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T R A N S M I T T A L F O R M

DATE : **June 9, 2005**

TO : **Mr. Dirk de Meester**

COMPANY : EBI Food Safety
Gerrit van der Veenlaan 2
3743 DN Baarn
The Netherlands,

FROM : Beth Landis

Transmitted herewith are the following:

☐ DRAFT REPORT (s)	☑ FINAL REPORT	☐ INVOICE (s)
☐ RAW DATA	☐ PROTOCOL SIGNATURE PAGE (s)	
☐ SPECIMEN DISPOSITION MEMO	☐ OTHER	

PROTOCOL	STUDY TITLE	TEST ARTICLE	MB PROJECT #
Cient	Oral Toxicity	P-100 Phage, PBS (control)	05-13221.01

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Study Title	:	Repeated Dose Oral Toxicity in Rats
Test Article	:	P-100 Phage (P1-5)
Control	:	Phosphate Buffered Saline (C1-5); hereinafter referred to as PBS
Author	:	Daniel R. Cerven, M.S., Study Director
Study Completed On	:	June 8, 2005
Performing Laboratory	:	MB Research Laboratories 1765 Wentz Road P.O. Box 178 Spinnerstown, PA 18968
MB Research Project #	:	MB 05-13221.01
MB Research Protocol #	:	Client - James W. Barnett, JR., Ph.D., DABT AAC Consulting Group
Sponsor	:	Exponential Biotherapies Inc. B.V. Meibergdreef 59 1105 BA Amsterdam The Netherlands
Citation	:	Daniel R. Cerven, M.S. (2005) Unpublished Report by MB Research Laboratories

GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

This study meets the Good Laboratory Practice requirements of EPA 40 CFR parts 792 and 160, FDA 21 CFR Part 58 and as specified in The Testing of Chemicals, published by the Organization for Economic Cooperation & Development (OECD), 1997 with the following exception:

The test article characterization was not supplied by the sponsor prior to study initiation.

STUDY DIRECTOR : 8-2-05

Daniel R. Cerven, M.S. Date
MB RESEARCH LABORATORIES

Repeated Dose Oral Toxicity in Rats

ABSTRACT

METHOD

Ten healthy male and ten healthy female Wistar albino rats, were randomly selected and assigned to two groups of five males and five females/group. Animals in Group 1 were dosed orally with 1.0 ml of P-100 Phage using a syringe and 16 gauge ball-tipped feeding needle once daily for five consecutive days. According to the sponsor's analysis (Appendix A, Table 1), daily doses ranged from a mean of 4.58×10^{11} to 5.10×10^{11} phage/ml. Animals in Group 2 were dosed in the same manner but with 1.0 ml of PBS.

Body weights were recorded pretest and prior to termination. The animals were observed once daily for toxicity and pharmacological effects and twice daily for morbidity and mortality. Food consumption was calculated at the end of the study. On day 8, all animals were anesthetized with ether and exsanguinated.

All animals were examined for gross pathology. The esophagus, stomach, duodenum, jejunum, ileum, cecum and colon were preserved in 10% neutral buffered formalin. Histopathologic preparation and microscopic analysis were performed by W. Ray Brown, DVM, Ph.D., DACVP, Research Pathology Services, Inc., New Britain, Pa.

All results were evaluated based on the relationship between the dose levels and incidence or severity of responses. Appropriate statistical evaluations were performed using Instat® statistics Version #2.0 software.

RESULTS

There was no mortality noted during the study. There were no abnormal physical signs noted in any animal and body weight changes were normal. There were no significant ($p \leq 0.05$) differences in mean body weights or food consumption between the groups. Necropsy results were normal in all animals except animal #C8945-F (Group 1, 1.0 ml/day of P-100 Phage) which exhibited an intestinal abnormality.

Histopathologic evaluation of the esophagus and gastrointestinal tract revealed no changes attributable to administration of the test article.

CONCLUSION

Oral administration of P-100 Phage for five consecutive days followed by a two-day recovery period in male and female Wistar albino rats revealed no in-life effects attributable to the test material. Histopathologic evaluation of the esophagus and gastrointestinal tract revealed no changes attributable to administration of the test article.

OBJECTIVE

To provide information on the possible health hazards that might arise from repeated oral administration of the test article.

TEST ARTICLE

Test Article Identity	:	P-100 Phage (P1-5) – (See Appendix A for Bacteriophage analysis)
Source	:	Exponential Biotherapies Inc. B.V.
Test Article Characterization	:	Not Supplied by the sponsor by study initiation.
Date Received	:	3/01/05
Description	:	Cloudy liquid
Sample Preparation	:	Used as received and shaken prior to individual dosing.
Storage	:	Stored in the refrigerator at 2 - 8° C.

VEHICLE

Control	:	Phosphate Buffered Saline (PBS)
Source	:	Exponential Biotherapies Inc. B.V.
Test Article Characterization	:	Not Supplied by the sponsor by study initiation.
Date Received	:	3/01/05
Description	:	Clear liquid
Sample Preparation	:	Used as received and shaken prior to individual dosing
Storage	:	Stored in the refrigerator at 2 - 8°C

EXPERIMENTAL DESIGN

Test Dates

Study Initiation	(date protocol signed):	:	3/14/05
Experimental Start Date	(1st exposure to test/control article)	:	3/14/05
Experimental Term Date	(Last date data collected)	:	3/21/05
Draft Report Signed	(if applicable)	:	4/26/05
Second Draft Report Signed		:	5/04/05
Final Report Signed	(study completion)	:	6/08/05

EXPERIMENTAL DESIGN (cont'd)

Test Animals

Wistar albino rats were received from Ace Animals, Boyertown, PA on 3/08/05. The animals were born 1/6, 1/7, 1/18 and 1/19/05 and equilibrated for six days. From the available pool of healthy animals, ten animals of each sex were randomly selected and assigned to treatment groups using a computer randomization program.

The pretest body weight range for males was 202 - 231 grams and for females 193 - 214 grams. The pretest mean body weights were not significantly different ($p \leq 0.05$) using the analysis of variance (ANOVA) module of Instat® Version #2.0 software.

The animals were housed 1/cage in suspended stainless steel wire bottom cages and individually identified by a uniquely numbered eartag. The individual cages were identified with a cage card indicating the MB project number, test article, dose level, date of study initiation, animal number and sex. ANIPADS™ paper bedding, placed beneath the cages, was changed at least three times/week. Fresh PMI Rodent Chow Diet #5001 was provided ad libitum except for the fasting period on the day prior to sacrifice. Fresh water was available ad libitum. The animal room, reserved exclusively for rats, was temperature controlled, had a 12-hour light/dark cycle and was kept clean and vermin free.

Dosing Procedures

The test article or control substance was administered orally, once daily over a five-day period, by gavage using a syringe and 16 gauge ball-tipped feeding needle as follows:

GROUP	DOSE (mg/kg)	VOLUME (ml)	# of Animals
1	P-100 Phage	1.0	5 Males - 5 Females
2	Control (PBS)	1.0	5 Males - 5 Females

The animals were dosed for 5 days over a five day period. The test article was administered at similar times each day.

EXPERIMENTAL DESIGN (cont'd)

Type and Frequency of Observations

In vivo -All animals were observed once daily for toxicity and pharmacological effects and twice daily for morbidity and mortality.

Body weights were recorded on the day following receipt, immediately pretest and at termination.

Measured amounts of diet were presented immediately pretest. The amount consumed was calculated at the end of the study.

Post Mortem - On day 8, all animals were humanely sacrificed using ether and exsanguination. Each animal underwent a gross necropsy, which included examination of the external surfaces of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents.

The following tissues and organs from all animals were preserved in 10% neutral buffered formalin and examined microscopically::

esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, all gross lesions and masses

Histopathologic preparation and microscopic analyses were performed by W. Ray Brown, DVM, Ph.D., DACVP, Research Pathology Services Inc., New Britain, PA. Histopathology results are included as Appendix B.

Analysis and Evaluation

The data was evaluated based on the relationship between the dose of the test article and the presence or absence of abnormalities.

Retention of Data

The raw data is filed at MB Research by MB Project number. The final report is filed at MB Research by sponsor name and MB project number.

The remaining test article will be returned to the sponsor upon submission of the report. Aliquots of the control and test article were taken, frozen and shipped to the sponsor following study termination. Tissues, blocks and slides will be stored at MB Research by project number and sponsor name for a period of one year. Following the one year period, the sponsor will be notified for final disposition.

Amendment to the Protocol

There were no amendments to the protocol. The entire protocol for this study is included herein as Appendix C.

Deviation to the Good Laboratory Practices

Although the complete chemical characteristics of this test material was not available prior to the start of this study, the unique nature of this material, i.e. bacteriophage, made a complete analysis unnecessary to the overall mission. Sponsor's analytical report is attached as Appendix A.

RESULTS and DISCUSSION

Mortality:

There was no mortality noted during the observation period.

Systemic Observations (Table 1):

There were no abnormal physical signs noted in any animal at any observation period.

Body Weights (Table 2):

Body weight changes were normal. There were no significant differences ($p \leq 0.05$) in mean body weights between control and test article animals.

Food Consumption (Table 3)

Food consumption was normal. There were no significant differences ($p \leq 0.05$) in mean food consumption between control and test article animals.

Necropsy Observations (Table4):

Necropsy results were normal in 19/20 animals. A red area was noted in the small intestines of a Group 1 animal #C8945-F.

Bacteriophage Analysis (Appendix A):

See Appendix A for analysis.

Histopathology (Appendix B):

There were no test article related microscopic changes observed in the esophagus and gastrointestinal tract in any of the male or female rats dosed with 1.0 ml of P-100 Phage. The few miscellaneous microscopic changes noted were typical of those that occur spontaneously and were not related to test article administration.

Protocol (Appendix C):

See Appendix C for protocol in its entirety.

CONCLUSION

Oral administration of P-100 Phage for five consecutive days followed by a two-day recovery period in male and female Wistar albino rats revealed no in-life effects attributable to the test material. Histopathologic evaluation of the esophagus and gastrointestinal tract revealed no changes attributable to administration of the test article.

