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CORNING Hazleton

Sponsor:

3M
St. Paul, Minnesota

FINAL REPORT

Study Title:

Single-Dose Intravenous Pharmacokinetic Study of T-6564 in Rabbits

Author:

F. Bud W. McDonald

Study Completion Date:

April 18, 1997

Performing Laboratory:

Corning Hazleton Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

CHW 6329-186

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 Study	
From	To	Phase	Director	Date to Management
06/27/96	06/27/96	Protocol Review	06/27/96	06/27/96
09/03/96	09/03/96	Sample Collection	09/03/96	09/03/96
10/15/96	10/15/96	Protocol Amendment	10/15/96	10/15/96
12/03/96	12/05/96	Data/Report Review	12/05/96	12/05/96

Rebecca I. Snow — 4-18-97

Representative, Quality Assurance Unit — Date

STUDY IDENTIFICATION

Single-Dose Intravenous Pharmacokinetic
Study of T-6564 in Rabbits

Test Material	T-6564
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	August 2, 1996
Experimental (In-life) Start Date	August 6, 1996
In-life End Date	September 3, 1996
Experimental Termination Date	April 18, 1997
Study Completion Date	April 18, 1997

KEY PERSONNEL

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Pathology Data

CONTENTS

SUMMARY

This study was done to assess the level of systemic exposure of T-6564 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)	Sterile Water	0.0	4	4
2	T-6564	3.0	4	4
3	T-6564	10.0	4	4
4	T-6564	100.0	4	4
5	T-6564	300.0	4[c]	4

a Administered at a dose volume of 0.5 mL/kg.
b Two animals/sex/dose level were sacrificed on Day 14. The remaining animals (two animals/sex/dose level) were sacrificed on Day 29.
c One male died approximately 20 minutes after treatment (possibly due to the volume and rate of injection). A replacement animal was then selected and treated in the same manner.

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 Sterile Water for Injection (sterile water) to a specific concentration for each dose level and administered at a dose volume of 0.5 mL/kg of body weight. One of the initial males treated at 300 mg/kg (Group 5) died approximately 20 minutes after treatment. This animal, discarded without an abbreviated necropsy examination or tissue collection, was replaced with another animal and treated in the same manner. Two animals/sex/dose level were sacrificed on Day 14 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, and 4 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 -6 for randomization

purposes, before test or control material administration (Day 1), and at the scheduled sacrifice interval (Day 14 or Day 29). A blood sample (approximately 4 mL) was collected from each animal four days before control or test material administration with the exception of the one Group 5 replacement male which had a blood sample collected on Day 1 (before test material administration). Blood samples were also collected from the animals at approximately 4-, 8-, 12-, 24-, and 48-hours post-injection, and on Day 8. An exception to these intervals was the replacement animal (Group 5 male), from which blood samples were collected at approximately 3.5-, 6.5-, and 10.5-hours postdose at the 4-, 8-, and 12-hour postdose intervals, respectively. An approximate 4-mL blood sample was also collected on Days 14 and 22 from each animal scheduled for sacrifice on Day 29. In addition, at the time of the scheduled sacrifice (Day 14 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 with the exception of one Group 4 female on Day 14 from which only approximately 12 mL of blood was collected. 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 14 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-6564 or the sterile water control, all animals appeared clinically normal throughout the study with the exception of the one Group 5 male (300 mg/kg) that died within 20 minutes of dose administration. In addition, one Group 4 female (100 mg/kg) and one Group 5 female (300 mg/kg) exhibited a slight loss in weight at their respective termination interval (Day 14).

OBJECTIVE

The objective of this study was to assess the level of systemic exposure to the test material, T-6564, 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-6564 and described as a clear colorless liquid. The control material was Sterile Water for Injection, USP (Abbott Laboratories, Lot No. 10-382-JT; Expiration November 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 or requested by the Sponsor. The purity and stability of the control material were considered to be adequate for the purposes of this study.

Storage and Retention

The test material was stored at room temperature. The control material was stored refrigerated. 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 in accordance with the Wisconsin facility of Corning Hazleton (CHW) Standard Operating Procedure (SOP) and then discarded. The test material reserve sample and unused test material were 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 June 26, 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. 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 have interfered with or affected 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 -6. A Group 5 replacement male (Animal No. F60093) was arbitrarily selected from the extra animals acclimated for the study.

