

**Summary**

(In accordance with 40 CFR Part 152, this summary is available for  
public release after registration)

**Study Title**

**PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1  
MICE**

**Test Guidelines**

OECD Guideline No. 401 Acute Oral Toxicity, 1987.  
EPA Health Effects Test Guidelines; OPPTS 870.1100, 1998.  
Japan MAFF Acute Oral Toxicity Study, 1985.  
EEC Methods Number B.1 Acute Toxicity (Oral), 1992.

**Author(s)**

[REDACTED]

**Study Completion Date**

26 January 2000

**Contact Phone**

(317) 337-4450

**Sponsor**

Dow AgroSciences (DAS) LLC  
9330 Zionsville Rd., Indianapolis, IN 46268

**Performing Laboratory**

Toxicology & Environmental Research and Consulting  
The Dow Chemical Company  
Midland, Michigan 48674

### SUMMARY

PAT Microbial Protein (FL), which was 84% pure microbial protein, was evaluated for acute oral toxicity. Five male and five female CD-1 mice received 6000 mg/kg of the test material (containing approximately 5000 mg/kg PAT) as a 25% w/v suspension in aqueous 0.5% methylcellulose. Because the volume of the test material in methylcellulose exceeded 2 ml/100g body weight, the test material suspension was administered as two fractional gavage doses given approximately one hour apart. Parameters evaluated during the two-week observation period included body weights and detailed clinical observations. All animals were examined for gross pathological changes.

All mice survived to the end of the two-week observation period. There were no treatment-related clinical observations. All mice except one female gained body weight over the duration of the study. There were no gross pathologic lesions for any animal on study.

Under the conditions of this study, the acute oral LD<sub>50</sub> of PAT Microbial Protein (FL) in male and female CD-1 mice was greater than 6000 mg/kg.

**STUDY TITLE**

**PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1  
MICE**

**Test Guidelines**

OECD Guideline No. 401 Acute Oral Toxicity, 1987.  
EPA Health Effects Test Guidelines; OPPTS 870.1100, 1998.  
Japan MAFF Acute Oral Toxicity Study, 1985.  
EEC Methods Number B.1 Acute Toxicity (Oral), 1992.

**Author(s)**

[REDACTED]

**Study Completion Date**

26 January 2000

**Contact Phone**

(317) 337-4450

**Sponsor**

Dow AgroSciences (DAS) LLC  
9330 Zionsville Rd., Indianapolis, IN 46268

**Performing Laboratory**

Toxicology & Environmental Research and Consulting  
The Dow Chemical Company  
Midland, Michigan 48674

**Laboratory Project Study ID**

991249

**STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS**

Compound: PAT MICROBIAL PROTEIN (FL)

Title: PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN  
CD-1 MICE

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C) †.

Company:

Dow AgroSciences

Company Agent:

[Redacted Signature]

1/20/2000  
(Date)

Regulatory Manager

†In the United States, the above statement supersedes all other statements of confidentiality that may occur elsewhere in this report.

**THESE DATA MAY BE CONSIDERED CONFIDENTIAL IN COUNTRIES  
OUTSIDE THE UNITED STATES.**

## COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Compound: PAT MICROBIAL PROTEIN (FL)

Title: PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

All phases of this study were conducted in compliance with the following Good Laboratory Practice Standards.

US Environmental Protection Agency-FIFRA GLPS  
Title 40 CFR, Part 160-Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule


Japan Ministry of Agriculture, Forestry and Fisheries (MAFF)  
Good Laboratory Practice Standards for Toxicological Studies on Agricultural Chemicals


Organization for Economic Co-Operation and Development (OECD)  
OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM(98)17


European Community (EC)  
EC Directive 99/11/EC of 8 March 1999 (OJ No. L 77/8-21, 23/3/1999)

Exceptions: The test material was not characterized by GLP standards; however, a purity analysis was conducted. The dose suspension was not analyzed for homogeneity or dose confirmation.

 26 JAN. 2000  
(Date)  
Study Director

 1-25-00  
(Date)  
Manager  
Toxicology & Environmental  
Research and Consulting

Sponsored By:  
 1/20/2000  
(Date)  
Regulatory Manager  
Dow AgroSciences

Submitted By:  
 1/20/2000  
(Date)  
Regulatory Manager  
Dow AgroSciences

## QUALITY ASSURANCE STATEMENT

Compound: PAT MICROBIAL PROTEIN (FL)


Title: PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN  
CD-1 MICE

This study was examined for conformance with Good Laboratory Practices as published by the U.S. Environmental Protection Agency. The final report was determined to be an accurate reflection of the data obtained. The dates of Quality Assurance activities on this study are listed below.