FINAL REPORT

Approved by: _____ 8-2-05

Daniel R. Cerven, M.S. Date
Study Director

QUALITY ASSURANCE EVALUATION

The Quality Assurance Unit (QAU) has inspected in-life phases of this study, audited the raw data and the report and determined that the methods and results contained herein accurately reflect the raw data. No deviations from the approved protocol or Standard Operating Procedures were made without proper authorization and documentation. A summary of the compliance inspections performed is presented below:

Date of Inspection	Phase	Performed By	Date Findings Reported to	
			Mgmt.	Sty. Dir.
3/14/05	Dosing administration	Erin Range	06/08/05	06/08/05
3/31/05	Raw data audit	Erin Range	06/08/05	06/08/05
4/26/05	Draft report audit	Erin Range	06/08/05	06/08/05
5/04/05	Second draft report audit	Betty Salyer	06/08/05	06/08/05
06/08/05	Final report audit	Erin Range	06/08/05	06/08/05

Erin Range 6/9/05

Erin Range, B.S. Date
Quality Assurance Unit

Project Number: MB 05-13221.01
Protocol Number: Client-J. Barnett

Table 1: Systemic Observations

Group 1 - P-100 Phage (P1-5)

TIME PERIODS	ANIMAL # & SEX									
	C8961-M	C8941-M	C8942-M	C8943-M	C-8944-M	C8945-F	C8960-F	C8947-F	C8948-F	C8949-F
Day 1	NO ENTRY INDICATES ANIMALS APPEARED NORMAL AT THAT OBSERVATION PERIOD.									
Day 2										
Day 3										
Day 4										
Day 5										
Day 6										
Day 7										
Day 8										

Group 2 - PBS (C1-5)

TIME PERIODS	ANIMAL # & SEX									
	C8950-M	C8951-M	C8962-M	C8953-M	C-8954-M	C8955-F	C8956-F	C8963-F	C8958-F	C8959-F
Day 1	NO ENTRY INDICATES ANIMALS APPEARED NORMAL AT THAT OBSERVATION PERIOD.									
Day 2										
Day 3										
Day 4										
Day 5										
Day 6										
Day 7										
Day 8										

TABLE 2

BODY WEIGHTS (g)

Group 1: P-100 Phage (P1-5)

Animal #/Sex	Day 1	Day 8
C8961-M	214	261
C8941-M	220	263
C8942-M	222	274
C8943-M	222	274
C8944-M	202	254
Mean	**216**	**265**
S.D.	**8.5**	**8.7**
C8945-F	207	234
C8960-F	206	235
C8947-F	205	231
C8948-F	214	236
C8949-F	194	212
Mean	**205**	**230**
S.D.	**7.2**	**10.0**

Group 2: PBS (C1-5)

Animal #/Sex	Day 1	Day 8
C8950-M	231	279
C8951-M	222	267
C8962-M	222	272
C8953-M	225	271
C8954-M	230	278
Mean	**226**	**273**
S.D.	**4.3**	**5.0**
C8955-F	198	229
C8956-F	204	228
C8963-F	193	219
C8958-F	201	221
C8959-F	202	217
Mean	**200**	**223**
S.D.	**4.3**	**5.4**

TABLE 3

FOOD CONSUMPTION (g)

Group 1: P-100 Phage (P1-5)		Group 2: PBS (C1-5)	
Animal #/Sex	**Day 7**	**Animal #/Sex**	**Day 7**
C8961-M	158.3	C8950-M	164.1
C8941-M	156.7	C8951-M	177.3
C8942-M	172.0	C8962-M	168.2
C8943-M	173.1	C8953-M	161.8
C8944-M	170.8	C8954-M	164.3
Mean	**166.2**	**Mean**	**167.1**
S.D.	**7.99**	**S.D.**	**6.13**
C8945-F	155.3	C8955-F	137.2
C8960-F	133.3	C8956-F	128.6
C8947-F	141.3	C8963-F	138.8
C8948-F	143.7	C8958-F	121.9
C8949-F	117.4	C8959-F	116.8
Mean	**138.2**	**Mean**	**128.7**
S.D.	**14.04**	**S.D.**	**9.51**

Table 4

NECROPSY OBSERVATIONS

GROUP	ANIMAL #SEX	DAY OF DEATH	CIRCUMSTANCE OF DEATH	OBSERVATION
1 - P-100 Phage (P1-5)	C8961-M	8	S	Normal
	C8941-M	8	S	Normal
	C8942-M	8	S	Normal
	C8943-M	8	S	Normal
	C8944-M	8	S	Normal
	C8945-F	8	S	Ileum/Jejunum - red area
	C8960-F	8	S	Normal
	C8947-F	8	S	Normal
	C8948-F	8	S	Normal
	C8949-F	8	S	Normal

S = sacrifice

Table 4

NECROPSY OBSERVATIONS

GROUP	ANIMAL #SEX	DAY OF DEATH	CIRCUMSTANCE OF DEATH	OBSERVATION
2 - PBS (C1-5)	C8950-M	8	S	Normal
	C8951-M	8	S	Normal
	C8962-M	8	S	Normal
	C8953-M	8	S	Normal
	C8954-M	8	S	Normal
	C8955-F	8	S	Normal
	C8956-F	8	S	Normal
	C8963-F	8	S	Normal
	C8958-F	8	S	Normal
	C8959-F	8	S	Normal

Report confidential

To	EBI food safety
From	CatchMabs b.v.
Date	22/4/05
Subject	**Gavage study**

CatchMabs

Gavage study with Listeria bacteriophage P100 on rats.

Client EBI food safety

Assignment Production, down stream processing, storage, shipment and titer-determination of Listeria specific P100 bacteriophages.