Study Design
Animals weighing from 2,613 to 2,998 g 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)	Sterile Water	0.0	4	4
2	T-6564	3.0	4	4
3	T-6564	10.0	4	4
4	T-6564	100.0	4	4
5	T-6564	300.0	4[c]	4

a Administered at a dose volume of 0.5 mL/kg.
b Two animals/sex/dose level were sacrificed on Day 14. The remaining animals (two animals/sex/dose level) were sacrificed on Day 29.
c One male died approximately 20 minutes after treatment (possibly due to the volume and rate of injection). A replacement animal was then selected and treated in the same manner.

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 sterile water to achieve a specific concentration for each dose level in Groups 2 to 5. An individual dose of each respective control material or test solution was calculated for each animal based on its body weight on the day of treatment. The control material or respective test solution was administered by intravenous injection into the marginal ear vein of the right ear over a period of 34 to 52 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 predose and at approximately 0.5, 2, and 4 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 14 or 29).

Body weights were determined on Day -6 for randomization purposes, before test or control material administration (Day 1), and at the scheduled sacrifice interval (Day 14 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, with the exception of Animal No. F60093 (Group 5 replacement male) which had a blood sample collected from the left ear on Day 1 (before test material administration). Blood samples (approximately 4 mL) were then collected from a marginal ear vein (left ear) of each animal at approximately 4-, 8-, 12-, 24-, and 48-hours post-injection, and on Day 8. An exception to these intervals was the replacement animal (Group 5 male), from which blood samples were collected at approximately 3.5-, 6.5-, and 10.5-hours postdose at the 4-, 8-, and 12-hour postdose intervals, respectively. An approximate 4-mL blood sample was also collected on Days 14 and 22 from each animal scheduled for sacrifice on Day 29. In addition, at the time of the scheduled sacrifice (Day 14 or Day 29) 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, with the exception of Animal No. F60085 (Group 4 female) from which only approximately 12 mL of blood was obtained. 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 through Day 14 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 21. The serum and cellular fraction samples obtained on Days 22 and 29 were stored at CHW and shipped to the Sponsor six days after experimental (in-life) termination in the same manner as the samples obtained prior to Day 22.

Pathology
The Group 5 animal that died shortly after dose administration was discarded without an abbreviated necropsy examination or tissue collection. On Day 14, 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 14.

Shipment of Tissues

The tissues (whole livers and kidneys) and bile collected on Days 14 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) one week after collection 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 4 female (100 mg/kg) and one Group 5 female (300 mg/kg), sacrificed at Day 14. which exhibited weight losses of 125 g and 13 g, respectively.

Clinical Observations

Individual clinical signs are in Table 2. All animals appeared normal throughout the study with the exception of the Group 5 male (Animal No. F60071) that died approximately twenty minutes postdose. The death of this animal is believed due to the rate and volume of the administered dose.

Pathology
Individual animal tissue weights and bile volumes are in Table 3. The animals were not examined grossly, although tissues were saved (with the exception of the Group 5 male that died shortly after dosing - no tissues were saved on this animal).

DISCUSSION

The level of systemic exposure of T-6564 was evaluated in male and female albino rabbits when administered as a single intravenous injection at levels of 3.0, 10.0, 100.0, and 300.0 mg/kg. All animals appeared clinically normal throughout the study with the exception of the one Group 5 male (300.0 mg/kg) that died within 20 minutes of dose administration (believed due to the rate and volume of the dose). All surviving animals gained weight during the study with the exception of one Group 4 female (100 mg/kg) and one Group 5 female (300 mg/kg) that were sacrificed at Day 14.

SIGNATURE

F. Bud W. McDonald — 4-18-97

F. Bud W. McDonald
Study Director
Acute Studies

Date

Table 1

Individual Body Weights (g)

Sex	Animal Number	Randomization (Day -6)	Initial (Day 1)	Terminal (Day 14)	Terminal (Day 29)
		Group 1 (Control) - Sterile Water (0.0 mg/kg)			
Male	F60052	2,643	2,724	2,919	*
	F60053	2,752	2,788	2,884	*
	F60054	2,653	2,686	-	2,833
	F60055	2,591	2,685	-	2,907
Female	F60072	2,828	2,904	2,914	*
	F60073	2,774	2,881	3,008	*
	F60074	2,797	2,805	-	3,207
	F60075	2,777	2,880	-	3,229
		Group 2 - T-6564 (3.0 mg/kg)			
Male	F60056	2,898	2,962	3,012	*
	F60057	2,798	2,851	2,937	*
	F60058	2,716	2,790	-	3,024
	F60059	2,821	2,940	-	3,149
Female	F60076	2,823	2,924	3,122	*
	F60077	2,698	2,873	3,141	*
	F60078	2,699	2,799	-	3,082
	F60079	2,882	2,950	-	3,242
		Group 3 - T-6564 (10.0 mg/kg)			
Male	F60060	2,738	2,822	2,924	*
	F60061	2,634	2,667	2,822	*
	F60062	2,716	2,912	-	3,293
	F60063	2,832	2,749	-	2,984
Female	F60080	2,732	2,797	2,923	*
	F60081	2,520	2,613	2,818	*
	F60082	2,769	2,838	-	3,147
	F60083	2,786	2,795	-	3,108

\- Not required.