Study Initiation Date: 14 December 1999

<u>TYPE OF AUDIT:</u>	<u>DATE OF AUDIT:</u>	<u>DATE FINDINGS REPORTED TO STUDY DIRECTOR/MANAGEMENT:</u>
Final protocol	14 December 1999	14 December 1999
Study conduct	21 December 1999	21 December 1999
Protocol, data, and draft report	18 January 2000	20 January 2000
Final report	The date of the signature below is the date of the final report audit.	

The final report accurately reflects the raw data of the study.

 20-JAN-2000  
(Date)  
Quality Assurance  
Toxicology & Environmental Research and Consulting  
The Dow Chemical Company  
1803 Building  
Midland, Michigan 48674

**SIGNATURE PAGE**

Compound: PAT MICROBIAL PROTEIN (FL)

Title: PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN  
CD-1 MICE

[Redacted Signature]

26 JAN. 2000

(Date)

Study Director

Reviewed by:

[Redacted Signature]

26 January 2000

(Date)

(Diplomate, American College of Veterinary Pathologists)

TABLE OF CONTENTS

	Page
STUDY TITLE .....	1
STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS.....	2
COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS.....	3
QUALITY ASSURANCE STATEMENT .....	4
SIGNATURE PAGE .....	5
TABLE OF CONTENTS.....	6
SUMMARY .....	8
INTRODUCTION .....	9
GLP Standards.....	9
Quality Assurance .....	9
Archiving.....	9
TEST MATERIAL INFORMATION .....	9
Test Material.....	9
Supplier (Lot #) .....	9
Reference Number.....	9
Physical Properties .....	10
TEST ANIMALS.....	10
Animal Welfare .....	11
STUDY DESIGN.....	11
Experimental Design .....	11
Pathology .....	11
Statistics.....	12
RESULTS .....	12
ACKNOWLEDGEMENTS.....	13
REFERENCES .....	14
TABLE 1. MORTALITY - MALE AND FEMALE RATS.....	15
TABLE 2. DETAILED CLINICAL OBSERVATIONS - DAY 1 .....	16
TABLE 3. INDIVIDUAL ANIMAL DETAILED CLINICAL OBSERVATIONS .....	17
TABLE 4. DETAILED CLINICAL OBSERVATIONS - SUMMARY DATA.....	19
TABLE 5. BODY WEIGHTS (G) - MALES.....	35
TABLE 6. BODY WEIGHTS (G) - FEMALES .....	36
TABLE 7. INDIVIDUAL ANIMAL PATHOLOGY REPORTS.....	37



TABLE OF CONTENTS (continued)

	Page
APPENDIX TABLE 1. DETAILED CLINICAL OBSERVATION PARAMETERS .....	39
APPENDIX TABLE 2. EXPLICITLY DEFINED DETAILED CLINICAL OBSERVATIONS .....	40

### SUMMARY

PAT Microbial Protein (FL), which was 84% pure microbial protein, was evaluated for acute oral toxicity. Five male and five female CD-1 mice received 6000 mg/kg of the test material (containing approximately 5000 mg/kg PAT) as a 25% w/v suspension in aqueous 0.5% methylcellulose. Because the volume of the test material in methylcellulose exceeded 2 ml/100g body weight, the test material suspension was administered as two fractional gavage doses given approximately one hour apart. Parameters evaluated during the two-week observation period included body weights and detailed clinical observations. All animals were examined for gross pathological changes.

All mice survived to the end of the two-week observation period. There were no treatment-related clinical observations. All mice except one female gained body weight over the duration of the study. There were no gross pathologic lesions for any animal on study.

Under the conditions of this study, the acute oral LD<sub>50</sub> of PAT Microbial Protein (FL) in male and female CD-1 mice was greater than 6000 mg/kg.

## INTRODUCTION

The purpose of this study was to determine the median oral lethal dose (LD<sub>50</sub>) of PAT Microbial Protein (FL).

### GLP Standards

This study was conducted in accordance with the US Environmental Protection Agency-FIFRA GLPS Title 40 CFR, Part 160-Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), Good Laboratory Practice Standards (Final Rule); the Japan Ministry of Agriculture, Forestry and Fisheries (MAFF) Good Laboratory Practice Standards for Toxicological Studies on Agricultural Chemicals; the Organization for Economic Co-Operation and Development (OECD) OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1, OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM(98)17; the European Community (EC) EC Directive 99/11/EEC of 8 March 1999 (OJ No. L 77/8-21, 23/3/1999); and the Standard Operating Procedures of Toxicology & Environmental Research and Consulting, The Dow Chemical Company.

