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CORNING Hazleton

Sponsor:

3M
St. Paul, Minnesota

FINAL REPORT

Study Title:

5-Daily Dose Dermal Absorption/Toxicity Study of T-6564 in Rabbits

Author:

F. Bud W. McDonald

Study Completion Date:

April 28, 1997

Performing Laboratory:

Corning Hazleton Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

CHW 6329-185

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 Standards, 21 CFR 58. The following inspections were conducted and findings reported to the Study Director and management. Written status reports of inspections and findings are issued to Corning Hazleton management according to standard operating procedures.

Inspection Dates			Date Reported to	Date Reported to
From	To	Phase	Study Director	Management
09/30/96	09/30/96	Protocol Review	10/01/96	10/01/96
12/02/96	12/02/96	Sample Handling	12/02/96	12/02/96
01/03/97	01/03/97	Protocol Amendment	01/03/97	01/03/97
01/13/97	01/14/97	Data/Report Review	01/14/97	01/14/97

Susan K Snatch
Representative, Quality Assurance Unit

4-28-97
Date

STUDY IDENTIFICATION

5-Daily Dose Dermal Absorption/Toxicity 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	October 25, 1996
Experimental (In-life) Start Date	October 28, 1996
In-life End Date	November 25, 1996
Experimental Termination Date	April 28, 1997
Study Completion Date	April 28, 1997

KEY PERSONNEL

Acute Studies

Steven M. Glaza
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CONTENTS

SUMMARY

This study was done to assess the systemic absorption/toxicity and relative skin irritancy of T-6564 when applied to the skin of rabbits for 5 consecutive days.

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

Group	Control/ Test Material	Dose Level [a] (mg/kg/day)	Number of Animals	
			Males	Females
1 (Control)	Distilled water	0.0	3	3
2	T-6564	10.0	3	3

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

The back of each rabbit was clipped free of hair and a single dose of the respective control or test material was administered to the skin of the rabbits for 5 consecutive days. The treatment sites remained intact. The area of application was covered with a gauze patch secured with paper tape around all edges and overwrapped with Saran Wrap® and Elastoplast® tape to provide an occlusive dressing for each approximate 23-hour exposure period.

Clinical observations were conducted predose on Day 1 and at approximately 1, 2.5, and 4 hours after each control or test material administration on Days 1 through 5. Mortality checks were conducted twice daily on Days 2 through 29 (a.m. mortality check only on Day 29). Additional clinical observations were conducted daily on Days 6 through 29. Body weights were determined on Day -5 for randomization purposes, before the first control or test material administration (Day 1), and at the scheduled sacrifice interval (Day 29). The initial dermal irritation reading was made before the first control or test material administration (recorded as the Day 1 reading). Subsequent readings of dermal irritation were made after each patch removal on Days 2 through 6 and on Day 13. A blood sample (approximately 4 mL) was collected from each animal on Days -3, 8, 15, and 22. In addition, at the time of necropsy (Day 29), approximately 20-40 mL of blood was obtained from each control animal and approximately 20 mL of blood was obtained

from each Group 2 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 29, the animals were anesthetized with sodium pentobarbital, bled via the posterior vena cava, exsanguinated and necropsied. The whole liver, bile, an approximate 1-cm x 1-cm section of the dermal application site, and both kidneys (collected as one sample) were collected from all animals, weighed (volume only determined for bile), and sent frozen to the Sponsor.

Application of T-6564 did not result in any test material-related changes in body weight gain or macroscopic findings at necropsy. All animals appeared normal throughout the study. In the distilled water control animals, no dermal irritation was observed in one animal and slight to severe dermal irritation was observed in the other five animals. In the animals treated with T-6564, slight dermal irritation was seen in three animals, and moderate to severe dermal irritation was seen in the other three animals. The reason for the unexpected dermal irritation in the distilled water control group was not determined.

OBJECTIVE

The objective of this study was to assess the systemic absorption/toxicity and relative skin irritancy of a test material when applied to the skin of rabbits for 5 consecutive days.

REGULATORY COMPLIANCE

This study was conducted in accordance with the United States Food and Drug Administration Good Laboratory Practice Regulations for Nonclinical Laboratory Studies, 21 CFR 58, with the exception that analysis of the test material 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. All procedural times presented in this report fall within the acceptable ranges as specified in the Wisconsin facility of Corning Hazleton Inc. (CHW) Standard Operating Procedure (SOP).

TEST AND CONTROL MATERIALS

Identification

The test material was identified as T-6564 and was described as a clear, colorless liquid.