* Animal sacrificed on Day 14.

Table 1 (Continued)

Individual Body Weights (g)

Sex	Animal Number	Randomization (Day -6)	Initial (Day 1)	Terminal (Day 14)	Terminal (Day 29)
		Group 4 - T-6564 (100.0 mg/kg)			
Male	F60064	2,686	2,697	2,836	*
	F60065	2,726	2,792	2,874	*
	F60066	2,740	2,734	-	3,071
	F60067	2,745	2,750	-	2,962
Female	F60084	2,538	2,653	2,789	*
	F60085	2,632	2,696	2,571	*
	F60086	2,728	2,825	-	3,137
	F60087	2,892	2,998	-	3,289
		Group 5 - T-6564 (300.0 mg/kg)			
Male	F60068	2,774	2,865	3,128	*
	F60069	2,590	2,722	2,784	*
	F60070	2,917	2,936	-	3,217
	F60071	2,633	2,707	-	a
	F60093[b]	2,989	2,951	-	3,174
Female	F60088	2,837	2,872	3,106	*
	F60089	2,661	2,737	2,724	*
	F60090	2,913	2,953	-	3,269
	F60091	2,711	2,781	-	3,070

- Not required.
* Animal sacrificed on Day 14.
a Animal died approximately 20 minutes postdose.
b Replacement animal for Animal No. F60071.

Table 2

Individual Clinical Signs

Sex	Animal Number	Observation	Hour (Day 1)[a] 0.5	2.0	4.0	Days 2-8	9-14	15-29
Group 1 (Control) - Sterile Water (0.0 mg/kg)								
Male	F60052	Appeared Normal	✓	✓	✓	✓	✓	*
	F60053	Appeared Normal	✓	✓	✓	✓	✓	*
	F60054	Appeared Normal	✓	✓	✓	✓	✓	✓
	F60055	Appeared Normal	✓	✓	✓	✓	✓	✓
Female	F60072	Appeared Normal	✓	✓	✓	✓	✓	*
	F60073	Appeared Normal	✓	✓	✓	✓	✓	*
	F60074	Appeared Normal	✓	✓	✓	✓	✓	✓
	F60075	Appeared Normal	✓	✓	✓	✓	✓	✓
Group 2 - T-6564 (3.0 mg/kg)								
Male	F60056	Appeared Normal	✓	✓	✓	✓	✓	*
	F60057	Appeared Normal	✓	✓	✓	✓	✓	*
	F60058	Appeared Normal	✓	✓	✓	✓	✓	✓
	F60059	Appeared Normal	✓	✓	✓	✓	✓	✓
Female	F60076	Appeared Normal	✓	✓	✓	✓	✓	*
	F60077	Appeared Normal	✓	✓	✓	✓	✓	*
	F60078	Appeared Normal	✓	✓	✓	✓	✓	✓
	F60079	Appeared Normal	✓	✓	✓	✓	✓	✓
Group 3 - T-6564 (10.0 mg/kg)								
Male	F60060	Appeared Normal	✓	✓	✓	✓	✓	*
	F60061	Appeared Normal	✓	✓	✓	✓	✓	*
	F60062	Appeared Normal	✓	✓	✓	✓	✓	✓
	F60063	Appeared Normal	✓	✓	✓	✓	✓	✓
Female	F60080	Appeared Normal	✓	✓	✓	✓	✓	*
	F60081	Appeared Normal	✓	✓	✓	✓	✓	*
	F60082	Appeared Normal	✓	✓	✓	✓	✓	✓
	F60083	Appeared Normal	✓	✓	✓	✓	✓	✓

a Each animal appeared normal prior to control or test material administration.

* Animal sacrificed on Day 14.

✓ 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	9-14	15-29
		Group 4 - T-6564 (100.0 mg/kg)						
Male	F60064	Appeared Normal	✓	✓	✓	✓	✓	•
	F60065	Appeared Normal	✓	✓	✓	✓	✓	•
	F60066	Appeared Normal	✓	✓	✓	✓	✓	✓
	F60067	Appeared Normal	✓	✓	✓	✓	✓	✓
Female	F60084	Appeared Normal	✓	✓	✓	✓	✓	•
	F60085	Appeared Normal	✓	✓	✓	✓	✓	•
	F60086	Appeared Normal	✓	✓	✓	✓	✓	✓
	F60087	Appeared Normal	✓	✓	✓	✓	✓	✓
		Group 5 - T-6564 (300.0 mg/kg)						
Male	F60068	Appeared Normal	✓	✓	✓	✓	✓	•
	F60069	Appeared Normal	✓	✓	✓	✓	✓	•
	F60070	Appeared Normal	✓	✓	✓	✓	✓	✓
	F60093	Appeared Normal	✓	✓	✓	✓	✓	✓
Female	F60088	Appeared Normal	✓	✓	✓	✓	✓	•
	F60089	Appeared Normal	✓	✓	✓	✓	✓	•
	F60090	Appeared Normal	✓	✓	✓	✓	✓	✓
	F60091	Appeared Normal	✓	✓	✓	✓	✓	✓

a Each animal appeared normal prior to control or test material administration.

• Animal sacrificed on Day 14.

✓ 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

ORGAN ABBREVIATION: LI - LIVER, KD - KIDNEY, BI - BILE (ML)

SEX	DOSE GROUP	ANIMAL NUMBER	LI (g)	KD (g)	BI (mL)	SEX	DOSE GROUP	ANIMAL NUMBER	LI (g)	KD (g)	BI (mL)
					Dose Level - 0.0 mg/kg						
M	1	F60052[a]	90.3302	14.6620	1.1000	F	1	F60072[a]	79.9848	15.7815	0.8000
M	1	F60053[a]	73.7863	16.7332	0.7000	F	1	F60073[a]	82.8800	14.6450	0.8000
M	1	F60054[b]	48.1630	15.2486	1.1000	F	1	F60074[b]	71.9963	15.1548	0.7000
M	1	F60055[b]	76.1902	14.6761	1.0000	F	1	F60075[b]	92.6407	18.5087	1.5000
					Dose Level - 3.0 mg/kg						
M	2	F60056[a]	81.8360	15.1615	0.3000	F	2	F60076[a]	91.0291	15.8879	0.4000
M	2	F60057[a]	80.8815	15.8406	0.7000	F	2	F60077[a]	101.0659	15.9660	1.2000
M	2	F60058[b]	77.3457	17.9012	0.2000	F	2	F60078[b]	80.7474	13.9903	1.1000
M	2	F60059[b]	90.2474	16.7928	1.0000	F	2	F60079[b]	92.7357	19.1657	1.4000
					Dose Level - 10.0 mg/kg						
M	3	F60060[a]	74.6403	15.0898	0.7000	F	3	F60080[a]	86.8055	16.3950	1.6000
M	3	F60061[a]	75.0707	16.0551	0.1000	F	3	F60081[a]	69.6640	16.0235	0.7000
M	3	F60062[b]	93.3989	16.2391	1.6000	F	3	F60082[b]	99.7506	18.3869	0.8000
M	3	F60063[b]	68.3306	15.0642	0.6000	F	3	F60083[b]	89.2276	15.7710	0.1000
					Dose Level - 100.0 mg/kg						
M	4	F60064[a]	63.9420	15.2450	1.5000	F	4	F60084[a]	69.7363	14.4089	0.8000
M	4	F60065[a]	81.3660	15.6753	0.6000	F	4	F60085[a]	80.6815	13.6620	0.9000
M	4	F60066[b]	74.4153	15.4621	0.9000	F	4	F60086[b]	88.9171	13.2784	1.0000
M	4	F60067[b]	66.7900	14.1424	0.3000	F	4	F60087[b]	87.2201	16.3357	0.9000
					Dose Level - 300.0 mg/kg						
M	5	F60068[a]	99.4912	15.2020	1.1000	F	5	F60088[a]	91.9828	15.3894	1.5000
M	5	F60069[a]	81.8480	17.1618	0.9000	F	5	F60089[a]	73.9520	12.3374	0.6000
M	5	F60070[b]	75.3822	16.5091	0.4000	F	5	F60090[b]	91.9623	18.2069	2.0000
M	5	F60093[b]	80.5678	16.9704	0.9000	F	5	F60091[b]	100.2084	17.9672	2.5000

a Sacrificed on Day 14.
b Sacrificed on Day 29.

CHW 6329-186

APPENDIX A

Protocol Deviations
Protocol TP6350
Amendment No. 1 To The Protocol

Protocol Deviations

Protocol	Actual Procedure
Page 8, 7. Experimental Design, D. Observation of Animals, (3) Blood Sample Collections, (b) Method of Collection/Number of Animals, Second Paragraph, Last Two Lines. "approximately 20 mL of blood will be obtained from each Group 2-5 animal sacrificed on Days 14 or 29."	Only approximately 12 mL of blood was collected from one Group 4 female (Animal No. F60085) at the Day 14 sacrifice interval.
Page 8, 7. Experimental Design, D. Observation of Animals, (3) Blood Sample Collections, (a) Frequency, Second Line. "administration to Day 1), 4-, 8-, 12-, 24-, and 48-hours post-".	The blood samples collected from the replacement animal (Animal No. F60093) at the 4-, 8-, and 12-hour postdose intervals were collected 3-hours/21 minutes, 6-hours/41 minutes, and 10-hours/28 minutes postdose, respectively.

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

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CORNING Hazleton

Sponsor:

3M
St. Paul, Minnesota

PROTOCOL TP6350

Study Title:

Single-Dose Intravenous Pharmacokinetic Study
of T-6564 in Rabbits

Date:

August 2, 1996

Performing Laboratory:

Corning Hazleton Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

CHW 6329-186

STUDY IDENTIFICATION

Single-Dose Intravenous Pharmacokinetic Study
of T-6564 in Rabbits

CHW No.	6329-186
Test Material	T-6564
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	August 6, 1996
Experimental Termination Date	September 3, 1996
Draft Report Date	October 15, 1996

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-6564

   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**
Sterile Water for Injection (sterile water)

B. **Physical Description**
Clear colorless liquid

C. **Purity and Stability**
The purity and stability of this USP grade material is considered adequate for the purposes of this study.

D. **Storage**
Refrigerated

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**
As 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 screen-bottom 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-6564	3.0	4	4
3	T-6564	10.0	4	4
4	T-6564	100.0	4	4
5	T-6564	300.0	4	4

a Administered at a dose volume of 0.5 mL/kg.
b Two animals/sex/dose level will be sacrificed on Day 14. The remaining animals (two animals/sex/dose level) will be sacrificed on Day 29.

C. Dosing Procedures

(1) Dosing Route
Intravenous injection into the marginal ear vein of the right ear 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 sterile water at a dose volume of 0.5 mL/kg. The test material will be diluted with sterile water 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 14 or Day 29). The animals sacrificed on Days 14 and 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 14 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), 4-, 8-, 12-, 24-, and 48-hours post-injection, on Days 8, 14, 22, and at the scheduled sacrifice interval (Day 14 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 14 and 22, additional blood samples (approximately 4 mL) will be collected from the marginal ear vein (left ear) 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 14 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 14 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 14 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 14 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 14, 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

8-8-96
Date

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

8-2-96
Date

Representative
Quality Assurance Unit
Corning Hazleton Inc.

8-2-96
Date

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CORNING Hazleton

AMENDMENT NO. 1 TO THE PROTOCOL

PROTOCOL TP6350

Single-Dose Intravenous Pharmacokinetic Study of
T-6564 in Rabbits

CHW 6329-186

Sponsor	Testing Facility
3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220	Corning Hazleton Inc. 3301 Kinsman Boulevard Madison, WI 53704
Sponsor's Representative	**Study Director**
Roger G. Perkins, PhD	F. Bud W. McDonald

This amendment modifies the following portion of the protocol:

Effective August 6, 1996

1. **Page 7, 7. Experimental Design, C. Dosing Procedures.** Add the following section to the protocol:

   (5) **Died on Test/Replacement Animals**
   Animal No. F60071 (Group 5 male) died approximately twenty minutes after treatment. Discard the animal without an abbreviated gross necropsy examination or tissue collection. Arbitrarily select a replacement animal (male), weighing from 2.5 to 3.5 kg, from the extra animals and obtain a predose (Day 1) 4-mL blood sample. Process the sample according to the procedure used for Day -4 samples. Treat the replacement animal on Day 1 in the same manner used for the initial animal and follow all subsequent study procedures as indicated in the protocol.

Reason for above change: To address the procedures associated with the death and replacement of the male treated at 300.0 mg/kg of body weight (Group 5).

PROTOCOL AMENDMENT APPROVAL

Roger G. Perkins
Sponsor's Representative
3M

10/29/96
Date

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

Oct. 25, 1996
Date

Representative
Quality Assurance Unit
Corning Hazleton Inc.

10.25.96
Date

(6329-186.Am1/HD)