### Quality Assurance

The study conduct, data, protocol, protocol changes/revisions, and final report were inspected by the Quality Assurance Unit, Toxicology & Environmental Research and Consulting, The Dow Chemical Company. Results of the inspections were reported to management and the study director.

### Archiving

The data, protocol, protocol changes/revisions, and final report are archived at Toxicology & Environmental Research and Consulting, The Dow Chemical Company.

## TEST MATERIAL INFORMATION

### Test Material

PAT MICROBIAL PROTEIN (FL)

### Supplier (Lot #)

Dow AgroSciences, 1669-66-124

### Reference Number

TSN 101850

### Physical Properties

Solid (powder) white

The test sample contained 84% pure PAT microbial protein (FL) and 16% proprietary ingredients (Young, 2000).

### **TEST ANIMALS**

CD-1 mice obtained from Charles River Laboratories Inc. (Portage, MI) and weighing 29.4-32.1 grams at study start, were used for this study. The mice were born on 25 October 1999, dosed on 21 December 1999, and were necropsied on 04 January 2000. Animal care facilities are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. Upon arrival at the laboratory the mice were examined for health status by the laboratory veterinarian. The animal rooms of the testing facility were designed to maintain adequate environmental conditions concerning temperature, humidity, photocycle and air exchanges. The relative humidity and room temperature were maintained within a range of 40-70% and 22±3°C, respectively. A 12-hour light/dark photocycle was maintained for all animal rooms with lights on at 6:00 a.m. and off at 6:00 p.m. Room air was exchanged approximately 12-15 times/hour, and the water lines automatically bled every six hours.

Animals were provided Purina Certified Rodent Lab Diet #5002 (Purina Mills, Inc., St. Louis, MO) in pelleted form. Feed and municipal water were provided ad libitum. Analysis of the feed was performed by Purina Mills Inc. to confirm the diet provided adequate nutrition and to quantify the levels of selected contaminants. Drinking water obtained from the municipal water source was periodically analyzed for chemical parameters and biological contaminants by the municipal water department. In addition, specific analyses for chemical contaminants were conducted at periodic intervals by an independent testing facility. Copies of these analyses are maintained at Toxicology & Environmental Research and Consulting, The Dow Chemical Company. Animals were acclimated to the laboratory environment for at least two weeks prior to study start. Animals were identified via a code number transmitted by a subcutaneously implanted transponder.

### Animal Welfare

In response to the Final Rules amending the U.S. Animal Welfare Act that were promulgated by the U.S. Department of Agriculture effective October 30, 1989, the Animal Care and Use Activities (ACUA) that were required for the conduct of this study were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). The IACUC determined that the proposed Activities were in full accordance with these Final Rules.

## STUDY DESIGN

### Experimental Design

Five male and five female CD-1 mice received 6000 mg PAT Microbial Protein (FL) per kg body weight as a 25% w/v suspension in aqueous 0.5% methylcellulose. Since the volume of the test material in the suspension exceeded 2 ml/100 g body weight, the test material suspension was administered as two fractional gavage doses approximately one hour apart. A detailed clinical observation (DCO) was conducted for all mice prior to test material administration for comparison with the observations recorded throughout the study. Animals were observed a minimum of 2 times on the day of treatment. A DCO was done each day (including weekends and holidays) during the study. Hand-held and open-field observations included a careful physical examination according to an established format (Appendix Table 1). For scored DCO's only observations other than typically expected were recorded. Observations were dictionary based, and the dictionary contained most of the common physical and neurologic abnormalities seen in toxicity studies. Since not all potential observations were contained in the dictionary, free-field descriptions also were allowed. Details of the specific observations, definitions of the ranks used for ranked observations and explanations of the categorical data can be found in Appendix Table 2. Each animal was weighed prestudy, the day of treatment, and on test days 2, 8, and 15. A necropsy was performed on all animals.

### Pathology

All rats submitted alive for necropsy were anesthetized by inhalation of methoxyflurane vapors and were euthanized by decapitation after clamping of the trachea. A complete necropsy was conducted on all animals by a veterinary pathologist assisted by a team of trained individuals. The eyes were examined *in situ* using a moistened glass microscope slide applied to the corneal surface. Following inspection of the externum and body

orifices, the nasal, cranial, oral, thoracic, and abdominal cavities were opened and the visceral organs were examined both *in situ* and following dissection, and tissues were not saved.