The control material was distilled water (Neenah Springs; Expiration May 20, 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. A reserve sample of the control and test materials was taken and stored in a freezer set to maintain a temperature of -20°C ±10°. The control material reserve sample will be retained at CHW for one year in accordance with CHW SOP. The test material reserve sample was sent to the Sponsor. Any unused test material was returned to the Sponsor according to CHW SOP. Any remaining control material may be used for other testing and will not be discarded after issuance of the final report.

Safety Precautions

The control and test 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 October 16, 1996.

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. 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. In cases where variations from the required temperature and humidity 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 using a stratified body weight randomization program. The randomization body weights were determined on Day -5.

Study Design

Animals weighing from 2,038 to 2,270 g and approximately 14 to 18 weeks of age at initiation of treatment were placed into the following study groups:

Group	Control/ Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg/day)	Number of Animals	
				Males	Females
1 (Control)	Distilled water	0.0	0.5	3	3
2	T-6564	10.0	0.5	3	3

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

Preparation of Exposure Area

On the day before the first control or test material application, the back and, if necessary (to obtain unblemished skin), the flanks of each rabbit was clipped free of hair with an

electric clipper. The clipped area made up approximately 20% of the total body surface area. The test sites (intact skin) were inspected for interfering lesions, irritation, or defects that would preclude the use of any of the animals. The animals were clipped as needed throughout the study (Days 4, 13 and 29).

Dose Administration

All animals received five consecutive days of administration of the respective control or test material. The first day of treatment was designated as Day 1.

Group 1. An individual dose was calculated and measured based on each animal's body weight on the first day of treatment. The control material (distilled water) was applied to the test site at a dose volume of 0.5 mL/kg in a thin and uniform layer. The rate of application was approximately 0.04 mL/cm^2.

Group 2. An individual dose of the test material mixture (T-6564 and distilled water) at a concentration of 20 mg/mL was calculated and measured based on each animal's body weight on the first day of treatment. The test material mixture was applied to the test site at a dose volume of 0.5 mL/kg of body weight in a thin and uniform layer. The rate of application was approximately 0.04 mL/cm^2.

Each area of application in Groups 1 and 2 was covered with a 4-ply 5-cm x 5-cm gauze patch. Each gauze patch was secured with paper tape around all edges and overwrapped with Saran Wrap® and Elastoplast® tape to provide an occlusive dressing. Collars were used to restrain the animals during each approximate 23-hour exposure period.

Approximately 23 hours after each control or test material application, the restraining collars and patches were removed and the application sites were rinsed with tap water and dried with disposable paper towels.

Reason for Route of Administration

The dermal route is a potential route of exposure in humans.

Observations of Animals

Clinical observations were conducted predose on Day 1 and at approximately 1, 2.5, and 4 hours after each control or test material administration on Days 1 through 5. Mortality checks were conducted twice daily on Days 2 through 29 (a.m. mortality check only on Day 29). Additional clinical observations were conducted daily on Days 6 through 29.

Body weights were determined on Day -5 for randomization purposes, before the first control or test material administration (Day 1), and at the scheduled sacrifice interval (Day 29).

The initial dermal irritation reading was made before the first control or test material application according to the Draize[1] technique (recorded as the Day 1 reading). Subsequent readings of dermal irritation were made within 30 to 37 minutes after each patch removal (Days 2 through 6) and on Day 13.

Blood Sample Collections/Shipment

A blood sample (approximately 4 mL) was collected from a marginal ear vein of each animal on Days -3, 8, 15, and 22. In addition, at the time of necropsy (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 animal via the posterior vena cava. All samples were stored at room temperature until centrifuged. After centrifugation, separate samples of serum and cellular fractions were obtained and stored in a freezer set to maintain a temperature of -20°C ±10°C until shipped to the Sponsor. The serum and cellular fraction samples obtained on Days -3, 8, and 15 were 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 17. The serum and cellular fraction samples obtained on Days 22 and 29 were shipped to the Sponsor one week after the experimental (in-life) date of termination in the same manner as the samples obtained prior to Day 22. 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.

Pathology

At termination of the in-life experimental phase (Day 29), animals were anesthetized with sodium pentobarbital, bled via the posterior vena cava, exsanguinated, and necropsied in random order. The sites of control or test material application were washed with warm tap water before the necropsy procedure. All animals were subjected to an abbreviated gross necropsy examination and any abnormalities were recorded. The whole liver, bile, an approximate 1-cm x 1-cm section of the dermal application site, and both kidneys (collected as one sample) were collected from all animals, weighed (volume only determined for bile), and immediately placed in a freezer set to maintain a temperature of -20°C ±10°. After necropsy, the animals were discarded.

