COVANCE
THE DEVELOPMENT SERVICES COMPANY

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3M
St. Paul, Minnesota

FINAL REPORT

Study Title:

5-Daily Dose Dermal Absorption/Toxicity Study
of T-6684 in Rabbits

Author:

F. Bud W. McDonald

Study Completion Date:

December 31, 1997

Testing Facility:

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Testing Facility Project Identification:

Covance 6329-200

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Covance Laboratories 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. Written status reports of inspections and findings are issued to Covance management according to standard operating procedures.

Inspection Dates			Date Reported to
From	To	Phase	Study Director and Management
04/15/97	04/15/97	Protocol Review	04/15/97
05/05/97	05/05/97	Dose Administration	05/05/97
05/21/97	05/21/97	Protocol Amendment	05/21/97
07/14/97	07/15/97	Data/Report Review	07/15/97

Randy Leesty
Representative, Quality Assurance Unit

12-31-97
Date

STUDY IDENTIFICATION

5-Daily Dose Dermal Absorption/Toxicity Study
of T-6684 in Rabbits

Test Material	T-6684
Sponsor	3M Toxicology Services 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 Services 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 Covance Laboratories Inc. P.O. Box 7545 Madison, WI 53707-7545 608.242.7901
Testing Facility	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, WI 53704
Study Timetable	
Study Initiation Date	April 18, 1997
Experimental (In-life) Start Date	May 5, 1997
In-life End Date	June 2, 1997
Experimental Termination Date	June 2, 1997
Study Completion Date	December 31, 1997

KEY PERSONNEL

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CONTENTS

SUMMARY

This study was done to assess the systemic absorption/toxicity and relative skin irritancy of T-6684 when applied to the skin of rabbits for five 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 (mg/kg/day)	Number of Animals	
			Males	Females
1 (Control)	Dimethyl Sulfoxide	0.0[a]	3	3
2	T-6684	3.0[a]	3	3

a Administered at a dose volume of 0.1 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 only and at approximately 1, 2.5, and 4 hours after each control or test material administration on Days 1 through 5. Additional clinical observations were conducted daily thereafter through Day 29. Mortality checks were conducted twice daily on Days 2 through 29 (a.m. mortality check only on Day 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 23. 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. 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-6684 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 with the exception of one Group 1 animal and two Group 2 animals which had decreased feed consumption for one to three days within the first nine days of the study. Slight dermal irritation was observed in one control animal while slight to moderate dermal irritation was observed in the other five control animals treated with dimethyl sulfoxide (DMSO). In the animals treated with T-6684, slight dermal irritation was observed in one animal while slight to moderate dermal irritation was observed in the other five animals. No macroscopic lesions were seen in any of the animals at necropsy.

OBJECTIVE

The objective of this study was to assess the systemic absorption/toxicity and relative skin irritancy of test materials when applied to the skin of rabbits for five 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 Covance Laboratories Inc. (Covance) Standard Operating Procedures (SOPs).

TEST AND CONTROL MATERIALS

Identification

The materials were identified and described as follows:

Identification	Physical Description
T-6684 (test)	off-white liquid
DMSO (control)	Clear colorless liquid

Dimethyl sulfoxide was manufactured by Mallinckrodt Chemical.

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 each the control and test 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 Covance for one 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 control and test material handling procedures were according to Covance SOPs and policies.

TEST SYSTEM

Test Animal

Adult albino rabbits of the Hra:(NZW)SPF strain were received from Covance Research Products Inc., Kalamazoo, Michigan on April 16, 1997.

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 16 to 22°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 obtained on Day -5.

Study Design

Animals weighing from 2,067 to 2,500 g and approximately 15 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)	Dimethyl Sulfoxide	0.0	0.1	3	3
2	T-6684	3.0	0.1	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 3, 6, 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 (dimethyl sulfoxide) was applied to the test site at a dose volume of 0.1 mL/kg in a thin and uniform layer.

Group 2. An individual dose of the test material mixture (T-6684 and dimethyl sulfoxide) at a concentration of 30 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 0.1 mL/kg of body weight in a thin and uniform layer.

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. One Group 2 male was found without its collar on at the a.m. mortality check on Day 3. The collar was placed back on the animal at this time (see protocol deviations page). Approximately 23 hours after each control or test material application, the restraining collars and patches were removed and any residual material removed from the application sites using tap water and 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 only and at approximately 1, 2.5, and 4 hours after each control or test material application on Days 1 through 5. Additional clinical observations were conducted daily thereafter through Day 29. Mortality checks were conducted twice daily on Days 2 through 29 (a.m. mortality check only on Day 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 23. 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 16. The serum and cellular fraction samples obtained on Days 23 and 29 were shipped to the Sponsor the day after the experimental (in-life) date of termination in the same manner as the samples obtained prior to Day 23. The Sponsor is responsible for the retention and disposition of the samples. Covance

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 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 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. Covance 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 Covance in accordance with Covance SOP.

RESULTS

Body Weights

Individual and mean body weights are in Table 1. All animals, with the exception of one control animal, exhibited a weight loss from randomization to initiation (in-life) which can be attributed to the acclimation of the animals to restraining collars conducted for 3 days (approximately 21-23 hours/day) before the initial control and test material administration. All animals gained weight from Day 1 to Day 29.

Clinical Observations

Individual clinical signs are in Table 2. All animals appeared normal throughout the study with the exception of one Group 1 animal and two Group 2 animals which had decreased feed consumption for one to three days within the first nine days of the study.

Dermal Irritation

Individual dermal irritation scores are in Tables 3 and 4. Slight erythema, edema and fissuring reactions were observed in one control animal while slight to moderate erythema and edema and slight desquamation reactions were observed in the other five control animals treated with dimethyl sulfoxide. Of these five control animals, two animals also exhibited slight coriaceousness reactions. In the animals treated with T-6684, one exhibited slight erythema, edema and fissuring reactions, one exhibited slight erythema and slight to moderate edema reactions, one exhibited slight to moderate erythema and slight edema and fissuring reactions, one exhibited slight to moderate erythema and slight fissuring reactions, one exhibited slight to moderate erythema and edema and slight desquamation and coriaceousness reactions, and one exhibited slight to moderate erythema and edema and slight desquamation, coriaceousness, and fissuring reactions.

Pathology

Individual animal pathology data (tissue weights, bile volumes, and gross pathology observations) are presented in Appendix A. No macroscopic lesions were observed in the animals at necropsy.

DISCUSSION

The acute systemic absorption/toxicity and relative skin irritancy of T-6684 was evaluated in male and female albino rabbits when administered dermally for five 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 with the exception of one Group 1 animal and two Group 2 animals which had decreased feed consumption for one to three days within the first nine days of the study. Slight dermal irritation was observed in one control animal while slight to moderate dermal irritation was observed in the other five control animals treated with dimethyl sulfoxide. In the animals treated with T-6684, slight dermal irritation was observed in one animal while slight to moderate dermal irritation was observed in the other five animals.

SIGNATURE

F. Bud W. McDonald 12/31/97

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) - Dimethyl Sulfoxide (0.0 mg/kg/day)							
F63011	2.315	2.277	2.529	F63017	2.424	2.333	2.755
F63012	2.430	2.500	2.766	F63018	2.380	2.277	2.783
F63013	2.447	2.388	2.708	F63019	2.263	2.244	2.619
Mean	2.397	2.388	2.668	Mean	2.356	2.285	2.719
Group 2 - T-6684 (3.0 mg/kg/day)							
F63014	2.404	2.402	2.722	F63020	2.262	2.067	2.506
F63015	2.485	2.444	2.754	F63021	2.438	2.429	2.804
F63016	2.447	2.390	2.805	F63022	2.395	2.321	2.735
Mean	2.445	2.412	2.760	Mean	2.365	2.272	2.682

Table 2

Individual Clinical Signs

Animal Number	Sex	Observation	Day 1[a] 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	9	10-15	16-29
Group 1 (Control) - Dimethyl Sulfoxide (0.0 mg/kg/day)																								
F63011	M	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-	-	✓	✓
		Decreased food consumption	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	✓	-	-
F63012	M	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
F63013	M	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
F63017	F	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
F63018	F	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
F63019	F	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Group 2 - T-6684 (3.0 mg/kg/day)																								
F63014	M	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
F63015	M	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-	-	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
		Decreased food consumption	-	-	-	-	-	-	-	-	-	✓	✓	✓	-	-	-	-	-	-	-	-	-	-
F63016	M	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
F63020	F	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-	-	✓	✓	✓	✓	✓	-	✓	✓	✓	✓
		Decreased food consumption	-	-	-	-	-	-	-	-	-	✓	✓	✓	-	-	-	-	-	✓	-	-	-	-
F63021	F	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
F63022	F	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

a Each animal appeared normal prior to control or test material administration.
✓ Condition existed.
- Condition not evident.