### Statistics

Means and standard deviations were calculated for body weights. The data were evaluated for statistical outliers by a sequential test (Grubbs, 1969), however, outliers were not routinely excluded from statistical analysis.

## RESULTS

Mortality results of male and female mice are presented in Table 1. There was no mortality.

Detailed clinical observations for multiple observation times on test day 1 are presented in Table 2, individual animal detailed clinical observations for the entire study are presented in Table 3, and summary data for detailed clinical observations are presented in Table 4. There were no treatment related clinical observations observed throughout the two-week study. One female mouse had increased pupil size from day -1 through day 6.

Mean and individual body weights are presented in Tables 5 and 6. All mice had a decrease in body weight between days 1 and 2. The body weight losses were minor, transient, and typical of high-volume gavage procedures and effects were not attributed to test material. All mice except one female gained body weight between days 2 and 15. One female lost 0.5 grams over the duration of the study.

Individual gross pathologic observations are presented in Table 7. There were no gross pathologic lesions in any animal on study.

Under the conditions of this study, the acute oral LD<sub>50</sub> of PAT Microbial Protein (FL) in male and female CD-1 mice was greater than 6000 mg/kg.

### ACKNOWLEDGEMENTS

The author gratefully acknowledges the contribution of the following individuals to the conduct of the study and report preparations:

A. K. Andrus, D. A. Keeley

Animal Husbandry, Weights and Data  
Collection

R. S. Drury

Document Management

T. J. Bell, B. L. Stieve

Quality Assurance Unit

## REFERENCES

EEC (1992). Official Journal of the European Economic Community: Methods for the Determination of Toxicity, Acute Toxicity (oral), Directive 92/69/EEC. Part B.

Environmental Protection Agency. Health Effects Test Guidelines, OPPTS 870.1100, Acute Oral Toxicity, August 1998.

EPA-FIFRA. US Environmental Protection Agency, Title 40 CFR, Part 160-Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule.

European Community (EC) EC Directive 99/11/EC of 8 March 1999 (OJ No. L 77/8-21, 23/3/1999).

Grubbs, F. E. (1969). Procedures For Detecting Outlying Observations In Samples. *Technometrics* 11(1): 1-21.

MAFF. Japan Ministry of Agriculture, Forestry and Fisheries, Good Laboratory Practice Standards for Toxicological Studies on Agricultural Chemicals.

Japan MAFF (1985). Ministry of Agriculture, Forestry and Fisheries, Requirements for Safety Evaluation of Agricultural Chemicals, 59 NohSan, Acute Oral Toxicity Study, 1985.

OECD (1987). Organization for Economic Co-operation and Development-Guideline for Testing of Chemicals, Acute Oral Toxicity 401, 24 February, 1987.

Organization for Economic Co-Operation and Development (OECD) OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM(98)17.

Young, Debra. Dow AgroSciences Non-GLP Purity Analysis, Bldg. 306, B2/819, notebook page E0666-30, 1/12/00.



PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 1. MORTALITY - MALE AND FEMALE RATS

Dose (mg/kg)	#/Sex/Dose	#Dead		Approximate Observed Time of Death (Day)	
		Males	Females	Males	Females
6000	5	0	0	---	---

---No deaths noted.

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 2. DETAILED CLINICAL OBSERVATIONS - DAY 1

Approx. time post dosing	MALE	FEMALE
1 hour	<u>Survivors-5</u> normal-5	<u>Survivors-5</u> normal-4 increased pupil size bilateral-1
2.5 hours	<u>Survivors-5</u> normal-5	<u>Survivors-5</u> normal-4 increased pupil size bilateral-1

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 3. INDIVIDUAL ANIMAL DETAILED CLINICAL OBSERVATIONS-MALES

Dose	Animal Number	Day Observed		Observation/Comment
		First	Last	
-----				
6000 mg/kg	6499	-1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	6500	-1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	6501	-1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	6502	-1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	6503	-1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 3. INDIVIDUAL ANIMAL DETAILED CLINICAL OBSERVATIONS (continued)-FEMALES

Dose	Animal Number	Day Observed		Observation/Comment
		First	Last	
-----				
6000 mg/kg				
	6504	-1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	6505	-1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	6506	-1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	6507	-1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	6508	-1	6	DCO, Pupil Size, Bilateral, Increase - Moderate
		7	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 4. DETAILED CLINICAL OBSERVATIONS - SUMMARY DATA

STUDY DAY: -1

SEX: DOSE (MG/KG): NUMBER OF ANIMALS OBSERVED: -----	MALES FEMALES	
	6000	6000
	5	5
-----		
All Categories		
Within Normal Limits	5	4
Scored Observations		
Within Normal Limits	5	4
Pupil Size, Bilateral, Increase - Moderate	0	1

Data are the number of animals with the specified observation.  
See Study Design section of the text for details of observation parameters.