Shipment of Bile and Tissues

One week after the date of in-life experimental termination, the tissues (whole livers, dermal application sites, and kidneys) and bile 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), along with documentation of their corresponding weights or volumes. 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 and mean body weights are in Table 1. All animals, with the exception of Animal No. F60987 (Group 2 male), exhibited a weight loss from randomization to initiation (in-life). This weight loss can be attributed to the acclimation of the animals to restraining collars conducted for 3 days (approximately 23 hours/day) before the initial control and test material administration. All animals gained weight from initiation (Day 1) to termination (Day 29).

Clinical Observations

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

Dermal Irritation

Individual dermal irritation scores are in Tables 3 and 4. The control material (distilled water) did not produce dermal irritation in one animal. Four other control animals exhibited slight to moderate erythema, slight edema and/or slight desquamation reactions. Slight to severe erythema and edema reactions and slight desquamation were observed in the other control animal. No explanation for the unexpected dermal irritation in the distilled water control group was found. The test material, T-6564, produced slight to moderate erythema and edema reactions in five animals. One of these animals also had slight desquamation on the application site. Slight to severe erythema and edema reactions (with subcutaneous hemorrhaging), slight desquamation and coriaceousness, and moderate fissuring were observed in the other animal treated with T-6564.

Pathology

Individual animal pathology data (tissue weights, bile volumes, and gross pathology observations) are presented in Appendix A. Observed at necropsy on the liver of one Group 2 female was a single, firm, green, pedunculated mass (0.7 x 0.3 x 0.3 cm) attached to the ventral surface of the right median lobe near the gallbladder. The findings

in this animals was not considered to be test material related. No visible lesions were seen in any of the other animals at necropsy.

DISCUSSION

The acute systemic absorption/toxicity and relative skin irritancy of T-6564 was evaluated in male and female albino rabbits when administered dermally for 5 consecutive days. Application of this material did not result in any test material-related changes in body weight gain or macroscopic findings at necropsy. All animals appeared normal throughout the study. Slight to severe dermal irritation was observed in the distilled water control animals and in the animals treated with T-6564.

SIGNATURE

F. Bud W. McDonald — 4-28-97

F. Bud W. McDonald
Study Director
Acute Studies

Date

REFERENCE

1. Draize, J. H., "Acute Dermal Toxicity (Single Exposure)," In: *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics - Dermal Toxicity*, Association of Food and Drug Officials of the U.S., pp. 54-56 (1959).

Table 1

Individual and Mean Body Weights (g)

Males				Females			
Animal Number	Random -ization	Day 1	Day 29	Animal Number	Random -ization	Day 1	Day 29
Group 1 (Control) - Distilled Water (0.0 mg/kg/day)							
F60982	2,213	2,113	2,764	F60988	2,297	2,142	2,714
F60983	2,203	2,097	2,673	F60989	2,246	2,101	2,677
F60984	2,258	2,102	2,646	F60990	2,298	2,114	2,783
Mean	2,225	2,104	2,694	Mean	2,280	2,119	2,725
Group 2 - T-6564 (10.0 mg/kg/day)							
F60985	2,196	2,038	2,583	F60991	2,210	2,068	2,676
F60986	2,199	2,086	2,597	F60992	2,181	2,047	2509
F60987	2,263	2,270	2,823	F60993	2,269	2,207	2,835
Mean	2,219	2,131	2,668	Mean	2,220	2,107	2,673

Table 2

Individual Clinical Signs

Animal Number	Sex	Observation	Day 1* Hour 1	2.5	4	Day 2 Hour 1	2.5	4	Day 3 Hour 1	2.5	4	Day 4 Hour 1	2.5	4	Day 5 Hour 1	2.5	4	Day(s) 6	7	8-11	12	13-15	16-29
Group 1 (Control) - Distilled Water (0.0 mg/kg/day)																							
F60982	M	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
F60983	M	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
F60984	M	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
F60988	F	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
F60989	F	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
F60990	F	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Group 2 - T-6564 (10.0 mg/kg/day)																							
F60985	M	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
F60986	M	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
F60987	M	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
F60991	F	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
F60992	F	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
F60993	F	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

* Each animal appeared normal prior to control or test material administration.
✓ Condition exists.

Table 3

Individual Dermal Irritation Scores -
Group 1 (Control) - Distilled Water (0.0 mg/kg/day)

	Males							Females						
	Study Day							Study Day						
Dermal Reaction	1	2	3	4	5	6	13	1	2	3	4	5	6	13
	Animal No. F60982							Animal No. F60988						
Erythema	0	1	1	1	2	2	1	0	1	1	1	2	2	1
Edema	0	1	1	1	1	1	1	0	0	1	1	1	1	0
Atonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Animal No. F60983							Animal No. F60989						
Erythema	0	0	0	0	0	0	0	0	1	1	1	1	2	1
Edema	0	0	0	0	0	0	0	0	0	1	1	1	1	0
Atonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Animal No. F60984							Animal No. F60990						
Erythema	0	0	0	1	2	2	1	0	1	1	1	2	3	1
Edema	0	0	0	0	1	1	0	0	0	1	1	3	3	1
Atonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	1	0	0	0	0	0	0	1
Coriaceousness	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4