Table 3

Individual Dermal Irritation Scores - Group 1 (Control) - Dimethyl Sulfoxide (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. F63011							Animal No. F63017						
Erythema	0	0	0	1	2	2	2	0	0	1	2	2	2	1
Edema	0	0	0	1	2	2	2	0	0	0	1	2	2	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	0	0
Fissuring	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Animal No. F63012							Animal No. F63018						
Erythema	0	1	1	1	1	1	1	0	1	1	1	1	2	2
Edema	0	0	0	0	0	1	1	0	1	1	1	2	2	1
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	1
Coriaceousness	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Fissuring	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	Animal No. F63013							Animal No. F63019						
Erythema	0	1	1	1	1	2	1	0	1	1	1	2	2	2
Edema	0	0	0	1	2	2	1	0	1	1	1	1	2	2
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-6684 (3.0 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. F63014							Animal No. F63020						
Erythema	0	0	1	1	1	1	1	0	0	1	2	2	2	1
Edema	0	0	1	1	2	2	0	0	0	0	1	2	2	1
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	1
Coriaceousness	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Fissuring	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	Animal No. F63015							Animal No. F63021						
Erythema	0	1	1	1	1	1	1	0	1	2	2	2	2	2
Edema	0	0	1	1	1	1	1	0	1	1	1	2	2	1
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	1
Coriaceousness	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Fissuring	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	Animal No. F63016							Animal No. F63022						
Erythema	0	1	1	1	1	2	1	0	0	1	1	2	2	1
Edema	0	0	0	0	1	1	1	0	0	1	1	1	2	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	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	1

APPENDIX A

Individual Animal Pathology Data

ANIMAL NUMBER: F63011 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 06/02/97 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 06/02/97 8:59 PROSECTOR: DAVID SCHUETTE RECORDER: KEVIN BILLINGS
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: JOHN S. HALFORD

ORGAN NAME	ABSOLUTE ORGAN WEIGHT (GRAMS)	ORGAN WEIGHT RELATIVE TO BODY WEIGHT (%)	ORGAN TO BRAIN WEIGHT RATIO	ORGAN STATUS
DERMAL APP. SITE (IS)	1.2216	--------	--------	WEIGHT TAKEN
KIDNEY (KD)	15.3938	--------	--------	WEIGHT TAKEN
LIVER (LI)	67.8896	--------	--------	WEIGHT TAKEN
BILE (VOLUME) (BI)	0.5000	--------	--------	WEIGHT TAKEN

* * * GROSS PATHOLOGY OBSERVATIONS * * *

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

ANIMAL NUMBER: F63012 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 06/02/97 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 06/02/97 8:35 PROSECTOR: DAVID SCHUETTE RECORDER: KEVIN BILLINGS
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: JOHN S. HALFORD

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
DERMAL APP. SITE (IS)	1.1614	--------	--------	WEIGHT TAKEN
KIDNEY (KD)	16.1562	--------	--------	WEIGHT TAKEN
LIVER (LI)	61.6643	--------	--------	WEIGHT TAKEN
BILE (VOLUME) (BI)	0.5000	--------	--------	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)	-NO MACROSCOPIC LESIONS -ABBR NEC PERFORM.	

ANIMAL NUMBER: F63013 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 06/02/97 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 06/02/97 9:05 PROSECTOR: JILL PAUS RECORDER: KEVIN BILLINGS
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: JOHN S. HALFORD

ORGAN NAME	ABSOLUTE ORGAN WEIGHT (GRAMS)	ORGAN WEIGHT RELATIVE TO BODY WEIGHT (%)	ORGAN TO BRAIN WEIGHT RATIO	ORGAN STATUS
DERMAL APP. SITE (IS)	0.7738			WEIGHT TAKEN
KIDNEY (KD)	15.1059			WEIGHT TAKEN
LIVER (LI)	73.3509			WEIGHT TAKEN
BILE (VOLUME) (BI)	0.6000			WEIGHT TAKEN

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

ANIMAL NUMBER: F63014 SEX: MALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 06/02/97 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 06/02/97 8:17 PROSECTOR: JILL PAUS RECORDER: KEVIN BILLINGS
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: JOHN S. HALFORD

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
DERMAL APP. SITE (IS)	0.4833	--------	--------	WEIGHT TAKEN
KIDNEY (KD)	12.5487	--------	--------	WEIGHT TAKEN
LIVER (LI)	68.0339	--------	--------	WEIGHT TAKEN
BILE (VOLUME) (BI)	0.9000	--------	--------	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)	-NO MACROSCOPIC LESIONS -ABBR NEC PERFORM.	

Corning Hazleton Inc.
Madison, Wisconsin USA

ANIMAL NUMBER: F63015 SEX: MALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 06/02/97 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 06/02/97 8:25 PROSECTOR: DAVID SCHUETTE RECORDER: KEVIN BILLINGS
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: JOHN S. HALFORD

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
DERMAL APP. SITE (IS)	1.0044	--------	--------	WEIGHT TAKEN
KIDNEY (KD)	15.9025	--------	--------	WEIGHT TAKEN
LIVER (LI)	74.7248	--------	--------	WEIGHT TAKEN
BILE (VOLUME) (BI)	1.9000	--------	--------	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)	-NO MACROSCOPIC LESIONS -ABBR NEC PERFORM.	

Corning Hazleton Inc.
Madison, Wisconsin USA

ANIMAL NUMBER: F63016 SEX: MALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 06/02/97 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 06/02/97 8:56 PROSECTOR: JILL PAUS RECORDER: KEVIN BILLINGS
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: JOHN S. HALFORD

ORGAN NAME	ABSOLUTE ORGAN WEIGHT (GRAMS)	ORGAN WEIGHT RELATIVE TO BODY WEIGHT (%)	ORGAN TO BRAIN WEIGHT RATIO	ORGAN STATUS
DERMAL APP. SITE (IS)	0.6073	--------	--------	WEIGHT TAKEN
KIDNEY (KD)	14.9890	--------	--------	WEIGHT TAKEN
LIVER (LI)	61.8841	--------	--------	WEIGHT TAKEN
BILE (VOLUME) (BI)	0.6000	--------	--------	WEIGHT TAKEN

* * * GROSS PATHOLOGY OBSERVATIONS * * *

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

Appendix A

Individual Animal Pathology Data

Corning Hazleton Inc.
Madison, Wisconsin USA

ANIMAL NUMBER: F63017 SEX: FEMALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 06/02/97 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 06/02/97 8:47 PROSECTOR: DAVID SCHUETTE RECORDER: KEVIN BILLINGS
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: JOHN S. HALFORD

ORGAN NAME	ABSOLUTE ORGAN WEIGHT (GRAMS)	ORGAN WEIGHT RELATIVE TO BODY WEIGHT (%)	ORGAN TO BRAIN WEIGHT RATIO	ORGAN STATUS
DERMAL APP. SITE (IS)	0.9965			WEIGHT TAKEN
KIDNEY (KD)	14.5047			WEIGHT TAKEN
LIVER (LI)	55.4966			WEIGHT TAKEN
BILE (VOLUME) (BI)	1.4000			WEIGHT TAKEN

* * * GROSS PATHOLOGY OBSERVATIONS * * *

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

Corning Hazleton Inc.
Madison, Wisconsin USA

ANIMAL NUMBER: F63018 SEX: FEMALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 06/02/97 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 06/02/97 8:14 PROSECTOR: DAVID SCHUETTE RECORDER: KEVIN BILLINGS
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: JOHN S. HALFORD

ORGAN NAME	ABSOLUTE ORGAN WEIGHT (GRAMS)	ORGAN WEIGHT RELATIVE TO BODY WEIGHT (%)	ORGAN TO BRAIN WEIGHT RATIO	ORGAN STATUS
DERMAL APP. SITE (IS)	0.8229	--------	--------	WEIGHT TAKEN
KIDNEY (KD)	13.4273	--------	--------	WEIGHT TAKEN
LIVER (LI)	63.0821	--------	--------	WEIGHT TAKEN
BILE (VOLUME) (BI)	1.6000	--------	--------	WEIGHT TAKEN

* * * GROSS PATHOLOGY OBSERVATIONS * * *

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

Corning Hazleton Inc.
Madison, Wisconsin USA

ANIMAL NUMBER: F63019 SEX: FEMALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 06/02/97 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 06/02/97 8:23 PROSECTOR: JILL PAUS RECORDER: KEVIN BILLINGS
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: JOHN S. HALFORD

ORGAN NAME	ABSOLUTE ORGAN WEIGHT (GRAMS)	ORGAN WEIGHT RELATIVE TO BODY WEIGHT (%)	ORGAN TO BRAIN WEIGHT RATIO	ORGAN STATUS
DERMAL APP. SITE (IS)	0.6150	--------	--------	WEIGHT TAKEN
KIDNEY (KD)	14.0486	--------	--------	WEIGHT TAKEN
LIVER (LI)	51.5804	--------	--------	WEIGHT TAKEN
BILE (VOLUME) (BI)	0.6000	--------	--------	WEIGHT TAKEN

* * * GROSS PATHOLOGY OBSERVATIONS * * *

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

Individual Animal Pathology Data

Corning Hazleton Inc.
Madison, Wisconsin USA

ANIMAL NUMBER: F63020 SEX: FEMALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 06/02/97 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 06/02/97 8:49 PROSECTOR: JILL PAUS RECORDER: KEVIN BILLINGS
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: JOHN S. HALFORD

ORGAN NAME	ABSOLUTE ORGAN WEIGHT (GRAMS)	ORGAN WEIGHT RELATIVE TO BODY WEIGHT (%)	ORGAN TO BRAIN WEIGHT RATIO	ORGAN STATUS
DERMAL APP. SITE (IS)	0.6370	--------	--------	WEIGHT TAKEN
KIDNEY (KD)	13.4440	--------	--------	WEIGHT TAKEN
LIVER (LI)	52.3928	--------	--------	WEIGHT TAKEN
BILE (VOLUME) (BI)	0.8000	--------	--------	WEIGHT TAKEN

* * * GROSS PATHOLOGY OBSERVATIONS * * *

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

Corning Hazleton Inc.
Madison, Wisconsin USA

ANIMAL NUMBER: F63021 SEX: FEMALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 06/02/97 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 06/02/97 8:37 PROSECTOR: JILL PAUS RECORDER: KEVIN BILLINGS
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: JOHN S. HALFORD

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
DERMAL APP. SITE (IS)	0.8344	--------	--------	WEIGHT TAKEN
KIDNEY (KD)	17.0195	--------	--------	WEIGHT TAKEN
LIVER (LI)	53.0880	--------	--------	WEIGHT TAKEN
BILE (VOLUME) (BI)	1.5000	--------	--------	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)	-NO MACROSCOPIC LESIONS -ABBR NEC PERFORM.	

ANIMAL NUMBER: F63022 SEX: FEMALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 06/02/97 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 06/02/97 9:07 PROSECTOR: DAVID SCHUETTE RECORDER: KEVIN BILLINGS
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. TOM PALMER WEIGHER: JOHN S. HALFORD

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
DERMAL APP. SITE (IS)	1.0944	--------	--------	WEIGHT TAKEN
KIDNEY (KD)	14.2647	--------	--------	WEIGHT TAKEN
LIVER (LI)	60.3910	--------	--------	WEIGHT TAKEN
BILE (VOLUME) (BI)	1.6000	--------	--------	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)	-NO MACROSCOPIC LESIONS -ABBR NEC PERFORM.	

APPENDIX B

Protocol Deviation
Protocol TP6798
Amendment No. 1 To The Protocol

Protocol Deviation

Protocol	Actual Procedure
Page 7, 7. Experimental Design, B. Dose Administration, (3) Dose Administration, Ninth Sentence: The rabbits will be collared during each approximate 23-hour application period.	Animal No. F63016 (Group 2 Male) was found without its collar on at the a.m. mortality check on Day 3. The collar was placed back on the animal at this time. The patch appeared to have remained in place on the application site

This deviation is not considered to have had an adverse effect on the outcome of the study.

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

Sponsor:

3M
St. Paul, Minnesota

PROTOCOL TP6798

Study Title:

5-Daily Dose Dermal Absorption/Toxicity Study of T-6684 in Rabbits

Date:

April 18, 1997

Testing Facility:

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Testing Facility Project Identification:

Covance 6329-200

STUDY IDENTIFICATION

5-Daily Dose Dermal Absorption/Toxicity Study
of T-6684 in Rabbits

Covance No.	6329-200
Test Material	T-6684
Sponsor	3M Toxicology Services 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 Services 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 Covance Laboratories Inc. P.O. Box 7545 Madison, WI 53707-7545 608.242.7901
Testing Facility	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, WI 53704
Proposed Study Timetable	
Experimental Start Date	Week of May 5, 1997
Experimental Termination Date	Week of June 2, 1997
Draft Report Date	Week of July 14, 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 Covance Laboratories Inc. (Covance) Standard Operating Procedures (SOPs) and policies.

5. **Test Material**

   A. **Identification**
      T-6684

   B. **Physical Description**
      Off-white liquid

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.

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 Covance 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.

G. **Safety Precautions**
As required by Covance SOPs and policies

6. **Control Material**

A. **Identification**
Dimethyl Sulfoxide (DMSO)

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

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

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 Covance 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 Covance SOPs and policies

7. Experimental Design

A. **Animals**

(1) **Species**
Rabbit

(2) **Strain/Source**
Hra:(NZW)SPF/Covance Research Products 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 periodically analyzed for specified microorganisms and environmental contaminants.

(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 Covance 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)	DMSO	0.0*	3	3
2	T-6684	3.0*	3	3

* To be administered at a dose volume of 0.1 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 0.1 mL/kg. The Group 2 test material will be diluted with DMSO and applied at a dose volume of 0.1 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 substance, if necessary. The sites of test and control material application will be washed with warm tap water on Day 29 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 starting on Day 2 and continuing to 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 each) 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 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 (via computer-generated random numbers) 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 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) collected at the scheduled sacrifice will be sent frozen on dry ice to the Sponsor within one week after collection. Samples collected at unscheduled sacrifices and deaths (if applicable) will be sent with and in the same manner as the samples from the scheduled sacrifice. 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 Covance 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 Covance 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 Covance Archives for an additional period of time and Covance will charge a storage fee. If the Sponsor chooses to have Covance 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 Covance 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 O. Perkins, PhD, DABT
Sponsor's Representative
3M

4/23/97
Date

F. Bud W. McDonald
Study Director
Acute Studies
Covance Laboratories Inc.

4-18-97
Date

Representative
Quality Assurance Unit
Covance Laboratories Inc.

4-18-97
Date

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 TP6798

5-Daily Dose Dermal Absorption/Toxicity Study
of T-6684 in Rabbits

Covance 6329-200

Sponsor

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

Testing Facility

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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 May 20, 1997

The observance of Memorial Day coincides with Day 22 of the study. To avoid having to conduct the Day 22 bleeds on a holiday, modify the following two sections of the protocol to move the Day 22 bleeds to Day 23.

1. **Page 8, 7. Experimental Design, C. Observation of Animals, (4) Blood Sample Collections, (a) Frequency.** Modify this section with the following underlined change:

> Predose (anytime from up to four days before the initial dose administration to Day 1), on Days 8, 15, 23, and at experimental termination (Day 29)

Amendment No. 1

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Page 2

2. **Page 9, 7. Experimental Design, C. Observation of Animals, (4) Blood Sample Collections, (b) Method of Collection/Number of Animals.** Modify the first paragraph in this section with the following underlined change:

> Blood samples (approximately 4 mL each) will be collected from a marginal ear vein of all animals at the intervals predose through Day 23.

PROTOCOL AMENDMENT APPROVAL

Roger O. Perkins, PhD, DABT — Date 6/4/97
Sponsor's Representative
3M

F. Bud W. McDonald — Date 5-21-97
F. Bud W. McDonald
Study Director
Acute Studies
Covance Laboratories Inc.

Representative — Date 5.21.97
Quality Assurance Unit
Covance Laboratories Inc.

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