Deliverables

1. $1.5*10^{13}$ viable P100 phages divided over 5 tubes each containing $3*10^{12}$ PFU's in 12 ml PBS ($2.5*10^{11}$ PFU/ml; indicated as vial P1, P2, P3, P4 and P5).
2. 60ml PBS containing divided over 5 tubes (each 12ml) (indicated as vial C1, C2, C3, C4 and C5).
3. Titer determination of deliverable **1.)** and **2).** vials 1 to 5 (P and C-series) in subsequent days in parallel to the gavage study.
4. Titer determination of deliverable **1.)** and **2).** vials P1, P5, C1 and C5 from top, middle and bottom fluid levels (taken at gavage location) from the delivered tubes.

Performance of deliverables

For this study CatchMabs provided:
1. $1.5*10^{13}$ viable P100 phages have been successfully produced in a Tsunami Bioreactor (internal batch CM100.005) followed by current appropriate down stream processing and concentrations elements. Minimum filtration diameter is 0.2 m.

2. One batch of PBS was used throughout the whole assignment. Sterile tubes were filled with PBS or phage solutions in an appropriate sterile environment (class II laminar flow) and stored at 4°C until testing or shipment.

3. Titer was determined for all samples. From all the provided tubes a 30 l sample was stored at $+4^0$C at CatchMabs. The phage and control samples corresponding to the day of gavage are titrated for plaque forming phage particles. The determining host strain *Listeria innocua* 2627 was delivered by EBI. For the titration the samples were diluted in 100 fold steps to obtain 10^{-8} and 10^{-10} dilutions. Both dilutions are titrated in duplo. Control samples were not diluted. All phage plaque counts were determined and the average and standard deviation was calculated (see table 1 and figure 1).

4. Setup identical as previous setup for titer determination. After receival, all tubes were stored at 4°C until use (see table 2).

Results

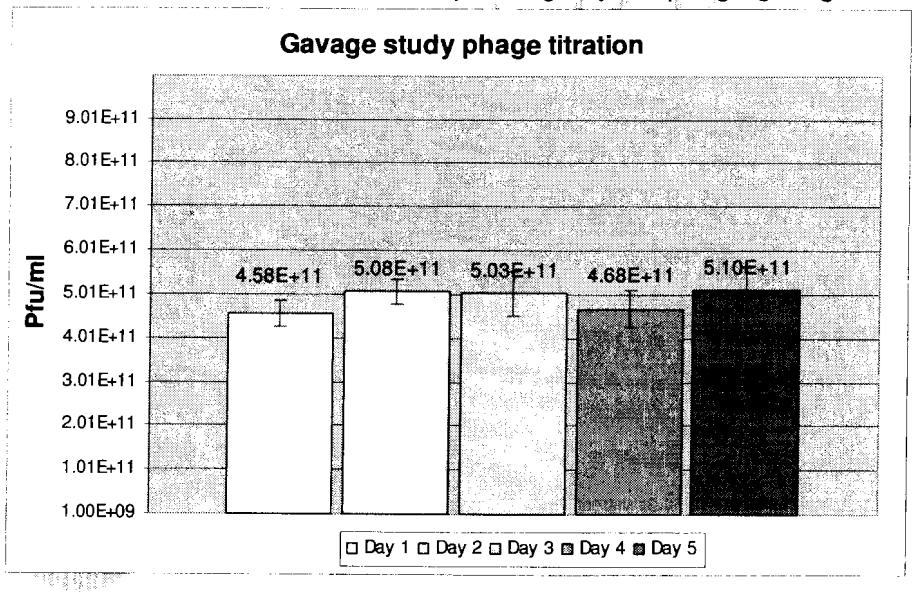
Table 1: Titration during gavage study

Day/tube	Average phage titer (pfu/ml)	SD (pfu/ml)	Day/tube	Phage-titer
1 / P1	$4.58x10^{11}$	$2.99 x10^{10}$	1 / C1	0
2 / P2	$5.08 x10^{11}$	$2.75 x10^{10}$	2 / C2	0
3 / P3	$5.03 x10^{11}$	$4.92 x10^{10}$	3 / C3	0
4 / P4	$4.68 x10^{11}$	$4.27 x10^{10}$	4 / C4	0
5 / P5	$5.10 x10^{11}$	$5.29 x10^{10}$	5 / C5	0

Table 2: Titration of samples sent back to CatchMabs

Sample	Phage titer (pfu/ml)	Day / tube	Phage titer
P1 top	$5.6 x10^{6}$	C1 top	0
P1 middle	$4.5 x10^{6}$	C1 middle	0
P1 bottom	$4.4 x10^{6}$	C1 bottom	0
P5 top	$5.1 x10^{3}$	C5 top	0
P5 middle	$1.1 x10^{8}$	C5 middle	0
P5 bottom	$2x10^{8}$	C5 bottom	0

Figure 1: Titration results on corresponding days of phage gavage.



Conclusion and discussion

The titration of the sample from the tubes sent to EBI (USA) showed identical phage titers for all days with a small standard deviation. Titration of viable phage particles has been determined as a internal quality control issue just before shipment. The phage counts at the moment of shipment were exactly as requested. It should be noted that it appeared that the number of viable phage particles were on average 2 times higher when determined during the gavage study. This phenomenon is frequently observed but not understood yet (has been discussed previously with parties).

From these results it can be conclude that the number of infective phage particles was stable during the gavage study. As expected, blank control samples did not contain any infective phages.

Phage titer determination of the returned samples indicated a phage count reduction of the samples by 3-7 logs. Blank control samples did not contain any infective phages.

This document with confidential information is covered by the manufacturing contract entered as of 6 Februari,2005 and signed by EBI food safety.

Author of Document:
Sander Huurman
Richard van der Linden
Date 19 April, 2005

Signature

Chief Scientific Officer: Erwin Houtzager

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June 2, 2005

TO: Daniel R. Cerven, MS
MB Research Laboratories
1765 Wentz Road
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FROM: W. Ray Brown, DVM, PhD, DACVP
Veterinary Pathologist

SUBJECT: Repeated Dose Oral Toxicity Study in Rats
MB Project Number: 05-13221.01
Histopathology Report - Amended

METHOD:

Microscopic examination was made of sections of the gastrointestinal tract and esophagus of twenty Wistar Albino rats equally divided by sex into two groups used in a repeated dose oral toxicity study. Rats in Group 2 were given ~1ml/animal of PBS while the Group 1 rats were given ~1ml/animal of the test article (P-100 Phage). The rats were given the test or control article once daily for five consecutive days and then were maintained without treatment for an additional three days.

On Day 8 of the study, the rats were necropsied and the esophagus, stomach, duodenum, jejunum, ileum, cecum and colon from each rat were preserved in 10% neutral buffered formalin. The in-life portion of the study was performed by the staff of MB Research Laboratories.

The preserved tissues were submitted to Research Pathology Services, Inc. for tissue processing, microscopic slide preparation and histopathologic evaluation. Samples of the esophagus, stomach (forestomach and fundic and pyloric regions of the glandular stomach), duodenum, jejunum, ileum, cecum and colon were routinely processed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic examination. Cross and longitudinal sections of the esophagus (when possible) and all areas of the intestine specified for examination were prepared and examined.

Daniel R. Cerven, MS

RESULTS:

The type and incidence of microscopic observations in the tissues specified for evaluation are presented in Table 1. The individual histomorphologic observations for each rat are listed in Table 2.

There were no test article-related microscopic changes observed in any of the male or female rats given P-100 Phage, orally, for five consecutive days.

There were only a very few microscopic changes observed in the rats of this study. Dilatation of mucosal glands in the stomach was observed in two male and two female T.A. rats and one male and one female rat of the control group (Table 1). The only other microscopic observation was minimal chronic mucosal inflammation in the cecum of one control female rat (Table 1).

T.A. Group female rat C8945 was noted at necropsy to have a red area at the junction of the jejunum and ileum. Multiple sections were examined from this area of the intestinal tract and all were within normal histologic limits with no microscopic change to correlate with the gross observation.

The histomorphologic observations in the male and female rats of both groups of this study were typical of those which occur spontaneously in laboratory rats of this strain and age and test article administration had no effect on the type or incidence of these findings.

SUMMARY:

Microscopic examination was made of the esophagus and gastrointestinal tract (stomach, duodenum, jejunum, ileum, cecum and colon) from 10 male and 10 female Wistar Albino rats used in a repeated dose oral toxicity study of P-100 Phage. The rats had been given approximately 1 ml of the control article (PBS) or test article (P-100 Phage), once daily, for five consecutive days and then were maintained without dosing for an additional three days. All rats were necropsied on Day 8 of the study.

There were no test article-related microscopic changes observed in any of the male or female rats given approximately 1 ml of the test article (P-100 Phage).

There were only a very few miscellaneous microscopic changes observed and they were typical of those that occur spontaneously and were not related to administration of the test article.

W. Ray Brown 06-02-05

W. Ray Brown, D.V.M., Ph.D., D.A.C.V.P.
Veterinary Pathologist

WRB:kwh

REPEATED DOSE ORAL TOXICITY STUDY IN RATS
STUDY NUMBER 05-13221.01

Table 1
Incidence of Histomorphologic Observations

Dose Group:	1	2	1	2
Sex:	M	M	F	F
Number of Animals/Group:	5	5	5	5
STOMACH:				
NO. EXAMINED	5	5	5	5
NO. NORMAL	3	4	3	4
-dilatation, mucosal glands	2	1	2	1
ESOPHAGUS:				
NO. EXAMINED	5	5	5	5
NO. NORMAL	5	5	5	5
DUODENUM:				
NO. EXAMINED	5	5	5	5
NO. NORMAL	5	5	5	5
JEJUNUM:				
NO. EXAMINED	5	5	5	5
NO. NORMAL	5	5	5	5
CECUM:				
NO. EXAMINED	5	5	5	5
NO. NORMAL	5	5	4	5
-inflammation, mucosa, chronic	0	0	1	0
COLON:				
NO. EXAMINED	5	5	5	5
NO. NORMAL	5	5	5	5
ILEOJEJUNAL JUNCTION:				
NO. EXAMINED	0	0	1	0
NO. NORMAL	0	0	1	0

REPEATED DOSE ORAL TOXICITY STUDY IN RATS
STUDY NUMBER 05-13221.01

Table 2
Histomorphologic Observations

Dose Group:	1	1	1	1	1	1	1	1	1	1
Animal Number:	C8961	C8941	C8942	C8943	C8944	C8945	C8960	C8947	C8948	C8949
Sex:	M	M	M	M	M	F	F	F	F	F
STOMACH:										
-dilatation, mucosal glands	1	2	-	1	-	1	-	-	2	-
ESOPHAGUS:	-	-	-	-	-	-	-	-	-	-
DUODENUM:	-	-	-	-	-	-	-	-	-	-
ILEUM:	-	-	-	-	-	-	-	-	-	-
JEJUNUM:	-	-	-	-	-	-	-	-	-	-
CECUM:										
-inflammation, mucosa, chronic	-	-	-	-	-	1	-	-	-	-
COLON:	-	-	-	-	-	-	-	-	-	-
ILEOJEJUNAL JUNCTION:						<->				

Dose Group:	2	2	2	2	2	2	2	2	2	2
Animal Number:	C8950	C8951	C8962	C8953	C8954	C8955	C8956	C8963	C8958	C8959
Sex:	M	M	M	M	M	F	F	F	F	F
STOMACH:										
-dilatation, mucosal glands	-	-	2	-	-	-	-	-	-	1
ESOPHAGUS:	-	-	-	-	-	-	-	-	-	-
DUODENUM:	-	-	-	-	-	-	-	-	-	-
ILEUM:	-	-	-	-	-	-	-	-	-	-
JEJUNUM:	-	-	-	-	-	-	-	-	-	-
CECUM:	-	-	-	-	-	-	-	-	-	-
COLON:	-	-	-	-	-	-	-	-	-	-

KEY: - = Not remarkable (within normal limits or indicated change not present)
1 = Minimal degree or amount of indicated change
2 = Mild degree or amount of indicated change
3 = Moderate degree or amount of indicated change
4 = Marked degree or amount of indicated change
P = Indicated change or lesion present

MB RESEARCH LABORATORIES
CLIENT PROTOCOL
J. BARNETT

1.0 TITLE OF STUDY: REPEATED DOSE ORAL TOXICITY STUDY IN RATS

2.0 OBJECTIVE: To provide information on the possible health hazards likely to arise from repeated oral administration of the test.

3.0 TEST ARTICLE:

3.1: Source: Phage suspension test article will be supplied by the sponsor. Prior to the initiation of the study, there should be a characterization of the test substance, including phage concentration (CFU/mL) based on colony forming units/mL. Analyses of test article for homogeneity and concentrations are the responsibility of the sponsor.

3.2: Label: Each test article tube will be identified by source, name and/or code number, date of receipt at MB Research, and MB Project Number.

3.3: Storage: The test and control substances will be stored at 2 - 8°C prior to dosing administration.

3.4: Hazards: Based on the information provided by the sponsor, appropriate routine safety precautions will be exercised in the handling of the test article.

3.5: Vehicle: Will be provided by the sponsor and indicated in section 13.3.4.

4.0 GENERAL TEST SYSTEM PARAMETERS:

4.1: Animal Requirements:

4.1.1: Number of Animals in Equilibration	:	24
4.1.2: Number of Animals on Study	:	20
4.1.3: Number of Groups	:	1 Test Article Group & 1 Control Group
4.1.4: No. Animals/Group	:	10
4.1.5: Sex	:	Equal #'s Male & Female (nulliparous & non-pregnant)
4.1.6: Species/Strain	:	Rat/Wistar Albino
4.1.7: Weight @ study initiation	:	Rats will be ordered to be approximately equivalent weights at the start of dosage

STUDY TITLE: Repeated Dose Oral Toxicity - Rats

4.2: Justification for Species and Number of Animals:

4.2.1: Species: The rat is the system of choice because it has been shown to be sensitive to toxic effects of a variety of chemicals, and is a standard animal model for the sub-chronic oral toxicity test.

4.2.2: Number of Animals: Ten animals is necessary to provide reliable interpretation of the data.

4.3: Husbandry:

4.3.1: Housing: Animals will be housed 1/cage in suspended cages which conform to the size recommendations in Guide for the Care and Use of Laboratory Animals DHEW (NIH). ANIPADS™ or other comparable product will be placed beneath the cage and changed at least three times/week. Feed containers will be changed and cleaned every week. The animal room, reserved exclusively for rats, is temperature controlled, and is equipped with a 12-hour light/dark cycle. Temperature and humidity will be continuously recorded using automatic recording devices.

4.3.2: Equilibration: The test animals will be conditioned to the housing facilities for at least 5 days prior to experimental start.

4.3.2.1: Equilibration Observations: Each rat will be observed once daily for general health. Body weights will be taken and recorded within one day of receipt and immediately prior to experimental start.

4.3.3: Food: PMI Rodent Chow (Diet #5001) is available ad libitum.

4.3.4: Water: Water is available ad libitum.

4.3.4.1: Analysis of Water and Acceptable Levels of Contaminants: Analysis of water is performed 4 times per year and results are compared against a list of acceptable levels of contaminants as provided by the water testing laboratory.

4.4: Control of Bias: From the available pool of animals, free from any evidence of disease or abnormality, and of the sex and weight range specified herein, rats will be selected and assigned to groups using a computer program which generates random numbers.

STUDY TITLE: Repeated Dose Oral Toxicity - Rats

4.5: Identification:

4.5.1: Cage: Each cage is identified by a cage tag indicating the test article identification, MB project number, dose level, number and sex of animals.

4.5.2: Animal: Each animal is identified by a uniquely numbered metal eartag

5.0 EXPERIMENTAL DESIGN:

5.1: Route of Administration: The test article is administered orally by gavage.

5.1.1: Justification for Route of Administration: The oral route of administration is chosen because it is the intended route of human exposure.

5.2: Dose Schedule: One test article treated group and one control will be used. Animals in the control group will be dosed in a manner and volume identical to the test article group.