Individual Dermal Irritation Scores -
Group 2 - T-6564 (10.0 mg/kg/day)

	Males							Females						
	Study Day							Study Day						
Dermal Reaction	1	2	3	4	5	6	13	1	2	3	4	5	6	13
	Animal No. F60985							Animal No. F60991						
Erythema	0	0	1	1	1	1	0	0	0	1	1	1	1	0
Edema	0	0	0	0	1	1	0	0	0	0	0	0	1	0
Atonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Animal No. F60986							Animal No. F60992						
Erythema	0	0	0	1	2	2	1	0	1	2	2	3	3[a]	2
Edema	0	0	0	0	2	2	1	0	1	1	2	3	3	1
Atonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	1	0	0	0	0	0	0	1
Coriaceousness	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Fissuring	0	0	0	0	0	0	0	0	0	0	0	0	0	2
	Animal No. F60987							Animal No. F60993						
Erythema	0	0	1	1	1	1	0	0	1	2	2	2	2	1
Edema	0	0	0	1	1	1	0	0	1	1	1	1	1	0
Atonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0	0	0	0	0	0	0

a Subcutaneous hemorrhaging.

APPENDIX A

Individual Animal Pathology Data

APPENDIX A

Individual Animal Pathology Data

Corning Hazleton Inc.
Madison, Wisconsin USA

PAGE: 1

ANIMAL NUMBER: F60982 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11/25/96 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 11/25/96 14:22 PROSECTOR: DAVID SCHUETTE RECORDER: STEVE VAN ADESTINE
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: STEVE VAN ADESTINE

ORGAN NAME	ABSOLUTE ORGAN WEIGHT (GRAMS)	ORGAN WEIGHT RELATIVE TO BODY WEIGHT (%)	ORGAN TO BRAIN WEIGHT RATIO	ORGAN STATUS
DERM APPL. SITE (IS)	0.9747	--------	--------	WEIGHT TAKEN
KIDNEY (KD)	15.2166	--------	--------	WEIGHT TAKEN
LIVER (LI)	86.3171	--------	--------	WEIGHT TAKEN
BILE (ML) (BI)	1.6	--------	--------	WEIGHT TAKEN

* * * GROSS PATHOLOGY OBSERVATIONS * * *

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
GENERAL COMMENT (GC)	-ABBR NEC PERFORM -NO MACROSCOPIC LESIONS	

APPENDIX A

Individual Animal Pathology Data

Corning Hazleton Inc.
Madison, Wisconsin USA

ANIMAL NUMBER: F60983 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11/25/96 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 11/25/96 14:23 PROSECTOR: DEBBIE PIRKEL RECORDER: STEVE VAN ADESTINE
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: STEVE VAN ADESTINE

ORGAN NAME	ABSOLUTE ORGAN WEIGHT (GRAMS)	ORGAN WEIGHT RELATIVE TO BODY WEIGHT (%)	ORGAN TO BRAIN WEIGHT RATIO	ORGAN STATUS
DERM APPL. SITE (IS)	1.2753	--------	--------	WEIGHT TAKEN
KIDNEY (KD)	14.0416	--------	--------	WEIGHT TAKEN
LIVER (LI)	83.7942	--------	--------	WEIGHT TAKEN
BILE (ML) (BI)	0.6	--------	--------	WEIGHT TAKEN

* * * GROSS PATHOLOGY OBSERVATIONS * * *

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
GENERAL COMMENT (GC)	-ABBR NEC PERFORM -NO MACROSCOPIC LESIONS	

APPENDIX A

Individual Animal Pathology Data

Corning Hazleton Inc.
Madison, Wisconsin USA

PAGE: 3

ANIMAL NUMBER: F60984 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11/25/96 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 11/25/96 14:34 PROSECTOR: DAVID SCHUETTE RECORDER: STEVE VAN ADESTINE
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: STEVE VAN ADESTINE

ORGAN NAME	ABSOLUTE ORGAN WEIGHT (GRAMS)	ORGAN WEIGHT RELATIVE TO BODY WEIGHT (%)	ORGAN TO BRAIN WEIGHT RATIO	O R G A N S T A T U S
DERM APPL. SITE (IS)	0.8539	--------	--------	WEIGHT TAKEN
KIDNEY (KD)	15.5098	--------	--------	WEIGHT TAKEN
LIVER (LI)	86.3868	--------	--------	WEIGHT TAKEN
BILE (ML) (BI)	0.6	--------	--------	WEIGHT TAKEN

