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

CORNING Hazleton

Sponsor:

3M
St. Paul, Minnesota

FINAL REPORT

Study Title:

Acute Oral Toxicity Study of T-6669 in Rats
(OECD Guidelines)

Author:

Steven M. Glaza

Study Completion Date:

January 10, 1997

Performing Laboratory:

Corning Hazleton Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

CHW 61001760

COMPLIANCE STATEMENT

Acute Oral Toxicity Study of T-6669 in Rats
(OECD Guidelines)

This study was conducted in accordance with the Organisation for Economic Cooperation and Development and Principles of Good Laboratory Practice, C(81)30(Final) with the exception that analysis of the test material mixtures for concentration, homogeneity/solubility and stability was not conducted.

Steven M. Glaza
Study Director
Acute Studies
Corning Hazleton Inc.

1-10-97
Date

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Corning Hazleton Inc., in accordance with the Organisation for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice, C(81)30(Final). The following inspections were conducted and findings reported to the Study Director and management.

Inspection Dates From	To	Phase	Date Reported to Study Director	Date to Management
10/24/96	10/24/96	Necropsy	10/24/96	10/24/96
12/29/96	12/30/96	Data/Report Review	12/30/96	12/30/96

Susan K. Swatek
Representative, Quality Assurance Unit

1-10-97
Date

STUDY IDENTIFICATION

Acute Oral Toxicity Study of T-6669 in Rats
(OECD Guidelines)

Test Material	T-6669
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	Steven M. Glaza Corning Hazleton Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Corning Hazleton Inc. 3301 Kinsman Boulevard Madison, WI 53704
Study Timetable	
Study Initiation Date	October 9, 1996
Experimental (In-life) Start Date	October 10, 1996
In-life End Date	November 14, 1996
Experimental Termination Date	January 10, 1997
Study Completion Date	January 10, 1997

KEY PERSONNEL

Acute Studies

Steven M. Glaza
Study Director
Manager

Steven R. Sorenson
Study Coordinator

Jeffrey B. Hicks
In-life Supervisor

Rose M. Bridge
Administrative Supervisor

Toxicology Support

Kathy Myers
Manager

Calvin L. Horton
Supervisor

Quality Assurance

Sherry R. W. Petsel
Manager

Laboratory Animal Medicine

Cindy J. Cary, DVM
Diplomate, ACLAM
Supervisor

Anatomical Pathology

Thomas E. Palmer, PhD
Anatomical Pathologist

Deborah L. Pirkel/
Jack Serfort
Supervisors
Necropsy

Anne Mosher
Supervisor
Pathology Data

CONTENTS

OBJECTIVE

The objective of this study was to assess the acute oral toxicity produced when the test material is administered by the oral route (gavage) to rats.[1]

All procedural times presented in this report fall within the acceptable ranges as specified in the Wisconsin facility of Corning Hazleton (CHW) Inc. Standard Operating Procedure (SOP).

TEST MATERIAL

Identification

The test material was identified as T-6669 and described as a white powder.

Purity and Stability

The Sponsor assumes responsibility for purity and stability determinations (including under test conditions). Analysis of the test material mixture for concentration, homogeneity/solubility and stability was not conducted

Storage and Retention

The test material was stored at room temperature. Any unused test material will be returned to the sponsor after issuance of the final report according to CHW SOP.

Safety Precautions

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

TEST SYSTEM

Test Animal

Young adult albino rats of the Crl:CD (SD)BR strain were procured from Charles River Laboratories, Inc. on September 23, October 7, October 21, 1996 (Portage, Michigan facility) and on October 1 and October 15, 1996 (Kingston, New York facility).

Housing

After receipt, the animals were acclimated for a period of at least 7 days. During acclimation and throughout the study, the animals were separated by sex and group housed in screen-bottom stainless steel cages. Environmental controls for the animal room were set to maintain a temperature of 19° to 25°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark lighting cycle. In cases where variations from these conditions existed, they were documented and considered to have had no adverse effect on the study outcome.