GROUP	DOSE (ml)	# OF ANIMALS
Control[1]	1.0 ml/animal	5 Male & 5 Female
Test Article	1.0 ml/animal	5 Male & 5 Female

[1] Vehicle or Negative control – refer to section 13.0
[2] Equal to that of the test article group

5.3: Frequency: Once daily, (Monday through Friday) for 5 consecutive days.

6.0 DOSING PROCEDURE:

6.1: Sample Preparation: All sample preparation procedures will be fully documented on the data collection forms.

6.2: Sample Description: The observable physical properties of the test article are recorded.

6.3: Treatment: The test article or control will be measured by syringe and dosed via syringe and a dosing needle. The dose will be 1.0 ml for all animals. Animals will be dosed at similar times each day.

6.4: Control Group: The control group will be handled in the same manner as described for the test article group, but will receive a volume of the control substance equal to the volume of the test article group.

6.5: Homogeneity Samples: 0.5 mL retention samples from the top, middle and bottom of test and control articles will be collected on the first and last day of dosage. Samples will be stored at 2 - 8°C and shipped on ice to the sponsor on study day 8.

6.6: Concentration Verification: Verification samples (0.5 mL) will be collected on the first and last day of dosage. Samples will be stored at 2 - 8°C and shipped on ice to the sponsor on study day 8.

STUDY TITLE: Repeated Dose Oral Toxicity - Rats

7.0 TYPE & FREQUENCY OF OBSERVATIONS:

7.1: In Vivo:

7.1.1: Routine Clinical Observations: Animals will be observed and observations recorded once daily for toxicological and pharmacological signs for 8 days (5 dosing days and 3 additional observation days). All animals will be observed twice daily for mortality. Moribund animals will be humanely sacrificed. (Refer to Section 7.2 herein.)

7.1.2: Food Consumption: Measured amounts of food will be presented and recorded at the study term.

7.1.3: Body Weights are recorded immediately pretest and at study term.

7.2 Post Mortem:

7.2.1: Spontaneous Deaths will be recorded, necropsied and tissues preserved.

7.2.2: Sacrifice: Animals showing severe and enduring signs of distress and pain or animals in a moribund condition and not expected to survive until the next observation interval will, if deemed appropriate by the Study Director, be humanely sacrificed using ether and exsanguination, necropsied and tissues preserved.

7.2.3: Termination of Survivors: At the end of the study, survivors will be humanely sacrificed using ether and exsanguination, necropsied and tissues preserved.

7.2.4: Necropsy: On day 8, all survivors will be subjected to gross necropsy which will include examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents.

7.2.5: Tissues: The following tissues and organs from all animals will be preserved in 10% neutral buffered formalin.

Digestive System:
Esophagus
Stomach
Duodenum
Jejunum
Ileum
Cecum
Colon

Other:
All gross lesions and masses

7.2.7: Histopathology: All tissues listed in section 7.2.5 from animals in both the test article group and control group will be examined microscopically. Histopathology examinations will be performed by W. Ray Brown, D.V.M., Ph.D., Research Pathology Services, Inc., New Britain, PA.

STUDY TITLE: Repeated Dose Oral Toxicity - Rats

8.0 TEST DURATION: The duration of this study is 8 days. However, at the sponsor's option, the study may be extended at additional cost.

9.0 ANALYSIS OF DATA:

Evaluation will include the relationship between the dose of the test article and the presence or absence, the incidence and severity of abnormalities, including behavioral and clinical abnormalities, gross lesions, identified target organs, body weight changes, effects on mortality and any other general or specific toxic effects.

10.0 REVISION OF THE PROTOCOL:

Any amendment to or deviation from this protocol will be fully documented in the study file, including the reason for the change, the authority for said change and the date thereof.

11.0 RECORDS TO BE MAINTAINED:

11.1: Collection of Data: All data generated during the conduct of this study will be recorded in ink on worksheets. All entries will be dated, initialed and verified by another person.

11.2: Reports:

11.2.1: Draft Report: A draft report will be submitted prior to submission of the final report.

11.2.2: Final Report: Following approval by the sponsor of the draft report, the final report will be submitted and will include, but not be limited to:

- Identification, manufacturer, source, lot/batch number of the test article
- Identification and composition of vehicles, if any, used in administration of the test article
- Species, strain, sex, age, source and number of test animals
- Equilibration, housing conditions including number of animals/cage, bedding material and diet
- Method of randomization
- Test procedures
- Tabulated data of individual clinical observations, body weights, and food consumption
- Description of toxic effects
- Gross necropsy findings
- Histopathological findings

STUDY TITLE: Repeated Dose Oral Toxicity - Rats

11.3: Retention of Data: All data generated as a result of the conduct of this study will be retained in the archives at MB Research for an indefinite period of time, but not less than 10 years from the date of the final report of this study.

After 10 years, the sponsor will be contacted in writing to determine final disposition of the records. If the sponsor fails to respond within 90 days, MB will dispose of these records.

11.3.1: Raw Data will be filed at MB Research by project number.

11.3.2: Final Reports will be filed at MB Research by sponsor name and MB project number.

11.3.3: Test Article: Any remaining test article will be treated with twice the amount of 95% EtOH, transferred to red bags, sealed and disposed of in Biohazard containers.

11.3.4: Tissues, blocks and slides will be stored at MB Research by sponsor name and MB project number for one year following submission of the final report. After one year, the sponsor will be contacted to determine final disposition.

12.0 GOOD LABORATORY PRACTICES:

This study will not be conducted under Good Laboratory Practices.

STUDY TITLE: Repeated Dose Oral Toxicity - Rats

MB RESEARCH LABS
PROTOCOL NO: CLIENT-J.BARNETT
PAGE NO: 7 of 8

13.0 SPONSOR REQUEST:

13.1: The sponsor requests that this protocol be implemented:
☒ As written (or) ☐ Amended per attached description of amendments

13.2: Will report be submitted to a regulatory agency? ☐ No ☒ Yes FDA (agency)

13.3: Test Article will be identified in the report and supporting documentation exactly as indicated below:

13.3.1: Identity: The test article is identified as follows: P-100 / HAGE

pH (when applicable): ~7 Lot/Batch #: AS MAILED / 1D

13.3.2: Dose (mg/kg): Test Article Group: ~1ml/animal Control Group: 1ml/animal

13.3.3: Dose Preparation: Should dosing preparation be adjusted for purity/active ingredient/strength?
☒ NO ☐ YES **If yes**, what correction factor should be used? _____

13.3.3.1: Analysis of aliquots of dosing mixture(s) required? ☐ No ☒ Yes Frequency: 1, 5

** 13.3.4: Vehicle or Negative Control _____

*** 13.3.5: Test Article Characterization is required in support of data submissions and must be reviewed by the Study Director and included in the final report. (EPA 40 CFR 160.105 and 792.105; FDA 21 CFR 58.105, OECD 6.2). This information is:
☒ included (or) ☐ Not available

*** 13.3.6: Material Safety Data Sheet Supplied: ☒ Yes ☐ No

13.3.7: DOT Hazardous Material: ☒ No ☐ Yes (indicate DOT shipping Name) _____
EPA Hazardous Waste: ☒ No ☐ Yes (indicate EPA Waste Number) _____

13.3.8: Shipping Instructions for Return of Residual Test Article: (Call or refer to Study Initiation Information for costs)
☐ UPS / Ambient temperature *(no charge)*
☐ Overnight carrier / Dry Ice
☐ Express carrier / Ambient temperature
☒ Overnight carrier / Ice packs

13.4: Authorization Statement: This protocol is authorized for implementation at MB Research. This study is necessary to estimate the toxic effects of the test compound. To the best of my knowledge and information, this test is not an unnecessary duplication of any previous studies.

13.4.1: Confidentiality: Study results and reports will be released only to the below named sponsor representative unless other sponsor representatives are identified below.

TA recd 3/1/05

BY: James W. Barnett Jr (signature)
JAMES W. BARNETT, JR (type/print name)
SR. REG. SPEC (title)

*** FOR: _____ (company) (date)
(address)
city) (st) (zip)
(phone) (fax)

Optional: Other Sponsor Representative: _____

**** - Per Jim Barnett - Co. Name on report S/B Experimental Biotherapies Inc. 3/31/05 BK

*** TAC and MSDS not received at time of logging in, Per Jim Barnett - he does not have them at this time. MSDS recd 3/15/05 BK
BK 3/16/05

1765 wentz road
phone: (215) 536-4110
spinnerstown, pa 18968
fax: (215) 536-1816

t:\bonnie\protocol\client\barnett-oral 2-18-05.doc

* store test article @ 2-8°C per J. Barnett 3-1-05

** Per attached email, the vehicle control is PBS (supplied by Sponsor) TAV 3-1-05

STUDY TITLE: Repeated Dose Oral Toxicity - Rats

MB RESEARCH LABS
PROTOCOL NO: CLIENT-J.BARNETT
PAGE NO: 8 of 8

14.0 MB RESEARCH ACKNOWLEDGMENT: Request for implementation of this protocol and receipt of the test article is acknowledged by MB Research.

14.1 Test Article Identity: P-100 Phage, PBS

14.1.1: Date Received: 3-1-05

14.1.2: Physical Description: Cloudy liquid, clear liquid

14.1.3: Test Article Characterization:

14.1.3.1: ☑ Not supplied by Sponsor, or

14.1.3.2: ☐ Received and Reviewed by Study Director:

14.2: MB Project Number assigned to this study: 05-13221.01

14.3: Animal Supplier: The Licensed USDA. animal supplier is: [illegible]

14.4: Proposed Study Dates:

14.4.1: Experimental Start Date: 14 Mar 05

14.4.2: Experimental Term Date: 21 Mar 05

14.4.3: Study Completion Date (Submission of Report): The target date for report submission is 5-6 weeks following Experimental Term Date.

14.5: Approval: There are currently no suitable non-animal alternatives to this study as determined according to MB Research SOP Vol. III A. This protocol is designed to avoid or minimize discomfort. The procedures will be performed by personnel thoroughly trained in the humane care and use of laboratory animals. If pain does occur as a result of the nature of the test article being used, it will be addressed according to MB SOP Vol. III A. This protocol is approved for implementation at MB Research by the below named MB Study Director.

[signature] 14 Mar 05

BY:
Study Director (date)
Testing Facility MB Research Laboratories
1765 Wentz Road, P. O. Box 178
Spinnerstown, PA 18968

This protocol was originally reviewed by the Institutional Animal Care and Use Committee (IACUC) of MB Research on the date indicated below and found to be in compliance with acceptable standards of animal welfare and humane care. The IACUC committee will review this protocol on an annual basis. This review will be documented in the IACUC minutes and included in the semi-annual report to the institutional official.

DATE: 2/22/05