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 4. DETAILED CLINICAL OBSERVATIONS - SUMMARY DATA (continued)

STUDY DAY: 1

	SEX: DOSE (MG/KG): NUMBER OF ANIMALS OBSERVED:	MALES 6000 5	FEMALES 6000 5
	-----	-----	-----
All Categories			
Within Normal Limits		5	4
Scored Observations			
Within Normal Limits		5	4
Pupil Size, Bilateral, Increase - Moderate		0	1

Data are the number of animals with the specified observation.  
 See Study Design section of the text for details of observation parameters.

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 4. DETAILED CLINICAL OBSERVATIONS - SUMMARY DATA (continued)

STUDY DAY: 2

SEX:	MALES	FEMALES
DOSE (MG/KG):	6000	6000
NUMBER OF ANIMALS OBSERVED:	5	5

All Categories

Within Normal Limits

5 4

Scored Observations

Within Normal Limits

5 4

Pupil Size, Bilateral, Increase - Moderate

0 1

Data are the number of animals with the specified observation.  
See Study Design section of the text for details of observation parameters.

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 4. DETAILED CLINICAL OBSERVATIONS - SUMMARY DATA (continued)

STUDY DAY: 3

SEX:	MALES	FEMALES
DOSE (MG/KG):	6000	6000
NUMBER OF ANIMALS OBSERVED:	5	5
-----	-----	-----

All Categories

Within Normal Limits

5 4

Scored Observations

Within Normal Limits

5 4

Pupil Size, Bilateral, Increase - Moderate

0 1

Data are the number of animals with the specified observation.  
See Study Design section of the text for details of observation parameters.



PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 4. DETAILED CLINICAL OBSERVATIONS - SUMMARY DATA (continued)

STUDY DAY: 4

	SEX:	MALES	FEMALES
	DOSE (MG/KG):	6000	6000
NUMBER OF ANIMALS OBSERVED:		5	5
-----			
All Categories			
Within Normal Limits		5	4
Scored Observations			
Within Normal Limits		5	4
Pupil Size, Bilateral, Increase - Moderate		0	1

Data are the number of animals with the specified observation.  
 See Study Design section of the text for details of observation parameters.

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 4. DETAILED CLINICAL OBSERVATIONS - SUMMARY DATA (continued)

STUDY DAY: 5

SEX: DOSE (MG/KG): NUMBER OF ANIMALS OBSERVED: -----	MALES FEMALES	
	6000 5	6000 5
-----		
All Categories		
Within Normal Limits	5	4
Scored Observations		
Within Normal Limits	5	4
Pupil Size, Bilateral, Increase - Moderate	0	1

Data are the number of animals with the specified observation.  
See Study Design section of the text for details of observation parameters.

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 4. DETAILED CLINICAL OBSERVATIONS - SUMMARY DATA (continued)

STUDY DAY: 6

	SEX: DOSE (MG/KG): NUMBER OF ANIMALS OBSERVED:	MALES 6000 5	FEMALES 6000 5
	-----	-----	-----
All Categories			
Within Normal Limits		5	4
Scored Observations			
Within Normal Limits		5	4
Pupil Size, Bilateral, Increase - Moderate		0	1

Data are the number of animals with the specified observation.  
See Study Design section of the text for details of observation parameters.

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 4. DETAILED CLINICAL OBSERVATIONS - SUMMARY DATA (continued)

STUDY DAY: 7

	SEX:	MALES	FEMALES
	DOSE (MG/KG):	6000	6000
NUMBER OF ANIMALS OBSERVED:		5	5
-----			

All Categories

Within Normal Limits

5 5

Data are the number of animals with the specified observation.  
See Study Design section of the text for details of observation parameters.

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 4. DETAILED CLINICAL OBSERVATIONS – SUMMARY DATA (continued)

STUDY DAY: 8

	SEX:	
	DOSE (MG/KG):	
NUMBER OF ANIMALS OBSERVED:	MALES	FEMALES
-----	6000	6000
	5	5
	-----	-----
All Categories		
Within Normal Limits	5	5

Data are the number of animals with the specified observation.  
See Study Design section of the text for details of observation parameters.