* * * G R O S S P A T H O L O G Y O B S E R V A T I O N S * * *

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
GENERAL COMMENT (GC)	-ABBR NEC PERFORM -NO MACROSCOPIC LESIONS	

APPENDIX A

Individual Animal Pathology Data

Corning Hazleton Inc.
Madison, Wisconsin USA

ANIMAL NUMBER: F60985 SEX: MALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11/25/96 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 11/25/96 14:59 PROSECTOR: DAVID SCHUETTE RECORDER: STEVE VAN ADESTINE
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: STEVE VAN ADESTINE

ORGAN NAME	ABSOLUTE ORGAN WEIGHT (GRAMS)	ORGAN WEIGHT RELATIVE TO BODY WEIGHT (%)	ORGAN TO BRAIN WEIGHT RATIO	O R G A N S T A T U S
DERM APPL. SITE (IS)	0.8180	--------	--------	WEIGHT TAKEN
KIDNEY (KD)	13.2012	--------	--------	WEIGHT TAKEN
LIVER (LI)	62.4555	--------	--------	WEIGHT TAKEN
BILE (ML) (BI)	0.8	--------	--------	WEIGHT TAKEN

* * * G R O S S P A T H O L O G Y O B S E R V A T I O N S * * *

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
GENERAL COMMENT (GC)	-ABBR NEC PERFORM -NO MACROSCOPIC LESIONS	

APPENDIX A

Individual Animal Pathology Data

Corning Hazleton Inc.
Madison, Wisconsin USA

ANIMAL NUMBER: F60986 SEX: MALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11/25/96 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 11/25/96 15:00 PROSECTOR: DEBBIE PIRKEL RECORDER: STEVE VAN ADESTINE
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: STEVE VAN ADESTINE

ORGAN NAME	ABSOLUTE ORGAN WEIGHT (GRAMS)	ORGAN WEIGHT RELATIVE TO BODY WEIGHT (%)	ORGAN TO BRAIN WEIGHT RATIO	ORGAN STATUS
DERM APPL. SITE (IS)	1.8205	--------	--------	WEIGHT TAKEN
KIDNEY (KD)	13.4319	--------	--------	WEIGHT TAKEN
LIVER (LI)	57.2256	--------	--------	WEIGHT TAKEN
BILE (ML) (BI)	0.5	--------	--------	WEIGHT TAKEN

* * * GROSS PATHOLOGY OBSERVATIONS * * *

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
GENERAL COMMENT (GC)	-ABBR NEC PERFORM -NO MACROSCOPIC LESIONS	

APPENDIX A

Individual Animal Pathology Data

Corning Hazleton Inc.
Madison, Wisconsin USA

ANIMAL NUMBER: F60987 SEX: MALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11/25/96 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 11/25/96 15:08 PROSECTOR: DEBBIE PIRKEL RECORDER: STEVE VAN ADESTINE
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: STEVE VAN ADESTINE

ORGAN NAME	ABSOLUTE ORGAN WEIGHT (GRAMS)	ORGAN WEIGHT RELATIVE TO BODY WEIGHT (%)	ORGAN TO BRAIN WEIGHT RATIO	O R G A N S T A T U S
DERM APPL. SITE (IS)	0.7972	--------	--------	WEIGHT TAKEN
KIDNEY (KD)	15.8230	--------	--------	WEIGHT TAKEN
LIVER (LI)	90.8606	--------	--------	WEIGHT TAKEN
BILE (ML) (BI)	0.2	--------	--------	WEIGHT TAKEN

* * * G R O S S P A T H O L O G Y O B S E R V A T I O N S * * *

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
GENERAL COMMENT (GC)	-ABBR NEC PERFORM -NO MACROSCOPIC LESIONS	

APPENDIX A

Individual Animal Pathology Data

Corning Hazleton Inc.
Madison, Wisconsin USA

ANIMAL NUMBER: F60988 SEX: FEMALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11/25/96 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 11/25/96 14:35 PROSECTOR: DEBBIE PIRKEL RECORDER: STEVE VAN ADESTINE
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: STEVE VAN ADESTINE

ORGAN NAME	ABSOLUTE ORGAN WEIGHT (GRAMS)	ORGAN WEIGHT RELATIVE TO BODY WEIGHT (%)	ORGAN TO BRAIN WEIGHT RATIO	O R G A N S T A T U S
DERM APPL. SITE (IS)	2.0758	--------	--------	WEIGHT TAKEN
KIDNEY (KD)	16.3783	--------	--------	WEIGHT TAKEN
LIVER (LI)	82.5349	--------	--------	WEIGHT TAKEN
BILE (ML) (BI)	1.6	--------	--------	WEIGHT TAKEN

