

The test material reserve sample(s) will be stored at CHW in a freezer set to maintain a temperature of -20°C ±10°C and then returned to the Sponsor after completion of the in-life phase of the study.

F. Retention
Any unused test material will be returned to the Sponsor after completion of all related in-life testing.

G. Safety Precautions
As required by CHW SOPs and policies

6. Control Material

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

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

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

D. Storage
Room temperature

E. Reserve Samples
Reserve sample(s) of each batch/lot of control material will be taken for this study.

The control material reserve sample(s) will be stored at CHW in a freezer set to maintain a temperature of -20°C ±10°C.

F. Retention
Any remaining control material may be used for other testing and will not be discarded after issuance of the final report.

G. Safety Precautions
required by CHW 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
2.5 to 3.5 kg

(5) Number and Sex
20 males and 20 females

(6) Identification
Individual numbered ear tag

(7) Husbandry

(a) Housing
Individually, in suspended stainless steel cages

(b) **Food**
A measured amount of Laboratory Rabbit Diet HF #5326 (PMI Feeds, Inc.). 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 for total dissolved solids, specified microbiological content, 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°C to 23°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark cycle. The dark cycle may be interrupted due to in-life procedures.

(f) **Acclimation**
At least 7 days

(8) **Selection of Test Animals**
Based on health and body weight according to CHW SOPs. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test. The animals will be placed into study groups using a stratified body weight randomization program within nine days of study initiation.

(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	Control/Test Material	Dose Level (mg/kg)[a]	Number of Animals[b] Male	Female
1 (Control)	Sterile Water	0.0	4	4
2	T-6684	1.0	4	4
3	T-6684	3.0	4	4
4	T-6684	10.0	4	4
5	T-6684	30.0	4	4

a Administered at a dose volume of 0.5 mL/kg.
b Two animals/sex/dose level will be sacrificed on Day 15. The remaining animals (two animals/sex/dose level) will be sacrificed on Day 29.

C. Dosing Procedures

(1) Dosing Route
Intravenous injection into the right marginal ear vein over approximately 30 to 60 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 day of treatment will be designated as Day 1. Individual doses will be calculated based on the animal's body weight taken on Day 1. The Group 1 animals will be treated with DMSO at a dose volume of 0.5 mL/kg. The test material will be diluted with DMSO to achieve a specific concentration for each dose level in Groups 2-5 and administered

at a dose volume of 0.5 mL/kg. The prepared test mixtures will be stored at room temperature until administered.

D. Observation of Animals

(1) Clinical Observations

Before test or control material administration, at approximately 0.5, 2.0, and 4.0 hours post-injection (Day 1) for clinical signs, daily thereafter for clinical signs, and twice daily (a.m. and p.m.) for mortality until the scheduled sacrifice interval (Day 15 or Day 29). The animals sacrificed on Days 15 or 29 will be observed only once for mortality on those respective days. Observations may be extended when directed by the study director.

(2) Body Weights

For randomization, before test or control material injection (Day 1), at the scheduled sacrifice interval (Day 15 or Day 29), or at unscheduled death and sacrifices (when survival exceeds 1 day)

(3) Blood Sample Collections

(a) Frequency

Pre-injection (anytime from up to four days before test material administration to Day 1), at approximately 4-, 8-, 12-, 24-, and 48-hours post-injection, on Days 8, 15, 22, and at the scheduled sacrifice interval (Day 15 or Day 29)

(b) Method of Collection/Number of Animals

Blood samples (approximately 4 mL) will be collected from the marginal ear vein (either ear) of all animals on the day before test or control material injection, and from the marginal ear vein (left ear) at 4-hours postdose through Day 8. On Days 15 and 22, additional blood samples (approximately 4 mL) will be collected from the marginal ear vein (left ear if possible) of the animals scheduled for sacrifice on Day 29.

From the posterior vena cava, approximately 20 mL of blood will be obtained from each animal sacrificed in a moribund condition (if possible), approximately 20 to 40 mL of blood will be obtained from each control animal sacrificed on Days 15 or 29 (the maximum volume possible will be obtained), and approximately

20 mL of blood will be obtained from each Group 2-5 animal sacrificed on Days 15 or 29.

The samples will be stored at room temperature and then centrifuged, and the separate serum and cellular fractions stored in a freezer set to maintain a temperature of -20°C ±10°C. The serum and cellular fractions obtained through Day 15 will be sent frozen on dry ice to the Sponsor one to two weeks prior to in-life termination. The serum and cellular fractions obtained after Day 15 will be sent frozen on dry ice to the Sponsor within one week after in-life termination. The Sponsor is responsible for the retention and disposition of the samples.

The serum and cellular fraction samples will be shipped to:

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

James D. Johnson or his alternate will be notified regarding the shipment of the samples.