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 4. DETAILED CLINICAL OBSERVATIONS - SUMMARY DATA (continued)

STUDY DAY: 9

	SEX:	MALES	FEMALES
	DOSE (MG/KG):	6000	6000
NUMBER OF ANIMALS OBSERVED:		5	5
-----			

All Categories

Within Normal Limits

5 5

Data are the number of animals with the specified observation.  
See Study Design section of the text for details of observation parameters.

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 4. DETAILED CLINICAL OBSERVATIONS - SUMMARY DATA (continued)

STUDY DAY: 10

	SEX:	MALES	FEMALES
	DOSE (MG/KG):	6000	6000
NUMBER OF ANIMALS OBSERVED:		5	5
-----			

All Categories

Within Normal Limits

5 5

Data are the number of animals with the specified observation.  
See Study Design section of the text for details of observation parameters.

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 4. DETAILED CLINICAL OBSERVATIONS - SUMMARY DATA (continued)

STUDY DAY: 11

	SEX:	MALES	FEMALES
	DOSE (MG/KG):	6000	6000
NUMBER OF ANIMALS OBSERVED:		5	5
-----			

All Categories

Within Normal Limits

5 5

Data are the number of animals with the specified observation.  
See Study Design section of the text for details of observation parameters.



PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 4. DETAILED CLINICAL OBSERVATIONS - SUMMARY DATA (continued)

STUDY DAY: 12

	SEX:	MALES	FEMALES
	DOSE (MG/KG):	6000	6000
NUMBER OF ANIMALS OBSERVED:		5	5
-----		-----	-----

All Categories

Within Normal Limits

5 5

Data are the number of animals with the specified observation.  
See Study Design section of the text for details of observation parameters.

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 4. DETAILED CLINICAL OBSERVATIONS - SUMMARY DATA (continued)

STUDY DAY: 13

SEX: DOSE (MG/KG): NUMBER OF ANIMALS OBSERVED:	MALES FEMALES	
	6000	6000
-----	5	5
-----		
All Categories		
Within Normal Limits	5	5

Data are the number of animals with the specified observation.  
See Study Design section of the text for details of observation parameters.

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 4. DETAILED CLINICAL OBSERVATIONS - SUMMARY DATA (continued)

STUDY DAY: 14

	SEX:	
	DOSE (MG/KG):	MALES FEMALES
		6000 6000
NUMBER OF ANIMALS OBSERVED:		5 5
-----		-----

All Categories

Within Normal Limits

5 5

Data are the number of animals with the specified observation.  
See Study Design section of the text for details of observation parameters.

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 4. DETAILED CLINICAL OBSERVATIONS - SUMMARY DATA (continued)

STUDY DAY: 15

SEX: DOSE (MG/KG): NUMBER OF ANIMALS OBSERVED:	MALES FEMALES	
	6000	6000
-----	5	5
All Categories		
Within Normal Limits	5	5
Disposition		
Scheduled Necropsy	5	5

Data are the number of animals with the specified observation.  
See Study Design section of the text for details of observation parameters.

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 5. BODY WEIGHTS (G) - MALES

DOSE MG/KG	ANIMAL NUMBER	DAYS ON TEST				
		-1	1	2	8	15
6000	6499	30.0	31.6	30.8	32.1	33.8
	6500	30.3	32.1	32.1	33.6	34.6
	6501	29.8	31.5	31.1	33.3	36.2
	6502	30.4	31.7	31.2	33.0	34.0
	6503	28.9	29.8	30.1	31.9	33.6
	MEAN	29.9	31.3	31.1	32.8	34.4
	S.D.	0.6	0.9	0.7	0.7	1.1
	N=	5	5	5	5	5

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 6. BODY WEIGHTS (G) - FEMALES

DOSE MG/KG	ANIMAL NUMBER	DAYS ON TEST				
		-1	1	2	8	15
6000	6504	30.5	31.6	31.5	31.6	31.1
	6505	28.6	29.4	29.3	29.8	31.5
	6506	30.3	31.5	30.9	30.9	32.0
	6507	29.9	30.6	30.0	30.0	30.8
	6508	29.1	30.5	29.6	29.3	31.1
	MEAN	29.7	30.7	30.3	30.3	31.3
	S.D.	0.8	0.9	0.9	0.9	0.5
	N=	5	5	5	5	5

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 7. INDIVIDUAL ANIMAL PATHOLOGY REPORTS