* * * G R O S S P A T H O L O G Y O B S E R V A T I O N S * * *

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
GENERAL COMMENT (GC)	-ABBR NEC PERFORM -NO MACROSCOPIC LESIONS	

APPENDIX A

Individual Animal Pathology Data

Corning Hazleton Inc.
Madison, Wisconsin USA

PAGE: 8

ANIMAL NUMBER: F60989 SEX: FEMALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11/25/96 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 11/25/96 14:49 PROSECTOR: DEBBIE PIRKEL RECORDER: STEVE VAN ADESTINE
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: STEVE VAN ADESTINE

ORGAN NAME	ABSOLUTE ORGAN WEIGHT (GRAMS)	ORGAN WEIGHT RELATIVE TO BODY WEIGHT (%)	ORGAN TO BRAIN WEIGHT RATIO	ORGAN STATUS
DERM APPL. SITE (IS)	1.1923	----------	----------	WEIGHT TAKEN
KIDNEY (KD)	16.1610	----------	----------	WEIGHT TAKEN
LIVER (LI)	77.6085	----------	----------	WEIGHT TAKEN
BILE (ML) (BI)	1.8	----------	----------	WEIGHT TAKEN

* * * GROSS PATHOLOGY OBSERVATIONS * * *

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
GENERAL COMMENT (GC)	-ABBR NEC PERFORM -NO MACROSCOPIC LESIONS	

APPENDIX A

Individual Animal Pathology Data

Corning Hazleton Inc.
Madison, Wisconsin USA

ANIMAL NUMBER: F60990 SEX: FEMALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11/25/96 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 11/25/96 14:48 PROSECTOR: DAVID SCHUETTE RECORDER: STEVE VAN ADESTINE
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: STEVE VAN ADESTINE

ORGAN NAME	ABSOLUTE ORGAN WEIGHT (GRAMS)	ORGAN WEIGHT RELATIVE TO BODY WEIGHT (%)	ORGAN TO BRAIN WEIGHT RATIO	ORGAN STATUS
DERM APPL. SITE (IS)	0.7232	--------	--------	WEIGHT TAKEN
KIDNEY (KD)	13.4790	--------	--------	WEIGHT TAKEN
LIVER (LI)	93.1001	--------	--------	WEIGHT TAKEN
BILE (ML) (BI)	1.1	--------	--------	WEIGHT TAKEN

* * * GROSS PATHOLOGY OBSERVATIONS * * *

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
GENERAL COMMENT (GC)	-ABBR NEC PERFORM -NO MACROSCOPIC LESIONS	

APPENDIX A

Individual Animal Pathology Data

Corning Hazleton Inc.
Madison, Wisconsin USA

ANIMAL NUMBER: F60991 SEX: FEMALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11/25/96 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 11/25/96 15:09 PROSECTOR: DAVID SCHUETTE RECORDER: STEVE VAN ADESTINE
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: STEVE VAN ADESTINE

ORGAN NAME	ABSOLUTE ORGAN WEIGHT (GRAMS)	ORGAN WEIGHT RELATIVE TO BODY WEIGHT (%)	ORGAN TO BRAIN WEIGHT RATIO	ORGAN STATUS
DERM APPL. SITE (IS)	1.0478	---	---	WEIGHT TAKEN
KIDNEY (KD)	13.4087	---	---	WEIGHT TAKEN
LIVER (LI)	85.4184	---	---	WEIGHT TAKEN
BILE (ML) (BI)	0.2	---	---	WEIGHT TAKEN

* * * GROSS PATHOLOGY OBSERVATIONS * * *

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
GENERAL COMMENT (GC)	-ABBR NEC PERFORM	
LIVER (LI)	-MASS(ES)	-ATTACHED TO VENTRAL SURFACE OF RIGHT MEDIAN LOBE NEAR GALL BLADDER: SINGLE PEDUNCULATED MASS, 0.7 X 0.3 X 0.3 CM, GREEN, FIRM; SAME ON CUT SURFACE; REGIONAL LN (HEPATIC) NOT IDENTIFIED

APPENDIX A

Individual Animal Pathology Data

Corning Hazleton Inc.
Madison, Wisconsin USA

ANIMAL NUMBER: F60992 SEX: FEMALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11/25/96 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 11/25/96 15:25 PROSECTOR: DEBBIE PIRKEL RECORDER: STEVE VAN ADESTINE
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: STEVE VAN ADESTINE

ORGAN NAME	ABSOLUTE ORGAN WEIGHT (GRAMS)	ORGAN WEIGHT RELATIVE TO BODY WEIGHT (%)	ORGAN TO BRAIN WEIGHT RATIO	ORGAN STATUS
DERM APPL. SITE (IS)	1.3648	--------	--------	WEIGHT TAKEN
KIDNEY (KD)	13.5655	--------	--------	WEIGHT TAKEN
LIVER (LI)	82.5047	--------	--------	WEIGHT TAKEN
BILE (ML) (BI)	0.5	--------	--------	WEIGHT TAKEN