Animal Diet

The animals were provided continuous access to Laboratory Rodent Diet #5001, PMI Feeds, Inc., and water except for approximately 17 to 20 hours before test material administration when food, but not water, was withheld. The feed is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed by CHW. There were no known contaminants in the feed or water at levels that could be expected to interfere with or affect the results of the study.

Animal Selection and Grouping

Ten male and 10 female healthy, acclimated rats, weighing from 208 to 269 g and approximately 8 to 12 weeks of age, were assigned to two treatment groups of 250 and 500 mg/kg of body weight. Each dose level consisted of 5 male and 5 female rats. The animals were identified by animal number and corresponding ear tag throughout the study.

Justification for Species Selection

Historically, rats have been used as a representative of a rodent species and are preferred by various regulatory agencies.

PROCEDURES

Preparation and Administration of Test Material

The test material was mixed with distilled water to a concentration of 0.025 g/mL for the 250 mg/kg dose level and 0.050 g/mL for the 500 mg/kg dose level. The prepared test material mixtures appeared to be solutions. An individual dose of the respective test mixture was calculated for each animal based on its fasted body weight and administered by gavage at a volume of 10 mL/kg of body weight. The test material mixtures were stored at room temperature until administered.

Reason for Route of Administration

Historically, the oral route has been the route of choice for administering a known amount of test material.

Observations

Clinical observations and mortality checks were conducted at 1, 2.5, and 4 hours after test material administration and daily thereafter for 14 days. Mortality checks were conducted twice a day (morning and afternoon) for 13 days after test material administration and again the morning of Day 14.

Body weights were determined before test material administration (Day 0). Additional body weights were determined at Day 7, at termination of the in-life phase (Day 14), or at death when survival exceeded 1 day.

Pathology

At termination of the respective in-life phase for each dose level, all surviving animals were euthanized. All animals, whether found dead during the study or euthanized, were

subjected to an abbreviated gross necropsy examination and any abnormalities were recorded. After necropsy, the animals were discarded and no tissues were saved.

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

Mortality

A summary of the observed mortality is in Table 1. No mortality was observed at the 250 mg/kg dose level. Two males and all five females treated at 500 mg/kg were found dead within 4 days of test material administration. No other mortality was observed. Based on the observed mortality, the estimated oral LD_{50} values were determined to be greater than 500 mg/kg for males and between 250 and 500 mg/kg for females.

Body Weights

Individual and mean body weights and body weight gains are in Table 2. All surviving animals exhibited body weight gain throughout the study.

Clinical Signs

Individual clinical signs are in Table 3. All animals treated at 250 mg/kg appeared normal during the study with the exception of two females which exhibited red-stained face and/or wet urogenital area within 24 hours of test material administration. Clinical signs of toxicity observed in the animals treated at 500 mg/kg included red-stained face, yellow-stained or wet urogenital area, hypoactivity, hunched posture, staggered gait, excessive

salivation, and death. The surviving animals treated at 500 mg/kg returned to a normal appearance by Day 7.

Pathology

Individual gross necropsy pathology findings are in Table 4. A summary report by the study pathologist is on Page 14. There were no test material related lesions observed at necropsy.

DISCUSSION

The acute oral toxicity of T-6669 was evaluated in male and female rats when administered as a single gavage dose at levels of 250 and 500 mg/kg of body weight. Based on the observed mortality, the estimated oral LD_{50} values for male rats was determined to be greater than 500 mg/kg and between 250 and 500 mg/kg for females. All mortality occurred at the 500 mg/kg dose level within 4 days of test material administration. All animals treated at 250 mg/kg appeared normal during the study with the exception of two females which exhibited red-stained face and/or wet urogenital area within 24 hours of treatment. Clinical signs of toxicity observed in the animals treated at 500 mg/kg included red-stained face, yellow-stained or wet urogenital area, hypoactivity, hunched posture, staggered gait, and excessive salivation. Animals surviving to the end of the observation period exhibited body weight gain during the study. The gross necropsy examinations did not reveal any test material related lesions.

SIGNATURE

Steven M. Glaza
Study Director
Acute Studies

1-10-97
Date

REFERENCE

1. "Acute Oral Toxicity," *Organisation for Economic Cooperation and Development Guidelines for Testing of Chemicals*, Section 4, Health Effects, Number 401, Paris Cedex (February 24, 1987).

PATHOLOGY REPORT

There were 10 rats (five male, five female) each from dose levels of 250 and 500 mg/kg necropsied. All animals given 500 mg/kg, except for three males, died on test. The surviving animals were euthanized and necropsied at the termination of the study. The dose level, day of death, and gross observations recorded for each animal are in the Individual Pathology Comments that follow this report.

At necropsy, there were few findings and all of these were considered incidental and unrelated to the test material. In the animals that died on test, one male was partially cannibalized, one female had multiple, dark brown areas of variable size in the glandular mucosa of the stomach, and both horns of the uterus in another female were filled with clear fluid. The pelvis of both kidneys in one female given 250 mg/kg was observed to be large. There were no visible lesions in the remaining animals.

Thomas E. Palmer, PhD
Pathologist

1-10-97
Date

(61001760.fin)
121696

Table 1

Mortality Summary

Dose Level (mg/kg)	Sex	Mortality Results No. Died/ No. Dosed*
250	M	0/5
250	F	0/5
500	M	2/5, Days 3^1, 4^1
500	F	5/5, Days 0^1, 1^1, 3^1, 4^2

* Superscript number indicates number of animals found dead on the indicated day.

Table 2

Individual and Mean Body Weights/ Body Weight Gains (g)

Animal Number	Day 0 Weight	Day 7 Weight	Day 7 Gain*	Day 14 Weight	Day 14 Gain*
		Males (250 mg/kg)			
C12551	269	330	61	383	114
C12550	269	325	56	339	70
C12611	248	307	59	375	127
C12612	259	296	37	354	95
C12613	253	296	43	375	122
Mean	260	311	51	365	106
		Females (250 mg/kg)			
C12371	261	294	33	315	54
C12367	248	285	37	294	46
C12368	235	280	45	285	50
C12369	259	303	44	309	50
C12370	252	287	35	288	36
Mean	251	290	39	298	47

* Gain from Day 0 body weight.

Table 2 (Continued)

Individual and Mean Body Weights/ Body Weight Gains (g)

Animal Number	Day 0 Weight	Day 7 Weight	Day 7 Gain*	Day 14 Weight	Day 14 Gain*
		Males (500 mg/kg)			
C12231	213	249	36	302	89
C12227	221	(167)[4]	-	-	-
C12228	208	(168)[3]	-	-	-
C12229	217	271	54	320	103
C12230	215	274	59	334	119
Mean	215	265	50	319	104
		Females (500 mg/kg)			
C12276	248	(231)[3]	-	-	-
C12277	223	(175)[4]	-	-	-
C12278	224	†	-	-	-
C12279	246	(234)[1]	-	-	-
C12280	236	(193)[4]	-	-	-
Mean	235	-	-	-	-

* Gain from Day 0 body weight.

† Animal was found dead on the day of dosing. No body weight required.

( ) Value in parentheses is a dead body weight and was not considered in calculating the mean. Superscript number indicates the day the animal was found dead.

\- Not applicable.

Table 3

Individual Clinical Signs

Animal Number	Observation	Hour			Day													
		1.0	2.5	4.0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
					Males (250 mg/kg)													
C12551	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C12550	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C12611	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C12612	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C12613	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
					Females (250 mg/kg)													
C12371	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C12367	Appeared normal	✓	✓	-	-	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Red-stained face	-	-	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	-
C12368	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C12369	Appeared normal	✓	-	-	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Red-stained face	-	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Wet urogenital area	-	-	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C12370	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ Condition existed.
- Condition not evident.

Table 3 (Continued)

Individual Clinical Signs

Animal Number	Observation	Hour			Day												
		1.0	2.5	4.0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	Males (500 mg/kg)																	
C12231	Appeared normal	✓	✓	✓	-	-	-	-	-	-	✓	✓	✓	✓	✓	✓	✓	✓
	Red-stained face	-	-	-	✓	✓	✓	✓	-	-	-	-	-	-	-	-	-	-
	Yellow-stained urogenital area	-	-	-	-	-	-	-	✓	✓	-	-	-	-	-	-	-	-
C12227	Appeared normal	✓	✓	✓	-	-	-											
	Red-stained face	-	-	-	✓	✓	✓											
	Hypoactivity	-	-	-	-	✓	✓											
	Hunched posture	-	-	-	-	-	✓											
	Staggered gait	-	-	-	-	-	✓											
	Found dead	-	-	-	-	-	-	✓										
C12228	Appeared normal	✓	✓	✓	-	-												
	Red-stained face	-	-	-	✓	✓												
	Hypoactivity	-	-	-	-	✓												
	Yellow-stained urogenital area	-	-	-	-	✓												
	Found dead	-	-	-	-	-	✓											
C12229	Appeared normal	✓	✓	✓	-	-	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Red-stained face	-	-	-	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-
C12230	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ Condition existed.
- Condition not evident.

Table 3 (Continued)

Individual Clinical Signs

Animal Number	Observation	Hour 1.0	Hour 2.5	Hour 4.0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
	Females (500 mg/kg)																	
C12276	Appeared normal	✓	✓	✓	-	-												
	Red-stained face	-	-	-	✓	✓												
	Wet urogenital area	-	-	-	✓	✓												
	Hypoactivity	-	-	-	-	✓												
	Hunched posture	-	-	-	-	✓												
	Found dead	-	-	-	-	-	✓											
C12277	Appeared normal	✓	✓	✓	-	-	-											
	Red-stained face	-	-	-	✓	✓	✓											
	Wet urogenital area	-	-	-	✓	✓	✓											
	Hypoactivity	-	-	-	-	✓	✓											
	Hunched posture	-	-	-	-	✓	✓											
	Found dead	-	-	-	-	-	-	✓										
C12278	Appeared normal	✓	✓															
	Excessive salivation	✓	✓															
	Found dead	-	-	✓														
C12279	Appeared normal	✓	✓	✓	-													
	Red-stained face	-	-	-	✓													
	Wet urogenital area	-	-	-	✓													
	Hypoactivity	-	-	-	✓													
	Staggered gait	-	-	-	✓													
	Found dead (after clinical observation)	-	-	-	✓													
C12280	Appeared normal	✓	✓	✓	-	-	-											
	Red-stained face	-	-	-	✓	✓	✓											
	Wet urogenital area	-	-	-	✓	✓	✓											
	Hypoactivity	-	-	-	-	-	✓											
	Hunched posture	-	-	-	-	-	✓											
	Staggered gait	-	-	-	-	-	✓											
	Found dead	-	-	-	-	-	-	✓										

✓ Condition existed.
- Condition not evident.

Table 4

Individual Pathology Comments

Dose Level: 250 mg/kg of Body Weight

Animal Number	Sex	Test Day		Necropsy Observation
		Died	Sacrificed	
C12551	M	-	14	No visible lesions.
C12550	M	-	14	No visible lesions.
C12611	M	-	14	No visible lesions.
C12612	M	-	14	No visible lesions.
C12613	M	-	14	No visible lesions.
C12371	F	-	14	Both of the kidneys have a large pelvis.
C12367	F	-	14	No visible lesions.
C12368	F	-	14	No visible lesions.
C12369	F	-	14	No visible lesions.
C12370	F	-	14	No visible lesions.

- Not applicable.

Table 4 (Continued)

Individual Pathology Comments

Dose Level: 500 mg/kg of Body Weight

Animal Number	Sex	Test Day		Necropsy Observation
		Died	Sacrificed	
C12231	M	-	14	No visible lesions.
C12227	M	4	-	The right flank was cannibalized.
C12228	M	3	-	No visible lesions.
C12229	M	-	14	No visible lesions.
C12230	M	-	14	No visible lesions.
C12276	F	3	-	The glandular mucosa of the stomach has multiple dark brown areas, up to 2 x 1 mm.
C12277	F	4	-	No visible lesions.
C12278	F	0	-	No visible lesions.
C12279	F	1	-	The lumen in the bilateral horns of the uterus is filled with clear fluid.
C12280	F	4	-	No visible lesions.

- Not applicable.

APPENDIX

Protocol TP2069
Protocol Amendment No. 1
Protocol Amendment No. 2
Protocol Amendment No. 3

12-02 MEDICAL 2E 02 008

Sample Submittal Form

This form is to be used when submitting samples for routine acute testing. Special testing needs can be easily arranged by contacting the Acute Studies Department at (608) 241-7282.

CHW Study No. 61001760
Enclose with samples and send to:
Corning Hazleton Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Submitted by: ROGER G. PERKINS
Date Sample Sent: (FAXED 10/8/96) 10/8/96
Company: 3M
Number of Reports Required: 3

Full GLP Compliance: ✓ Yes ___ No
___ FDA (21 CFR 58) ___ EPA (TSCA—40 CFR 792)
___ EPA (FIFRA—40 CFR 160) X OECD
___ MAFF ___ MOHW

Sample Name: T-6669
Physical Description: WHITE SOLID
Special Handling Precautions: SEE MSDS 10-3803-2
Test material purity and stability information (including under test conditions) on file with Sponsor: X Yes ___ No
Test mixture analysis for concentration/homogeneity/stability to be conducted: ___ Yes* X by Sponsor ___ by CHW
X No
Sample Disposal: ✓ Return to Sponsor at following address:

L. J. PICKETT
3M SCD
2453-3S-02 3M CENTER
ST. PAUL, MN 55144-1000

___ Dispose of according to CHW SOPs

Sample Storage Requirements:
X Room temperature
___ Refrigerated
___ Other ___
* At additional cost to Sponsor (CHW will contact Sponsor as to these additional charges).

Tests

Acute Oral Toxicity in Rats

___TP8084 Up and down LD50 procedure
___TP3206 FHSA screen; 5M-5F at 5.0 g/kg
___ Conduct defined study if death occurs at 5.0 g/kg
___TP3013 EPA screen; 5M-5F at 5.0 g/kg
___ Conduct defined study if death occurs at 5.0 g/kg
X TP2069 OECD screen; 5M-5F at 5.0 g/kg
___ Conduct defined study if death occurs at 5.0 g/kg

Special Instructions: ___

Acute Dermal Toxicity in Rabbits

___TP3207 FHSA screen; 5M-5F at 2.0 g/kg
___TP3016 EPA screen; 5M-5F at 2.0 g/kg
___ Conduct defined study if death occurs at 2.0 g/kg
___TP2070 OECD screen; 5M-5F at 2.0 g/kg
___ Conduct defined study if death occurs at 2.0 g/kg

Special Instructions: ___

Primary Skin Irritation

___TP3208 FHSA; 6 rabbits-1 abraded, 1 intact site/rabbit
___TP3014 EPA; 6 rabbits-1 intact site/rabbit
___TP2071 OECD; 3 rabbits-1 intact site/rabbit
___TP4206 DOT corrosivity; 6 rabbits-1 intact site/rabbit
___TP7145 Phototoxicity; 6 rabbits-2 intact sites/rabbit
(one site with UVA exposure)

Special Instructions: ___

Primary Eye Irritation

___TP6380 Low-volume procedure; 6 rabbits unwashed
___TP3209 FHSA; 6 rabbits unwashed
___TP2012 1978 EPA; 6 rabbits unwashed, 3 washed
___TP3015 1982 EPA; 6 rabbits unwashed
___TP2072 OECD; 3 rabbits unwashed
___ 3 rabbits washed at 4 seconds
___ 3 rabbits washed at 30 seconds

Special Instructions: ___

Guinea Pig Sensitization

___TP2017 EPA Magnusson-Kligman maximization
___TP6164.EC OECD/EC Magnusson-Kligman maximization
___TP2008 Buehler sensitization
___TP6289 Photoallergenic contact dermatitis (Armstrong)

Special Instructions: ___

For CHW Use Only
Protocol Issue Date: 10-9-96
Study Director: Steven M. Ellis

White copy—CHW Yellow copy—Submitter

HAZLETON
WISCONSIN
POST OFFICE BOX 7545
MADISON, WISCONSIN 53707-7545

a CORNING Laboratory Services Company

Sponsor:

3M
St. Paul, Minnesota

PROTOCOL TP2069

Study Title:

Acute Oral Toxicity Study in Rats
(OECD Guidelines)

Date:

June 1, 1993

Performing Laboratory:

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

Laboratory Project Identification:

HWI 61001760

STUDY IDENTIFICATION

Acute Oral Toxicity Study in Rats
(OECD Guidelines)

HWI No.	61001760
Test Material	(See sample submittal form)
Sponsor	3M Toxicology Services 220-2E-02 3M Center St. Paul, MN 55144
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Services 220-2E-02 3M Center St. Paul, MN 55144 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Proposed Study Timetable	
Experimental Start Date	Week of 10-7-96
Experimental Termination Date	Week of 12-9-96
Final Report Date	Week of 12-9-96

1. Study
Acute Oral Toxicity Study in Rats (OECD Guidelines)

2. Purpose
To assess the acute oral toxicity produced when the test material is administered by the oral route (gavage) to rats

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

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

All procedures in this protocol are in compliance with the Animal Welfare Act Regulations. In the opinion of the Sponsor and study director, the study does not unnecessarily duplicate any previous work.

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

5. Test Material

A. Identification
(See sample submittal form)

B. Physical Description
(See sample submittal form)

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

D. Storage
(See sample submittal form)

E. Reserve Samples
Studies of less than 4 weeks in experimental duration will not have reserve samples retained.

Reserve sample(s) of each batch/lot of test material will be taken if this study is more than 4 weeks in experimental duration.

The test material reserve sample will be stored at HWI in a freezer set to maintain a temperature of below 0°C for 10 years per HWI SOP. The Sponsor will be contacted after 10 years for disposition in accordance with the appropriate regulatory Good Laboratory Practices.

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

G. Safety Precautions
As required by HWI SOPs and policies

6. Experimental Design

A. Animals

(1) Species
Rat

(2) Strain/Source
[X] Crl:CD®BR/Charles River Laboratories, Inc.
[ ] Hsd:Sprague Dawley SD®/Harlan Sprague Dawley, Inc.

(3) Age at Initiation
Young adult

(4) Weight at Initiation
200 to 300 g

(5) Number and Sex
5 males and 5 females for the initial dose level
5 males and/or 5 females for any additional dose levels (if required)

(6) Identification
Individual numbered ear tag

(7) Husbandry

(a) Housing
Separated by sex and group housed in screen-bottom stainless steel cages (heavy gauge)

(b) Food
Rodent Chow® #5001 (Purina Mills, Inc.) *ad libitum* except for overnight before test material administration. The food is routinely analyzed by the manufacturer for nutritional components and environmental contaminants.

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

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

(e) Environment
Environmental controls for the animal room will be set to maintain a temperature of 19 to 25°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark cycle.

(f) Acclimation
At least 7 days

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

(9) Justification for Species Selection
Historically, rats have been used as representative of a rodent species and are preferred by various regulatory agencies.

B. Dose Administration

(1) Dose Level
A single dose of 5,000 mg/kg of body weight will be administered to five males and five females. If no test material-related mortality is produced at this level, no

further testing will be required. If any mortality occurs at the 5,000 mg/kg dose level, additional dose levels may be added at the direction of the study director in order to meet the objectives of the study.

(2) Dose Preparation and Administration
All animals will receive the same concentration of test mixture per dose level. If a solid, the test material will be suspended in an appropriate vehicle. If a liquid, the test material will be dosed undiluted, using the bulk density to determine the dose volume. If the material is an aerosol, it will be discharged into a beaker and administered as a liquid. Individual doses will be based upon the animal's body weight taken just before test material administration, and administered by gavage. The animals will have food withheld for 17 to 20 hours before test material administration. The prepared test mixtures will be stored at room temperature until administration. After administration, any remaining test mixtures will be discarded.

(3) Reason for Route of Administration
Historically, the oral route has been the route of choice for administering a known amount of test material.

C. Observation of Animals

(1) Clinical Observations
At approximately 1, 2.5, and 4 hours after test material administration and daily thereafter for at least 14 days for clinical signs and twice daily (a.m. and p.m.) for mortality. Observations may be extended when directed by the study director.

(2) Body Weights
Before experimental initiation, at 7 and 14 days after test material administration, and at death (when survival exceeds 1 day)

D. Pathology
At termination of the experimental phase, surviving animals will be euthanized. All animals, whether dying during the study or euthanized, will be subjected to an abbreviated gross necropsy examination and all abnormalities will be recorded. After necropsy, the animals will be discarded and no tissues will be saved.

E. Statistical Analyses
Other than LD_{50} calculations (when applicable) no statistical analyses are required.

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

Description of the test material
Description of the test system
Procedures
Dates of experimental initiation and termination
Tabulation of mortality data by sex and dose level
Description of any toxic effects
Tabulation of mean body weights by sex and dose level
LD_{50} calculations for each sex with 95% confidence intervals (when applicable)
Gross pathology findings/gross pathology report (when applicable)

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

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

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

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

PROTOCOL APPROVAL

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

July 22, 1993
Date

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

6-1-93
Date

Rebecca S. Nelson
Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

6/1/93
Date

(TP2069.3M)

CHW No. 61001760

PROTOCOL AMENDMENTS

Amendment No. 1

Effective October 9, 1996

Portion of Protocol Being Modified: Applicable sections of the protocol.

Reason for Modification: To identify the location where the study will be conducted and to reflect a company name change from Hazleton Wisconsin, Inc. (HWI) to Corning Hazleton Inc. (CHW), replace wherever applicable the following changes

Modification: Corning Hazleton Inc. (CHW)
3301 Kinsman Boulevard.
Madison, WI 53704

Study Director Approval: Steven M. Yoder 10-9-96

(G21/01-07-91)

CHW No. 61001760

PROTOCOL AMENDMENTS

Amendment No. 2

Effective October 9, 1996

Portion of Protocol Being Modified: Page 4, 6. Experimental Design: A. Animals

(2) Strain/Source

Reason for Modification: To correctly identify the nomenclature used for animals received from Charles River Laboratories, Inc.

Modification: Replace this section with the following change:

(2) Strain/Source

☒ Crl:CD®(SD)BR/Charles River Laboratories, Inc.
☐ Hsd:Sprague Dawley SD®/Harlan Sprague Dawley, Inc.

Study Director Approval: Steven M. Hoyt 10-9-96

(G21/01-07-91)

CHW No. 61001760

PROTOCOL AMENDMENTS

Amendment No. 3

Effective October 9, 1996

Portion of Protocol Being Modified: Page 5, 6, Experimental Design: B. Dose Administration: (1) Dose Level.

Reason for Modification: The Sponsor requested that a level of 500 mg/kg be treated initially.

Modification: Replace 5,000 mg/kg with 500 mg/kg wherever listed in this section.

Study Director Approval: 10-9-96

(G21/01-07-91)