E. Termination

(1) Unscheduled Sacrifices and Deaths

Any animal dying during the study or sacrificed in a moribund condition will be subjected to an abbreviated gross necropsy examination and all abnormalities will be recorded. Animals in a moribund condition will be anesthetized with sodium pentobarbital (via injection in the marginal ear vein), bled via the vena cava, and exsanguinated. Tissues, as described in section 7.E.(3) Sample Collection, will be collected from any animal dying during the study or sacrificed in a moribund condition. After necropsy, the animals will be discarded.

(2) Scheduled Sacrifices

On Day 15, the first two animals/sex assigned to each dose level (based on the group assignment randomization) will be anesthetized with sodium pentobarbital (via injection in the marginal ear vein), bled via the vena cava, and exsanguinated. The remaining two animals/sex/dose

level will be anesthetized with sodium pentobarbital (via injection in the marginal ear vein), bled via the vena cava, and exsanguinated on Day 29. An abbreviated gross necropsy examination will not be done, however, tissues [as described in section 7.E.(3) Sample Collection] will be collected.

(3) **Sample Collection**
The whole liver, bile, and both kidneys (collected as one sample) from each animal will be collected, weighed (volume only determined for bile), and immediately placed into a freezer set to maintain a temperature of -20°C ±10°C. After sample collection, the animals will be discarded.

The samples (liver, bile, and kidneys) will be sent frozen on dry ice to the Sponsor within one week after collection. The samples and their corresponding weights or volumes will be shipped to the person listed in Section 7.D.(3)(b). The Sponsor is responsible for the retention and disposition of the samples.

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 and control materials
Description of the test system
Procedures
Dates of experimental initiation and termination
Description of any toxic effects
Gross pathology findings (if applicable)
Gross pathology report (if applicable and requested by the study director)
Individual animal tissue weights and bile volumes

9. **Location of Raw Data, Reserve Sample(s), Records, and Final Report**
Original data, or copies thereof, will be available at CHW to facilitate auditing the study during its progress and before acceptance of the final report. When the final report is completed, control material reserve sample(s), all original paper data, including those items listed below will be retained in the archives of CHW for a period of one year following signing of the final report. One year after signing of the final report, all of the aforementioned materials will be sent to the Sponsor and a return fee will be charged. The Sponsor may elect to have the materials retained in the CHW Archives for an additional period of time and CHW will charge a storage fee. If the Sponsor chooses to have CHW dispose of the materials, a disposal fee will be charged.

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

The following supporting records will be retained at CHW 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

Roger G. Perkins, PhD, DABT
Sponsor's Representative
3M

1/6/97
Date

F. Bud W. McDonald
Study Director
Acute Studies
Corning Hazleton Inc.

1-3-97
Date

Representative
Quality Assurance Unit
Corning Hazleton Inc.

1-3-97
Date

COVANCE
THE DEVELOPMENT SERVICES COMPANY

Covance Laboratories Inc.
P.O. Box 7545
Madison, Wisconsin 53707-7545
Packages: 3301 Kinsman Boulevard
Madison, Wisconsin 53704
Tel: 608/241-4471
Fax: 608/241-7227

AMENDMENT NO. 1 TO THE PROTOCOL

PROTOCOL TP6797

Single-Dose Intravenous Pharmacokinetic Study of T-6684 in Rabbits

CHW 6329-199

Sponsor

3M
Toxicology Service
Medical Department
3M Center, Bldg. 220-2E-02
P.O. Box 33220
St. Paul, MN 55133-3220

Testing Facility

Corning Hazleton Inc.
3301 Kinsman Boulevard
Madison, WI 53704

Sponsor's Representative

Roger G. Perkins, PhD, DABT

Study Director

F. Bud W. McDonald

This amendment modifies the following portions of the protocol:

Effective January 3, 1997

1. **Page 7, 7. Experimental Design, B. Dose Administration, (1) Test Groups.** To correctly identify the control material to be used in this study, which was listed incorrectly in the table at the time the protocol was issued by the study director, replace **sterile water** with **DMSO**.

Amendment No. 1

Effective January 14, 1997

2. **Page 5, 7. Experimental Design, A. Animals, (4) Weight at Initiation.** To accommodate the use of lighter animals available for use in the study, modify the body weight range with the following underlined change:

2.3 to 3.5 kg

PROTOCOL AMENDMENT APPROVAL

Roger G. Perkins, PhD, DABT — Date: 26 FEB 97
Sponsor Representative
3M

F. Bud W. McDonald — Date: 18 FEB 97
Study Director
Acute Studies
Corning Hazleton Inc.

Representative — Date: 18 Feb 1997
Quality Assurance Unit
Corning Hazleton Inc.