* * * GROSS PATHOLOGY OBSERVATIONS * * *

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
GENERAL COMMENT (GC)	-ABBR NEC PERFORM -NO MACROSCOPIC LESIONS	

APPENDIX A

Individual Animal Pathology Data

Corning Hazleton Inc.
Madison, Wisconsin USA

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ANIMAL NUMBER: F60993 SEX: FEMALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 11/25/96 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 11/25/96 15:30 PROSECTOR: DEBBIE PIRKEL RECORDER: STEVE VAN ADESTINE
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: STEVE VAN ADESTINE

ORGAN NAME	ABSOLUTE ORGAN WEIGHT (GRAMS)	ORGAN WEIGHT RELATIVE TO BODY WEIGHT (%)	ORGAN TO BRAIN WEIGHT RATIO	ORGAN STATUS
DERM APPL. SITE (IS)	0.9460	---	---	WEIGHT TAKEN
KIDNEY (KD)	15.0657	---	---	WEIGHT TAKEN
LIVER (LI)	91.6304	---	---	WEIGHT TAKEN
BILE (ML) (BI)	0.4	---	---	WEIGHT TAKEN

* * * GROSS PATHOLOGY OBSERVATIONS * * *

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
GENERAL COMMENT (GC)	-ABBR NEC PERFORM -NO MACROSCOPIC LESIONS	

APPENDIX B

Protocol TP6351
Amendment No. 1 to the Protocol

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

PROTOCOL TP6351

Study Title:

5-Daily Dose Dermal Absorption/Toxicity Study of T-6564 in Rabbits

Date:

October 25, 1996

Performing Laboratory:

Corning Hazleton Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

CHW 6329-185

STUDY IDENTIFICATION

5-Daily Dose Dermal Absorption/Toxicity Study of T-6564 in Rabbits

CHW No.	6329-185
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	Week of October 28, 1996
Experimental Termination Date	Week of November 25, 1996
Draft Report Date	Week of January 6, 1997

1. **Study**
   5-Daily Dose Dermal Absorption/Toxicity Study in Rabbits

2. **Purpose**
   To assess the systemic absorption and toxicity and relative skin irritancy of a test material when applied to the skin of rabbits for five consecutive days

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 until 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**
Distilled water

B. **Physical Description**
Clear, colorless liquid

C. **Purity and Stability**
The purity and stability of this commercially available material is considered to be adequate for the purposes of this study.

D. **Storage Conditions**
Room temperature

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

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**
2.0 to 3.0 kg

(5) **Number and Sex**
6 males and 6 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/day)	Number of Animals Males	Females
1 (Control)	Distilled Water	0.0*	3	3
2	T-6564	10.0**	3	3

* To be administered at a dose volume of 0.5 mL/kg of body weight
** To be administered at a dose volume of 0.01 mL/kg of body weight

(2) Preparation of Exposure Area

On the day before the initial test and control material application, the back and, if necessary (to obtain unblemished skin), the flanks of each rabbit will be clipped free of hair with an electric clipper. The shaved area will constitute approximately 20% of the total body surface area. The treatment sites (intact skin) will be inspected for interfering lesions, irritation, or defects that would preclude the use of any of the animals. The animals will be clipped as needed throughout the study.

(3) Dose Administration

All animals will receive five consecutive days of administration of the respective control or test material applied to the same respective application site each day. The test material/vehicle mixture will be prepared fresh each day of administration. The first day of treatment will be designated as Day 1. The control material will be applied undiluted at a dose volume of 0.5 mL/kg. The Group 2 test material will be diluted with distilled water and applied at a dose volume of 0.01 mL/kg. The doses for the animals in Groups 1 and 2 will be based on the animal's body weight taken on Day 1 before the first administration and applied to the area of exposure in a thin and uniform layer. The area of application (Groups 1 and 2) will be completely covered with a 4-ply 5.0-cm x 5.0-cm gauze patch. All patches will be secured with paper tape around all edges and overwrapped with Saran Wrap® and Elastoplast® tape to provide an occlusive dressing. The rabbits will be collared during each approximate 23-hour application period. The prepared test mixtures will be stored at room temperature until administration.

(4) **Reason for Route of Administration**
The dermal route is a potential route of exposure in humans.

(5) **Removal of Test Material**
Approximately 23 hours after each test or control material application, the patches and collars will be removed and the residual material will be removed using water or an appropriate solvent, if necessary. The sites of test and control material application will be washed with warm tap water prior to the tissue collection procedure.