Group: 1   Dose: 6000 mg/kg   Sex: Male

Animal Number	Mode Of Death	Death		Observation(s)
		Day	(Week)	
6499	SCHEDULED NECROPSY	15	(3)	No Visible Lesions
6500	SCHEDULED NECROPSY	15	(3)	No Visible Lesions
6501	SCHEDULED NECROPSY	15	(3)	No Visible Lesions
6502	SCHEDULED NECROPSY	15	(3)	No Visible Lesions
6503	SCHEDULED NECROPSY	15	(3)	No Visible Lesions

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

TABLE 7. INDIVIDUAL ANIMAL PATHOLOGY REPORTS (continued)

-----  
Group: 1    Dose: 6000 mg/kg    Sex: Female

Animal Number	Mode Of Death	Death Day    (Week)	Observation(s)
6504	SCHEDULED NECROPSY	15    (3)	No Visible Lesions
6505	SCHEDULED NECROPSY	15    (3)	No Visible Lesions
6506	SCHEDULED NECROPSY	15    (3)	No Visible Lesions
6507	SCHEDULED NECROPSY	15    (3)	No Visible Lesions
6508	SCHEDULED NECROPSY	15    (3)	No Visible Lesions



PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

APPENDIX TABLE 1. DETAILED CLINICAL OBSERVATION PARAMETERS

<u>Cage-side Observations</u>	<u>Recorded As</u>
Abnormal movements or behavior	See Categorical
Resistance to removal from cage	Scored
<u>Hand-held Observations</u>	<u>Recorded As</u>
Palpebral closure	Scored
Lacrimation (non-colored periocular wetness)	Scored
Pupil Size	Scored
Salivation (non-colored perioral wetness)	Scored
Muscle tone	Scored
Extensor-thrust response	Scored
Reactivity to handling	Scored
<u>Open-field observations</u>	<u>Recorded As</u>
Responsiveness to touch	Scored
Gait evaluation	Scored
<u>Categorical observations (anytime during the DCO)</u>	<u>Recorded As</u>
Abnormal behavior	Positive finding
Abnormalities of the eye	Positive finding
Abnormal urine or feces	Positive finding
Abnormalities of the gastrointestinal (GI) tract	Positive finding
Injury	Positive finding
Missing extremity	Positive finding
Abnormal muscle movements	Positive finding
Palpable mass/swellings	Positive finding
Abnormal posture	Positive finding
Abnormalities of the reproductive system	Positive finding
Abnormal respiration	Positive finding
Abnormal skin or hair-coat/mucous membranes	Positive finding
Excessive soiling	Positive finding
General abnormalities	Positive finding

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

APPENDIX TABLE 2. EXPLICITLY DEFINED DETAILED CLINICAL OBSERVATIONS

**Clinical Examination Conduct**

The clinical examination is conducted in a careful and systematic format. The examination begins at the head of the animal and gradually works towards the tail as outlined below.

A. Cageside observations are made first.

1. Categorical observations includes: unusual body movements (e.g. tremors, convulsion), abnormal behavior (e.g. circling, stereotypy) and posture.
2. Resistance to Removal: The degree to which an animal attempts to escape capture is scored. The observer will slowly present his/her gloved hand into the cage and the capture technique will consist in grasping the animal over the shoulder area or by grasping the tail.
  - 1 = Decrease - pronounced; no resistance to capture and removal
  - 2 = Decrease - moderate; clearly less effort to avoid capture and is not aggressive
  - 3 = Typical; actively avoids capture but is not aggressive
  - 4 = Increase - moderate; clearly more effort to avoid capture and may be mildly aggressive
  - 5 = Increase - pronounced; very difficult to capture and is very aggressive (i.e. tries to bite)

B. Eye observations: Both eyes are examined for these categories, however, if a unilateral observation is made, a concurrent observation is not made for the other eye if it is within normal limits.

Palpebral closure:

- 1 = closed
- 2 = 50% closed
- 3 = open
- 4 = protruding eyes

Pupil size (aided by pen light): Under typical exam conditions (white light) the normal appearance of the pupils in albino animals is complete constriction. Therefore a decrease in pupil size cannot be observed.

- 0 = Unable to evaluate
- 1 = Decrease - pronounced; decreased pupil size (completely constricted)
- 2 = Decrease - moderate; decreased pupil size (moderately constricted)

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

APPENDIX TABLE 2. EXPLICITLY DEFINED DETAILED CLINICAL OBSERVATIONS (continued)

- 3 = Typical pupil size
- 4 = Increase - moderate; increased pupil size (moderately dilated)
- 5 = Increase - pronounced; increased pupil size (completely dilated)

Lacrimation (clear wetness): Under typical exam conditions, corneal dryness is not observable in rodents.

- 1 = Decrease - pronounced; very dry appearance of cornea
- 2 = Decrease - moderate; dry appearance of cornea
- 3 = Typical; glistening cornea
- 4 = Increase - moderate; wet around the eyes
- 5 = Increase - pronounced; extensively wet around the eyes

C. The degree of salivation: Under typical exam conditions, dryness of the oral cavity is not observable in rodents.

- 1 = Decrease - pronounced; extensive oral dryness
- 2 = Decrease - moderate; oral dryness
- 3 = Typical; no perioral wetness
- 4 = Increase - moderate; wet around the mouth
- 5 = Increase - pronounced; extensively wet around the mouth or drop(s) of fluid in or around mouth

D. Muscle tone: An assessment of muscle tone at the time of hand-held observations.

- 1 = Decrease - pronounced; limp
- 2 = Decrease - moderate; less muscle tone, relaxed, "soft"
- 3 = Typical; animal is neither very relaxed nor tense
- 4 = Increase - moderate; more muscle tone, animal feels slightly rigid or tense
- 5 = Increase - pronounced, very rigid or stiff

E. Extensor-thrust response: Extent of reflex response to brisk pushes (by finger) on the plantar surface of the hind-feet.

- 1 = Decrease - pronounced; no extensor thrust response
- 2 = Decrease - moderate; less detectable response
- 3 = Typical; clearly detectable
- 4 = Increase - moderate; more detectable, pushes vigorously

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

APPENDIX TABLE 2. EXPLICITLY DEFINED DETAILED CLINICAL OBSERVATIONS (continued)

5 = Increase - pronounced; extreme thrust response, greater than number 4

F. Reactivity to handling: The degree to which an animal struggles to get free from hand-held restraint is ranked.

- 1 = Decrease - pronounced; none, no struggling
- 2 = Decrease - moderate; clearly less struggling
- 3 = Typical; some struggling with little or no vocalization
- 4 = Increase - moderate; clearly more struggling, animal may vocalize
- 5 = Increase - pronounced; aggressive escape behavior, tries to bite observer and usually vocalizes

G. Observations made in the open-field.

1. Responsiveness to touch: the ventral aspect of the tail is lightly stroked using a finger. Typically, the animal will spontaneously lift its tail (wrap around finger) when lightly touched.

- 1 = Decrease - pronounced; no response to touch
- 2 = Decrease - moderate; clearly less response, does not spontaneously lift tail; but briefly holds tail in the air when manually lifted
- 3 = Typical; spontaneously lifts tail when touched
- 4 = Increase - moderate; clearly more response, spontaneously lifts tail and acts startled
- 5 = Increase - pronounced; pronounced response, turns towards finger in an attack response

2. Gait evaluation: Open-field observations are used for gait evaluation. The animal may be forced to walk on it's forelegs while the hindlegs are held off the floor of the observation box ("the wheel-barrow test").

- 1 = Typical; smooth and coordinated gait
- 2 = Slight knuckling of the paws and/or slight tendency to stumble
- 3 = Moderate; clear knuckling and stumbling, appears to be incoordinated
- 4 = Pronounced; to include but not limited to: poor coordination, falling, and/or clear and repetitive knuckling of paws, and/or dragging of one or more limbs
- 5 = Exaggerated; animal is unable to walk

PAT MICROBIAL PROTEIN (FL): ACUTE ORAL TOXICITY STUDY IN CD-1 MICE

APPENDIX TABLE 2. EXPLICITLY DEFINED DETAILED CLINICAL OBSERVATIONS (continued)

H. Categorical Observations: These observations can be made at anytime during the DCO. For the categories listed below the observer directly records the positive observation.

1. Abnormal behavior	Positive finding
2. Abnormalities of the eye	Positive finding
3. Abnormal urine or feces	Positive finding
4. Abnormalities of the gastrointestinal (GI) tract	Positive finding
5. Injury	Positive finding
6. Missing extremity	Positive finding
7. Abnormal muscle movements	Positive finding
8. Palpable mass/swellings	Positive finding
9. Abnormal posture	Positive finding
10. Abnormalities of the reproductive system	Positive finding
11. Abnormal respiration	Positive finding
12. Abnormal skin or hair-coat/mucous membranes	Positive finding
13. Excessive soiling	Positive finding
14. General abnormalities	Positive finding