C. **Observation of Animals**

(1) **Clinical Observations**
Before control or test material administration, at approximately 1, 2.5, and 4 hours after each administration of control and test material on Days 1-5 for clinical signs, daily thereafter for clinical signs, and twice daily (a.m. and p.m.) for mortality through Day 29 (a.m. mortality only on Day 29). Observations may be extended when directed by the study director.

(2) **Reading of Dermal Irritation**
Before the initial control or test material administration, the dermal irritation reading will be made and recorded as the Day 1 reading (Attachment 1). Additional dermal irritation readings will be made within 30 to 60 minutes after each patch removal (Days 2-6) and on Study Day 13. Individual dermal irritation records will be maintained for each animal.

(3) **Body Weights**
For randomization, before the initial test or control material application (Day 1), on Day 29, or at death (when survival exceeds 1 day)

(4) **Blood Sample Collections**

(a) **Frequency**
Predose (anytime from up to four days before the initial dose administration to Day 1), on Days 8, 15, 22, and at experimental termination (Day 29)

(b) **Method of Collection/Number of Animals**
Blood samples (approximately 4 mL) will be collected from a marginal ear vein of all animals at the intervals predose through Day 22.

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 Day 29 (the maximum volume possible will be obtained), and approximately 20 mL of blood will be obtained from each Group 2 animal sacrificed on Day 29.

The blood samples will be stored at room temperature and then centrifuged, and the separate serum and cellular fractions stored in a freezer set to maintain -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.

D. Pathology

(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. The whole liver, bile (all available), an approximate 1-cm x 1-cm section of the dermal application site and both kidneys (collected as one sample) will be collected from all animals dying during the study or sacrificed in a moribund condition, weighed (volume only determined for bile), and immediately placed into a freezer set to maintain a temperature of -20°C ±10°C. After necropsy, the animals will be discarded.

(2) Scheduled Sacrifice

On Day 29, the animals will be anesthetized with sodium pentobarbital (via injection in the marginal ear vein), bled via the vena cava, exsanguinated, and subjected to an abbreviated gross necropsy examination. The animals will be necropsied in random order and all abnormalities will be recorded. The whole liver, bile (all available), an approximate 1-cm x 1-cm section of the dermal application site, and both kidneys (collected as one sample) 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 necropsy, the animals will be discarded.

(3) Tissue Sample Shipment

The samples (liver, bile, dermal application site, 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.C.(4).(b). The Sponsor is responsible for the retention and disposition of the samples.

E. 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
Tabulation of mortality data by sex and dose level
Description of any toxic effects/dermal irritation
Tabulation of mean body weights by sex and dose level
Gross pathology findings
Gross pathology report (if requested by the Study Director)
Individual animal tissue weights and bile volumes

9. **Location of Raw Data, Records, Reserve Sample(s), 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
  Randomization data
  Dose administration
  Observations
Anatomical pathology records
Sample collection records
Shipping 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

4/7/97
Date

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

Oct. 25, 1996
Date

Representative
Quality Assurance Unit
Corning Hazleton Inc.

10.25.96
Date

(6329-185)HD

Attachment 1

Scoring Scale for Acute Dermal Reactions

Erythema
0 - None
1 - Slight
2 - Moderate
3 - Severe

Edema
0 - None
1 - Slight (barely perceptible to well defined by definite raising)
2 - Moderate (raised approximately 1 mm)
3 - Severe (raised more than 1 mm)

Atonia
0 - None
1 - Slight (slight impairment of elasticity)
2 - Moderate (slow return to normal)
3 - Marked (no elasticity)

Desquamation
0 - None
1 - Slight (slight scaling)
2 - Moderate (scales and flakes)
3 - Marked (pronounced flaking with denuded areas)

Coriaceousness
0 - None
1 - Slight (decrease in pliability)
2 - Moderate (leathery texture)
3 - Marked (tough and brittle)

Fissuring
0 - None
1 - Slight (definite cracks in epidermis)
2 - Moderate (cracks in dermis)
3 - Marked (cracks with bleeding)

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AMENDMENT NO. 1 TO THE PROTOCOL

Protocol No.: TP6351

5-Daily Dose Dermal Absorption/Toxicity Study of T-6564 in Rabbits

CHW 6329-185

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, DABT	F. Bud W. McDonald

This amendment modifies the following portion of the protocol:

Effective October 28, 1996

1. **Page 7, 7. Experimental Design, B. Dose Administration, (1) Test Groups.** The test material can not be administered at a dose level of 10.0 mg/kg using a dose volume of 0.01 mL/kg. To be able to administer the test material at 10.0 mg/kg, increase the dose volume to 0.5 mL/kg. Replace the table in this section of the protocol with the following: