

FINAL REPORT

**28-DAY REPEATED DOSE TOXICITY STUDY OF TOBACCO BLEND AND
AQUEOUS TOBACCO EXTRACT IN CD-1 MICE**

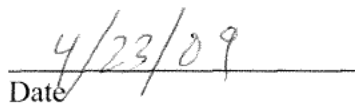
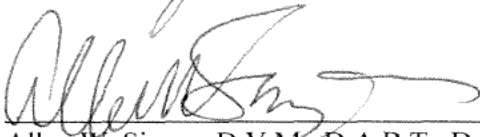
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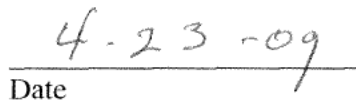
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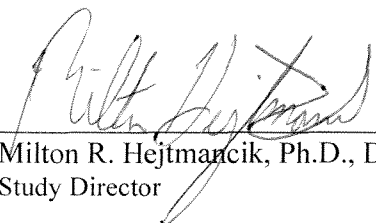

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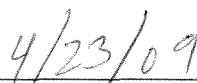
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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with the Food and Drug Administration's (FDA) Good Laboratory Practice (GLP) regulations (21 CFR Part 58), for the conduct of non-clinical laboratory studies with the following exceptions: plasma analysis was conducted using a non-validated bioanalytical method; characterization and stability analysis of bulk test articles ([Appendix B](#)) and serology analyses ([Appendix I](#)) were conducted, as intended, under non-GLP development procedures.



Milton R. Hejtmancik, Ph.D., D.A.B.T.
Study Director

Date

QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to the Study Director and management as follows:

Phase Inspected	Date Inspected	Date Reported to Study Director and Management
Protocol review	06/02/2008	06/02/2008
Formulation preparation	06/20/2008	06/20/2008
Dispensing	06/20/2008	06/20/2008
Animal room inspection	06/20/2008	06/20/2008
Formulation analysis	06/23/2008	06/23/2008
Group assignment	06/27/2008	06/27/2008
Test system identification	06/27/2008	06/27/2008
Animal room inspection	06/27/2008	06/27/2008
Body weights	07/01/2008	07/02/2008
Clinical observations	07/01/2008	07/02/2008
Veterinary evaluation	07/01/2008	07/02/2008
Quarantine release	07/01/2008	07/02/2008
Animal room inspection	07/01/2008	07/02/2008
Dispensing	07/01/2008	07/02/2008
Food consumption measurements	07/01/2008	07/02/2008
Test article administration - dosed feed	07/01/2008	07/02/2008
Functional observational battery	07/16/2008	07/17/2008
Anesthetization	07/24/2008	07/25/2008
Blood collection	07/24/2008	07/25/2008
Euthanasia	07/24/2008	07/25/2008
Centrifugation	07/24/2008	07/25/2008
Audit study file	07/24/2008	07/24/2008
Protocol amendment review	07/25/2008	07/25/2008
Fasting	07/30/2008	07/30/2008
Anesthetization	07/30/2008	07/30/2008
Blood collection	07/30/2008	07/30/2008
Euthanasia	07/30/2008	07/30/2008
Necropsy/tissue collection	07/30/2008	07/30/2008
Organ weights	07/30/2008	07/30/2008
Clinical lab blood processing/analysis	07/30/2008	07/30/2008
Protocol amendment review	08/05/2008	08/05/2008
Animal room formulation analysis	08/14/2008	08/15/2008
Audit study file	08/29/2008	08/29/2008
Audit study file	09/03/2008	09/03/2008

QUALITY ASSURANCE STATEMENT

Phase Inspected	Date Inspected	Date Reported to Study Director and Management
Audit analytical report	09/03/2008	09/03/2008
Audit study file	09/10/2008	09/10/2008
Audit study file	09/16/2008	09/16/2008
Audit neurobehavioral report	09/16/2008	09/16/2008
Audit study file	09/30/2008	09/30/2008
Audit study file	10/01/2008	10/01/2008
Audit study file	10/03/2008	10/03/2008
Audit study file	10/08/2008	10/08/2008
Audit study file	10/10/2008	10/10/2008
Audit bioanalytical and kinetics report	11/19/2008	11/19/2008
Audit report tables and/or appendices	11/21/2008	11/21/2008
Audit draft final report	11/25/2008	11/25/2008
Audit draft final report	03/23/2009	03/23/2009
Audit final report	04/21/2009	04/21/2009

 4/23/09

Quality Assurance Unit Date
Battelle

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SUMMARY

The objective of this study was to compare the short-term toxicity of a tobacco blend, aqueous tobacco extract, and appropriate controls (nicotine tartrate positive control and diet negative control) in rodents. The following evaluations were performed: clinical observations, body weights, food consumption, clinical pathology, functional observational test battery (FOB), gross necropsy, and selected organ weights. Toxicokinetic evaluations of plasma for nicotine and cotinine were performed on Days 14, 15, and 28 under continuous dosed feed exposure. The overall summary of the study design and the estimated nicotine doses and test article concentrations for nicotine tartrate, tobacco blend, and aqueous tobacco extract are listed below (Text Table 1):

Text Table 1. Study Design for the 28-Day Repeated Dose Toxicity Study of Tobacco Blend and Aqueous Tobacco Extract in CD-1 Mice

Group	Target Dosage of Nicotine (mg/kg/BW*/day)	Test Article Concentration** (mg/kg)	
		Males	Females
1 - Control	0	0	0
2 - Nicotine Tartrate (NT) High Dose	200	4988	4275
3 - Tobacco Blend Low Dose	2	666	571
4 - Tobacco Blend Intermediate Dose 1	20	6659	5708
5 - Tobacco Blend Intermediate Dose 2	80	26636	22831
6 - Tobacco Blend High Dose	200	66589	57077
7 - Tobacco Extract Low Dose	2	761	653
8 - Tobacco Extract Intermediate Dose 1	20	7612	6525
9 - Tobacco Extract Intermediate Dose 2	80	30448	26098
10 - Tobacco Extract High Dose	200	76120	65246

* BW = Body weight.

** Quantity of test article or positive control per kg feed.

Dosing concentrations were derived based on the information provided in [Text Table 2](#).

Text Table 2. Nicotine Concentrations in Tobacco Blend, Tobacco Extract, and Nicotine Tartrate Formulations

Sex	Nicotine Dose (mg/kg BW ^a /day)	Food Consumption (kg/day)	Body Weight (kg)	Nicotine Needed (mg)	Nicotine ^b (mg/kg)	Tobacco ^c Blend (mg/kg)	Tobacco ^c Extract (mg/kg)	Nicotine Tartrate ^c (mg/kg)
M	2	0.004	0.035	0.07	17.5	666	761	--
M	20	0.004	0.035	0.7	175	6659	7612	--
M	80	0.004	0.035	2.8	700	26636	30448	--
M	200	0.004	0.035	7	1750	66589	76120	4988
F	2	0.004	0.03	0.06	15	571	653	--
F	20	0.004	0.03	0.6	150	5708	6525	--
F	80	0.004	0.03	2.4	600	22831	26098	--
F	200	0.004	0.03	6	1500	57077	65246	4275

Tobacco = 26.28 mg/g of nicotine; 38.051 mg of tobacco blend for every mg of nicotine.

Extract = 22.99 mg/g of nicotine; 43.497 mg of extract for every mg of nicotine.

Nicotine Tartrate = 35% nicotine; 2.85 mg of nicotine hydrogen tartrate for every mg of nicotine.

a. BW = body weight.

b. Quantity of nicotine per kg feed.

c. Quantity of test article or positive control per kg of feed.

Abbreviations used throughout this report to designate the dosage groups of male and female mice are listed below (Text Table 3):

Text Table 3. Abbreviations Used Throughout this Report to Designate the Dose Groups for Male and Female Mice

Group	Males	Females
1	CM	CF
2	NT200M	NT200F
3	B2M	B2F
4	B20M	B20F
5	B80M	B80F
6	B200M	B200F
7	E2M	E2F
8	E20M	E20F
9	E80M	E80F
10	E200M	E200F

1.0 INTRODUCTION

The objective of this study was to evaluate the short-term toxicity of a tobacco blend and aqueous tobacco extract in comparison to the nicotine tartrate positive control and diet negative control in CD-1 mice. Plasma was analyzed at the approximately mid-point of the study and Study Day 28 to measure nicotine and cotinine concentrations from animals fed nicotine containing test articles in the diet.

R.J. Reynolds Tobacco Company was the Sponsor of the study. Dr. Suzana Theophilus was designated as the Sponsor Monitor and approved the study protocol.

The study was conducted at Battelle under the direction of Dr. Milton R. Hejtmancik. The in-life portion of the study began with exposure initiation on July 1, 2008 and ended with final necropsy on August 1, 2008.

2.0 EXPERIMENTAL DESIGN

Five hundred ninety-eight male and female mice were randomized into ten dose groups. The study consisted of a 28-day toxicity study that included a neurotoxicity component and a toxicokinetic study. Endpoints used to evaluate the potential toxicity of tobacco blend and aqueous tobacco extract were clinical observations, body weights, body weight changes, food consumption, clinical pathology, neurological and behavioral toxicology, and gross necropsy including selected organ weights. Toxicokinetic evaluations of nicotine tartrate, tobacco blend, and aqueous tobacco extract groups were performed on Days 14, 15, and 28. The general study design is listed below.

Group	Target Dosage of Nicotine (mg/kg/day)	Number of Mice			
		Males		Females	
		Core	TK ^a	Core	TK ^a
1 - Control	0	10	5	10	5
2 - Nicotine Tartrate (NT) High Dose	200	10	23	10	23
3 - Tobacco Blend Low Dose	2	10	23	10	23
4 - Tobacco Blend Intermediate Dose 1	20	10	23	10	23
5 - Tobacco Blend Intermediate Dose 2	80	10	23	10	23
6 - Tobacco Blend High Dose	200	10	--	10	--
7 - Tobacco Extract Low Dose	2	10	23	10	23
8 - Tobacco Extract Intermediate Dose 1	20	10	23	10	23
9 - Tobacco Extract Intermediate Dose 2	80	10	23	10	23
10 - Tobacco Extract High Dose	200	10	23	10	23

a. Nicotine/cotinine analysis.

TK = Toxicokinetic.

3.0 METHODS

3.1 Protocol and Amendments

The study protocol, amendments to the protocol and deviations from the protocol are provided in [Appendix A](#). There were no deviations that occurred in the conduct of the study that were considered to significantly affect the quality or integrity of the study.

3.2 Test Articles (Tobacco Blend, Aqueous Tobacco Extract) and Positive Control Article (Nicotine Hydrogen Tartrate Salt)

Test articles, a natural tobacco blend containing no additives and an aqueous tobacco extract, were supplied by R.J. Reynolds Tobacco Company and were received on May 7, 2008 in good condition. A total of approximately 1278 lbs of tobacco blend was received in 71 containers (18 lbs per bucket) and a total of approximately 1105.5 lbs of tobacco extract was received in 33 containers (33.5 lbs per bucket). Test articles were provided by the Sponsor in plastic buckets and were stored frozen (-30 to -15°C). Nicotine hydrogen tartrate salt (Batch No. 028K0705) was supplied by Sigma-Aldrich. A total of approximately 1.8 kg of nicotine tartrate was received on May 20, 2008 (expiration date May 20, 2009) in good condition and was stored at room temperature. The certificates of analysis for test articles and the nicotine tartrate positive control are provided in [Appendix B](#). The identity, strength, purity, composition, stability, and methods of synthesis of test articles were the responsibility of the Sponsor.

Reserve samples of each set of the tobacco blend, aqueous tobacco extract, and the nicotine tartrate control article used to formulate the animal diets were collected under design form CN49730A-TASTAB. Reserve samples of the tobacco blend and tobacco extract will be maintained frozen (-30 to -15°C) and a reserve sample of the nicotine tartrate will be maintained at room temperature until submission of the chronic study final report.

3.3 Chemical Analysis of Formulations

3.3.1 Pre-Dosing

Samples of formulations from the dose preparation of tobacco blend, tobacco extract, and nicotine tartrate were analyzed at Battelle for verification of nicotine concentrations based on

methods provided by the Sponsor. The detailed analytical results are provided in [Appendix F](#). All pre-dose formulations of tobacco blend, tobacco extract, and nicotine tartrate that were analyzed for nicotine concentration met acceptance criteria (within 10 percent of the target concentrations; relative standard deviation [RSD] less than or equal to 10 percent).

Homogeneity studies were performed to support the 28-day study (CN49730A-FORMPRE). The tobacco extract and nicotine tartrate formulations met all design form acceptance criteria for homogeneity. The tobacco blend formulations met the design form acceptance criteria for homogeneity for grand % RSD (RSDs were less than 10%). Relative errors (RE) were all more than 10% above target, suggesting the nicotine content of this test article may exceed the labeled concentration.

3.3.2 Post-Dosing

Post-dosing (animal room) formulations met the criteria for concentration (RE within 10% of target; $RSD \leq 10\%$), except for the following:

- NT200F (4275 mg/kg) formulation had an RE of -16.0%.
- NT200M (4988 mg/kg) formulation had an RE of -13.9%.
- E20F (6525 mg/kg) formulation had an RE of -14.7%.

There was no impact of these failures to meet acceptance criteria on the study. Post-dose samples are collected from the animal room feeders. They have been exposed to the animal and are subject to the impact from this exposure on concentration. Possibilities include selective eating of the feed or analyte from the formulation by the animal; contamination of the formulation by urine, feces, bedding, or other materials; and exposure of the formulation to the animal room environment. For these reasons, animal room samples should be used only to and/or indicate general trends that may result from exposure to the animal's room environment.

In general, the post-dose animal room concentrations were lower than the pre-dose concentrations. The tobacco blend animal room samples were ~12% lower on average;

tobacco extract animal room samples were ~3% lower on average; and nicotine tartrate animal room samples were ~9% lower on average.

3.4 Experimental Animals

A total of 644 male and female CD-1 mice were required for the study; however, due to mortality in the B200M and B200F core groups, 46 TK B200M and B200F mice (23/sex) were removed from the study. A sufficient number of animals were obtained from Charles River Laboratories (Portage, MI) to provide the required number of healthy animals for testing. The mice were approximately four to five weeks of age and ranged in body weight from approximately 19 to 32 grams at Day 1 of the study. Mice were housed in Room 7C-068 except when neurobehavioral exams were conducted in Rooms 7C-089 and 7C-085.

The mouse was chosen as the test system because considerable scientific documentation of the mouse as a predictive animal model for humans exists, and there are no *in vitro* or computer models that can replace the integrative function of the whole animal model. The Battelle Institutional Animal Care and Use Committee approved the proposed activities before implementation of this study.

3.4.1 Animal Housing and Environmental Conditions

All animals were received, quarantined, and housed in polycarbonate cages with hardwood bedding according to testing facility standard operating procedures (SOPs). Male mice were housed in individual cages and female mice were housed up to 4 per cage. All housing and animal care and maintenance conformed to the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) recommendations, current requirements stated in the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996), and the U.S. Department of Agriculture through the Animal Welfare Act, as amended.

The environmental conditions of the animal study rooms conformed to the following: (1) the light/dark cycle was held at approximately 12 hours of light and 12 hours of dark each day during the study using fluorescent lighting, starting at approximately 6:00 AM each day; (2) the room temperature and relative humidity controls were set to provide from 64 to 79°F and

30 to 70 percent, respectively, and were monitored for conformance; and (3) fresh air was supplied to the room at a rate providing a minimum of ten changes of room air per hour.

3.4.2 Diet

Animals were fed powdered NTP-2000 rodent diet *ad libitum*, according to facility SOP, except when fasted prior to scheduled necropsy. The control group was fed the diet without test article and treated animals were fed the diet with the specified quantity of test article required to maintain their designated doses. Analysis reports of each feed lot were supplied by the vendor and were maintained by Battelle. There were no known or reported contaminants in the feed that would have any impact on study results or interpretations.

3.4.3 Water

Fresh water from the city of Columbus municipal water supply was provided *ad libitum* via automatic watering system and water bottles. The water supply was monitored under Battelle SOPs.

Water samples from Room 7C-068 were collected on October 23, 2008 for analysis. Results were available on October 31, 2008 and indicated there were no known or reported contaminants in the water that would have any impact on study results or interpretations.

3.5 Treatment Group Allocation and Animal Identification

Animals were identified by pre-study numbers on cage cards during quarantine and acclimation. Following group assignment, the mice were individually identified by tail tattoo.

Prior to the initiation of exposures, animals were assigned to study groups using the PATH/TOX SYSTEM (version 4.2.2, Xybion Medical Systems Corporation, Cedar Knolls, NJ). The PATH/TOX SYSTEM software algorithm ensures homogeneity of group variances with respect to body weight across all groups. The following were the group assignments and animal identification numbers:

Animal Identification Numbers				
Group	Males Core	TK	Females Core	TK
1	101-110	111-115 ^a	151-160	161-165 ^a
2	201-210	211-233	251-260	261-283
3	301-310	311-333	351-360	361-383
4	401-410	411-433	451-460	461-483
5	501-510	511-533	551-560	561-583
6	601-610	611-633 ^b	651-660	661-683 ^b
7	701-710	711-733	751-760	761-783
8	801-810	811-833	851-860	861-883
9	901-910	911-933	951-960	961-983
10	1001-1010	1011-1033	1051-1060	1061-1083
11	1101-1110	--	1151-1160	--

a. Animals not randomized by Xybion; assigned to CM and CF groups after randomization.

b. Randomized and tattooed but removed from study prior to TK Study Day 1.

Animals subjected to the FOB were identified such that the FOB was blind. Therefore, these animals were identified by tail tattoos using a unique number between 1 and 100 randomly assigned to each animal instead of each animal's identification number (listed above). Both the tail tattoo and the animal identification numbers were listed on the cage card for animals subjected to the FOB.

3.6 Experimental Design

Mice were randomized into 10 treatment groups and 1 sentinel group. The study consisted of a 28-day toxicity study including both a neurotoxicity component and a toxicokinetic study. Endpoints used to evaluate potential toxicity of tobacco blend, aqueous tobacco extract, and nicotine tartrate were clinical observations; body weights and body weight changes; food consumption; neurobehavioral/toxicology; and clinical and anatomic pathology including organ weights. Toxicokinetic evaluations of plasma nicotine and cotinine concentrations were performed on toxicokinetic animals.

3.7 Clinical Observations

Cage-side observations were made twice daily, for moribundity and mortality, once in the morning and once in the afternoon, throughout the duration of study. Clinical examinations were conducted on all core animals at weekly intervals. A final detailed clinical examination was conducted on each core study mouse on the day of scheduled necropsy.

3.8 Body Weights (Core and TK)

Individual body weights of animals were recorded on Day -5 (with respect to core males) for randomization and group assignment. After initiation of dosing, body weights for core study animals were recorded on Study Days 1, 4, 8, 11, 15, 18, 22, 25, and 29. Body weights for TK study animals were recorded on Study Days 1, 8, 14, 15, 22, and 28. Body weights were collected on Study Day 14 for TK animals (except B200F and CF) for potential calculation of plasma nicotine and cotinine concentrations per body weight.

3.9 Food Consumption (Core Animals)

Quantitative food consumption was measured for each core study animal bi-weekly starting on Day 1. A known amount of food was placed in the feed container and reweighed after three to four days. The difference in the weight of the food container was taken as a measurement of food consumed, and food consumption (g/day) was calculated.

3.10 Neurobehavioral Toxicology (Core Animals)

Five mice of each sex were randomly selected from each core study groups and were identified in such a manner that the conduct of the FOB was blind. Animals were subjected to the FOB on Study Days 16 and 17. Animals were individually housed the day prior to and the day of the neurobehavioral examination. At the completion of the FOB, animals were transported back to Room 7C-068 and housed in their home cages per protocol specifications.

3.11 Clinical Pathology (Core Animals)

Clinical chemistry and hematology assessments were performed on all surviving core study mice on the day of their scheduled necropsy. Core study mice were divided into two groups for clinical pathology blood collections. Blood from approximately half the animals were used in hematology analysis and blood from the remaining half were used for clinical chemistry. Blood samples were collected under CO₂/O₂ anesthesia from the retro-orbital sinus for hematology and serum chemistry determinations. The tubes contained ethylene diamine tetraacetic acid (EDTA) as an anticoagulant for blood samples collected for

hematology. The tubes used for serum chemistry determinations did not contain any anticoagulant, but did contain a serum separator gel.

3.11.1 Hematology

Hematologic parameters measured or calculated were as follows:

Erythrocyte count (RBC)	Mean corpuscular hemoglobin
Hemoglobin (HGB)	concentration (MCHC) (calculated)
Hematocrit (HCT) (calculated)	Reticulocyte count (RET) (absolute)
Mean corpuscular volume (MCV)	Platelet count (PLT)
Mean corpuscular hemoglobin (MCH)	Total leukocyte count (WBC)
(calculated)	WBC differential (absolute)

3.11.2 Serum Chemistries

Serum chemistries measured or calculated were as follows (listed in order of priority):

Aspartate aminotransferase (AST)	Cholesterol (total) (CHOL)
Direct bilirubin	Creatinine (CREA)
Total bilirubin (TBIL)	Total protein (TP)
Gamma-glutamyl transferase (GGT)	Urea nitrogen (BUN)
Albumin (ALB)	Calcium (total) (CA)
Globulin (calculated) (GLOB)	Chloride (CL)
Albumin/globulin ratio (calculated) (AGR)	Phosphorus (PHOS)
Alkaline phosphatase (ALP)	Potassium (K)
Glucose (GLU)	Sodium (NA)
Triglycerides (TRIG)	

3.12 Toxicokinetic Blood Collections (TK Animals)

The toxicokinetic portion consisted of two phases. Phase I determined an appropriate time to sample plasma for nicotine and cotinine from animals fed nicotine containing test articles in the diet. This was accomplished by determining the time course of plasma nicotine and cotinine at the approximate mid-point of the study. At Study Days 14 and 15, six specified time points were spread over a 20-hour time interval to determine the observed C_{\max} and T_{\max} values for nicotine and cotinine in male and female mice from up to eight specified treatment groups. Phase 2 was based on the information obtained from Phase 1 data, in which the most appropriate (estimated C_{\max} and T_{\max}) single collection time point was selected for

collecting samples from male and female mice. The data from Phase 2 was used to evaluate dose proportionality by sex and group.

3.12.1 Phase 1 Collections

From each dose group, 18 mice of each sex assigned to the toxicokinetics study were randomly subdivided into six subsets of animals. Each subset was used for blood collection at specific times in a manner that resulted in one blood collection/animal and in a manner that distributed each subset over the 20-hour time course. Each subset consisted of three males and three females from each of eight treatment groups that had blood drawn at a designated time point (10:00 PM, 2:00 AM, 6:00 AM, 10:00 AM, 2:00 PM, and 6:00 PM). This resulted in six time points for plasma nicotine and cotinine over a 20-hour time interval and allowed for the determination of the optimal time for collection in Phase 2 and subsequent studies.

TK study animals were anesthetized with CO₂/O₂ and blood was collected via cardiac puncture into tubes containing potassium EDTA as the anti-coagulant. Blood was placed on wet ice until centrifuged. Plasma was extracted and placed on dry ice until stored in a freezer (-60 to -80°C). After each blood collection the animal was placed in its home cage supplied with feed and water until the next available blood draw.

3.12.2 Phase 2 Collections

On Study Day 28, blood was collected from five male and five female TK animals in each of the ten treatment groups at 10:00 AM based on the results from Phase 1 toxicokinetics. After plasma collection, all animals in the TK subset were terminated with no further data collection. Plasma was collected from control animals (CM and CF) to measure background nicotine and cotinine plasma levels among study animals not exposed to the test article.

3.13 Necropsy and Organ Weights

After at least 28 days of dosing, all surviving core animals, excluding sentinels, were fasted overnight and humanely terminated using CO₂. Terminal body weights were determined and external features of the animals were evaluated prior to euthanasia, followed by necropsy.

Each necropsy included: examination of the external surface of the body; all orifices; the cranial, thoracic, abdominal and pelvic cavities and their contents; and collection of all tissues listed in the protocol, as well as gross findings. All scheduled necropsies were conducted under the supervision of a board-certified veterinary pathologist.

The following tissues were collected according to facility SOP. Tissues were fixed in 10 percent neutral buffered formalin (NBF), with the exception of testes, which were preserved in Bouin's fixative and subsequently transferred to 70 percent ethanol, and eyes with optic nerve which was fixed in Davidson's fixative and subsequently transferred to 10 percent NBF, per facility SOP.

Tissues Collected at Necropsy

Animal identification	Parathyroid/thyroid gland
Gross lesions	Pituitary gland
Adrenal glands	Preputial glands
Bone with articular surface and marrow (femur)	Prostate
Brain (cerebrum, cerebellum, medulla)	Salivary gland (mandibular)
Clitoral gland	Sciatic nerve
Epididymides	Seminal vesicles
Esophagus, pharynx	Skeletal muscle (biceps femoris)
Eye (with optic nerve)	Skin
Harderian glands	Small intestine (duodenum, jejunum, ileum)
Heart	Spinal cord (cervical, thoracic, lumbar)
Kidneys	Spleen
Large intestine (cecum, colon, rectum)	Sternum, bone marrow
Liver (median lobe and left lateral lobe)	Stomach (fore-stomach and glandular)
Lungs with bronchi	Testes
Mesenteric lymph node	Thymus
Mammary gland (females only)	Tongue
Nasal cavities and turbinates	Urinary Bladder
Ovaries (without oviduct)	Uterus
Oral cavity	Vagina
Pancreas	Zymbal glands

The following organs were weighed for core mice euthanized at scheduled necropsy.

Organs Weighed at Necropsy	
Brain	Spleen
Epididymides ^a	Testes (without epididymides) ^a
Heart	Thymus
Kidneys ^a	Salivary glands (mandibular)
Liver with gall bladder ^b	Uterus (with cervix)

a. Paired organs weighed together.

b. Gall bladder opened and bile drained before weighing.

3.14 Computer Systems for Data Management

(b) (4)

3.15 Data Analysis

(b) (4)

(b) (4)



4.0 RESULTS

4.1 Mortality

Mortality occurred in many high dose core study groups over the course of this study. For core study animals ([Table 1](#)), all B200M and B200F group mice, all E200M group mice, six out of ten E200F group mice, and four out of ten NT200M mice either died or were terminated in moribund condition prior to their scheduled termination. A mortality summary for mice in the toxicokinetic groups is included in [Table 2](#). All mice in the B200F toxicokinetic group were removed from the study due to initial mortality that was encountered in the B200F core study group. Mortality also occurred in 7/23 mice in the NT200M toxicokinetic group and 20/23 in the E200M toxicokinetic study group.

4.2 Clinical Observations (Core Animals)

Clinical signs of toxicity were apparent predominately in high dose mice as indicated in [Table 3](#) for males and [Table 4](#) for females. High dose mice generally appeared thin and showed signs indicative of inanition and lack of grooming. Other clinical signs in high dose mice included hunched posture, lethargy, paleness, rough hair coat, tremors, hindlimb weakness, and labored respiration. Rough hair coat was the most common observation in lower dose group animals (B80M, E2M, and E80M).

4.3 Body Weights

The group mean absolute body weights for male and female core study mice are included in [Tables 5](#) and [6](#). Treatment with the test articles and positive control was associated with a significant reduction in group mean body weight in the NT200M, B80M, and E80M groups of 30.2, 10.2, and 10.5%, respectively, relative to control (CM) on Study Day 29 ([Table 5](#)). Depressions in group mean body weight for the high dose groups were apparent (-23.6% relative to control) in the B200M group and E200M groups on Study Day 4 and the body weights of these groups never returned to their pre-test value. The three survivors in the E200M showed a group mean body weight value that was decreased 30.2% relative to the CM group on Study Day 11. There was a depression in group mean body weight of 2.7, 0.6, and 10.2% for the B2M, B20M, and B80M dose groups and 0.6, 1.8, and 10.5% for the E2M, E20M, and E80M dose groups, respectively, on Study Day 29. Over the course of this study,

the NT200F and E200F groups both showed depressions in group mean body weight of 19.1 and 17.1%, respectively, relative to control (CF) on Study Day 29. The B200F group showed a depression in group mean body weight of 25.8% relative to the CF group on Study Day 8.

The group mean absolute body weights for male and female mice in the toxicokinetic groups are included in [Tables 7 and 8](#), respectively. The B2M, B20M, B80M, E2M, E20M, and E80M groups showed dose-response decreases in group mean weight gain of 10.1, 13.1, 23.5, 9.5, 16.0, and 17.8%, respectively, relative to the CM group on Study Day 28. The NT200F, B2F, B20F, B80F, E2F, E20F, E80F, and E200F groups showed respective decreases in group mean body weight relative to control (CF) of 22.7, 13.6, 13.3, 12.3, 17.2, 13.0, 13.6, and 26.6%.

The group mean absolute body weights are also included for male and female mice in [Figures 1 and 2](#) for tobacco blend (TB) mice, [Figures 3 and 4](#) for tobacco extract (TE) mice, and [Figures 5 and 6](#) for mice exposed to nicotine tartrate (NT), respectively. The group mean absolute weight gain is also included for male and female mice in [Figures 7 and 8](#) for TB mice, in [Figures 9 and 10](#) for TE mice, and in [Figures 11 and 12](#) for mice exposed to NT, respectively.

4.4 Food Consumption

The mean food consumption values for the core male and female mice groups are shown in [Tables 9 and 10](#), respectively. The mean food consumption for male and female mice is also included in [Figures 13 and 14](#) for TB mice, [Figures 15 and 16](#) for TE mice, and [Figures 17 and 18](#) for mice exposed to NT, respectively. The NT200M and B80M dose groups showed a decrease in grand mean food consumption of 43.8% and 16.7% over the course of the study, respectively ([Table 9](#)). Large reductions in food consumption values occurred in the B200M group on Study Day 8 (81.6 %) and in the E200M group on Study Day 15 (35.6%) and indicated a lack of palatability of these groups for the dosed feed. The NT200F and B80F dose groups showed a decrease in grand mean food consumption of 29.5 and 18.2% over the course of the study. A large reduction in food consumption values occurred in the B200F group on Study Day 11 (42%) indicating a lack of palatability for the dosed feed. The few surviving mice in the E200F dose groups showed a decrease in grand food consumption of

25.0% over the course of the study. The B2F, B20F, and B80F dose groups showed a general dose-related decrease in food consumption of 9.1, 6.8, and 18.2% respectively. A similar trend occurred in the E80F and E200F dose groups (-11.4% and -25.0%, respectively).

4.5 Neurobehavioral Evaluation

The neurobehavioral effects narrative is provided in [Appendix G](#). Only data for the CM and CF groups; the NT200M and NT200F groups; the three TB male and female dose groups; and the three male and four female TE dose groups were available for evaluation (all male and female animals in the B200M/B200F groups and all male animals in the E200M group had died prior to FOB testing). Neurobehavioral testing revealed three findings in male mice that were considered to be treatment-related. The E80M dose group (the highest surviving extract dose group) showed increased home cage arousal (FOB Posture test) similar to that exhibited by the NT200M group. Similarly, the E80M group showed more arousal (less sleeping) than did the B80M group as measured by the FOB Home Cage Lethargy/Arousal test. The mean rectal temperature for the NT200M group (96.3°F) was significantly lower than the temperature of the CM group (101.8°F). Neurobehavioral testing revealed two findings in female mice that were considered to be treatment related. The E200F dose group exhibited an increased resistance to removal from the home cage relative to the CF group during the FOB Home Cage Ease of Removing test. The E2F group showed a decreased palpebral reflex sensitivity (sluggish closing of the eyelids) when compared to the response of the B2F group.

4.6 Toxicokinetics

The narrative for the toxicokinetic study is provided in [Appendix H](#). Phase 1 studies were conducted to determine the appropriate time to sample plasma for nicotine and cotinine from mice fed nicotine containing test substances in the diet. The T_{\max} was determined by selecting the most common Days 14 and 15 T_{\max} for nicotine and cotinine among male and female mice of all dose groups. There was high variability in the group mean C_{\max} determinations resulting in many dose groups with multiple timepoints that could be considered as potential T_{\max} values. The 10:00 AM time point was selected as it occurred with the highest frequency among the male and female dose groups. The 10:00 AM time

point also occurred with the highest frequency among the dose groups that had only one discernable T_{\max} value.

Evaluation of C_{\max} values for nicotine and cotinine on Study Day 28 revealed both gender- and dose-dependent effects but not formulation-dependent effects for the TB and TE groups. For the 80 and 200 mg/kg dose groups, male mice had higher C_{\max} values than the female mice. For the 2 and 20 mg/kg dose groups, there was no gender effect on nicotine or cotinine with either formulation. The C_{\max} values increased proportionally with an increase in dose for both the TE and TB groups from 2 to 20 mg/kg for nicotine and cotinine. Male mice had a greater than proportional increase in nicotine and cotinine C_{\max} when increasing from 20 to 80 mg/kg for both the TB and TE. Female mice had a greater than dose proportional increase in nicotine and cotinine C_{\max} for the 80 and 200 mg/kg TE groups, but the change from 20 to 80 mg/kg for the TB was slightly less than dose proportional. Among groups that could be compared, there were no formulation effects as tobacco extract and tobacco blends with the same nicotine dose had similar C_{\max} values for males and females.

4.7 Clinical Pathology

Group mean hematology data are presented in [Table 11](#) for male mice and in [Table 12](#) for female mice. Decreased hemoglobin in the NT200M, E80M and the E200F groups of 6, 3, and 4%, respectively, in conjunction with increased reticulocyte counts, were of uncertain relationship to treatment. The decrease in hemoglobin did not appear to be due to reduced red blood cell numbers, because red blood cell counts were not similarly affected, but rather were due to red blood cells being smaller and containing less hemoglobin, as indicated by the decreased mean corpuscular volume and mean corpuscular hemoglobin values found in these three groups, when compared to those of the controls. Statistical analysis showed that the mean corpuscular hemoglobin concentration result of the B20M was greater than that of the E20M group. However, both groups had adequate hemoglobin and red cell numbers, and their mean corpuscular hemoglobin concentration results, though different, were both within expected ranges for this parameter in CD-1 mice, so the statistical difference was interpreted to be spurious.

Group mean absolute white blood cell differential count data are included in [Table 13](#) for male mice and [Table 14](#) for female mice. The NT200M, E80M, and E200F groups had decreased total white cell counts and lymphocyte counts, when compared with results in same-sex controls. The decreases in total white cell count in the NT200M, B20M, B80M, E2M, E80M and the E200F groups amounted to 32, 34, 33, 45, 43 and 44%, respectively, when compared with same-sex controls. Similar comparisons of total lymphocyte count results in these six groups to control-group results showed decreases of 53, 16, 25, 43, 42, and 57%, respectively. Mean neutrophil count results were not decreased in the NT200M or NT200F groups, but were decreased in the other four referenced groups of males, relative to same-sex control results, by 48, 43, 49, and 53%, respectively. These decreased total white cell, lymphocyte, and neutrophil count results were treatment related, were of similar severity in all affected groups, and were interpreted to be stress-mediated. It was also not clear that the statistical difference between the neutrophil count results of the B80M group and control males and the lack of statistical difference from controls for the decreases in neutrophil counts in the B20M, E2M and E80M indicated any real treatment difference, since all these groups had what were considered to be similar stress-induced altered neutrophil (and other white cell) counts. Similarly, the statistical significance attached to the comparison of the relative decrease in lymphocytes of the E2M group when compared to the B2M group was not interpreted to be definite evidence of a greater effect on lymphocytes by the extract versus the blend. No other hematology results indicated any effects due to treatment.

Group mean serum chemistry parameters are summarized in [Table 15](#) for male mice and [Table 16](#) for female mice. Increased group mean aspartate aminotransferase and cholesterol levels in the NT200 males and females were sporadic and of uncertain relationship to treatment. Not all animals in these groups exhibited high aspartate aminotransferase levels, but NT200 male 207 and females 255 and 257 had the three highest aspartate aminotransferase results found in the study. Statistical comparisons across groups revealed a greater decrease in aspartate aminotransferase in the E2M group compared to control (26%) than the B2M group compared to control (5%), but overall the results from these groups were similar and did not indicate major release of this enzyme to serum from the liver. Similarly, a statistical indication of higher glucose in the E20M as compared to the B20M group was considered to

be spurious because both groups had glucose levels within expected ranges of variation for CD-1 mice. The relatively decreased globulin and increased albumin/globulin ratios of the E20M and E2F groups in comparison to other groups was also considered to be of little biological importance, because of the small size of the differences noted between groups in comparison to variation expected in globulin and albumin/globulin ratio in CD-1 mice. The statistically significant increase in alkaline phosphatase of the NT200 and E200F groups also were not interpreted to be indicative of treatment effects on these groups, because the alkaline phosphatase results of all treated and control mice of this study were within expected ranges of variation for untreated CD-1 mice. No other clinical chemistry results indicated any effects due to treatment.

4.8 Organ Weights

Group mean absolute organ weights are included in [Table 17](#) for male mice and [Table 18](#) for female mice. The absolute brain weights of the NT200M and NT200F groups and those of the E200F group were decreased (by 6, 10, and 8%, respectively) and were statistically different from the same-sex controls. Decreased absolute brain weights of 4% that were not statistically significant, compared with control, were found in the B80M group and the E80M group; these decreases were interpreted to be due to treatment. The absolute epididymis weights of the NT200M group were decreased by 35% and statistically different from those of the control males. Also noted with statistical significance were decreased group mean absolute heart weights, compared with same-sex controls, in the NT200M and NT200F groups, of 27 and 25%, respectively, and in the B80M group of 21%. Though not statistically significant, decreased mean heart weight in the E200F group of 17% was interpreted to be due to treatment. The absolute renal weights in the NT200M and NT200F groups were decreased by 32 and 27%, respectively, and the same parameter in the E200F group was decreased by 19%. Decreased mean absolute hepatic weight in the NT200M group of 22% was statistically significant. Though not statistically significant, decreased mean hepatic weight in the NT200F group and the E200F group of 13 and 15%, respectively, were interpreted to be due to treatment. Decreased absolute salivary gland weights in the NT200M and NT200F groups and the E80M group of 35, 29, and 17%, respectively, were statistically significant. Though not statistically significant, decreased mean absolute salivary gland weight in the E80F and

E200F groups of 9 and 12%, respectively, were interpreted to be due to treatment. Decreased group mean absolute splenic weights of 51 and 28%, respectively, compared with control, were statistically significant in the NT200M and NT200F groups, and a 31% decrease in mean absolute splenic weight in the E200F group (not statistically significant) all were interpreted to be due to treatment. Statistically significant decreased group mean absolute testicular weight (36%) was noted in the NT200M group. Decreased mean absolute thymic weight of 47% was noted with statistical significance in the NT200M group. Decreased absolute uterine weight of 53% in the NT200F group was statistically significant when compared with female controls.

Group mean organ weight to body weight values are included in [Table 19](#) for male mice and [Table 20](#) for female mice. Organ-to-body weight ratios reported as percent body weight included statistically significant increased brain/body weight ratios of 28 and 11%, respectively, in the NT200M and NT200F groups, and a 14% increase (not statistically significant) in the E200F group. The terminal body and absolute brain weights of these three groups were decreased due to treatment, when compared with those of control mice, but brain mass was conserved in these mice to a greater degree than that of other organs and body mass generally. When expressed as a portion of body weight, the weights of the epididymides, hearts, kidneys, salivary glands, spleens, testes, thymuses and uteruses of groups listed above as having test article-related decreased absolute weights were for the most part only a little decreased when compared to control, and not statistically different from control. However, these slight decreases, such as decreased heart/terminal body weight ratios in the NT200F group (8%) and the B80M group (13%), decreased group mean salivary gland/terminal body weight ratios in the NT200M and NT200F groups of 15 and 13%, respectively, and in the E80M group of 11%, decreased spleen/terminal body weight ratios in the NT200F group of 13% and in the E200F group of 18%, might indicate organ-specific effects. Also, splenic and thymic weight/terminal body weight ratios in the NT200M group were decreased by 36 and 31%, respectively, with statistical significance, compared with the male controls; and the renal/terminal body weight ratio of the NT200F group was decreased by 10%, with statistical significance, compared with female controls, possibly indicating nicotine-induced effects on these organs over and above generalized failure to grow. The liver/terminal body weight ratios

of the groups with test article-induced decreased absolute hepatic weights were slightly increased over same-sex control means, suggesting possibly a conservation of liver weight in conditions of generally low weight gain, but these differences were not statistically significant.

Group mean organ to brain weight values are included in [Table 21](#) for male mice and in [Table 22](#) for female mice. The organ-to-brain weight results served to highlight absolute organ weight decreases found in the NT200M and NT200F groups, but decreases in organ-to-brain weight ratio for some of these organs were also found in the E200F group, the E80M group and the B80M group. The toxicologic relevance of statistically significant increased liver/brain weight ratio (15%) in the B2F group was interpreted to be questionable, since similar increases were not seen in the groups fed higher amounts of tobacco blend.

4.9 Gross Lesions

The gross findings of this study were all considered to be incidental and unrelated to the test articles. The small thymus in NT200F 258 was probably related to stress. Gross examination did not reveal findings useful for establishing the cause of death/moribund condition in unscheduled-death mice, but observed clinical signs were consistent with tobacco/nicotine toxicity.

5.0 DISCUSSION

More lethality occurred in high dose (200 mg/kg) mice exposed to TE and TB than mice exposed to a comparable dose of NT. Male mice generally appeared to be more susceptible than female mice at comparable dose levels.

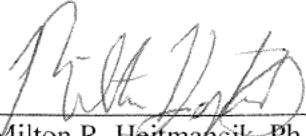
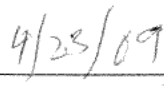
Exposure of the core study mice to the high doses of the test articles produced mortality in all mice that comprised the B200M, B200F, and E200M groups, 6/10 in the E200F group, and 4/10 in the NT200M. The high dose 200 mg/kg groups exhibited a decrease in food consumption, a decrease in body weight gain, and showed clinical signs of toxicity indicative of inanition and stress indicating these doses would be too high for 90-day study due to a lack of palatability for the dosed feed.

Neurobehavioral testing revealed more subtle changes at the lower dose levels. The E80M dose group showed increased home cage arousal (similar to the NT200M group on the FOB posture test); more arousal and less sleeping (than the B80M group on the FOB Home Cage Lethargy/Arousal test); and decrease in rectal temperature (which was significantly lower than that of the CM group). The increased arousal and emotionality are considered to be minimal neurobehavioral effects which can be attributed to the nicotine content in the TE diet. This finding was most evident in the FOB homecage observation tests, when treated mice were posturally erect while many CM/CF animals were supine or sleeping. Female mice also showed increased resistance to removal from the home cage when compared to the CF group. In female mice, there was a significant decrease in palpebral reflex responsivity in the E2F group (as compared to the B2F group). Indeed, all three of the tobacco extract groups exhibited numerically decreased palpebral reflex responsivity as compared to the comparable tobacco blend groups. This result would appear counterintuitive, as nicotine is known to generally increase reflexive responses ([Ikemoto, 2007](#)). However, Hall and Reit (1966) have reported that intraventricular injections in cats of nicotine induced persistent narrowing of the palpebral fissures (distance between the upper and lower eyelids), possibly interfering with the integrity of the palpebral reflex response. The NT200M group showed a reduction in mean rectal temperature relative to the CM group and the mean rectal temperatures of the B80M, E20M, and E80M groups were numerically lower than the CM group. This effect can be attributed to

the hypothemic effect of nicotine in rodents (Ruskin *et al.*, 2007) and may be related to a slight modification of neurotransmitter systems (e.g. serotonin) (Knapp *et al.*, 2000).

Mice exposed to 80 mg/kg TB or TE exhibited more moderate effects. The B80M and E80M groups both showed slight (~10%) depressions in group mean body weight relative to control group with decreases in food consumption of 16.7 and 8.3%, respectively. The B80F and E80F groups showed group mean body weights that were -1.2% and +0.8% of the CF group value. Food consumption values of the B80F and E80F were depressed 18.2 and 11.4%, respectively, relative to that of the CF group. Clinical pathology findings (NT200M, E80M, and E200F) revealed subtle changes including decreased hemoglobin and decreased total white cell counts and lymphocyte counts which were similar in magnitude in all affected groups and attributed to stress. Changes in clinical chemistry parameters were sporadic and of little biological significance. Changes in absolute organ weights and in the organ to body weight and brain weight values were attributed to decreases in body weight rather than to any specific direct organ toxicity due the absence of findings at necropsy. Consequently, doses of 6, 60, and 120 mg/kg are recommended as the exposure levels for the 90-day study.

6.0 REQUIRED SIGNATURES

Milton R. Hejtmancik, Ph.D. Date
Diplomate, A.B.T.
Study Director

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Ruskin D.N., Anand R., LaHoste G.J. (2007). Menthol and nicotine oppositely modulate body temperature in the rat. *Eur J Pharmacol.* 559:161-4.

7.0 SPECIMEN STORAGE AND RECORD ARCHIVES

The pertinent study records were maintained according to SOPs. The Battelle study records and final report were maintained under the direction of Battelle.

The final report, study files, records, wet tissues, clinical pathology slides, and archival samples will be maintained for a period of no less than one year after issuance of the final report. After one year, the Sponsor will provide authorization concerning the disposition of those items.

8.0 ACKNOWLEDGMENTS

Participant	Role
Milton R. Hejtmancik, Ph.D., D.A.B.T.	Study Director
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Daphne V. Vasconcelos, D.V.M., Ph.D., D.A.B.T., Diplomate, A.C.V.P.	Manager Pathology
Brian Burback, Ph.D.	Chemist
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Kevin Carrico, B.A.	Dose Formulations
Seth T. Gibbs, Ph.D.	Toxicokineticist
Jerry D. Johnson, Ph.D., D.A.B.T.	Toxicokineticist
Michael A. Hawk, M.S., D.A.B.T.	Safety Pharmacologist
Glenn D. Ritchie, Ph.D.	Neurobehavioral Scientist

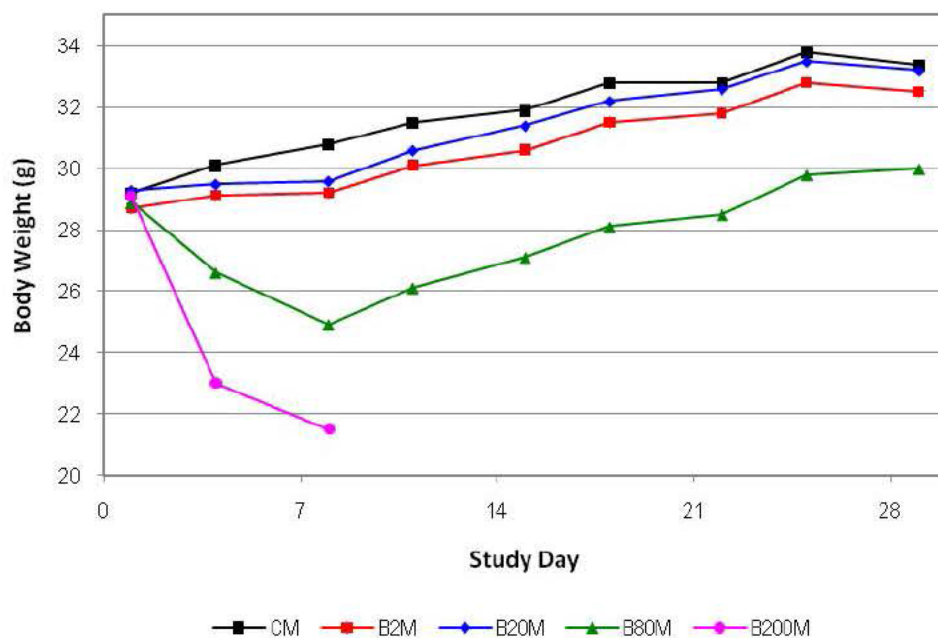


Figure 1. Group Mean Body Weight (g) Tobacco Blend – Males

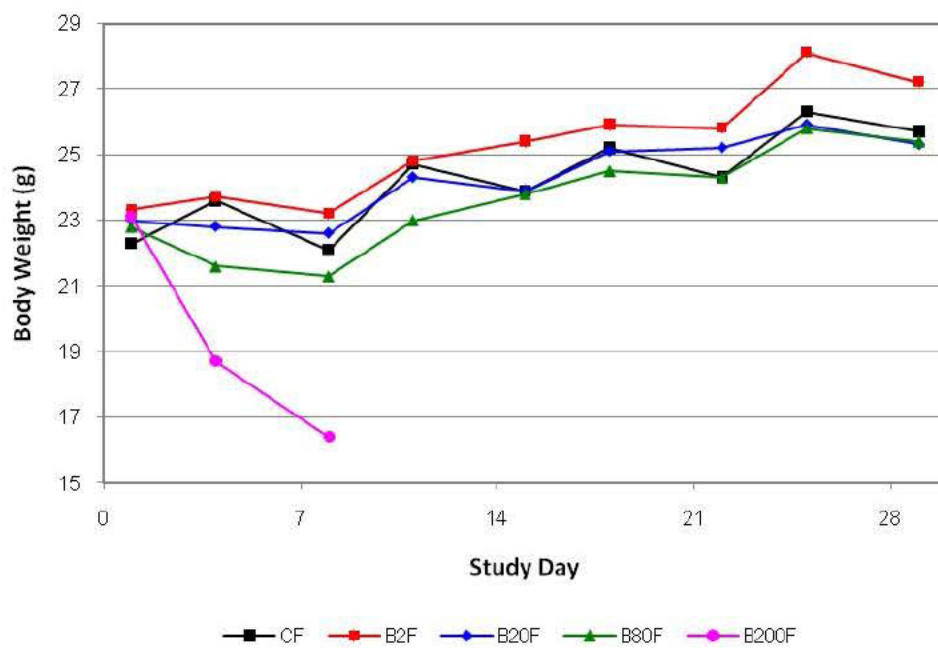


Figure 2. Group Mean Body Weight (g) Tobacco Blend – Females

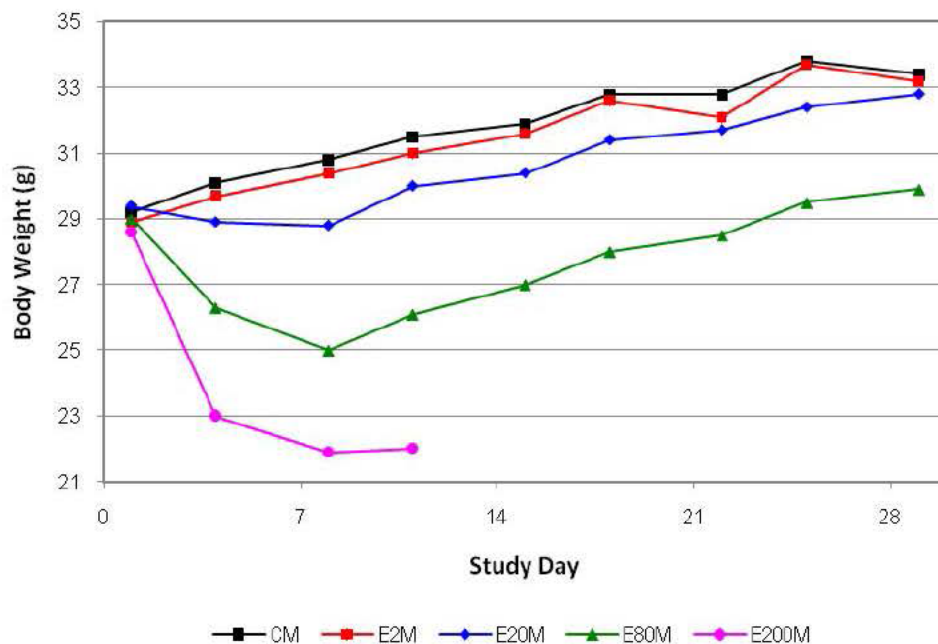


Figure 3. Group Mean Body Weights (g) Tobacco Extract – Males

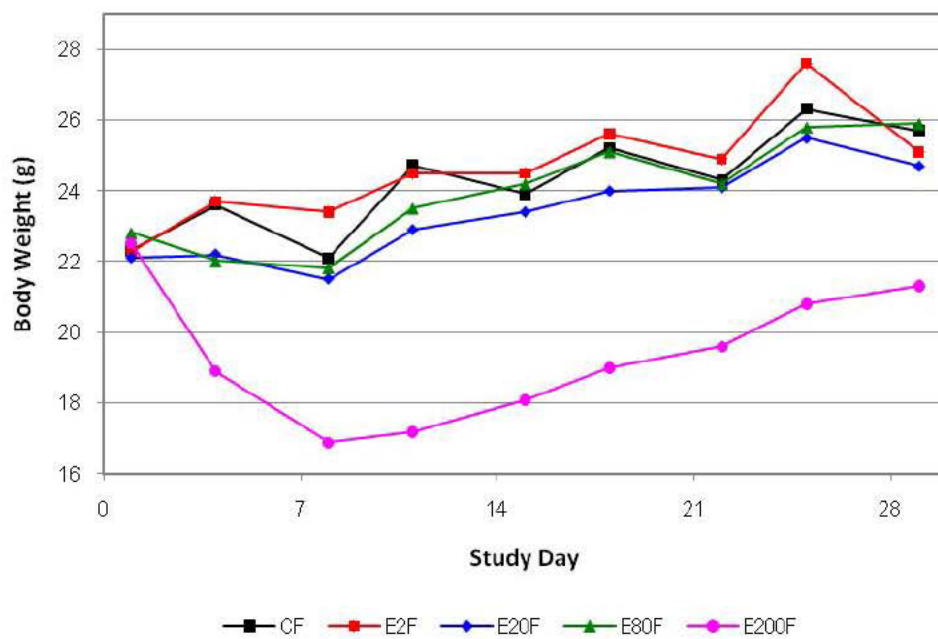


Figure 4. Group Mean Body Weights (g) Tobacco Extract – Females

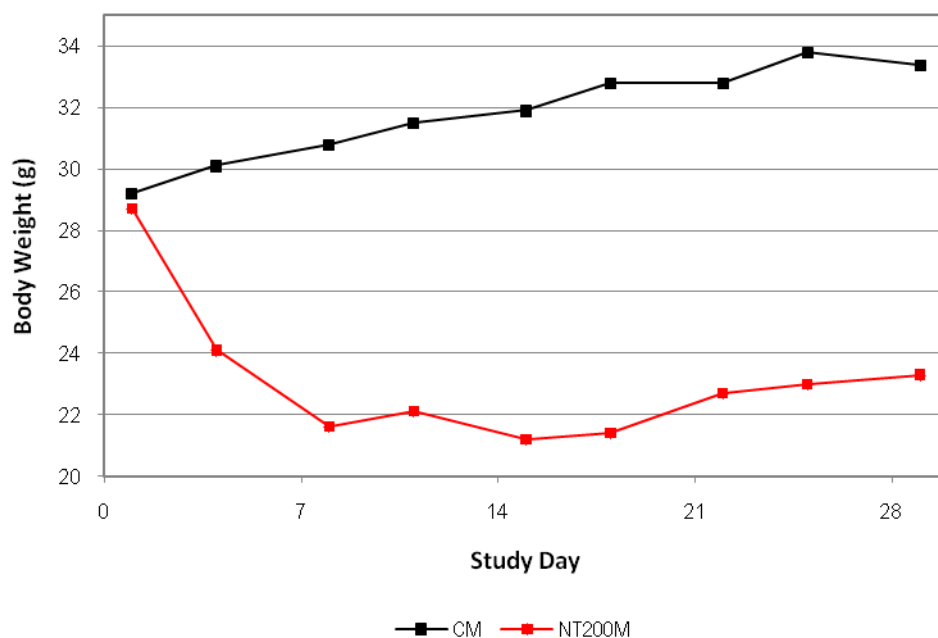


Figure 5. Group Mean Body Weight (g) Nicotine Tartrate – Males

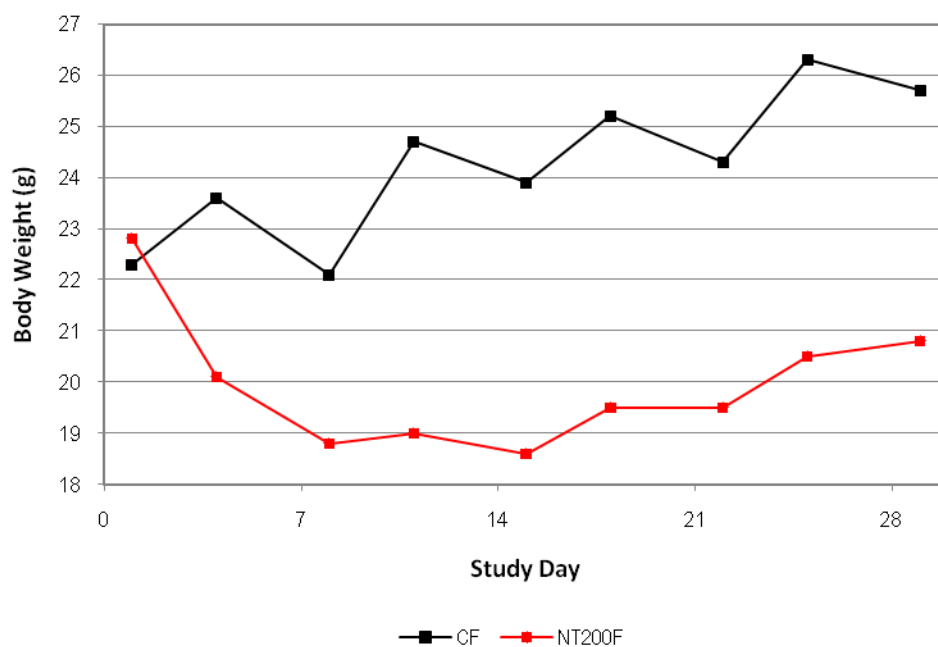


Figure 6. Group Mean Body Weight (g) Nicotine Tartrate – Females

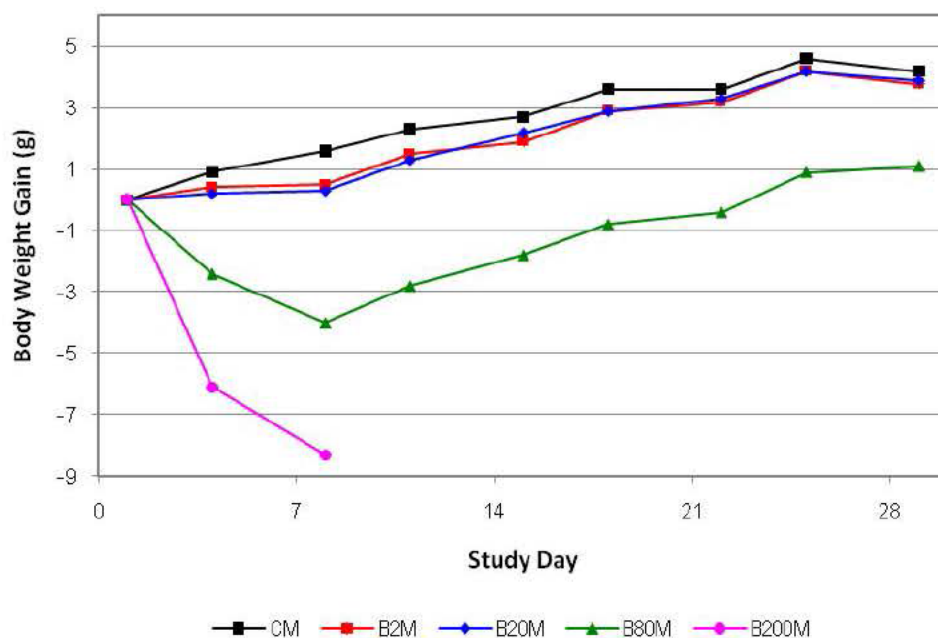


Figure 7. Group Mean Absolute Body Weight Gain (g) Tobacco Blend – Males

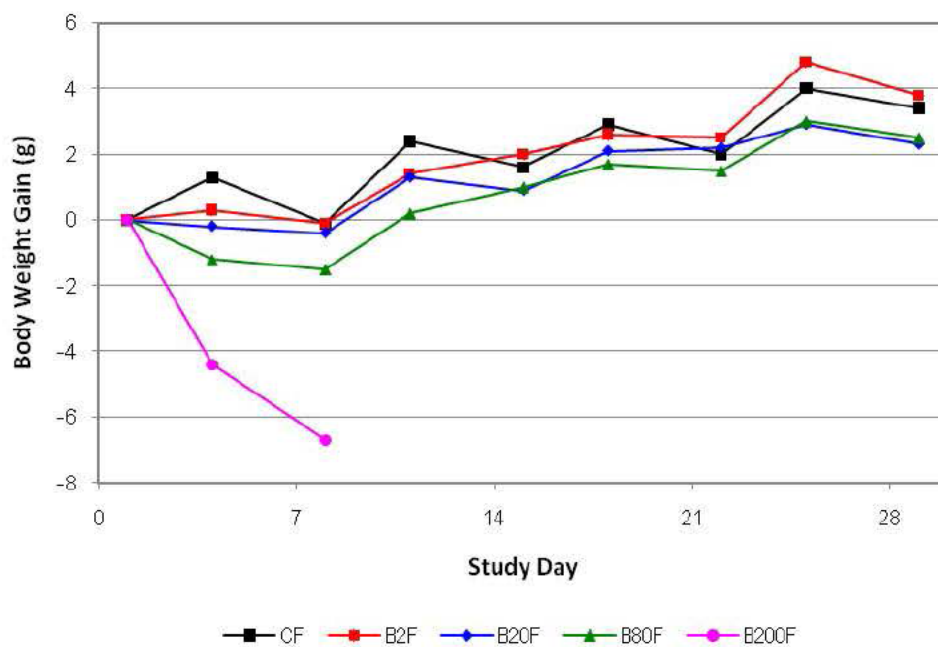


Figure 8. Group Mean Absolute Body Weight Gain (g) Tobacco Blend – Females

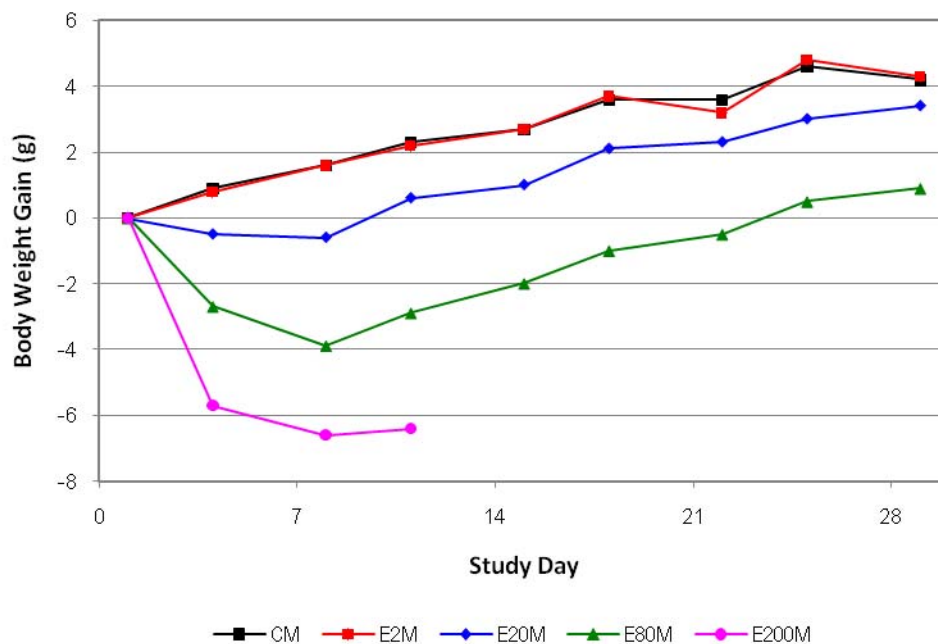


Figure 9. Group Mean Absolute Body Weight Gain (g) Tobacco Extract – Males

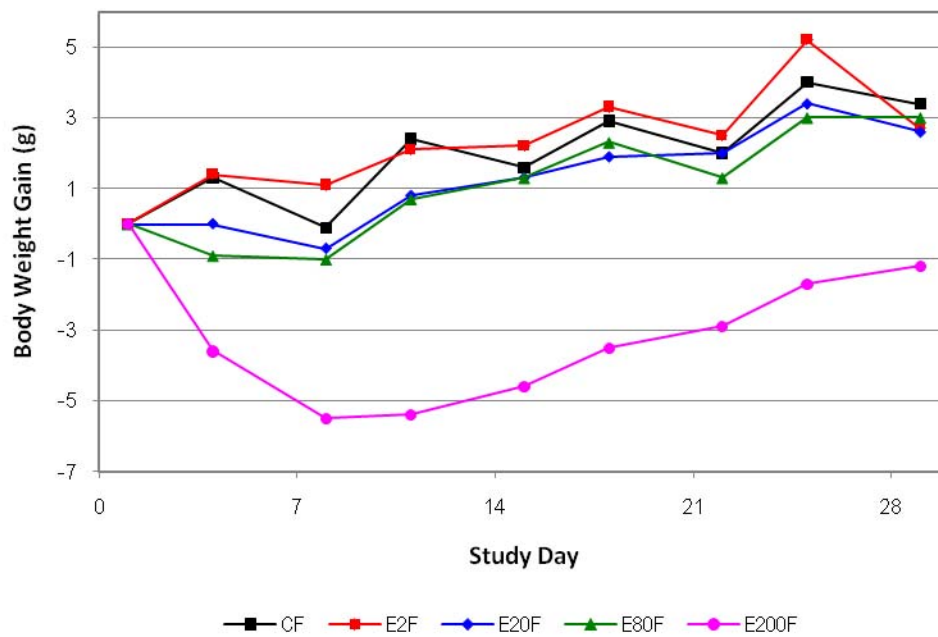


Figure 10. Group Mean Absolute Body Weight Gain (g) Tobacco Extract – Females

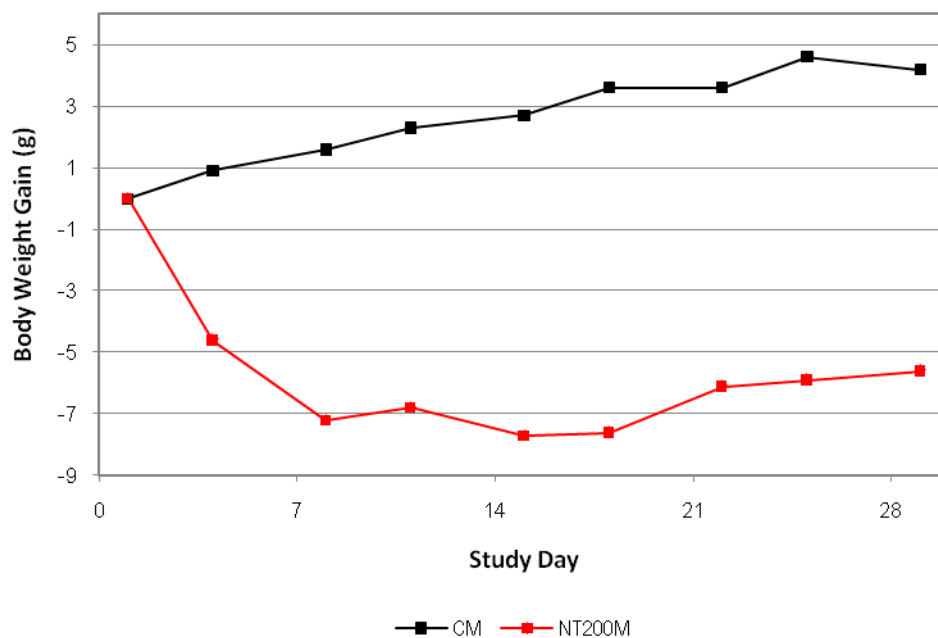


Figure 11. Group Mean Absolute Body Weight Gain (g) Nicotine Tartrate - Males

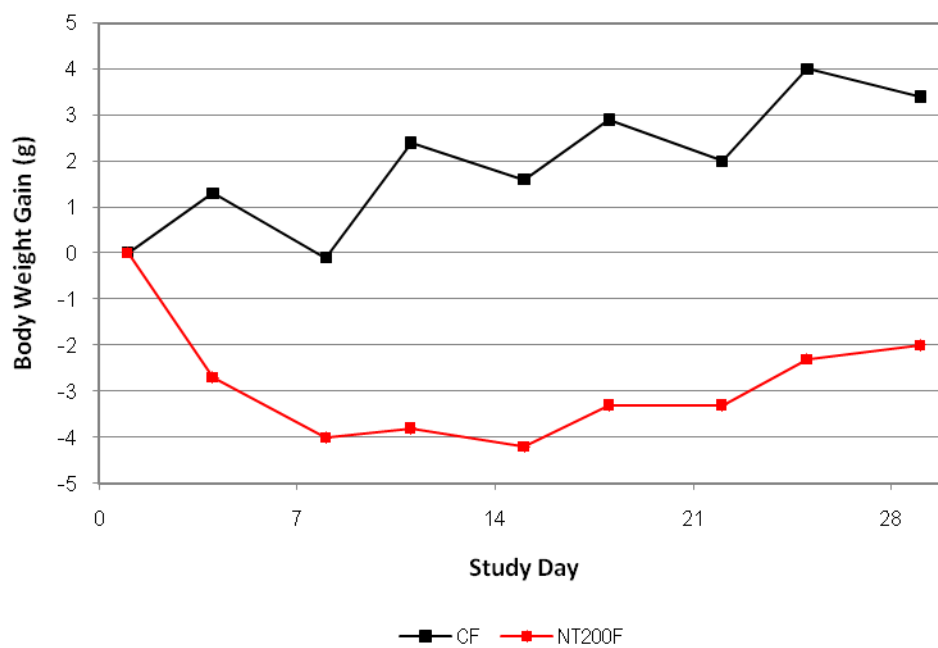


Figure 12. Group Mean Absolute Body Weight Gain (g) Nicotine Tartrate – Females

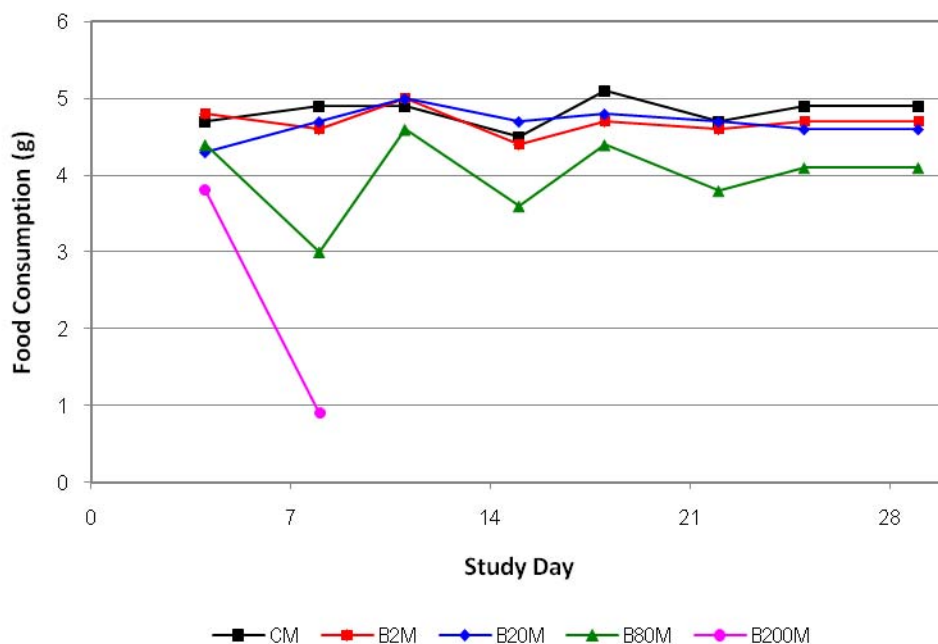


Figure 13. Group Mean Food Consumption (g) Tobacco Blend – Males

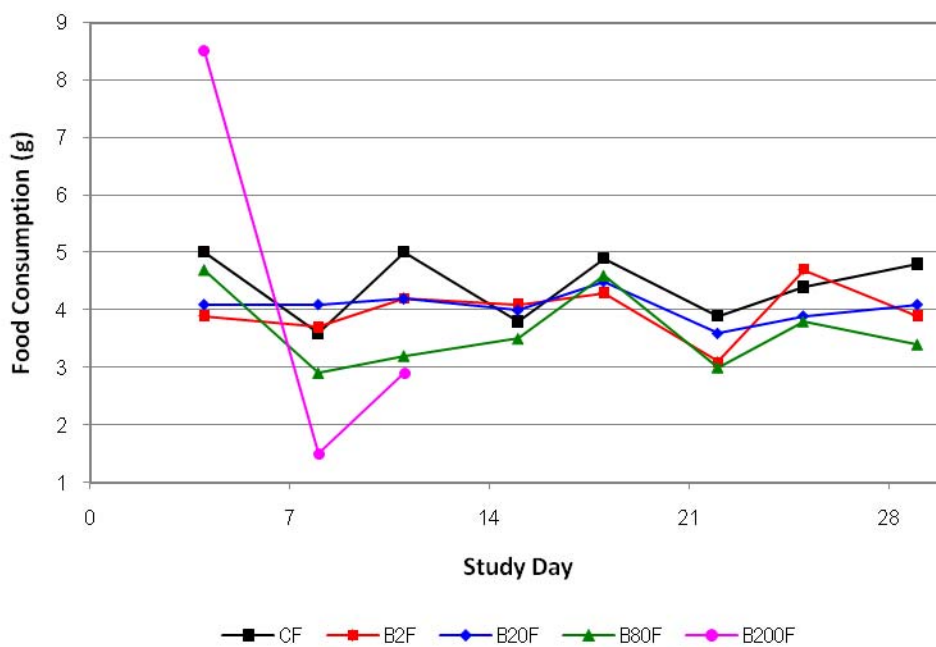


Figure 14. Group Mean Food Consumption (g) Tobacco Blend – Females

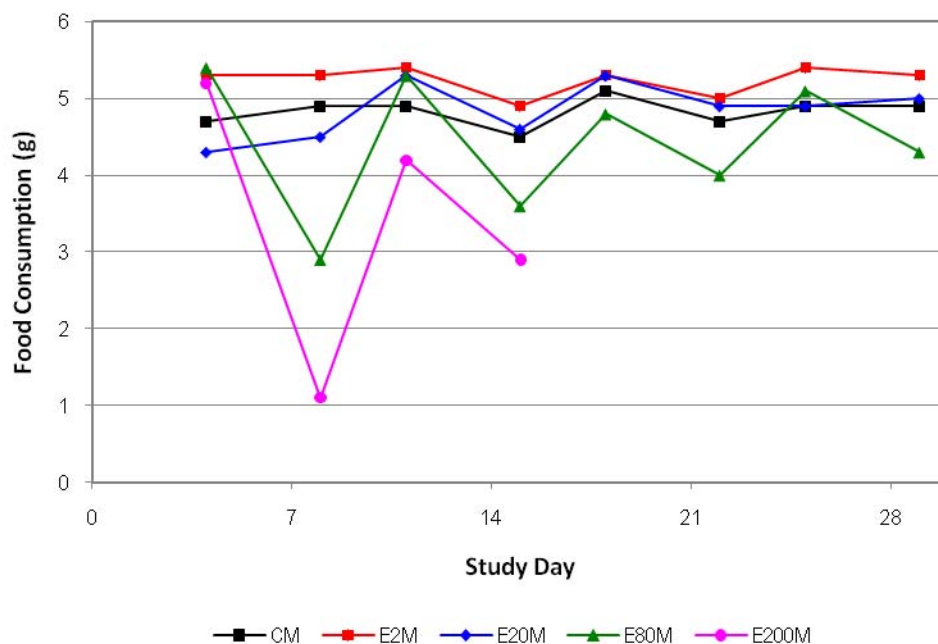


Figure 15. Group Mean Food Consumption (g) Tobacco Extract – Males

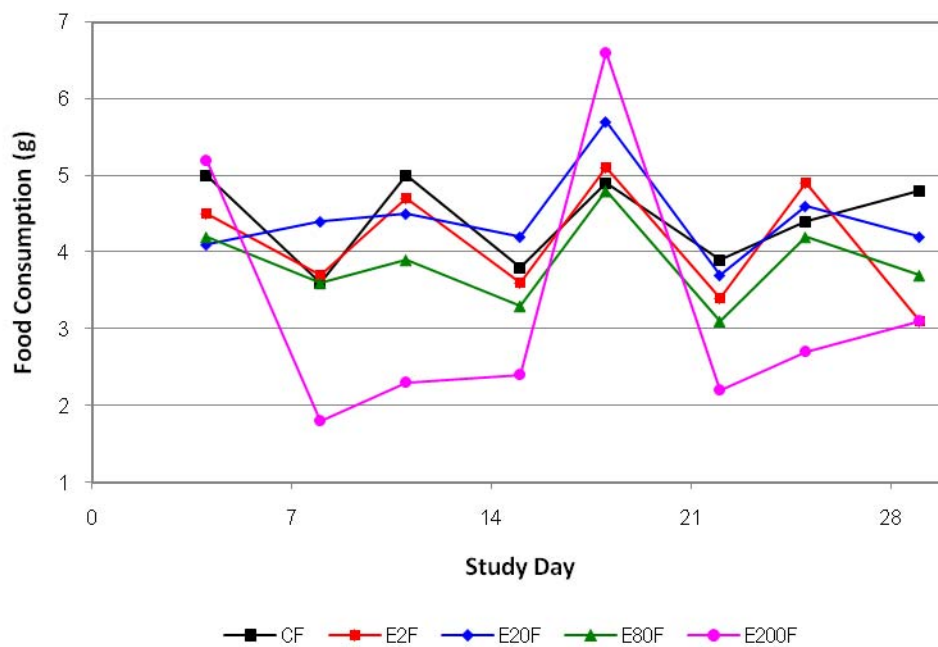


Figure 16. Group Mean Food Consumption (g) Tobacco Extract – Females

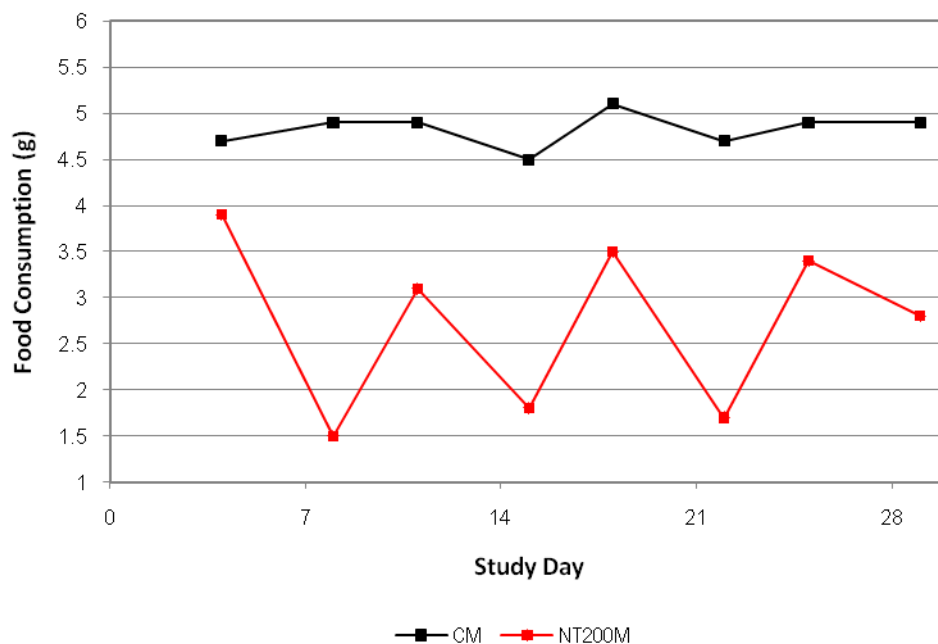


Figure 17. Group Mean Food Consumption (g) Nicotine Tartrate – Males

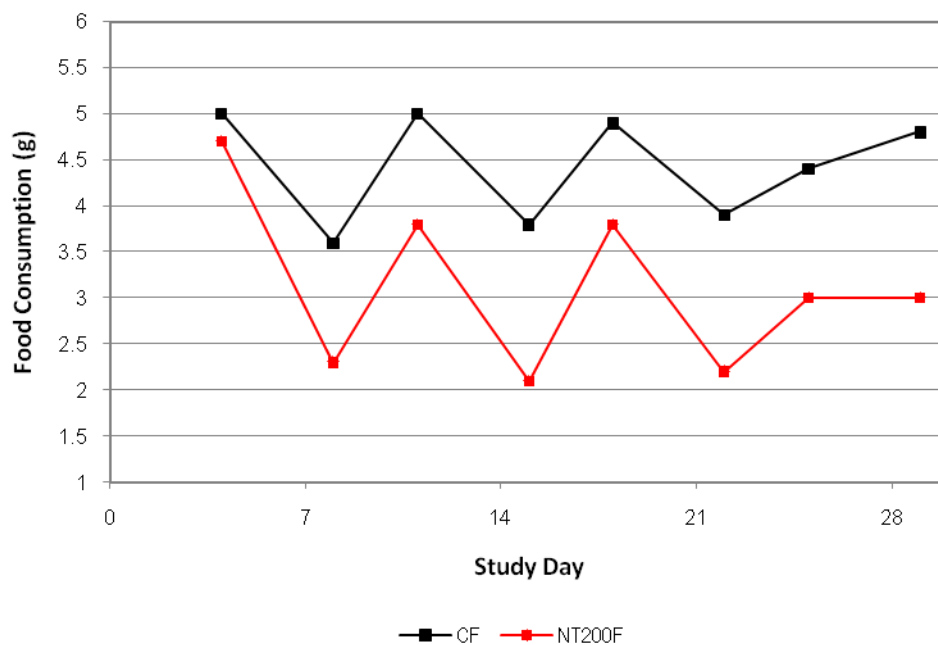


Figure 18. Group Mean Food Consumption (g) Nicotine Tartrate – Females

[illegible][illegible]

Table 2. Mortality Summary – TK Animals

Type of Sacrifice	Female Group									
	NT200F	B2F	B20F	B80F	B200F	E2F	E20F	E80F	E200F	CF
TERMINAL BLEED	23	23	23	23	--	23	23	23	23	5
REMOVED FROM STUDY	--	--	--	--	23	--	--	--	--	--
Grand Total:	23	23	23	23	23	23	23	23	23	5

Type of Sacrifice	Male Group^a								
	NT200M	B2M	B20M	B80M	E2M	E20M	E80M	E200M	CM
SACRIFICED MORIBUND	4	--	--	--	--	--	--	14	--
TERMINAL BLEED	16	23	23	22	23	23	23	3	5
FOUND DEAD	3	--	--	1	--	--	--	6	--
Grand Total:	23	23	23	23	23	23	23	23	5

a. All B200M group removed from study prior to TK bleed.

Table 3. Group Summary of Clinical Abnormalities – Males

Group	Observation	Animals Affected	Observed		Total Number
			First Day	Last Day	
NT200M	Malocclusion	1	15	22	2
	Hunched Posture	8	8	31	25
	Lethargic	4	8	21	5
	Pale	5	9	31	11
	Rough Coat	9	8	31	29
	Thin Appearance	1	8	8	2
	Labored Respiration	1	9	9	1
B80M	Rough Coat	2	8	29	2
B200M	Hunched Posture	6	7	8	8
	Lethargic	5	7	8	6
	Rough Coat	5	7	8	6
	Thin Appearance	1	8	8	2
	Ataxic (incoordination)	1	6	6	1
	Hindlimb Weakness	1	8	8	2
	Tremors	1	7	7	1
	Labored Respiration	1	6	6	1
E2M	Rough Coat	1	29	30	2
E80M	Rough Coat	2	29	29	2
E200M	Hunched Posture	6	7	14	10
	Lethargic	3	7	10	5
	Pale	3	14	14	3
	Rough Coat	6	7	14	9
	Thin Appearance	2	8	10	4
	Tremors	1	7	7	1
	Labored Respiration	1	10	10	1

Table 4. Group Summary of Clinical Abnormalities – Females

Group	Observation	Animals Affected	Observed		Total Number
			First Day	Last Day	
CF	Thin Appearance	1	31	31	1
NT200F	Hunched Posture	5	15	32	16
	Pale	1	22	32	3
	Rough Coat	4	15	32	12
	Thin Appearance	4	15	32	14
	Microphthalmia	2	10	10	2
B200F	Hunched Posture	10	8	11	20
	Lethargic	1	10	10	1
	Pale	5	10	11	5
	Rough Coat	9	8	11	15
	Thin Appearance	9	8	11	16
	Hindlimb Weakness	1	11	11	1
	Labored Respiration	2	10	10	2
	Hunched Posture	7	8	32	12
E200F	Lethargic	1	12	12	1
	Pale	4	10	16	5
	Rough Coat	6	8	32	10
	Thin Appearance	6	8	22	13
	Tremors	2	10	16	2
	Labored Respiration	2	10	10	2

Table 5. Group Mean Absolute Body Weight (g) Data – Males

[illegible]

Table 5. Group Mean Absolute Body Weight (g) Data – Males (Continued)

Group		Day								
		1	4	8	11	15	18	22	25	29
E80M	Mean	29.0	26.3 ^B	25.0 ^B	26.1 ^b	27.0 ^B	28.0 ^B	28.5 ^B	29.5 ^B	29.9 ^B
	SD	1.0	1.1	1.0	0.8	1.5	1.5	1.4	1.7	2.1
	N	10	10	10	10	10	10	10	10	10
E200M	Mean	28.6	23.0 ^B	21.9 ^B	22.0 ^b					
	SD	1.2	1.6	2.6	0.2					
	N	10	10	5	3					

Multiple comparisons were made according to the letters listed below. Capital letters indicate the comparison was significantly different at $p \leq 0.05$ with Dunnett's test of significance; lower case letters indicate comparisons were significantly different at $p \leq 0.05$ with Modified T test.

A = CM vs. NT200M

B = CM vs. B2M, B20M, B80M, B200M, E2M, E20M, E80M, E200M

C = NT200M vs. B200M, E200M

D = B2M vs. E2M

E = B20M vs. E20M

F = B80M vs. E80M

G = B200M vs. E200M

Table 6. Group Mean Absolute Body Weight (g) Data – Females

[illegible]

Table 6. Group Mean Absolute Body Weight (g) Data – Females (Continued)

Group		Day								
		1	4	8	11	15	18	22	25	29
E80F	Mean	22.8	22.0 ^b	21.8	23.5	24.2	25.1	24.2	25.8	25.9
	SD	0.8	1.2	1.4	1.2	1.6	1.3	1.3	1.3	1.3
	N	10	10	10	10	10	10	10	10	10
E200F	Mean	22.5	18.9 ^b	16.9 ^{B,C}	17.2 ^B	18.1 ^b	19.0 ^b	19.6 ^b	20.8 ^b	21.3
	SD	1.4	2.1	1.8	2.2	1.5	2.3	2.5	2.5	2.8
	N	10	10	10	8	5	4	4	4	4

Multiple comparisons were made according to the letters listed below. Capital letters indicate the comparison was significantly different at $p \leq 0.05$ with Dunnett's test of significance; lower case letters indicate comparisons were significantly different at $p \leq 0.05$ with Modified T test.

A = CF vs. NT200F

B = CF vs. B2F, B20F, B80F, B200F, E2F, E20F, E80F, E200F

C = NT200F vs. B200F, E200F

D = B2F vs. E2F

E = B20F vs. E20F

F = B80F vs. E80F

G = B200F vs. E200F

Table 7. TK Group Mean Absolute Body Weight (g) Data – Males*

Group		Day					
		1	8	14**	15	22	28
CM	Mean	34.2	35.3		36.0	37.7	38.8
	SD	1.0	1.4		1.4	1.3	1.3
	N	5	5		5	5	5
NT200M	Mean	30.5 ^A	23.4 ^A	22.4			
	SD	2.0	2.0	2.3			
	N	23	23	16			
B2M	Mean	30.3 ^B	31.4 ^B	31.5	33.3	34.2	34.9
	SD	1.9	2.4	2.2	2.7	2.5	2.5
	N	23	23	18	5	5	5
B20M	Mean	30.5 ^B	30.0 ^B	30.8	31.3 ^B	32.6 ^B	33.7 ^B
	SD	1.9	2.7	2.3	2.8	2.8	2.2
	N	23	23	18	5	5	5
B80M	Mean	30.6 ^B	25.5 ^B	25.7	25.2 ^B	27.9 ^B	29.7 ^B
	SD	1.5	1.5	2.1	1.8	1.3	1.6
	N	23	23	18	5	4	4
E2M	Mean	30.5 ^B	31.1 ^B	32.5	33.4	34.1	35.1
	SD	1.8	1.9	1.9	2.4	2.2	2.0
	N	23	23	18	5	5	5
E20M	Mean	29.6 ^B	29.9 ^B	31.0	30.6 ^B	30.7 ^B	32.6 ^B
	SD	1.6	1.6	1.4	2.4	2.8	3.2
	N	23	23	18	5	5	5

Table 7. TK Group Mean Absolute Body Weight (g) Data – Males* (Continued)

Group		Day					
		1	8	14**	15	22	28
E80M	Mean	29.4 ^{B,F}	26.3 ^B	27.2 ^F	27.5 ^B	29.0 ^B	31.9 ^B
	SD	1.7	2.1	2.4	3.0	2.7	3.9
	N	23	23	18	5	5	5
E200M	Mean	29.9 ^B	22.8 ^B	22.6			
	SD	1.0	1.9	0.9			
	N	23	9	3			

* B200M TK animals not included in Table 7 because they were removed from study prior to dosing based on the mortality and moribundity exhibited in B200M core animals.

** Body weights were collected on Day 14 for TK animals (excluding controls) for potential calculation of plasma nicotine and cotinine concentrations per unit body weight.

Multiple comparisons were made according to the letters listed below. Capital letters indicate the comparison was significantly different at $p \leq 0.05$ with Dunnett's test of significance; lower case letters indicate comparisons were significantly different at $p \leq 0.05$ with Modified T test.

A = CM vs. NT200M

B = CM vs. B2M, B20M, B80M, B200M, E2M, E20M, E80M, E200M

C = NT200M vs. E200M

D = B2M vs. E2M

E = B20M vs. E20M

F = B80M vs. E80M

Table 8. TK Group Mean Absolute Body Weight (g) Data – Females

Group		Day					
		1	8	14*	15	22	28
CF	Mean	26.4	27.7		29.1	29.1	30.8
	SD	1.1	0.7		1.5	1.1	1.1
	N	5	5		5	5	5
NT200F	Mean	24.2 ^A	21.1 ^a	21.0	20.0 ^A	22.8 ^A	23.8 ^A
	SD	1.6	2.5	2.8	1.9	1.5	1.7
	N	23	23	18	5	5	5
B2F	Mean	23.5 ^B	24.7 ^B	25.7	25.9 ^B	26.8	26.6 ^B
	SD	1.3	1.4	1.5	1.3	1.2	1.3
	N	23	23	18	5	5	5
B20F	Mean	23.6 ^B	24.0 ^B	25.5	25.3 ^B	26.8	26.7 ^B
	SD	1.2	1.3	1.2	1.4	1.8	1.8
	N	23	23	18	5	5	5
B80F	Mean	23.5 ^B	23.2 ^B	24.4	25.1 ^B	25.5 ^B	27.0
	SD	1.5	1.5	1.1	1.3	1.2	1.7
	N	23	23	18	5	5	5
B200F	Mean	23.0 ^{B,C}					
	SD	2.2					
	N	23					
E2F	Mean	23.0 ^B	22.5 ^{B,D}	24.2 ^D	25.1 ^B	24.2 ^{B,D}	25.5 ^B
	SD	1.3	1.9	2.1	1.5	1.6	2.9
	N	23	23	18	5	5	5
E20F	Mean	23.8 ^B	23.0 ^{B,E}	25.2	25.5 ^B	25.5 ^B	26.8 ^B
	SD	1.7	1.7	2.2	2.0	1.8	1.9
	N	23	23	18	5	5	5

Table 8. TK Group Mean Absolute Body Weight (g) Data – Females (Continued)

Group		Day					
		1	8	14*	15	22	28
E80F	Mean	23.8 ^B	22.8 ^B	24.2	25.3 ^B	25.0 ^B	26.6 ^B
	SD	1.7	1.6	1.6	1.3	1.0	2.4
	N	23	23	18	5	5	5
E200F	Mean	23.2 ^B	19.7 ^{B,C}	19.5	18.2 ^B	20.5 ^B	22.6 ^B
	SD	1.4	1.9	2.8	1.3	3.1	3.7
	N	23	23	18	5	5	5

* Body weights were collected on Day 14 for TK animals (excluding controls) for potential calculation of plasma nicotine and cotinine concentrations per unit body weight.

Multiple comparisons were made according to the letters listed below. Capital letters indicate the comparison was significantly different at $p \leq 0.05$ with Dunnett's test of significance; lower case letters indicate comparisons were significantly different at $p \leq 0.05$ with Modified T test.

A = CF vs. NT200F

B = CF vs. B2F, B20F, B80F, B200F, E2F, E20F, E80F, E200F

C = NT200F vs. B200F, E200F

D = B2F vs. E2F

E = B20F vs. E20F

F = B80F vs. E80F

G = B200F vs. E200F

Table 9. Group Mean Average Feed Consumed (g) per Day Data – Males

Group		Day								Grand Mean	Grand SD
		4	8	11	15	18	22	25	29		
CM	Mean	4.7	4.9	4.9	4.5	5.1	4.7	4.9	4.9	4.8	0.2
	SD	0.4	0.4	0.4	0.7	0.3	0.4	0.5	0.7		
	N	10	10	10	10	10	10	10	10		
NT200M	Mean	3.9	1.5 ^A	3.1 ^a	1.8 ^A	3.5	1.7 ^A	3.4 ^a	2.8 ^A	2.7	0.9
	SD	2.5	0.4	1.2	0.5	2.0	0.9	1.2	1.0		
	N	10	10	9	8	8	6	6	6		
B2M	Mean	4.8	4.6	5.0	4.4	4.7	4.6	4.7	4.7	4.7	0.2
	SD	0.7	0.6	0.5	0.5	0.6	0.5	0.2	0.6		
	N	10	10	10	10	10	10	10	10		
B20M	Mean	4.3	4.7	5.0	4.7	4.8	4.7	4.6	4.6	4.7	0.2
	SD	0.8	0.3	0.5	0.4	0.5	0.5	0.6	0.6		
	N	10	10	10	10	10	10	10	10		
B80M	Mean	4.4	3.0 ^b	4.6	3.6 ^B	4.4	3.8 ^B	4.1 ^b	4.1 ^B	4.0	0.5
	SD	1.2	0.6	0.9	0.7	0.6	0.5	0.3	0.5		
	N	10	10	10	10	10	10	10	10		
B200M	Mean	3.8	0.9 ^b							2.4	2.1
	SD	1.9	0.6								
	N	10	9								
E2M	Mean	5.3 ^b	5.3 ^{b,D}	5.4	4.9	5.3	5.0	5.4 ^d	5.3 ^D	5.2	0.2
	SD	0.8	0.5	0.6	0.6	0.7	0.4	0.9	0.5		
	N	10	10	10	10	10	10	10	10		
E20M	Mean	4.3	4.5	5.3	4.6	5.3	4.9	4.9	5.0	4.9	0.4
	SD	0.6	0.8	0.6	0.6	0.9	0.4	0.9	0.5		
	N	10	10	10	10	10	10	10	10		

Table 9. Group Mean Average Feed Consumed (g) per Day Data – Males (Continued)

Group		Day								Grand Mean	Grand SD
		4	8	11	15	18	22	25	29		
E80M	Mean	5.4	2.9 ^b	5.3	3.6 ^B	4.8	4.0 ^B	5.1 ^f	4.3	4.4	0.9
	SD	1.1	1.0	1.2	0.6	0.5	0.5	1.4	0.5		
	N	10	10	10	10	10	10	10	10		
E200M	Mean	5.2	1.1 ^b	4.2	2.9 ^B					3.4	1.8
	SD	1.8	0.8	2.7	1.3						
	N	10	10	5	3						

Multiple comparisons were made according to the letters listed below. Capital letters indicate the comparison was significantly different at $p \leq 0.05$ with Dunnett's test of significance; lower case letters indicate comparisons were significantly different at $p \leq 0.05$ with Modified T test.

A = CM vs. NT200M

B = CM vs. B2M, B20M, B80M, B200M, E2M, E20M, E80M, E200M

C = NT200M vs. B200M, E200M

D = B2M vs. E2M

E = B20M vs. E20M

F = B80M vs. E80M

G = B200M vs. E200M

Table 10. Group Mean Average Feed Consumed (g) per Day Data – Females

Group		Day								Grand Mean	Grand SD
		4	8	11	15	18	22	25	29		
CF	Mean	5.0	3.6	5.0	3.8	4.9	3.9	4.4	4.8	4.4	0.6
	SD	1.6	0.9	0.3	1.0	1.1	0.3	0.9	1.3		
	N	3	3	3	3	3	3	3	3		
NT200F	Mean	4.7	2.3	3.8	2.1	3.8	2.2 ^A	3.0	3.0	3.1	0.9
	SD	0.8	1.3	1.4	1.1	1.9	0.7	1.1	1.0		
	N	3	3	3	3	3	3	3	3		
B2F	Mean	3.9	3.7	4.2	4.1	4.3	3.1	4.7	3.9	4.0	0.5
	SD	0.2	0.8	0.8	0.3	0.5	1.4	0.5	0.3		
	N	3	3	3	3	3	3	3	3		
B20F	Mean	4.1	4.1	4.2	4.0	4.5	3.6	3.9	4.1	4.1	0.3
	SD	1.0	0.7	0.2	0.2	1.3	0.7	0.3	0.4		
	N	3	3	3	3	3	3	3	3		
B80F	Mean	4.7	2.9	3.2 ^B	3.5	4.6	3.0	3.8	3.4	3.6	0.7
	SD	1.1	0.9	0.3	0.3	0.3	0.8	0.0	0.6		
	N	3	3	3	3	3	3	3	3		
B200F	Mean	8.5	1.5 ^B	2.9 ^B						4.3	3.7
	SD	3.8	0.3	0.5							
	N	3	3	3							
E2F	Mean	4.5	3.7	4.7	3.6	5.1	3.4	4.9	3.1	4.1	0.8
	SD	0.7	0.1	0.2	0.4	0.5	0.2	0.9	1.0		
	N	3	3	3	3	3	3	3	3		
E20F	Mean	4.1	4.4	4.5	4.2	5.7	3.7	4.6	4.2	4.4	0.6
	SD	0.7	0.8	0.4	0.3	1.6	0.4	0.5	1.1		
	N	3	3	3	3	3	3	3	3		

Table 10. Group Mean Average Feed Consumed (g) per Day Data – Females (Continued)

Group		Day								Grand Mean	Grand SD
		4	8	11	15	18	22	25	29		
E80F	Mean	4.2	3.6	3.9 ^B	3.3	4.8	3.1	4.2	3.7	3.9	0.5
	SD	0.6	0.4	0.6	0.1	1.4	0.3	0.7	0.6		
	N	3	3	3	3	3	3	3	3		
E200F	Mean	5.2	1.8 ^B	2.3 ^B	2.4 ^B	6.6	2.2	2.7	3.1	3.3	1.7
	SD	0.6	0.5	0.2	0.3	4.4	0.5	0.2	0.7		
	N	3	3	3	3	3	2	2	2		

Multiple comparisons were made according to the letters listed below. Capital letters indicate the comparison was significantly different at $p \leq 0.05$ with Dunnett's test of significance; lower case letters indicate comparisons were significantly different at $p \leq 0.05$ with Modified T test.

A = CF vs. NT200F

B = CF vs. B2F, B20F, B80F, B200F, E2F, E20F, E80F, E200F

C = NT200F vs. B200F, E200F

D = B2F vs. E2F

E = B20F vs. E20F

F = B80F vs. E80F

G = B200F vs. E200F

Table 11. Group Mean Hematology Data – Males

Group		Red Blood Cell Count (10⁶/μL)	Hemoglobin (g/dL)	Hematocrit (%)	Mean Corpuscular Volume (fL)	Mean Corpuscular Hemoglobin (pg)
CM	Mean	10.87	16.3	50.1	46.2	15.0
	SD	0.85	0.9	2.7	1.5	0.5
	N	5	5	5	5	5
NT200M	Mean	10.41	15.4	46.7	44.9	14.8
	SD	0.78	1.7	4.4	1.7	0.6
	N	3	3	3	3	3
B2M	Mean	10.37	16.0	48.0	46.3	15.4
	SD	0.39	0.7	2.3	0.6	0.3
	N	4	4	4	4	4
B20M	Mean	10.50	16.6	48.9	46.7	15.8
	SD	0.56	0.8	1.6	1.7	0.8
	N	5	5	5	5	5
B80M	Mean	10.78	16.2	49.7	46.1	15.0
	SD	0.57	1.2	3.0	1.2	0.6
	N	5	5	5	5	5
E2M	Mean	10.56	16.4	49.4	46.8	15.6
	SD	0.40	0.6	1.9	1.6	0.6
	N	5	5	5	5	5
E20M	Mean	10.71	16.2	49.1	45.8	15.1
	SD	0.57	1.1	3.0	1.0	0.5
	N	5	5	5	5	5
E80M	Mean	10.73	15.8	48.6	45.3	14.8
	SD	0.81	1.0	3.3	0.7	0.3
	N	5	5	5	5	5

Table 11. Group Mean Hematology Data – Males (Continued)

		Mean Corpuscular Hemoglobin Concentration	Platelet Count	Reticulocytes
Group		(g/dL)	($10^3/\mu\text{L}$)	($10^3/\mu\text{L}$)
CM	Mean	32.5	882	358.2
	SD	0.2	335	28.2
	N	5	5	5
NT200M	Mean	33.0	1102	582.8
	SD	0.5	583	266.6
	N	3	3	3
B2M	Mean	33.4	1005	360.0
	SD	0.6	206	57.3
	N	4	4	4
B20M	Mean	33.8 ^B	818	343.1
	SD	0.8	212	22.6
	N	5	5	5
B80M	Mean	32.6	937	410.0
	SD	0.8	222	55.9
	N	5	5	5
E2M	Mean	33.3	942	337.1
	SD	0.8	255	35.4
	N	5	5	5

Table 11. Group Mean Hematology Data – Males (Continued)

Group		Mean Corpuscular Hemoglobin Concentration	Platelet Count	Reticulocytes
		(g/dL)	(10³/μL)	(10³/μL)
E20M	Mean	33.1	1000	357.0
	SD	0.7	92	57.0
	N	5	5	5
E80M	Mean	32.6	770	430.2
	SD	0.6	198	52.0
	N	5	5	5

Multiple comparisons were made according to the letters listed below. Capital letters indicate the comparison was significantly different at $p \leq 0.05$ with Dunnett's test of significance; lower case letters indicate comparisons were significantly different at $p \leq 0.05$ with Modified T test.

A = CM vs. NT200M

B = CM vs. B2M, B20M, B80M, B200M, E2M, E20M, E80M, E200M

C = NT200M vs. B200M, E200M

D = B2M vs. E2M

E = B20M vs. E20M

F = B80M vs. E80M

G = B200M vs. E200M

Table 12. Group Mean Hematology Data – Females

Group		Red Blood Cell Count (10⁶/μL)	Hemoglobin (g/dL)	Hematocrit (%)	Mean Corpuscular Volume (fL)	Mean Corpuscular Hemoglobin (pg)
CF	Mean	10.62	16.4	49.3	46.4	15.4
	SD	0.52	0.9	2.2	0.4	0.4
	N	5	5	5	5	5
NT200F	Mean	10.88	16.6	48.7	44.8	15.2
	SD	0.69	1.4	4.6	3.0	0.8
	N	5	5	5	5	5
B2F	Mean	10.38	16.4	48.6	46.9	15.8
	SD	0.76	1.3	2.3	1.6	0.5
	N	5	5	5	5	5
B20F	Mean	10.14	15.5	47.3	46.7	15.3
	SD	0.66	0.9	2.1	1.3	0.6
	N	5	5	5	5	5
B80F	Mean	10.93	17.3	51.7	47.3	15.8
	SD	0.69	1.4	3.9	1.2	0.4
	N	5	5	5	5	5
E2F	Mean	10.92	17.0	49.9	45.7	15.6
	SD	0.42	0.5	1.3	1.1	0.6
	N	4	4	4	4	4
E20F	Mean	10.63	16.9	49.8	46.9	15.8
	SD	0.85	1.6	3.6	1.0	0.4
	N	5	5	5	5	5
E80F	Mean	10.92	16.9	50.3	46.1	15.5
	SD	0.37	0.6	1.8	1.1	0.5
	N	5	5	5	5	5
E200F	Mean	11.01	15.7	48.8	44.5	14.3
	SD	0.83	0.1	1.0	4.2	1.1
	N	2	2	2	2	2

Table 12. Group Mean Hematology Data – Females (Continued)

		Mean Corpuscular Hemoglobin Concentration (g/dL)	Platelet Count (10³/μL)	Reticulocytes (10³/μL)
CF	Mean	33.3	560	314.6
	SD	0.7	353	62.6
	N	5	5	5
NT200F	Mean	34.1	799	445.3
	SD	0.7	332	204.0
	N	5	5	5
B2F	Mean	33.7	1027	332.8
	SD	1.1	276	113.7
	N	5	5	5
B20F	Mean	32.8	958	339.1
	SD	0.7	299	55.7
	N	5	5	5
B80F	Mean	33.4	742	324.9
	SD	0.3	204	91.1
	N	5	5	5
E2F	Mean	34.2	815	264.3
	SD	0.8	192	109.6
	N	4	4	4
E20F	Mean	33.8	890	364.9
	SD	1.1	366	128.3
	N	5	5	5

Table 12. Group Mean Hematology Data – Females (Continued)

		Mean Corpuscular Hemoglobin Concentration (g/dL)	Platelet Count (10³/μL)	Reticulocytes (10³/μL)
E80F	Mean	33.5	705	432.4
	SD	0.6	230	76.6
	N	5	5	5
E200F	Mean	32.2	1192	651.2
	SD	0.4	35	180.5
	N	2	2	2

Multiple comparisons were made according to the letters listed below. Capital letters indicate the comparison was significantly different at $p \leq 0.05$ with Dunnett's test of significance; lower case letters indicate comparisons were significantly different at $p \leq 0.05$ with Modified T test.

A = CF vs. NT200F

B = CF vs. B2F, B20F, B80F, B200F, E2F, E20F, E80F, E200F

C = NT200F vs. B200F, E200F

D = B2F vs. E2F

E = B20F vs. E20F

F = B80F vs. E80F

G = B200F vs. E200F

Table 13. Group Mean Absolute WBC Differential Count Data – Males

Group		White Blood	Neutrophils	Total	Monocytes	Eosinophils	Basophils
		Cell Count (10 ³ /μL)		Lymphocytes (10 ³ /μL)			
CM	Mean	4.91	1.06	3.73	0.02	0.10	0.01
	SD	1.89	0.58	1.26	0.03	0.15	0.01
	N	5	5	5	5	5	5
NT200M	Mean	3.33	1.57	1.74	0.00	0.02	0.00
	SD	2.36	2.14	2.04	0.01	0.03	0.00
	N	3	3	3	3	3	3
B2M	Mean	4.42	0.74	3.56	0.05	0.07	0.01
	SD	1.50	0.31	1.21	0.04	0.11	0.01
	N	4	4	4	4	4	4
B20M	Mean	3.75	0.55	3.15	0.02	0.04	0.00
	SD	0.63	0.36	0.48	0.02	0.04	0.00
	N	5	5	5	5	5	5
B80M	Mean	3.29	0.39 ^B	2.80	0.02	0.07	0.00
	SD	1.52	0.17	1.30	0.02	0.11	0.00
	N	5	5	5	5	5	5
E2M	Mean	2.72	0.54	2.14 ^D	0.01	0.02	0.00
	SD	0.57	0.25	0.39	0.01	0.02	0.00
	N	5	5	5	5	5	5

Table 13. Group Mean Absolute WBC Differential Count Data – Males (Continued)

Group		White Blood	Neutrophils	Total	Monocytes	Eosinophils	Basophils
		Cell Count (10³/μL)	(10³/μL)	Lymphocytes (10³/μL)	(10³/μL)	(10³/μL)	(10³/μL)
E20M	Mean	4.32	1.01	3.24	0.01	0.06	0.00
	SD	1.30	0.49	0.85	0.02	0.06	0.00
	N	5	5	5	5	5	5
E80M	Mean	2.80	0.50	2.18	0.03	0.08	0.00
	SD	1.15	0.34	0.69	0.06	0.11	0.00
	N	5	5	5	5	5	5

Multiple comparisons were made according to the letters listed below. Capital letters indicate the comparison was significantly different at $p \leq 0.05$ with Dunnett's test of significance; lower case letters indicate comparisons were significantly different at $p \leq 0.05$ with Modified T test.

A = CM vs. NT200M

B = CM vs. B2M, B20M, B80M, B200M, E2M, E20M, E80M, E200M

C = NT200M vs. B200M, E200M

D = B2M vs. E2M

E = B20M vs. E20M

F = B80M vs. E80M

G = B200M vs. E200M

Table 14. Group Mean Absolute WBC Differential Count Data – Females

		White Blood Cell Count (10³/μL)	Neutrophils (10³/μL)	Total Lymphocytes (10³/μL)	Monocytes (10³/μL)	Eosinophils (10³/μL)	Basophils (10³/μL)
Group							
CF	Mean	4.11	0.41	3.54	0.03	0.13	0.00
	SD	1.57	0.15	1.35	0.03	0.15	0.01
	N	5	5	5	5	5	5
NT200F	Mean	3.84	0.96	2.81	0.04	0.04	0.00
	SD	1.24	0.76	0.74	0.04	0.06	0.00
	N	5	5	5	5	5	5
B2F	Mean	4.92	0.81	3.70	0.05	0.35	0.01
	SD	3.37	0.52	2.74	0.06	0.39	0.01
	N	5	5	5	5	5	5
B20F	Mean	4.41	0.72	3.55	0.03	0.11	0.00
	SD	1.06	0.24	0.82	0.02	0.07	0.00
	N	5	5	5	5	5	5
B80F	Mean	4.88	0.73	4.06	0.00	0.08	0.00
	SD	1.98	0.54	1.66	0.01	0.08	0.00
	N	5	5	5	5	5	5
E2F	Mean	3.87	0.69	3.03	0.04	0.12	0.00
	SD	1.13	0.21	0.97	0.01	0.06	0.00
	N	4	4	4	4	4	4
E20F	Mean	4.45	0.78	3.59	0.01	0.07	0.01
	SD	1.87	0.44	1.74	0.01	0.07	0.01
	N	5	5	5	5	5	5

Table 14. Group Mean Absolute WBC Differential Count Data – Females (Continued)

Group		White Blood Cell Count	Neutrophils	Total Lymphocytes	Monocytes	Eosinophils	Basophils
		(10 ³ /μL)	(10 ³ /μL)	(10 ³ /μL)	(10 ³ /μL)	(10 ³ /μL)	(10 ³ /μL)
E80F	Mean	4.59	0.42	4.01	0.04	0.10	0.01
	SD	1.49	0.17	1.33	0.04	0.05	0.01
	N	5	5	5	5	5	5
E200F	Mean	2.29	0.70	1.53	0.02	0.04	0.00
	SD	0.06	0.65	0.63	0.02	0.06	0.00
	N	2	2	2	2	2	2

Multiple comparisons were made according to the letters listed below. Capital letters indicate the comparison was significantly different at $p \leq 0.05$ with Dunnett's test of significance; lower case letters indicate comparisons were significantly different at $p \leq 0.05$ with Modified T test.

A = CF vs. NT200F

B = CF vs. B2F, B20F, B80F, B200F, E2F, E20F, E80F, E200F

C = NT200F vs. B200F, E200F

D = B2F vs. E2F

E = B20F vs. E20F

F = B80F vs. E80F

G = B200F vs. E200F

Table 15. Group Mean Serum Chemistry Data – Males

Group		Alkaline Phosphatase (U/L)	Aspartate Aminotransferase (U/L)	Gamma Glutamyltransferase (U/L)	Total Bilirubin (mg/dL)
CM	Mean	101	73	1	0.15
	SD	33	18	0	0.03
	N	5	5	5	5
NT200M	Mean	121	142	1	0.15
	SD	52	93	1	0.04
	N	2	3	2	3
B2M	Mean	94	69	1	0.16
	SD	17	10	0	0.02
	N	5	5	5	5
B20M	Mean	90	82	1	0.16
	SD	14	35	0	0.02
	N	5	5	5	5
B80M	Mean	141	73	1	0.15
	SD	81	13	0	0.05
	N	5	5	5	5
E2M	Mean	79	54 ^d	1	0.16
	SD	12	3	0	0.02
	N	5	5	5	5
E20M	Mean	83	80	1	0.15
	SD	14	48	0	0.03
	N	5	5	5	5
E80M	Mean	112	72	1	0.16
	SD	28	20	1	0.03
	N	4	5	5	5

Table 15. Group Mean Serum Chemistry Data – Males (Continued)

Group		Direct Bilirubin (mg/dL)	Total Protein (g/dL)	Glucose (mg/dL)	Albumin (g/dL)
CM	Mean	0.05	5.8	108	3.8
	SD	0.01	0.1	24	0.1
	N	5	5	5	5
NT200M	Mean	0.05	5.9	127	4.1
	SD	0.02	0.4	11	0.2
	N	3	2	2	2
B2M	Mean	0.05	6.0	106	3.9
	SD	0.01	0.2	13	0.1
	N	5	5	5	5
B20M	Mean	0.05	5.9	107	4.0
	SD	0.01	0.2	11	0.2
	N	5	5	5	5
B80M	Mean	0.04	5.9	123	3.9
	SD	0.01	0.4	33	0.2
	N	5	5	5	5
E2M	Mean	0.05	5.7	102	3.8
	SD	0.01	0.2	7	0.1
	N	5	5	5	5
E20M	Mean	0.05	5.8	130 ^E	3.9
	SD	0.01	0.2	12	0.1
	N	5	5	5	5
E80M	Mean	0.04	5.8	131	4.0
	SD	0.02	0.2	22	0.2
	N	5	4	4	5

Table 15. Group Mean Serum Chemistry Data – Males (Continued)

Group		Globulin (g/dL)	Albumin/Globulin Ratio	Blood Urea Nitrogen (mg/dL)	Creatinine (mg/dL)
CM	Mean	2.1	1.84	19	0.5
	SD	0.1	0.07	3	0.0
	N	5	5	5	5
NT200M	Mean	1.9	2.35	24	0.5
	SD	0.6	0.92	8	0.1
	N	2	2	2	2
B2M	Mean	2.1	1.92	22	0.4
	SD	0.2	0.19	5	0.1
	N	5	5	5	5
B20M	Mean	2.0	2.02	22	0.4
	SD	0.1	0.10	3	0.1
	N	5	5	5	5
B80M	Mean	1.9	2.04	21	0.5
	SD	0.2	0.16	4	0.1
	N	5	5	5	5
E2M	Mean	1.9	2.01	19	0.5
	SD	0.1	0.15	2	0.1
	N	5	5	5	5
E20M	Mean	2.0	1.98	22	0.5
	SD	0.1	0.11	5	0.0
	N	5	5	5	5
E80M	Mean	1.8 ^B	2.20 ^B	21	0.5
	SD	0.1	0.17	4	0.1
	N	4	4	4	4

Table 15. Group Mean Serum Chemistry Data – Males (Continued)

Group		Triglycerides (mg/dL)	Cholesterol (mg/dL)	Calcium (mg/dL)	Phosphorus (mg/dL)
CM	Mean	72	176	10.0	8.5
	SD	24	28	0.3	0.3
	N	5	5	5	5
NT200M	Mean	94	225	10.1	9.2
	SD	79	73		2.9
	N	2	2	1	2
B2M	Mean	65	172	10.1	8.9
	SD	24	26	0.2	0.8
	N	5	5	5	5
B20M	Mean	61	168	10.2	9.0
	SD	33	43	0.4	0.7
	N	5	5	5	5
B80M	Mean	53	177	9.9	8.6
	SD	12	34	0.4	0.9
	N	5	5	5	5
E2M	Mean	63	166	10.1	9.8
	SD	14	36	0.3	1.3
	N	5	5	5	5
E20M	Mean	70	178	10.1	9.0
	SD	8	31	0.3	0.6
	N	5	5	5	5
E80M	Mean	118	188	9.9	8.1
	SD	123	21	0.2	1.0
	N	4	4	4	4

Table 15. Group Mean Serum Chemistry Data – Males (Continued)

Group		Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)
CM	Mean	154	7.6	114
	SD	3	0.7	4
	N	5	5	5
NT200M	Mean	154	8.4	115
	SD	3	0.8	7
	N	2	2	2
B2M	Mean	155	7.5	112
	SD	3	0.7	3
	N	5	5	5
B20M	Mean	155	7.7	113
	SD	3	0.5	3
	N	5	5	5
B80M	Mean	154	7.5	112
	SD	2	0.5	3
	N	5	5	5
E2M	Mean	154	7.6	112
	SD	3	0.4	3
	N	5	5	5

Table 15. Group Mean Serum Chemistry Data – Males (Continued)

Group		Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)
E20M	Mean	155	7.4	114
	SD	3	0.3	4
	N	5	5	5
E80M	Mean	153	7.3	113
	SD	5	0.3	5
	N	4	4	4

Multiple comparisons were made according to the letters listed below. Capital letters indicate the comparison was significantly different at $p \leq 0.05$ with Dunnett's test of significance; lower case letters indicate comparisons were significantly different at $p \leq 0.05$ with Modified T test.

A = CM vs. NT200M

B = CM vs. B2M, B20M, B80M, B200M, E2M, E20M, E80M, E200M

C = NT200M vs. B200M, E200M

D = B2M vs. E2M

E = B20M vs. E20M

F = B80M vs. E80M

G = B200M vs. E200M

Table 16. Group Mean Serum Chemistry Data – Females

Group		Alkaline Phosphatase (U/L)	Aspartate Aminotransferase (U/L)	Gamma Glutamyltransferase (U/L)	Total Bilirubin (mg/dL)
CF	Mean	94	86	1	0.12
	SD	18	14	0	0.01
	N	5	5	5	5
NT200F	Mean	140 ^A	143	1	0.12
	SD	34	53	1	0.06
	N	4	4	4	5
B2F	Mean	86	86	1	0.10
	SD	7	12	0	0.01
	N	4	5	4	5
B20F	Mean	131	112	1	0.11
	SD	37	27	0	0.02
	N	4	4	5	4
B80F	Mean	129	93	0	0.10
	SD	32	11	1	0.02
	N	5	5	5	5
E2F	Mean	90	94	1	0.12
	SD	21	23	0	0.04
	N	5	5	5	5
E20F	Mean	108	104	1	0.11
	SD	29	30	0	0.03
	N	5	5	5	5
E80F	Mean	99	107	1 ^F	0.11
	SD	16	21	0	0.02
	N	5	5	5	5
E200F	Mean	158	118	1	0.15
	SD	19	9	0	0.00
	N	2	2	2	2

Table 16. Group Mean Serum Chemistry Data – Females (Continued)

Group		Direct Bilirubin (mg/dL)	Total Protein (g/dL)	Glucose (mg/dL)	Albumin (g/dL)
CF	Mean	0.03	6.1	80	4.2
	SD	0.00	0.4	6	0.2
	N	5	4	4	5
NT200F	Mean	0.05	5.5	65	4.0
	SD	0.02	0.8	23	0.6
	N	4	3	4	4
B2F	Mean	0.03	5.9	86	3.9
	SD	0.01	0.3	17	0.3
	N	5	4	4	5
B20F	Mean	0.04	5.9	95	4.2
	SD	0.02	0.4	23	0.3
	N	4	4	4	5
B80F	Mean	0.03	5.6	69	4.0
	SD	0.00	0.2	19	0.2
	N	5	5	5	5
E2F	Mean	0.03	5.8	82	4.2
	SD	0.01	0.4	10	0.3
	N	5	5	5	5
E20F	Mean	0.04	6.0	75	4.2
	SD	0.01	0.4	14	0.3
	N	5	4	5	5
E80F	Mean	0.04	5.8	75	4.1
	SD	0.01	0.1	13	0.1
	N	5	5	5	5
E200F	Mean	0.05	5.6	64	4.1
	SD	0.01	0.5	7	0.4
	N	2	2	2	2

Table 16. Group Mean Serum Chemistry Data – Females (Continued)

Group		Globulin (g/dL)	Albumin/Globulin Ratio	Blood Urea Nitrogen (mg/dL)	Creatinine (mg/dL)
CF	Mean	1.9	2.29	12	0.5
	SD	0.3	0.34	2	0.1
	N	4	4	4	4
NT200F	Mean	1.6	2.50	32	0.5
	SD	0.1	0.39	21	0.1
	N	3	3	3	3
B2F	Mean	1.9	2.07	13	0.5
	SD	0.2	0.29	1	0.1
	N	4	4	4	4
B20F	Mean	1.7	2.50	15	0.5
	SD	0.2	0.16	4	0.1
	N	4	4	4	4
B80F	Mean	1.6	2.46	15	0.4
	SD	0.2	0.31	3	0.1
	N	5	5	5	5
E2F	Mean	1.5 ^D	2.77 ^D	13	0.5
	SD	0.2	0.28	3	0.1
	N	5	5	5	5
E20F	Mean	1.7	2.57	13	0.5
	SD	0.1	0.14	1	0.1
	N	4	4	4	3
E80F	Mean	1.7	2.49	13	0.5
	SD	0.2	0.28	2	0.0
	N	5	5	5	4
E200F	Mean	1.5	2.70	19	0.4
	SD	0.1	0.02	1	0.0
	N	2	2	2	2

Table 16. Group Mean Serum Chemistry Data – Females (Continued)

Group		Triglycerides (mg/dL)	Cholesterol (mg/dL)	Calcium (mg/dL)	Phosphorus (mg/dL)
CF	Mean	54	115	10.2	8.4
	SD	9	17	0.2	0.6
	N	4	4	4	4
NT200F	Mean	42	163 ^A	10.3	9.8
	SD	17	21	0.4	2.0
	N	3	3	3	3
B2F	Mean	49	111	10.1	8.6
	SD	6	19	0.1	1.4
	N	4	4	4	4
B20F	Mean	50	118	10.0	8.3
	SD	12	31	0.2	1.3
	N	4	4	4	4
B80F	Mean	48	107	10.3	9.1
	SD	8	20	0.2	1.6
	N	5	5	5	5
E2F	Mean	45	114	10.2	8.7
	SD	9	14	0.2	0.9
	N	5	5	5	5
E20F	Mean	50	132	10.2	8.1
	SD	15	16	0.2	1.0
	N	5	3	4	4
E80F	Mean	49	114	10.2	8.5
	SD	13	28	0.1	1.2
	N	5	5	5	4
E200F	Mean	63	144	10.0	9.9
	SD	6	4	0.4	1.1
	N	2	2	2	2

Table 16. Group Mean Serum Chemistry Data – Females (Continued)

Group		Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)
CF	Mean	155	7.1	114
	SD	2	0.3	3
	N	4	4	4
NT200F	Mean	154	7.0	117
	SD	3	0.3	1
	N	3	3	3
B2F	Mean	152	7.2	112
	SD	1	0.5	2
	N	4	4	4
B20F	Mean	155	6.9	116
	SD	1	0.3	2
	N	4	4	4
B80F	Mean	155	7.2	114
	SD	2	0.5	2
	N	5	5	5
E2F	Mean	154	6.7	114
	SD	2	0.3	2
	N	5	5	5
E20F	Mean	154	7.1	114
	SD	2	0.1	3
	N	4	4	4

Table 16. Group Mean Serum Chemistry Data – Females (Continued)

Group		Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)
E80F	Mean	155	7.0	115
	SD	2	0.3	1
	N	5	5	5
E200F	Mean	157	7.3	115
	SD	1	0.4	3
	N	2	2	2

Multiple comparisons were made according to the letters listed below. Capital letters indicate the comparison was significantly different at $p \leq 0.05$ with Dunnett's test of significance; lower case letters indicate comparisons were significantly different at $p \leq 0.05$ with Modified T test.

A = CF vs. NT200F

B = CF vs. B2F, B20F, B80F, B200F, E2F, E20F, E80F, E200F

C = NT200F vs. B200F, E200F

D = B2F vs. E2F

E = B20F vs. E20F

F = B80F vs. E80F

G = B200F vs. E200F

Table 17. Group Mean Absolute Organ Weights (g) – Males

Group		Brain	Epididymides	Heart	Kidneys	Liver	Salivary Gland
CM	Mean	0.493	0.1031	0.205	0.496	1.241	0.226
	SD	0.011	0.0176	0.046	0.063	0.118	0.028
	N	10	10	10	10	10	10
NT200M	Mean	0.465 ^a	0.0671 ^A	0.149 ^A	0.335 ^A	0.964 ^A	0.146 ^A
	SD	0.024	0.0182	0.028	0.073	0.229	0.038
	N	6	6	6	6	6	6
B2M	Mean	0.503	0.0970	0.198	0.476	1.212	0.211
	SD	0.025	0.0105	0.022	0.035	0.085	0.023
	N	10	10	10	10	10	10
B20M	Mean	0.481	0.0957	0.192	0.497	1.231	0.218
	SD	0.014	0.0137	0.033	0.056	0.125	0.024
	N	10	10	10	10	10	10
B80M	Mean	0.472 ^B	0.1026	0.163 ^B	0.448	1.178	0.197
	SD	0.014	0.0167	0.027	0.069	0.118	0.028
	N	10	10	10	10	10	10
E2M	Mean	0.486	0.0990	0.188	0.494	1.176	0.200
	SD	0.016	0.0158	0.018	0.049	0.075	0.029
	N	10	10	10	10	10	10
E20M	Mean	0.494	0.0990	0.199	0.488	1.209	0.213
	SD	0.021	0.0139	0.031	0.061	0.088	0.023
	N	10	10	10	10	10	10
E80M	Mean	0.475	0.0889 ^F	0.184	0.428 ^B	1.267	0.188 ^B
	SD	0.015	0.0088	0.038	0.057	0.244	0.022
	N	10	10	10	10	10	10

Table 17. Group Mean Absolute Organ Weights (g) – Males (Continued)

Group		Spleen	Testes	Thymus
CM	Mean	0.086	0.253	0.045
	SD	0.009	0.035	0.009
	N	10	10	10
NT200M	Mean	0.042 ^A	0.163 ^A	0.024 ^A
	SD	0.017	0.042	0.015
	N	6	6	6
B2M	Mean	0.091	0.238	0.044
	SD	0.013	0.034	0.009
	N	10	10	10
B20M	Mean	0.085	0.232	0.048
	SD	0.010	0.024	0.010
	N	10	10	10
B80M	Mean	0.076	0.232	0.046
	SD	0.014	0.020	0.007
	N	10	10	10
E2M	Mean	0.083	0.224	0.043
	SD	0.009	0.045	0.010
	N	10	10	10

Table 17. Group Mean Absolute Organ Weights (g) – Males (Continued)

Group		Spleen	Testes	Thymus
E20M	Mean	0.095	0.236	0.041
	SD	0.014	0.038	0.011
	N	10	10	10
E80M	Mean	0.078	0.231	0.043
	SD	0.018	0.017	0.016
	N	10	10	10

Multiple comparisons were made according to the letters listed below. Capital letters indicate the comparison was significantly different at $p \leq 0.05$ with Dunnett's test of significance; lower case letters indicate comparisons were significantly different at $p \leq 0.05$ with Modified T test.

A = CM vs. NT200M

B = CM vs. B2M, B20M, B80M, B200M, E2M, E20M, E80M, E200M

C = NT200M vs. B200M, E200M

D = B2M vs. E2M

E = B20M vs. E20M

F = B80M vs. E80M

G = B200M vs. E200M

Table 18. Group Mean Absolute Organ Weights (g) – Females

Group		Brain	Heart	Kidneys	Liver	Salivary Gland	Spleen
CF	Mean	0.491	0.151	0.333	0.981	0.142	0.090
	SD	0.025	0.031	0.033	0.116	0.028	0.013
	N	10	10	10	10	10	10
NT200F	Mean	0.442 ^A	0.113 ^A	0.244 ^A	0.857	0.101 ^A	0.065 ^A
	SD	0.030	0.024	0.041	0.154	0.024	0.021
	N	10	10	10	10	10	10
B2F	Mean	0.486	0.161	0.334	1.120 ^B	0.149	0.101
	SD	0.034	0.025	0.040	0.141	0.022	0.021
	N	10	10	10	10	10	10
B20F	Mean	0.485	0.154	0.312	0.955	0.136	0.087
	SD	0.017	0.021	0.042	0.101	0.021	0.017
	N	10	10	10	10	10	10
B80F	Mean	0.483	0.142	0.298	0.957	0.141	0.085
	SD	0.016	0.022	0.024	0.088	0.016	0.016
	N	10	10	10	10	10	10
E2F	Mean	0.489	0.178	0.310	0.968 ^D	0.145	0.081 ^D
	SD	0.021	0.026	0.032	0.106	0.024	0.018
	N	10	10	10	10	10	10
E20F	Mean	0.488	0.153	0.298	0.946	0.145	0.080
	SD	0.016	0.030	0.025	0.120	0.014	0.024
	N	10	10	10	10	10	10
E80F	Mean	0.487	0.143	0.309	0.992	0.129	0.087
	SD	0.028	0.026	0.025	0.095	0.021	0.011
	N	10	10	10	10	10	10
E200F	Mean	0.451 ^B	0.125	0.269 ^B	0.838	0.125	0.062
	SD	0.015	0.029	0.027	0.144	0.028	0.030
	N	4	4	4	4	4	4

Table 18. Group Mean Absolute Organ Weights (g) – Females (Continued)

Group		Thymus	Uterus
CF	Mean	0.047	0.142
	SD	0.010	0.037
	N	10	10
NT200F	Mean	0.041	0.067 ^A
	SD	0.019	0.027
	N	10	10
B2F	Mean	0.057	0.162
	SD	0.013	0.052
	N	10	10
B20F	Mean	0.046	0.204
	SD	0.013	0.061
	N	10	10
B80F	Mean	0.052	0.162
	SD	0.010	0.074
	N	10	10
E2F	Mean	0.046	0.150
	SD	0.014	0.079
	N	10	10
E20F	Mean	0.051	0.148 ^E
	SD	0.008	0.052
	N	10	10

Table 18. Group Mean Absolute Organ Weights (g) – Females (Continued)

Group		Thymus	Uterus
E80F	Mean	0.050	0.157
	SD	0.016	0.064
	N	10	10
E200F	Mean	0.035	0.128
	SD	0.015	0.102
	N	4	4

Multiple comparisons were made according to the letters listed below. Capital letters indicate the comparison was significantly different at $p \leq 0.05$ with Dunnett's test of significance; lower case letters indicate comparisons were significantly different at $p \leq 0.05$ with Modified T test.

A = CF vs. NT200F

B = CF vs. B2F, B20F, B80F, B200F, E2F, E20F, E80F, E200F

C = NT200F vs. B200F, E200F

D = B2F vs. E2F

E = B20F vs. E20F

F = B80F vs. E80F

G = B200F vs. E200F

Table 19. Group Mean Terminal Body Weights (g) and Percent Organ-to-Body Weight Ratios – Males

		Terminal Body Weight	Brain	Epididymides	Heart	Kidneys	Liver
Group							
CM	Mean	30.5	1.624	0.3395	0.672	1.632	4.069
	SD	2.3	0.130	0.0599	0.143	0.224	0.270
	N	10	10	10	10	10	10
NT200M	Mean	22.8 ^A	2.078 ^a	0.2906	0.654	1.458	4.183
	SD	3.6	0.289	0.0480	0.079	0.132	0.370
	N	6	6	6	6	6	6
B2M	Mean	29.9	1.685	0.3246	0.665	1.591	4.055
	SD	0.9	0.090	0.0339	0.083	0.106	0.258
	N	10	10	10	10	10	10
B20M	Mean	30.4	1.583	0.3147	0.633	1.629	4.037
	SD	1.7	0.082	0.0445	0.124	0.128	0.256
	N	10	10	10	10	10	10
B80M	Mean	28.0 ^B	1.698	0.3659	0.585	1.599	4.217
	SD	2.6	0.143	0.0466	0.084	0.178	0.320
	N	10	10	10	10	10	10
E2M	Mean	30.1	1.619	0.3294	0.627	1.638	3.904
	SD	2.0	0.104	0.0523	0.069	0.122	0.119
	N	10	10	10	10	10	10
E20M	Mean	30.1	1.650	0.3291	0.663	1.622	4.023
	SD	2.0	0.134	0.0399	0.112	0.154	0.173
	N	10	10	10	10	10	10
E80M	Mean	28.5	1.674	0.3123 ^f	0.643	1.503	4.423
	SD	2.2	0.105	0.0212	0.097	0.152	0.543
	N	10	10	10	10	10	10

Table 19. Group Mean Terminal Body Weights (g) and Percent Organ-to-Body Weight Ratios – Males (Continued)

Group	Terminal					
		Body Weight	Salivary Gland	Spleen	Testes	Thymus
CM	Mean	30.5	0.745	0.283	0.833	0.147
	SD	2.3	0.107	0.027	0.120	0.032
	N	10	10	10	10	10
NT200M	Mean	22.8 ^A	0.635	0.180 ^A	0.708	0.101 ^A
	SD	3.6	0.086	0.050	0.130	0.050
	N	6	6	6	6	6
B2M	Mean	29.9	0.706	0.304	0.798	0.147
	SD	0.9	0.083	0.036	0.112	0.029
	N	10	10	10	10	10
B20M	Mean	30.4	0.715	0.279	0.761	0.156
	SD	1.7	0.065	0.025	0.066	0.030
	N	10	10	10	10	10
B80M	Mean	28.0 ^B	0.704	0.270	0.835	0.163
	SD	2.6	0.074	0.034	0.097	0.015
	N	10	10	10	10	10
E2M	Mean	30.1	0.664	0.275 ^D	0.742	0.144
	SD	2.0	0.087	0.020	0.139	0.028
	N	10	10	10	10	10

Table 19. Group Mean Terminal Body Weights (g) and Percent Organ-to-Body Weight Ratios – Males (Continued)

Group	Terminal Body Weight Salivary Gland Spleen Testes Thymus					
E20M	Mean	30.1	0.710	0.315 ^E	0.787	0.136
	SD	2.0	0.087	0.037	0.137	0.033
	N	10	10	10	10	10
E80M	Mean	28.5	0.662	0.271	0.813	0.150
	SD	2.2	0.078	0.048	0.059	0.052
	N	10	10	10	10	10

Multiple comparisons were made according to the letters listed below. Capital letters indicate the comparison was significantly different at $p \leq 0.05$ with Dunnett's test of significance; lower case letters indicate comparisons were significantly different at $p \leq 0.05$ with Modified T test.

A = CM vs. NT200M

B = CM vs. B2M, B20M, B80M, B200M, E2M, E20M, E80M, E200M

C = NT200M vs. B200M, E200M

D = B2M vs. E2M

E = B20M vs. E20M

F = B80M vs. E80M

G = B200M vs. E200M

Table 20. Group Mean Terminal Body Weights (g) and Percent Organ-to-Body Weight Ratios – Females

		Terminal Body Weight	Brain	Heart	Kidneys	Liver	Salivary Gland
Group							
CF	Mean	23.2	2.126	0.648	1.439	4.228	0.612
	SD	1.8	0.165	0.120	0.116	0.330	0.113
	N	10	10	10	10	10	10
NT200F	Mean	18.9 ^A	2.366 ^A	0.596	1.288 ^A	4.516	0.533
	SD	2.9	0.264	0.104	0.079	0.427	0.096
	N	10	10	10	10	10	10
B2F	Mean	24.7	1.971	0.652	1.349	4.516	0.603
	SD	2.0	0.169	0.099	0.104	0.322	0.089
	N	10	10	10	10	10	10
B20F	Mean	23.1	2.102	0.666	1.350	4.132	0.588
	SD	1.0	0.118	0.077	0.168	0.380	0.079
	N	10	10	10	10	10	10
B80F	Mean	22.8	2.119	0.624	1.304	4.188	0.620
	SD	0.9	0.094	0.106	0.089	0.285	0.081
	N	10	10	10	10	10	10
E2F	Mean	23.4	2.093	0.759 ^D	1.322	4.122 ^D	0.619
	SD	1.6	0.128	0.103	0.091	0.257	0.089
	N	10	10	10	10	10	10
E20F	Mean	22.3	2.194	0.684	1.337	4.231	0.652
	SD	1.1	0.115	0.116	0.096	0.380	0.062
	N	10	10	10	10	10	10
E80F	Mean	23.1	2.114	0.618	1.341	4.296	0.560
	SD	1.4	0.190	0.097	0.130	0.391	0.107
	N	10	10	10	10	10	10
E200F	Mean	18.9 ^B	2.425 ^B	0.654	1.432 ^C	4.431	0.658 ^C
	SD	3.1	0.338	0.046	0.096	0.289	0.056
	N	4	4	4	4	4	4

Table 20. Group Mean Terminal Body Weights (g) and Percent Organ-to-Body Weight Ratios – Females (Continued)

Group	Terminal				
		Body Weight	Spleen	Thymus	Uterus
CF	Mean	23.2	0.387	0.203	0.610
	SD	1.8	0.053	0.041	0.142
	N	10	10	10	10
NT200F	Mean	18.9 ^A	0.338	0.209	0.343 ^A
	SD	2.9	0.095	0.084	0.106
	N	10	10	10	10
B2F	Mean	24.7	0.406	0.233	0.658
	SD	2.0	0.075	0.054	0.210
	N	10	10	10	10
B20F	Mean	23.1	0.375	0.198	0.880
	SD	1.0	0.080	0.058	0.254
	N	10	10	10	10
B80F	Mean	22.8	0.370	0.228	0.705
	SD	0.9	0.059	0.043	0.305
	N	10	10	10	10
E2F	Mean	23.4	0.344	0.195	0.626
	SD	1.6	0.066	0.053	0.286
	N	10	10	10	10
E20F	Mean	22.3	0.355	0.226	0.662
	SD	1.1	0.094	0.029	0.233
	N	10	10	10	10

Table 20. Group Mean Terminal Body Weights (g) and Percent Organ-to-Body Weight Ratios – Females (Continued)

Group	Terminal				
		Body Weight	Spleen	Thymus	Uterus
E80F	Mean	23.1	0.377	0.213	0.677
	SD	1.4	0.043	0.063	0.272
	N	10	10	10	10
E200F	Mean	18.9 ^B	0.316	0.180	0.630
	SD	3.1	0.100	0.061	0.453
	N	4	4	4	4

Multiple comparisons were made according to the letters listed below. Capital letters indicate the comparison was significantly different at $p \leq 0.05$ with Dunnett's test of significance; lower case letters indicate comparisons were significantly different at $p \leq 0.05$ with Modified T test.

A = CF vs. NT200F

B = CF vs. B2F, B20F, B80F, B200F, E2F, E20F, E80F, E200F

C = NT200F vs. B200F, E200F

D = B2F vs. E2F

E = B20F vs. E20F

F = B80F vs. E80F

G = B200F vs. E200F

Table 21. Group Mean Absolute Brain Weights (g) and Percent Organ-to-Brain Weight Ratios – Males

Group		Absolute Brain Weight	Epididymides	Heart	Kidneys	Liver	Salivary Gland
CM	Mean	0.493	20.924	41.59	100.88	252.10	45.91
	SD	0.011	3.585	9.33	14.20	27.21	5.53
	N	10	10	10	10	10	10
NT200M	Mean	0.465 ^a	14.332 ^A	31.85 ^A	71.78 ^A	206.50 ^A	31.39 ^A
	SD	0.024	3.482	5.19	14.49	45.59	7.78
	N	6	6	6	6	6	6
B2M	Mean	0.503	19.275	39.55	94.77	241.27	41.95
	SD	0.025	1.933	5.19	9.45	20.42	4.93
	N	10	10	10	10	10	10
B20M	Mean	0.481	19.899	39.98	103.15	255.75	45.25
	SD	0.014	2.805	7.39	10.21	23.66	4.60
	N	10	10	10	10	10	10
B80M	Mean	0.472 ^B	21.728	34.67	94.92	249.74	41.70
	SD	0.014	3.477	5.63	14.17	25.61	5.61
	N	10	10	10	10	10	10
E2M	Mean	0.486	20.364	38.80	101.48	241.84	41.13
	SD	0.016	3.108	4.35	8.93	13.82	5.78
	N	10	10	10	10	10	10
E20M	Mean	0.494	20.068	40.47	98.85	245.24	43.03
	SD	0.021	3.006	7.78	12.02	21.62	3.66
	N	10	10	10	10	10	10
E80M	Mean	0.475	18.715 ^F	38.76	90.11	265.99	39.61 ^B
	SD	0.015	1.684	7.61	10.69	44.36	4.40
	N	10	10	10	10	10	10

Table 21. Group Mean Absolute Brain Weights (g) and Percent Organ-to-Brain Weight Ratios – Males (Continued)

		Absolute Brain Weight	Spleen	Testes	Thymus
Group					
CM	Mean	0.493	17.48	51.29	9.07
	SD	0.011	1.86	6.69	1.88
	N	10	10	10	10
NT200M	Mean	0.465 ^a	9.02 ^A	34.74 ^A	5.13 ^A
	SD	0.024	3.46	7.92	3.25
	N	6	6	6	6
B2M	Mean	0.503	18.10	47.48	8.80
	SD	0.025	2.42	7.22	1.99
	N	10	10	10	10
B20M	Mean	0.481	17.66	48.17	9.86
	SD	0.014	1.87	4.64	1.96
	N	10	10	10	10
B80M	Mean	0.472 ^B	16.09	49.17	9.68
	SD	0.014	2.88	4.06	1.54
	N	10	10	10	10
E2M	Mean	0.486	17.05	45.95	8.93
	SD	0.016	1.61	8.94	1.93
	N	10	10	10	10

Table 21. Group Mean Absolute Brain Weights (g) and Percent Organ-to-Brain Weight Ratios – Males (Continued)

Group	Absolute Brain Weight				
			Spleen	Testes	Thymus
E20M	Mean	0.494	19.28	47.71	8.36
	SD	0.021	3.32	7.38	2.39
	N	10	10	10	10
E80M	Mean	0.475	16.38	48.65	9.00
	SD	0.015	3.69	3.53	3.36
	N	10	10	10	10

Multiple comparisons were made according to the letters listed below. Capital letters indicate the comparison was significantly different at $p \leq 0.05$ with Dunnett's test of significance; lower case letters indicate comparisons were significantly different at $p \leq 0.05$ with Modified T test.

A = CM vs. NT200M

B = CM vs. B2M, B20M, B80M, B200M, E2M, E20M, E80M, E200M

C = NT200M vs. B200M, E200M

D = B2M vs. E2M

E = B20M vs. E20M

F = B80M vs. E80M

G = B200M vs. E200M

Table 22. Group Mean Absolute Brain Weights (g) and Percent Organ-to-Brain Weight Ratios – Females

		Absolute Brain Weight	Heart	Kidneys	Liver	Salivary Gland	Spleen
Group							
CF	Mean	0.491	30.60	67.82	199.87	29.12	18.31
	SD	0.025	5.66	4.57	21.38	6.58	2.86
	N	10	10	10	10	10	10
NT200F	Mean	0.442 ^A	25.38 ^A	55.11 ^A	193.38	22.76 ^A	14.59 ^A
	SD	0.030	4.52	7.82	29.58	4.64	4.50
	N	10	10	10	10	10	10
B2F	Mean	0.486	33.10	69.07	230.83 ^B	30.65	20.72
	SD	0.034	4.38	9.58	27.63	4.46	4.02
	N	10	10	10	10	10	10
B20F	Mean	0.485	31.85	64.32	196.83	28.10	17.90
	SD	0.017	4.82	8.18	19.20	4.41	3.84
	N	10	10	10	10	10	10
B80F	Mean	0.483	29.48	61.68	198.15	29.20	17.52
	SD	0.016	5.00	5.20	17.76	3.18	3.06
	N	10	10	10	10	10	10
E2F	Mean	0.489	36.34	63.34	197.76 ^D	29.70	16.57 ^D
	SD	0.021	5.10	5.53	18.46	4.91	3.64
	N	10	10	10	10	10	10
E20F	Mean	0.488	31.37	61.07	193.66	29.77	16.33
	SD	0.016	6.31	4.90	23.71	3.05	4.96
	N	10	10	10	10	10	10
E80F	Mean	0.487	29.32	63.75	204.73	26.51	18.04
	SD	0.028	4.22	7.00	26.46	4.40	3.03
	N	10	10	10	10	10	10
E200F	Mean	0.451 ^B	27.60	59.58	185.75	27.73	13.71
	SD	0.015	6.24	5.61	31.70	5.91	6.64
	N	4	4	4	4	4	4

Table 22. Group Mean Absolute Brain Weights (g) and Percent Organ-to-Brain Weight Ratios – Females (Continued)

Group		Absolute Brain Weight	Thymus	Uterus
CF	Mean	0.491	9.64	28.71
	SD	0.025	2.31	6.33
	N	10	10	10
NT200F	Mean	0.442 ^A	9.16	15.00 ^A
	SD	0.030	4.04	5.66
	N	10	10	10
B2F	Mean	0.486	11.89	33.68
	SD	0.034	2.87	11.40
	N	10	10	10
B20F	Mean	0.485	9.47	42.08
	SD	0.017	2.81	12.69
	N	10	10	10
B80F	Mean	0.483	10.75	33.34
	SD	0.016	2.00	14.60
	N	10	10	10
E2F	Mean	0.489	9.42	30.34
	SD	0.021	2.86	14.76
	N	10	10	10
E20F	Mean	0.488	10.35	30.46 ^E
	SD	0.016	1.59	11.68
	N	10	10	10

Table 22. Group Mean Absolute Brain Weights (g) and Percent Organ-to-Brain Weight Ratios – Females (Continued)

Group	Absolute Brain Weight			
		Thymus	Uterus	
E80F	Mean	0.487	10.18	32.24
	SD	0.028	3.14	13.34
	N	10	10	10
E200F	Mean	0.451 ^B	7.70	28.02
	SD	0.015	3.24	21.88
	N	4	4	4

Multiple comparisons were made according to the letters listed below. Capital letters indicate the comparison was significantly different at $p \leq 0.05$ with Dunnett's test of significance; lower case letters indicate comparisons were significantly different at $p \leq 0.05$ with Modified T test.

A = CF vs. NT200F

B = CF vs. B2F, B20F, B80F, B200F, E2F, E20F, E80F, E200F

C = NT200F vs. B200F, E200F

D = B2F vs. E2F

E = B20F vs. E20F

F = B80F vs. E80F

G = B200F vs. E200F

APPENDIX A: PROTOCOL, AMENDMENTS, AND DEVIATIONS

STUDY PROTOCOL**28-DAY REPEATED DOSE TOXICITY STUDY OF
TOBACCO BLEND AND AQUEOUS TOBACCO EXTRACT IN CD-1 MICE**

**TESTING FACILITY:
BATTELLE COLUMBUS
505 KING AVENUE
COLUMBUS, OH 43201**

**SPONSOR:
R.J. REYNOLDS TOBACCO COMPANY
RESEARCH AND DEVELOPMENT
BOWMAN GRAY TECHNICAL CENTER
WINSTON-SALEM, NC 27102**

This protocol was approved by the Sponsor Study Monitor on 6/17/08 [Signature]
Date / Initials

AP PROVED, BATTELLE:

[Signature]
Milton R. Hejtmancik, Ph.D., D.A.B.T.
Study Director

6/17/08
Date

[Signature]
Allen W. Singer, D.V.M., D.A.C.V.P., D.A.B.T.
Toxicology Columbus Manager

6-17-08
Date

The protocol for the study, data, study conduct and the final report will be reviewed by Battelle's Quality Assurance Unit based upon current assurance principles and Good Laboratory Practices.

REVIEWED, BATTELLE:

[Signature]
Carrie James, RQAP-GLP
Battelle Quality Assurance

6/17/08
Date

AP PROVED, SPONSOR:

[Signature]
Suzana Theophilus, Ph.D., D.A.B.T.
Senior Staff Toxicologist

6/18/08
Date

To the best of our knowledge, this study does not unnecessarily duplicate any previous experiments.

1.0 PURPOSE

The purpose of this study is to compare the short-term toxicity of a tobacco blend, aqueous tobacco extract, and appropriate controls in rodents (nicotine tartrate positive control and diet negative control). The study will also determine plasma concentrations of nicotine and cotinine under the conditions of the study. This data will be used in the design of long-term studies.

2.0 REGULATORY COMPLIANCE

This study will be conducted in compliance with the current version of the United States Food and Drug Administration's (FDA) Good Laboratory Practice (GLP) Regulations, 21 CFR Part 58, for the conduct of nonclinical laboratory studies. This protocol will be listed in the Battelle total list of studies as "FDA GLP (non-regulated)."

All portions of this study to be performed at Battelle will adhere to the study protocol and any amendments, as well as to applicable Battelle facility Standard Operating Procedures (SOPs).

Portions of this study performed by the Sponsor or Sponsor's designee will be conducted according to SOPs of the performing laboratory. The conduct of such portions will be conducted in compliance with the current version of the United States Food and Drug Administration's (FDA) Good Laboratory Practice (GLP) Regulations, 21 CFR Part 58 for the conduct of nonclinical laboratory studies.

3.0 ROUTE AND DURATION OF ADMINISTRATION

The test articles will be administered orally mixed in the feed for a minimum of 28 days. This route of administration is chosen based upon human exposure via the oral route.

4.0 TESTING FACILITY

4.1 Testing Facility

Battelle Columbus
505 King Avenue
Columbus, Ohio 43201-2693

4.2 Study Director

Milton R. Hejtmancik, Ph.D., D.A.B.T.
 Tel: 614-424-4465
 Fax: 614-424-3171
 E-mail: hejtmman@battelle.org

5.0 SPONSOR AND STUDY MONITOR**5.1 Sponsor:**

R.J. Reynolds Tobacco Company
 Research and Development
 Bowman Gray Technical Center
 Winston-Salem, NC 27102

5.2 Sponsor's Study Monitor

Suzana Theophilus, Ph.D., D.A.B.T.
 R.J. Reynolds Tobacco Company
 Research and Development
 Bowman Gray Technical Center
 Winston-Salem, NC 27102
 Tel: 336-741-1536
 E-mail: theophe@rjrt.com

6.0 PROPOSED STUDY SCHEDULE

Proposed dates for the following study events are listed below. The actual dates will be documented in the study file.

Animal Receipt:	June 19, 2008
Animal Quarantine:	June 19, 2008 – June 30, 2008
First Day of Dosing (Core Study):	July 1, 2008
Initiate Part I (TK Study):	July 10, 2008 (M), July 11, 2008 (F)
Phase 1 Collection:	July 23, 2008 – July 25, 2008
Phase 2 Collection (Completion of In-Life):	August 6, 2008 (M), August 7, 2008 (F)
Neurobehavioral Testing:	July [14 16] ¹ , 2008 (M), July [15 17] ¹ , 2008 (F)
Clinical Pathology/Necropsy:	July 29, 2008 – August 1, 2008
Unaudited Data Submitted to RJRT (Tox Portion Only):	Week of August 11, 2008
Unaudited Data Submitted to RJRT (TK Portion):	As available

RJRT Approval to Proceed to 90-Day

Mouse Study:

Week of August 25, 2008

Draft Final Report:

Week of November 28, 2008

7.0 TEST SYSTEM

Species: Mouse

Strain: CD-1

Source: Charles River

Anticipated Age Range at Arrival: 4-5 weeks

Anticipated Body Weight Range at Arrival: 10-50 g

Number of Mice Required for Study: [634 598]¹ mice ([347 299]¹/sex), including sentinels. A sufficient number of extra mice will be ordered to provide the required number of mice for the study.

7.1 Test System Justification

The strain and species designated by the Sponsor are commonly used for chemical safety evaluation. At this time, studies in laboratory animals are required to support regulatory submissions. The number of mice is considered to be the minimum number necessary to yield meaningful results.

8.0 ANIMAL CARE, HOUSING, AND ENVIRONMENTAL CONDITIONS

General procedures for animal care and housing will meet or exceed current AAALAC recommendations, current requirements stated in the "Guide for Care and Use of Laboratory Animals" (National Research Council, 1996), and will conform to the Testing Facility Standard Operating Procedures (SOPs). The protocol will be reviewed and approved by Battelle's Institutional Animal Care and Use Committee (IACUC) and will be reviewed by the sponsor's IACUC, and Battelle will respond to any written comments and/or questions from the sponsor's IACUC regarding the protocol.

8.1 Quarantine and Acclimation

Mice will be quarantined and acclimated for not less than 7 days in accordance with facility SOP.

8.2 Animal Housing

All animal housing and environmental conditions will follow facility SOPs. Male mice will be individually housed and female mice will be housed 4 per cage in polycarbonate cages appropriate for the animals and the study. Sentinel mice for serological monitoring will be housed in the same rooms as study mice.

8.3 Feed

Mice will be fed powdered NTP-2000 rodent diet *ad libitum*, according to facility SOP, except when fasted prior to scheduled necropsy. The control group will be fed the diet without test article and the treated mice will be fed the diet with the specified quantity of test article required to maintain their designated doses. Analytical reports of each feed lot will be provided by the manufacturer. Analytical reports will be reviewed according to facility SOP to ensure acceptable standards, and freedom from levels of contaminants that may interfere with the purpose or conduct of the study. Copies of the analytical results will be retained in the study file.

8.4 Water

Fresh water from the Columbus municipal water supply will be provided *ad libitum* to the mice by an automatic watering system. The water supply will be analyzed within 6 months of the start of the study to ensure acceptable standards, and freedom from levels of contaminants that may interfere with the purpose or conduct of the study. A copy of the analytical results will be retained in the study file.

9.0 TEST ARTICLE AND CONTROL ARTICLE

Records of receipt and use of the test article and control article will be maintained.

9.1 Test Articles

9.1.1 Tobacco Blend

Description:	Natural tobacco blend containing no additives
Supplier:	R.J. Reynolds Tobacco Company
Characterization:	A Certificate of Analysis (CoA) and/or equivalent documentation of test article identity, strength, purity, composition and other defining characteristics was provided by the Sponsor. Documentation of synthesis will be maintained by the Sponsor. Lot number(s) and expiration date(s), if any, will be included in the final report and study files.
Stability:	Test article stability was provided by the Sponsor for inclusion in the final report.
Storage Conditions:	Suitable quantities of the test article were provided by the sponsor in plastic buckets. The test article will be stored frozen (-30 to -15°C). Any test article from a single-use container that is not used for the formulation task for

which it was aliquoted will be saved for emergency use only.

9.1.2 Aqueous Tobacco Extract

Description: Water extraction of tobacco test article

Supplier: R.J. Reynolds Tobacco Company

Characterization: A Certificate of Analysis (CoA) and/or equivalent documentation of test article identity, strength, purity, composition and other defining characteristics was provided by the Sponsor. Documentation of synthesis will be maintained by the Sponsor. Lot number(s) and expiration date(s) will be included in the final report.

Stability: Test article stability was provided by the Sponsor for inclusion in the final report.

Storage Conditions: Suitable quantities of the test article were provided by the sponsor in plastic buckets. The test article will be stored frozen (-30 to -15°C). Any test article from a single-use container that is not used for the formulation task for which it was aliquoted will be saved for emergency use only.

9.2 Positive Control Article

Name: Nicotine hydrogen tartrate salt

Description: Positive control article containing nicotine. The nicotine free base is 35.1% of the bulk salt (2.85 g of salt contains 1 g of free nicotine). Animal dosing will be based upon nicotine and not the bulk salt.

Supplier: Sigma-Aldrich

Characterization: Identity, lot number(s), purity, composition, stability and other defining characteristics was provided by the Supplier. A Certificate of Analysis and a Material Safety Data Sheet was obtained from the supplier and will be maintained in the study file by the conducting laboratory and provided to the sponsor.

Storage Conditions: The control article will be stored under conditions recommended by the supplier.

9.3 Reserve Samples

Archival samples (~100 g) of each set of the tobacco blend, aqueous tobacco extract, and ~5 g of the nicotine hydrogen tartrate positive control article used to formulate the animal diets were collected under design form CN49730 A-TASTAB. Reserve samples of the tobacco blend and tobacco extract shall be maintained frozen (-30 to -15 °C) and a reserve sample of the nicotine tartrate shall be maintained at room temperature until submission of the chronic study final report. At that time, reserve samples will be shipped to R.J. Reynolds Tobacco Company upon authorization by the Study Director. Samples will ship overnight on dry ice to:

R.J. Reynolds Tobacco Company
Research and Development
Bowman Gray Technical Center
Winston Salem, NC 27102

The Study Monitor will be notified of the date of shipment.

9.3.1 Disposition of Unused and Residual Test Article

Following the completion of in-life dosing, the sponsor will provide the laboratory authorization to either dispose of or directions to store unused test article or positive control for potential use in further studies. If for any reason, the subsequent studies are cancelled, the sponsor will provide Battelle authorization to either dispose of these materials or have them returned to the sponsor.

9.4 Formulation Preparation and Analysis

9.4.1 Formulation Preparation

Diet formulations will be prepared once according to a procedure developed by Battelle for this study, based on method(s) provided by the Sponsor. Exposure of the animals to the test articles and positive control will be by the NTP-2000 powdered feed. Formulations will be stored at room temperature prior to use and will be appropriately discarded on or after their expiration date.

9.4.2 Retention Samples

Duplicate samples, target 5 g, of the formulations will be taken from the formulation batches prepared for each diet at each dose will be stored frozen (-30 to -15° C). These retention samples will be discarded upon submission of the final report.

9.4.3 Formulation Analysis

Nicotine will be used as the tracking compound for dose preparation. All prepared formulations will be analyzed for nicotine content. Animal room samples will be collected on the last day of use of the first and only formulation preparation.

Results of analyses for concentration and homogeneity and an audited formulation analysis report will be included in the final report.

10.0 EXPERIMENTAL DESIGN

[~~Six hundred thirty-four~~ **Five hundred ninety-eight**]¹ mice will be assigned to 1 of 11 dose groups. The study will consist of 28-day toxicity study including a neurotoxicity component and a toxicokinetic study.

Ten mice per sex will be maintained with the study mice for serological monitoring. Serological monitoring will be conducted before dose initiation and at study termination according to facility SOP.

Endpoints used to evaluate the potential toxicity of tobacco blend and aqueous tobacco extract will be clinical observations, body weights and body weight changes, food consumption, neurological and behavioral toxicology, and clinical and anatomic pathology including organ weights. A subset of 23 mice per sex is included in each treatment group[, **excluding Group 6,**]¹ for determinations of plasma nicotine and cotinine concentrations (TK study). [~~No TK animals will be assigned to groups Five~~ **TK animals per sex will be assigned to Group 1**]¹ in which animals are fed the control diet [~~(Group 1)~~]¹.

The number of mice per group, and dosage levels, are as follows:

Group	Target Dosage of Nicotine (mg/kg/day) ^a	Number of Mice			
		Male		Female	
		Core	TK ^a	Core	TK ^a
1 - Control	0	10	[-5] ¹	10	[-5] ¹
2 - Nicotine Tartrate High Dose	200	10	23	10	23
3 - Tobacco Blend Low Dose	2	10	23	10	23
4 - Tobacco Blend Intermediate Dose 1	20	10	23	10	23
5 - Tobacco Blend Intermediate Dose 2	80	10	23	10	23
6 - Tobacco Blend High Dose	200	10	[23--] ¹	10	[23--] ¹
7 - Tobacco Extract Low Dose	2	10	23	10	23
8 - Tobacco Extract Intermediate Dose 1	20	10	23	10	23
9 - Tobacco Extract Intermediate Dose 2	80	10	23	10	23
10 - Tobacco Extract High Dose	200	10	23	10	23
11 - Sentinels	0	10	--	10	--

^aNicotine/cotinine analysis

10.1 Serology

The serology screen will be conducted according to facility SOP using 5 males and 5 females from the sentinel group soon after arrival. These animals will be necropsied to evaluate the internal organs for any signs of disease. Initiation of the study will be dependent on negative serology and no evidence of disease in the animals. This procedure will be repeated near or at termination of the study with the remaining 5 males and 5 females in the sentinel group.

Mouse serology endpoints are as follows:

Sendai virus	Mouse adenovirus (MAV) 1 & 2
Pneumonia virus of mice (PVM)	Epizootic diarrhea of infant mice virus (EDIM)
Mouse hepatitis virus (MHV)	Mouse cytomegalovirus (MCMV)
Minute virus of mice (MVM)	Hantaviruses (HANT)
GDVII (murine encephalomyelitis virus)	<i>Encephalitozoon cuniculi</i> (ECUN)
REO-3	Cilia associated respiratory bacillus (CARB)
<i>Mycoplasma pulmonis</i>	Mouse parvovirus (MPV) 1 & 2
Lymphocytic choriomeningitis virus (LCMV)	Mouse thymic virus (MTLV)
Electromelia (mousepox)	Murine norovirus (MNV)
K virus	
Polyoma virus	

10.2 Assignment to Groups

Mice will be assigned to dose groups by sex and body weight prior to the initiation of dosing using PATH/TOX SYSTEM 4.2.2 (Xybion Medical Systems Corp., Cedar Knolls, NJ), which ensures similar group mean body weights by sex. Mice whose body weights are outside a suitable range based on the mean body weights of the animals will not be assigned to the study upon the judgment of the study director. Animals whose behavior or clinical condition deviates from that typical of the species and strain should also be eliminated from use on the study. After randomization, the mean body weights of each study group should not be significantly different ($p \leq 0.05$). After assignment to groups, each mouse will be identified by tail tattoo with an animal identification number unique within the study. Each cage card will contain information including but not limited to study number, group assignment, and animal identification number.

Animal identification numbers will be assigned as follows:

Group	Color Code	Males		Females	
		Core	TK	Core	TK
1 - Control	White	101-110	[-111-115] ¹	151-160	[-161-165] ¹
2 - Nicotine Tartrate High Dose	Gray	201-210	211-233	251-260	261-283
3 - Tobacco Blend Low Dose	Lilac/Blue	301-310	311-333	351-360	361-383
4 - Tobacco Blend Intermediate Dose 1	Lilac/Green	401-410	411-433	451-460	461-483
5 - Tobacco Blend Intermediate Dose 2	Lilac/Yellow	501-510	511-533	551-560	561-583
6 - Tobacco Blend High Dose	Lilac/Red	601-610	[611-633--] ¹	651-660	[661-683--] ¹
7 - Tobacco Extract Low Dose	Tan/Blue	701-710	711-733	751-760	761-783
8 - Tobacco Extract Intermediate Dose 1	Tan/Green	801-810	811-833	851-860	861-883
9 - Tobacco Extract Intermediate Dose 2	Tan/Yellow	901-910	911-933	951-960	961-983
10 - Tobacco Extract High Dose	Tan/Red	1001-1010	1011-1033	1051-1060	1061-1083
11 - Serology Sentinels	Black	1101-1110	--	1151-1160	--

10.3 Clinical Observations

Cage-side observations for moribundity and mortality will be performed on all mice, twice daily, at least 6 hours apart, per facility SOP.

Detailed clinical examinations will be conducted on all mice, including those not subsequently assigned to study, prior to group assignment. During the in-life phase of the study detailed clinical examinations will be conducted weekly on all surviving core study mice. The final detailed clinical examination of each core study mice will be conducted on the day of its scheduled necropsy.

10.4 Body Weight

Individual animal body weights will be recorded for all mice pre-study for randomization and group assignment. After initiation of dosing, body weights for all core study mice will be recorded biweekly on Tuesdays and Fridays and at necropsy. Weekly body weights will be recorded for animals in the TK plasma analysis groups.

10.5 Food Consumption

Food consumption will be measured for all core animals on Tuesdays and Fridays. Food consumption will not be measured on TK animals or sentinels.

10.6 Neuro/Behavioral Toxicology

Five mice of each sex will be randomly selected from each core study group and will be identified such that the conduct of the functional observational test battery (FOB) will be blind. On Study Day [14 16]¹ (males) and [15 17]¹ (females), these mice will be subjected to the FOB to detect any signs of neuro/behavioral toxicology. The day prior to their scheduled FOB, female mice will be individually housed in polycarbonate cages with bedding and feed to be acclimated prior to the conduct of the test. Upon completion of the FOB, females mice will be housed 4 per cage.

~~[Food consumption will not be measured on Day 15 (Tuesday) for females subjected to the FOB.]~~¹ A copy of the neuro/behavioral findings will be included in the final report.

10.7 Toxicokinetics

Twenty-three (23) mice/sex ~~[are is]~~¹ included for each dose group, excluding controls (Group 1), **in which 5 mice/sex are included, Group 6,**¹ and sentinels (Group 11), for determinations of plasma nicotine and cotinine concentrations. The methodology for plasma nicotine and cotinine analysis will be validated under design form CN49730 A-BIOVAL.

The toxicokinetic portion will consist of 2 phases. Phase 1 will determine an appropriate time to sample plasma for nicotine and cotinine from animals fed

nicotine-containing test articles in the diet. This will be accomplished by determining the time course of plasma nicotine and cotinine at the approximate mid-point of the study. At Study Days 14 and 15, 6 specified time points will be spread over a 20-hour time interval to determine the observed C_{max} and T_{max} values for nicotine and cotinine in male and female mice from up to nine specified treatment groups. No compartmental modeling or non-compartmental analysis will be performed on these concentration time profiles. Phase 2 will be based on the information obtained from the Study Day 14 and 15 data, in which the most appropriate (estimated C_{max} and T_{max}) single collection time point will be selected for collecting samples for nicotine and cotinine analysis in male and female mice from up to nine specified treatment groups. The data from both study days will be used to evaluate dose proportionality and nicotine metabolism by sex and group and, if possible or if necessary, evaluate changes in C_{max} and T_{max} between time periods.

10.7.1 Phase 1 Toxicokinetics

For each dose group, [***excluding controls (Group 1)***]¹, 18 mice of each sex assigned to the toxicokinetics study will be randomly subdivided into 6 subsets of animals. Each subset will be used for blood collection at specific times in a manner that results in one blood collection/animal and in a manner that distributes each subset over the 20-hour time course. Each subset consists of 3 males and 3 females from each of [~~nine~~ ***eight***]¹ treatment groups that will each have blood drawn at a designated time point (10 PM, 2 AM, 6 AM, 10 AM, 2 PM, and 6 PM) ([~~27~~ ***24***]¹ total TK samples/sex/time point). This will result in 6 time points for plasma nicotine and cotinine over a 20-hour time interval and allow a determination of the optimal time for collection in Phase 2 and subsequent studies.

Toxicokinetic animals will be euthanized after the blood collection with no further data collected. TK mice will be anesthetized with CO₂/O₂ and blood will be collected [~~via cardiac puncture from the retroorbital cavity~~]¹ into tubes containing potassium EDTA as the [~~anti-~~]¹coagulant. The minimum quantity of blood required to yield 100 µl of plasma for analysis will be drawn at each time point using techniques according to facility SOPs. Samples will be placed on wet ice until centrifuged. Plasma will be transferred into appropriately labeled tubes and placed on dry ice until stored in a freezer set to maintain -60 to -80°C. Samples will be analyzed for nicotine and cotinine by Battelle using a previously validated method.

An audited bioanalytical report, together with appropriate QA documentation, will be provided to the Study Director for inclusion in the final report.

Toxicokinetic parameters to be evaluated will include but may not be limited to the observed C_{max} and observed T_{max} . An audited toxicokinetic report, together with appropriate QA documentation, will be provided to the Study Director for inclusion in the final report.

10.7.2 Phase 2 Toxicokinetics

On Study Day 28, blood will be collected from the remaining 5 TK males and 5 TK females in each of the nine treatment groups (Groups [1,]¹ 2, 3, 4, 5, [6]¹, 7, 8, 9, and 10) at a time selected based on the results of the Phase 1 toxicokinetics study (45 total TK samples/sex/time point). The blood will be used to harvest plasma and the plasma analyzed for nicotine and cotinine under the conditions described for the Phase 1 Toxicokinetics. After plasma collection, all animals in the Toxicokinetic subset will be terminated with no further data collection.

10.8 Clinical Pathology

Clinical chemistry and hematology assessments will be performed on all surviving core study mice on the day of their scheduled necropsy.

All mice will be fasted overnight prior to scheduled blood sampling for hematology and clinical chemistry determinations. Mice will be anesthetized and blood will be collected using an appropriate method. The tubes for hematology will contain EDTA as an anticoagulant. The tubes used for clinical chemistry determinations will not contain anticoagulant, but may contain serum separator gel.

Core study mice will be divided into 2 groups for clinical pathology blood collections. Blood from approximately half the animals will be used in hematology analysis and blood from the remaining half will be used for clinical chemistry. Target volumes of blood collections for clinical chemistry and hematology are 0.7 and 0.25 mL, respectively. In the event that blood volumes do not meet these suggested values, clinical chemistry parameters will be assigned priority based upon anticipated target organs (see below).

Clinical pathology results, and the clinical pathologist's report, will be included in the final report.

10.8.1 Clinical Chemistry Parameters

Clinical chemistry parameters to be evaluated are (listed in order of priority):

Aspartate aminotransferase	Cholesterol
Bilirubin, direct	Creatinine
Bilirubin, total	Protein, total
Gamma glutamyl transferase	Urea nitrogen
Albumin	Electrolytes:
Globulin	Calcium
Albumin/globulin ratio	Chloride
Alkaline phosphatase	Phosphorus
Glucose	Potassium
Triglycerides	Sodium

10.8.2 Hematologic Parameters

Hematologic parameters to be evaluated are:

Erythrocyte count	Mean corpuscular hemoglobin
Hematocrit	Mean corpuscular hemoglobin concentration
Hemoglobin	Mean corpuscular volume
Leukocyte count, total	Platelet count
Leukocyte differential	Reticulocyte count

10.9 Necropsy

10.9.1 Unscheduled Necropsy

Complete necropsies will be performed on all [*core*]¹ study mice that die or are terminated at an unscheduled interval. Terminal body weights will be recorded for moribund mice prior to euthanasia. Moribund mice will be euthanized using CO₂. Organ weights will not be taken for unscheduled deaths, and preservation of selected tissues will be at the discretion of the Study Director. Necropsy will not be conducted on sentinel mice.

10.9.2 Scheduled Necropsy

After at least 28 days of dosing, all surviving [*core*]¹ animals, excluding sentinels, will be fasted overnight and humanely terminated using CO₂. Terminal body weights will be determined and the external features of the animals will be evaluated followed by necropsy.

All scheduled necropsies will be conducted under the supervision of a board-certified veterinary pathologist. Each necropsy will include examination of the external

surface of the body and all orifices; the cranial, thoracic, abdominal and pelvic cavities and their contents; and collection of tissues.

Tissues listed below, when present, will be collected from all mice according to facility SOP. Tissues will be placed in 10% neutral buffered formalin (NBF), with the exceptions of testes, which will be preserved in Bouin's fixative and subsequently transferred to 70% ethanol, and eyes with optic nerve which will be fixed in Davidson's fixative and subsequently transferred to 10% NBF, per facility SOP.

Animal identification	Pancreas
Adrenal glands	Pituitary gland
Bone with articular surface and marrow (femur)	Preputial glands
Brain	Prostate gland
Clitoral gland	Salivary gland (mandibular)
Epididymides	Sciatic nerve
Esophagus, pharynx	Seminal vesicles
Eyes	Skeletal muscle (biceps femoris)
Gross lesions	Skin
Harderian glands	Spinal cord (cervical, thoracic, lumbar)
Heart	Spleen
Intestine, large (cecum, colon, rectum)	Sternum with bone marrow
Intestine, small (duodenum, jejunum, ileum)	Stomach (fore-stomach and glandular)
Kidneys	Testes
Liver (median lobe and left lateral lobe)	Thymus
Lungs with bronchi	Thyroid gland (with parathyroids, if present in routine section)
Lymph node (mesenteric)	Tongue
Mammary gland (females only)	Urinary bladder
Nose (nasal cavity and turbinates)	Uterus
Ovaries (without oviduct)	Vagina
Oral cavity	Zymbal glands

10.10 Organ Weights

The following organs, when present, will be weighed for all scheduled necropsies. Paired organs will be weighed together. Absolute weight, organ-to-body weight and organ-to-brain-weight will be reported. Organ weights will not be conducted on sentinel mice or mice found dead or euthanized in moribund condition.

Brain	Spleen
Epididymides	Testes (without epididymides)
Heart	Thymus
Kidneys	Salivary glands (mandibular)
Liver with gall bladder ^a	Uterus (with cervix)

^a Gall bladder opened and bile drained before weighing

11.0 COMPUTER SYSTEMS FOR DATA MANAGEMENT

(b) (4)

**12.0 STATISTICAL ANALYSIS**

(b) (4)



(b) (4)



13.0 REPORTING

A draft final report will be prepared and submitted to the Sponsor as a .pdf file via email. The Sponsor shall submit final comments, if any, on the draft report to the Study Director. After review and acceptance of the draft final report by the sponsor, Battelle will submit to the Sponsor a bound final report along with a .pdf file.

14.0 STORAGE OF STUDY MATERIALS AND RECORDS RETENTION

Except for analyses performed by the Sponsor or Sponsor's designated laboratory, all records required to reconstruct the study and the final report will be maintained under the direction of Battelle according to facility SOPs. The final report, study files, records and specimens will be stored in Battelle's archives for a period of no less than one year after issue of the final report. At the end of 1 year, the sponsor will provide authorization concerning the disposition of these items.

**AMENDMENT NUMBER 1 TO THE PROTOCOL FOR THE 28-DAY REPEATED DOSE
TOXICITY STUDY OF TOBACCO BLEND AND AQUEOUS TOBACCO EXTRACT IN
CD-1 MICE (CN49730-D)**

1. a. Page 4, Section 6.0, Proposed Study Schedule. The dates for the functional observational test battery (FOB) for male and female mice has changed from:

“July 14, 2008 (M) and July 15, 2008 (F)”

to:

“July 16, 2008 (M) and July 17, 2008 (F)”

b. The reason for the change is to conduct neurobehavioral exams on days other than Tuesday and/or Friday, when other events are scheduled at weekly intervals.

c. The effective date for this change is July 2, 2008.
2. a. Page 5, Section 7.0, Test System. The number of mice required for study has changed from:

“634 mice (317/sex), including sentinels.”

to:

“598 mice (299/sex), including sentinels.”

b. The reason for the change is that the high dose tobacco blend TK animals were removed from the study prior to dosing based on the mortality and moribundity exhibited in the high dose tobacco blend core animals, and that an additional five animals per sex were added as a TK control group to Group 1.

c. The effective date for this change is June 30, 2008 (for the decision to add an additional five animals per sex to Group 1), July 9, 2008 (removal of high dose tobacco blend TK males), and July 11, 2008 (removal of high dose tobacco blend TK females).
3. a. Page 9, Section 10.0, Experimental Design. The following sentence has changed from:

“Six hundred thirty-four mice will be assigned to 1 of 11 dose groups.”

to:

“Five hundred ninety-eight mice will be assigned to 1 of 11 dose groups.”

- b. The reason for the change is that the high dose tobacco blend TK animals were removed from the study prior to dosing based on the mortality and moribundity exhibited in the high dose tobacco blend core animals, and that an additional five animals per sex were added as a TK control group to Group 1.
 - c. The effective date for this change is June 30, 2008 (for the decision to add an additional five animals per sex to Group 1), July 9, 2008 (removal of high dose tobacco blend TK males), and July 11, 2008 (removal of high dose tobacco blend TK females).
4. a. Page 9, Section 10.0, Experimental Design. The following sentence has changed from:
- “A subset of 23 mice per sex is included in each treatment group for determinations of plasma nicotine and cotinine concentrations (TK study).”
- to:
- “A subset of 23 mice per sex is included in each treatment group, excluding Group 6, for determinations of plasma nicotine and cotinine concentrations (TK study).”
- b. The reason for this change is to remove the high dose tobacco blend TK animals from the study prior to dosing based on the mortality and moribundity exhibited in the high dose tobacco blend core animals.
 - c. The effective dates for this change are July 9, 2008 (males) and July 11, 2008 (females).
5. a. Page 9, Section 10.0, Experimental Design. The following sentence has been changed from:
- “No TK animals will be assigned to groups in which animals are fed the control diet (Group 1).”
- to:
- “Five TK animals per sex will be assigned to Group 1 in which animals are fed the control diet.”

- b. The reason for this change is to measure background nicotine and cotinine plasma levels among study animals not exposed to test article.
 - c. The effective date for this change is June 30, 2008.
- 6.
 - a. Page 10, Section 10.0, Experimental Design. Five male and 5 female mice have been assigned to the TK control group (Group 1).
 - b. The reason for this change is to measure background nicotine and cotinine plasma levels among study animals not exposed to test article.
 - c. The effective date for this change is June 30, 2008.
- 7.
 - a. Page 11, Section 10.0, Experimental Design. Twenty-three male and 23 female mice in Group 6 (Tobacco Blend High Dose) have been deleted from TK analysis.
 - b. The reason for this change is to remove the high dose tobacco blend TK animals from the study prior to dosing based on the mortality and moribundity exhibited in the high dose tobacco blend core animals.
 - c. The effective dates for this change are July 9, 2008 (males) and July 11, 2008 (females).
- 8.
 - a. Page 11, Section 10.2, Assignment to Groups. Animal identification numbers will be assigned to the TK control group (Group 1) and as follows:

"111-115" for males and "161-165" for females
 - b. The reason for this change is to measure background nicotine and cotinine plasma levels among study animals not exposed to test article.
 - c. The effective date for this change is June 30, 2008.
- 9.
 - a. Page 11, Section 10.2, Assignment to Groups. The following animal identification numbers will be deleted:

"611-633" for males and "661-683" for females
 - b. The reason for this change is to remove the high dose tobacco blend TK animals from the study prior to dosing based on the mortality and moribundity exhibited in the high dose tobacco blend core animals.

- c. The effective dates for this change are July 9, 2008 (males) and July 11, 2008 (females).
- 10.
 - a. Page 12, Section 10.6, Neuro/Behavioral Toxicology. The following sentence has changed from:

“On Study Day 14 (males) and 15 (females), these mice will be subjected to the FOB to detect any signs of neuro/behavioral toxicology.”

to:

“On Study Day 16 (males) and 17 (females), these mice will be subjected to the FOB to detect any signs of neuro/behavioral toxicology.”
 - b. The reason for the change is to conduct neurobehavioral exams on days other than Tuesday and/or Friday, when other critical phase events are scheduled at weekly intervals.
 - c. The effective date for this change is July 2, 2008.
- 11.
 - a. Page 12, Section 10.6, Neuro/Behavioral Toxicology. The following sentence has been deleted:

“Food consumption will not be measured on Day 15 (Tuesday) for females subjected to the FOB.”
 - b. The reason for the change is to continue food consumption measurements for females subjected to the FOB.
 - c. The effective date for this change is July 2, 2008.
- 12.
 - a. Page 12, Section 10.7, Toxicokinetics. The following paragraph has changed from:

“Twenty-three (23) mice/sex are included for each dose group, excluding controls (Group 1), and sentinels (Group 11), for determinations of plasma nicotine and cotinine concentrations. The methodology for plasma nicotine and cotinine analysis will be validated under design form CN49730 A-BIOVAL.”

to:

“Twenty-three (23) mice/sex is included in each dose group, excluding controls (Group 1) in which 5 mice/sex are included, Group 6, and sentinels (Group 11), for determinations of plasma nicotine and cotinine concentrations. The methodology for plasma nicotine and cotinine analysis will be validated under design form CN49730 A-BIOVAL.”

- b. The reason for the change is that the high dose tobacco blend TK animals were removed from the study prior to dosing based on the mortality and moribundity exhibited in the high dose tobacco blend core animals, and that an additional five animals per sex were added as a TK control group to Group 1.
 - c. The effective date for this change is June 30, 2008 (for the decision to add an additional five animals per sex to Group 1), July 9, 2008 (removal of high dose tobacco blend TK males), and July 11, 2008 (removal of high dosed tobacco blend TK females).
- 13.
 - a. Page 13, Section 10.7.1, Phase 1 Toxicokinetics. The following sentence has changed from:

“For each dose group, 18 mice of each sex assigned to the toxicokinetics study will be randomly subdivided into 6 subsets of animals.”

to:

“For each dose group, excluding controls (Group 1), 18 mice of each sex assigned to the toxicokinetics study will be randomly subdivided into 6 subsets of animals.”
 - b. The reason for this change is to clarify that the five TK mice per sex assigned to the control group will be used for study Day 28 TK blood collections only.
 - c. The effective date for this change is June 30, 2008.
- 14.
 - a. Page 13, Section 10.7.1, Phase 1 Toxicokinetics. The following sentence has changed from:

“Each subset consists of 3 males and 3 females from each of nine treatment groups that will each have blood drawn at a designated time point (10 PM, 2 AM, 6 AM, 10 AM, 2 PM, and 6 PM) (27 total TK samples/sex/time point).”

to:

“Each subset consists of 3 males and 3 females from each of eight treatment groups that will each have blood drawn at a designated time point (10 PM, 2 AM, 6 AM, 10 AM, 2 PM, and 6 PM) (24 total TK samples/sex/time point).”

- b. The reason for this change is to delete the tobacco blend high dose group (Group 6) from Phase I Toxicokinetics.
 - c. The effective date for this change is July 9, 2008.
- 15.
 - a. Page 13, Section 10.7.1, Phase I Toxicokinetics. The following sentence has changed from:

“TK mice will be anesthetized with CO₂/O₂ and blood will be collected from the retroorbital cavity into tubes containing potassium EDTA as the coagulant.”

to:

“TK mice will be anesthetized with CO₂/O₂ and blood will be collected via cardiac puncture into tubes containing potassium EDTA as the anti-coagulant.”
 - b. The reason for this change is to use a method for blood collection that would maximize the volume of blood collected for TK plasma analysis, and to correct an error in terminology.
 - c. The effective date for this change is July 23, 2008.
- 16.
 - a. Page 14, Section 10.7.2, Phase 2 Toxicokinetics. The following paragraph has changed from:

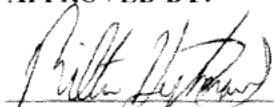
“On Study Day 28, blood will be collected from the remaining 5 TK males and 5 TK females in each of the nine treatment groups (Groups 2, 3, 4, 5, 6, 7, 8, 9, and 10) at a time selected based on the results of the Phase 1 toxicokinetics study (45 total TK samples/sex/time point).”

to:

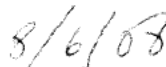
“On Study Day 28, blood will be collected from the remaining 5 TK males and 5 TK females in each of the nine treatment groups (Groups 1, 2, 3, 4, 5, 7, 8, 9, and 10) at a time selected based on the results of the Phase 1 toxicokinetics study (45 total TK samples/sex/time point).”
 - b. The reason for this change is to measure background nicotine and cotinine plasma levels among study animals not exposed to test article.
 - c. The effective date for this change is June 30, 2008.

17. a. Page 15, Section 10.9.1, Unscheduled Necropsy. The following sentence has changed from:
- “Complete necropsies will be performed on all study mice that die or are terminated at an unscheduled interval.”
- to:
- “Complete necropsies will be performed on all core study mice that die or are terminated at an unscheduled interval.”
- b. The reason for this change is to clarify that unscheduled necropsies will not be performed on toxicokinetic animals. Currently, this information is only provided in Section 10.7.1 Phase I Toxicokinetics in which the following sentence appears at the beginning of the second paragraph:
- “Toxicokinetic animals will be euthanized after the blood collection with no further data collected.”
- c. An effective date is not applicable.
18. a. Page 15, Section 10.9.2, Scheduled Necropsy. The following sentence has changed from:
- “After at least 28 days of dosing, all surviving animals will be fasted overnight and humanely terminated using CO₂.”
- to:
- “After at least 28 days of dosing all surviving core animals, excluding sentinels, will be fasted overnight and humanely terminated using CO₂.”
- b. The reason for this change is to clarify that scheduled necropsies will not be performed on sentinels or toxicokinetic animals, and to correct a grammatical error.
- c. The effective date is not applicable.

19. Revised pages 4, 5, 9, 10, 11, 12, 13, 14 and 15 of the protocol as changed in this amendment are attached.

APPROVED BY:

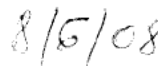
Milton Hejtmancik, Ph.D.
Diplomate, A.B.T.
Study Director



Date



Suzana Theophilus, Ph.D.
Diplomate, A.B.T.
Study Monitor
R.J. Reynolds Tobacco Company



Date

Battelle Study Number CN49730D

Page 1 of 2

**AMENDMENT NUMBER 2 TO THE PROTOCOL FOR THE 28-DAY REPEATED DOSE
TOXICITY STUDY OF TOBACCO BLEND AND AQUEOUS TOBACCO EXTRACT IN
CD-1 MICE (CN49730D)**

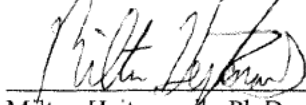
(b) (4)



(b) (4)

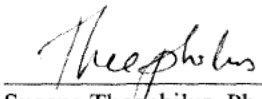
3. Revised pages 17 and 18 as changed in this amendment are attached.

APPROVED BY:


Milton Hejtmancik, Ph.D.
Diplomate, A.B.T.
Study Director

8/12/08

Date


Suzana Theophilus, Ph.D.
Diplomate, A.B.T.
Study Monitor
R.J. Reynolds Tobacco Company

8/13/08

Date

DEVIATION REPORT

CN49730D

28-Day Repeated Dose Toxicity Study of Tobacco Blend and Aqueous Tobacco Extract in
CD-1 Mice

Type of Deviation: GLP

Date of Deviation: July 23, 2008 – August 7, 2008

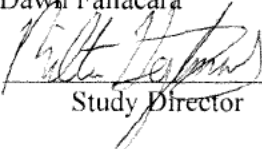
Nature of Deviation: Methodology for plasma nicotine and cotinine analysis were not validated
under design form CN49730A-BIOVAL

Cause of Deviation: Technical difficulties with the blank samples

Impact on the Study: No adverse effect

Corrective Action: A non-validated method was used for this 28-day study. Subsequent work
was required to get the desired 1 ng/ml LOQ but only by increasing the
acceptance criteria for the blank (from approximately 20-50% of the low
standard).

Prepared By: Dawn Fallacara

Approved By: 

Study Director

Date: 4/12/08

Original: Study File

Copies: M. Hejtmancik
Study Supervisor
C. James
B. Burback
8835 Files

DEVIATION REPORT

CN49730D

28-Day Repeated Dose Toxicity Study of Tobacco Blend and Aqueous Tobacco Extract in
CD-1 Mice

Type of Deviation: Protocol

Date of Deviation: July 24, 2008

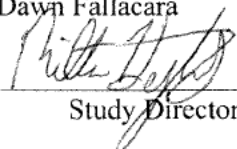
Nature of Deviation: A bucket sample was obtained from Tobacco Extract High Dose males
(Group 10) as opposed to an animal room sample.

Cause of Deviation: Contents of the feeders were discarded before an animal room sample
could be collected.

Impact on the Study: This deviation has minimal impact as a bucket sample was substituted for
the animal room sample. The feeders in question were filled with feed that
originated from this bucket and all feed was stored under similar
conditions.

Corrective Action: A bucket sample was analyzed in place of the animal room sample. Results
were similar (within 0.62%) to the pre-dosing concentration (1610 ppm) of
the 200 mg/kg exposure level for the aqueous tobacco extract formulation.

Prepared By: Dawn Fallacara

Approved By: 
Study Director

Date: 9/3/2008

Original: Study File
Copies: M. Hejtmanick
Study Supervisor
C. James
N. Detty
8835 Files

DEVIATION REPORT

CN49730D

28-Day Repeated Dose Toxicity Study of Tobacco Blend and Aqueous Tobacco Extract in
CD-1 Mice

Type of Deviation: Protocol

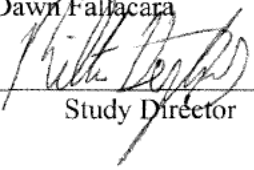
Dates of Deviation: July 30, 2008-August 1, 2008

Nature of Deviation: Lower priority clinical chemistry parameters were evaluated while higher
priority parameters were not analyzed.Cause of Deviation: Insufficient blood volumes at collection caused parameters requiring larger
volumes to be skipped.

Impact on the Study: This deviation has no impact on the study.

Corrective Action: The order of priority as dictated in the protocol changed based upon volume.

Prepared By: Dawn Fallacara

Approved By:  _____

Study Director

Date: 10/8/08

Original: Study File

Copies: M. Hejtmancik

Dawn Fallacara

C. James

M. Toy

T. Wheat

N. Detty

8835 Files

DEVIATION REPORT

CN49730D

28-Day Repeated Dose Toxicity Study of Tobacco Blend and Aqueous Tobacco Extract in CD-1 Mice

Type of Deviation: Protocol

Date of Deviation: August 1, 2008

Nature of Deviation: At the termination of the study, the remaining 5 male and 5 female mice assigned to the study as sentinels were subjected to a serology screen in which blood was collected and necropsies were performed to evaluate internal organs for any signs of disease. At the time of blood collection, 2 of the 5 female sentinels had tail tattoos with the same identification number (1156).

Cause of Deviation: Technician error.

Impact on the Study: No adverse effects.

Corrective Action: The tattoo error was acknowledged on the ^{IAPR} IANR Pathology sheet for the animal with the questionable tattoo number. Therefore, all appropriate documentation for this animal was labeled with the unique animal identification number 1157.

Prepared By: Dawn Fallacara

Approved By: 
Study Director

Date: 10/27/08

Original: Study File
Copies: M. Hejtmancik
Dawn Fallacara
C. James
M. Toy
T. Wheat
N. Detty
8835 Files

**APPENDIX B: CERTIFICATES OF ANALYSIS AND TEST ARTICLE
CHARACTERIZATION AND STABILITY**

RJReynolds

Bowman Gray Technical Center
960 Reynolds Boulevard
Winston-Salem, NC 27106
(336) 741-1836

(b) (4)



THE INFORMATION CONTAINED HEREIN IS, TO THE BEST OF OUR KNOWLEDGE, CORRECT. THE DATA OUTLINED AND THE STATEMENTS MADE ARE INTENDED AS A SOURCE OF INFORMATION.

CERTIFICATE OF ANALYSIS(b) (4)


THE INFORMATION CONTAINED HEREIN IS, TO THE BEST OF OUR KNOWLEDGE, CORRECT. THE DATA OUTLINED AND THE STATEMENTS MADE ARE INTENDED AS A SOURCE OF INFORMATION

RJReynolds

Bowman Gray Technical Center
950 Reynolds Boulevard
Winston-Salem, NC 27106
(336) 741-1536

CERTIFICATE OF ANALYSIS

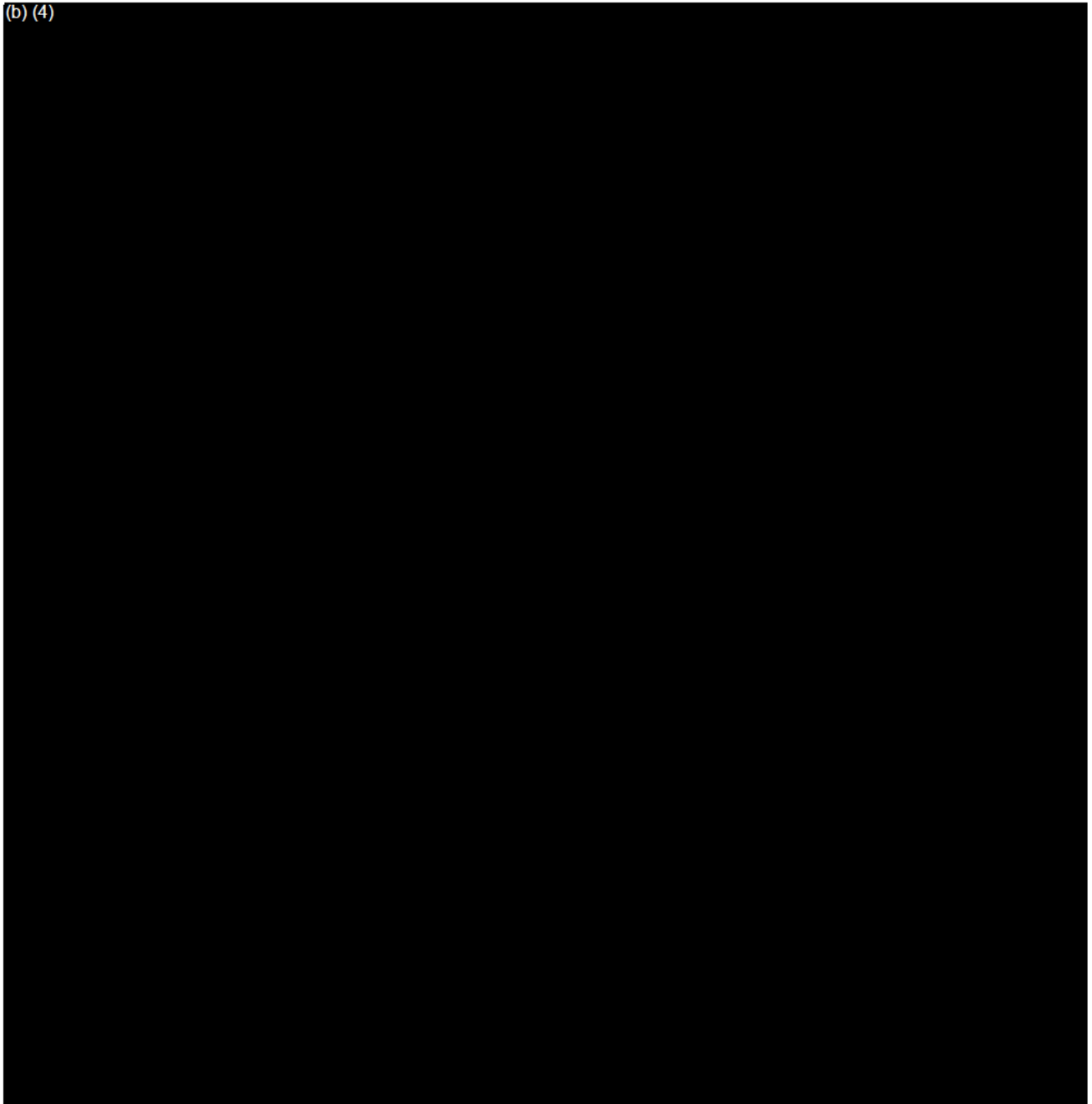
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THE INFORMATION CONTAINED HEREIN IS, TO THE BEST OF OUR KNOWLEDGE, CORRECT. THE DATA OUTLINED AND THE STATEMENTS MADE ARE INTENDED AS A SOURCE OF INFORMATION.

CERTIFICATE OF ANALYSIS

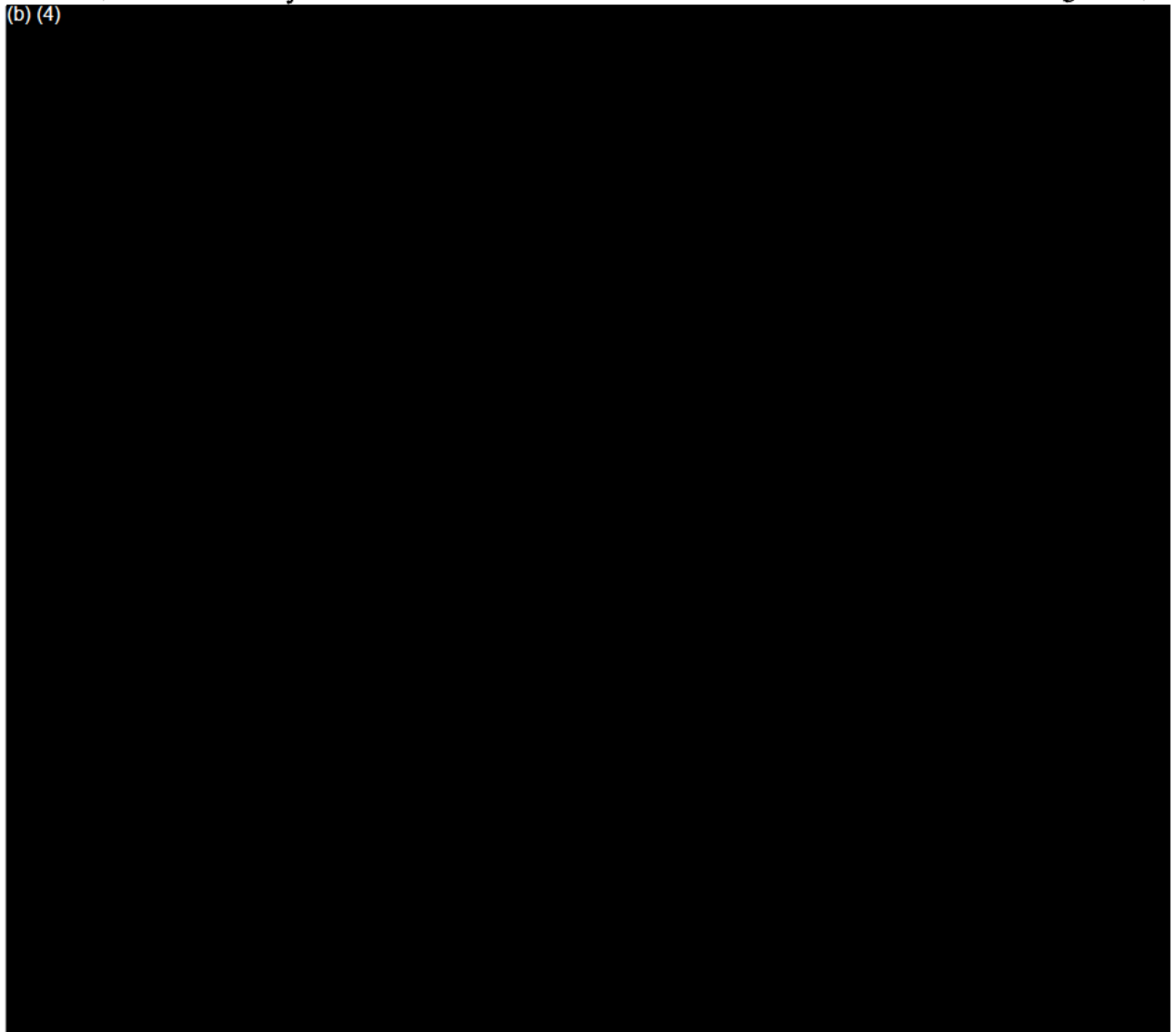
(b) (4)



THE INFORMATION CONTAINED HEREIN IS, TO THE BEST OF OUR KNOWLEDGE, CORRECT. THE DATA OUTLINED AND THE STATEMENTS MADE ARE INTENDED AS A SOURCE OF INFORMATION.

Certificate Of Analysis**Page 1 of 1**

(b) (4)



**RJRT Summary of
Initial Test Article Characterization and
Stability Data**

**Smokeless Tobacco and Extract
Feeding Studies**

8/27/08

Summary

(b) (4)



Test article characterization

Test design

Analyses were planned for 2008, 2009, 2010, and 2011 to span the full length of the toxicology studies and to determine the evolution of the measured endpoints for the test articles with time.

The test article characterization study had 2 main components:

- 1) Chemical analyses conducted at
 - a. RJRT
 - b. Labstat
 - c. Microbac
- 2) Microbial analyses conducted at
 - a. RJRT
 - b. Trilogy

The chemical and microbiological test article characterization and stability studies were designed to analyze various chemicals of interest and microbiological endpoints to determine the evolution of the test articles over time. The chemistry endpoints that were planned to be measured are presented in Table 1.

Table 1. Chemistry endpoints by evaluation site

Analyte	Site
pH	RJRT
% Dry matter	RJRT
% Moisture/water	RJRT
Nicotine	RJRT
Nornicotine	RJRT
Anabasine	RJRT
Myosamine	RJRT
Anatabine	RJRT
N'-Nitrosornicotine (NNN)	RJRT
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)	RJRT
N'-nitrosoanatabine (NAT)	RJRT
N'-nitrosoanabasine (NAB)	RJRT
Chloride	RJRT
Sugars (sucrose, fructose, glucose)	RJRT
Ammonia	RJRT
Hydroquinone	RJRT
Catechol	RJRT
Phenol	RJRT
M+p-Cresol	RJRT
Arsenic	RJRT
Cadmium	RJRT
Chromium	RJRT
Nickel	RJRT
Lead	RJRT
Formaldehyde	Labstat
Acrolein	Labstat
Benzo[a]pyrene	Labstat
Benzo[a]anthracene	Labstat
Benzo[b]fluoranthene	Labstat
Benzo[j] fluoranthene	Labstat
Benzo[k]fluranthene	Labstat
Dibenz[a,h]anthracene	Labstat
Indeno[1,2,3-cd]pyrene	Labstat
Fluorene	Labstat
Acenaphthylene	Labstat
Fluoranthene	Labstat
Acenaphthene	Labstat
Naphthalene	Labstat
Chrysene	Labstat

Analyte	Site
N-Nitrosodimethylamine (NDMA)	Labstat
N-Nitrosoethylmethylamine (NEMA)	Labstat
N-Nitrosopyrrolidine (NPYR)	Labstat
N-Nitrosodimethylpropylamine (NDPA)	Labstat
N-nitrosodimethylethylamine (NDEA)	Labstat
N-Nitrosodimethylbutylamine (NDBA)	Labstat
N-Nitrosopiperidine (NPIP)	Labstat
Nitrite	Labstat
Organochlorines	Microbac
Organophosphates	Microbac
Maleic hydrazide	Microbac
Dithiocarbamates (reported as mancozeb)	Microbac
N-methylcarbamates	Microbac
N-containing pesticides	Microbac
Herbicides	Microbac

The microbiological endpoints planned to be measured are presented in Table 2.

Table 2. Microbial endpoints by evaluation site

Endpoint	Site
Total bacteria	RJRT
Enteric bacteria (coliforms)	RJRT
Total yeast	RJRT
Total mold	RJRT
Water activity	RJRT
<i>Escherichia coli</i> type I	RJRT
<i>Streptococcus faecalis</i>	RJRT
<i>Thermophilic actinomycetes</i>	RJRT
<i>Aspergillus fumigatus</i> and other yeast/mold	RJRT
<i>Staphylococcus sp.</i> including <i>aureus</i>	RJRT
<i>Klebsiella spp.</i>	RJRT
<i>Salmonella</i>	RJRT
Aflatoxin B1	Trilogy
Aflatoxin B2	Trilogy
Aflatoxin G1	Trilogy
Aflatoxin G2	Trilogy
Ochratoxin A	Trilogy
T-2 toxin	Trilogy
Zearelenone	Trilogy
Sterigmatocystin	Trilogy
Deoxynivalenol	Trilogy
Diacetoxyscirpenol	Trilogy

For microbiological measurements, periodic samples were planned to be analyzed to construct a time course analysis. The frequency of measurement was planned to be

monthly or bimonthly for the first 6 months and once per quarter or biannually throughout the study (to be determined based on first 6 months of data). Microbial toxins were planned to be measured initially and yearly thereafter.

Besides the chemistry and microbiology core study plan, there were two additional studies. The first was to obtain 1-month stability data on a subset of key compounds (e.g., nicotine). The second was to obtain additional data on the blend. Due to the fact that the blend had to be ground finer to obtain homogenous diet mixtures for the animal studies, an additional analysis (measuring the same compounds of toxicological interest as for the original test articles) was conducted to demonstrate that the ground and non-ground blends were chemically equivalent.

Where appropriate, an additional reference smokeless tobacco (2S3, moist snuff) was included. The inclusion of this reference tobacco was designed mainly to help ensure that the methods used were working as expected.

Initial test article characterization Results summary: March-July 2008

1) Chemistry results

a) RJRT analyses

Full production test article characterization (initial time point)

(b) (4)



Table 3. RJRT analyses-full production test articles (blend, extract) and reference

<i>Analyte, Measurement unit</i>	<i>Test Articles</i>			<i>Comparisons</i>			
	<i>2S3 (R)</i>	<i>Blend (B)</i>	<i>Extract (E)</i>	<i>R vs. B</i>	<i>R vs. E</i>	<i>B vs. E</i>	<i>Ranking</i>
Total Solids, %			37.8	NA	NA	NA	NA
pH	7.32	5.45	5.19	B < R	E < R	E < B	E < B < R
Moisture, %	53.7	10.1		B < R	NA	NA	B < R
Nicotine, mg/g	15.1	26.3	23.0	R < B	R < E	E < B	R < E < B
Nicotine (colorimetric), %		2.57	2.40		NA	NA	NA
Nicotine, %	1.51	2.63	2.30	R < B	R < E	E < B	R < E < B
Normicotine, %	<0.010	0.068	0.057	R < B	R < E	E < B	R < E < B
Myosmine, %	0.0010	0.0015	0.0010	NA	NA	NA	NA
Anabasine, %	0.003	0.010	0.009	R < B	R < E	E < B	R < E < B
Anatabine, %	0.024	0.065	0.056	R < B	R < E	E < B	R < E < B
Total Alkaloids, %	<1.55	2.77	2.42	R < B	R < E	E < B	R < E < B
2 nd Total Alkaloids, %	<0.038	0.15	0.12	R < B	R < E	E < B	R < E < B
Fructose, %	0.16	1.01	0.96	R < B	R < E	E < B	R < E < B
Sucrose, %	<0.10	0.19	<0.08	R < B	NA	E < B	R, E < B
Glucose, %	<0.10	0.29	0.37	R < B	R < E	B < E	R < B < E
Ammonia, %	0.27	0.30	0.26	R < B	NS	E < B	R, E < B
Chloride, %	5.53	2.71	2.55	B < R	E < R	E < B	E < B < R
Hydroquinone, µg/g	BDL	BDL	BDL	NA	NA	NA	NA
Catechol, µg/g	12.30	14.42	21.06	R < B	R < E	B < E	R < B < E
Phenol, µg/g	5.35	BDL	BDL	B < R	E < R	NA	B, E < R
p,m-Cresol, µg/g	7.98	BDL	BDL	B < R	E < R	NA	B, E < R
NNN, µg/g	1.57	1.02	1.00	B < R	E < R	NS	B, E < R
NNK, µg/g	0.43	0.40	0.36	NS	NS	NS	NS
NAT, µg/g	1.09	0.68	0.68	B < R	E < R	NS	B, E < R
NAB, µg/g	<0.43	<0.43	<0.49	NA	NA	NA	NS
Arsenic, µg/g	0.252	0.308	0.111	R < B	E < R	E < B	E < R < B
Cadmium, µg/g	0.77	0.74	0.30	B < R	E < R	E < B	E < B < R
Chromium, µg/g	0.44	0.71	0.23	R < B	E < R	E < B	E < R < B
Lead, µg/g	0.220	0.283	0.065	R < B	E < R	E < B	E < R < B
Nickel, µg/g	1.38	1.89	0.99	R < B	E < R	E < B	E < R < B

<indicates <LOD except for cumulative endpoints like total alkaloids, where at least one component of the sum was <LOD (e.g., normicotine)

NA indicates non-applicable cases (e.g., only one replicate run such as nicotine, colorimetric assay; no significance test could be conducted for SD=0; comparisons of means with <LOD results)

NS indicates not statistically significant

b) Labstat analyses

The results for the analytes measured in the test articles and 2S3 reference (R) of the initial test article characterization work (2008 analysis) are presented in Table 4. The general trend for measured analytes is as follows: E < B < R. It is noteworthy that many PAHs are present at much lower levels in the blend and extract than in the reference tobacco.

Table 4. Labstat analyses-full test article production: blend, extract, and reference

Analyte, Measurement unit	Mean SD	Test Articles			Comparisons (% difference)			Ranking
		2S3 (R)	Blend (B)	Extract (E)	B vs. R	E vs. R	E vs. B	
Formaldehyde, µg/g	Mean SD	0.860 0.144	0.309 0.071	0.023 0.002	-64.0	-97.3	-92.4	E < B < R
Acrolein, µg/g	Mean SD	<0.016 0.000	<0.008 0.000	<0.001 0.000	NA	NA	NA	NA
Nitrite, µg/g	Mean SD	9.182 2.167	2.808 0.000	* 0.125 0.000	-69.4	-98.6	-95.5	E < B < R
NDMA, ng/g	Mean SD	* 7.553 1.745	* 2.835 0.000	<0.071 0.000	NA	-99.1	-97.5	E < B, R
NPYR, ng/g	Mean SD	* 7.213 0.000	* 4.010 0.000	* 0.216 0.000	NA	NA	-94.6	E < B < R
NEMA, ng/g	Mean SD	<2.980 0.000	<1.510 0.745	<0.081 0.000	NA	NA	NA	NA
NDEA, ng/g	Mean SD	<3.080 0.000	* 1.864 0.000	<0.084 0.000	NA	NA	NA	NA
NDPA, ng/g	Mean SD	<3.330 0.000	<1.690 0.000	<0.091 0.000	NA	NA	NA	NA
NDBA, ng/g	Mean SD	<4.650 0.000	<2.360 0.000	<0.127 0.000	NA	NA	NA	NA
NPIP, ng/g	Mean SD	<5.040 0.378	<2.560 0.089	<0.137 0.051	NA	NA	NA	NA
Naphthalene, ng/g	Mean SD	80.257 20.285	28.462 4.634	4.289 1.243	-64.5	-94.7	-84.9	E < B < R
Acenaphthylene, ng/g	Mean SD	58.486 7.660	2.003 0.247	0.074 0.008	-96.6	-99.9	-96.3	E < B < R
Acenaphthene, ng/g	Mean SD	77.822 11.786	5.960 0.625	0.723 0.207	-92.3	-99.1	-87.9	E < B < R
Fluorene, ng/g	Mean SD	495.400 53.837	8.973 0.933	0.409 0.076	-98.2	-99.9	-95.4	E < B < R
Phenanthrene, ^{&} ng/g	Mean SD	4747.210 268.135	65.110 8.068	2.760 0.586	-98.6	-99.9	-95.8	E < B < R
Fluoranthene, ng/g	Mean SD	1806.850 55.967	44.870 4.987	2.950 0.329	-97.5	-99.8	-93.4	E < B < R
Pyrene, ^{&} ng/g	Mean SD	1750.400 53.727	32.170 4.879	2.340 0.415	-98.2	-99.9	-92.7	E < B < R
Benzo(a)anthracene, ^{&} ng/g	Mean SD	343.677 17.607	4.041 0.691	0.290 0.053	-98.8	-99.9	-92.8	E < B < R
Chrysene, ng/g	Mean SD	496.849 21.811	10.707 1.381	0.947 0.119	-97.8	-99.8	-91.2	E < B < R
Benzo(b)fluoranthene, ng/g	Mean SD	77.915 4.635	2.983 0.273	0.276 0.030	-96.2	-99.6	-90.7	E < B < R
Benzo(k)fluoranthene, ng/g	Mean SD	27.482 2.343	1.536 0.128	0.137 0.029	-94.4	-99.5	-91.1	E < B < R
Benzo(j)fluoranthene, ng/g	Mean SD	38.042 1.997	1.792 0.151	0.176 0.029	-95.3	-99.5	-90.2	E < B < R
Benzo(e)pyrene, ^{&} ng/g	Mean SD	69.059 3.814	2.102 0.203	0.211 0.023	-97.0	-99.7	-90.0	E < B < R

Analyte, Measurement unit	Mean SD	Test Articles			Comparisons (% difference)			Ranking
		2S3 (R)	Blend (B)	Extract (E)	B vs. R	E vs. R	E vs. B	
Benzo(a)pyrene, ng/g	Mean SD	62.696 4.234	1.599 0.228	0.140 0.020	-97.5	-99.8	-91.2	E < B < R
Perylene, ^{&} ng/g	Mean SD	8.572 1.608	* 0.172 0.000	0.031 0.005	-98.0	-99.6	-81.9	E < B < R
Indeno(1,2,3,-cd)pyrene, ng/g	Mean SD	25.273 2.102	1.362 0.218	0.120 0.017	-94.6	-99.5	-91.2	E < B < R
Dibenz(a,h)anthracene, ng/g	Mean SD	7.131 1.324	* 0.310 0.104	* 0.033 0.013	-95.7	-99.5	-89.3	E < B < R
Benzo(g,h,i)perylene, ^{&} ng/g	Mean SD	27.156 2.003	1.612 0.256	0.170 0.024	-94.1	-99.4	-89.4	E < B < R
Dry Matter, %	Mean SD	45.462 0.057	89.589 0.071		97.1			R < B
Moisture, %	Mean SD	54.538 0.057	10.411 0.071		-80.9			B < R

< indicates all LOD values; * indicates some LOQ values, with midpoint value assigned

& indicates additional analytes not requested to be measured but measured and, therefore, reported

For the available values, the analyte levels measured for the 2S3 reference indicated that the methods worked as expected.

c) Microbac analyses

The following pesticides were measured (GN75387AB-blend, AC-extract): alachlor, aldrin, benfluralin, bifenthrin, butralin, camphechlor, captan, chinomethionate, chlordane, chlorothalonil, cyfluthrin, λ -cyhalothrin, cypermethrin, o,p-DDD, p,p-DDD, o,p-DDE, o,p-DDT, p,p-DDT, deltamethrin, dichloran, dieldrin, dinocap, endosulfan I, endosulfan II, endosulfan SO₄, endrin, esfenvalerate, fenvalerate, flucytrinate, flumetralin, folpet, α -HCH, β -HCH, δ -HCH, heptachlor, heptachlor epoxide, hexachlorobenzene, isopropalin, lindane (γ -HCH), methoxychlor, nitrofen, pendimethalin, permethrin, pyrethrins, trifluralin, EBDC (as mancozeb), maleic hydrazide, acephate, ethyl azinphos, methyl azinphos, methyl bromophos, chlorfenvinphos, chlorpyrifos, S-methyl demeton, diazinon, dichlorvos, dimefox, dimethoate, disulfoton, disulfoton sulfone, disulfoton sulfoxide, ethoprophos, fenamiphos, fenamiphos sulfoxide, fenamiphos sulfone, fenchlorphos, fenitrothion, fensulfothion, fenthion, fenthion sulfone, fenthion sulfoxide, fenophos, formothion, malathion, methamidophos, methidathion, mevinphos, monocrotophos, naled, parathion, methyl parathion, phorate, phosalone, phosphamidon, phoxim, methyl pirimiphos, profenofos, trebufos, trebufos sulfone, trebufos sulfoxide, tetrachlorvinphos, thionazin, trichlorfon, vamidothion, vamidothion sulfoxide, dicamba, 2,4-D, 2,4,5-T, aldicarb, aldicarb sulfone, aldicarb sulfoxide, benalaxyl, butylate, carbaryl, carbofuran, clomazone, diflubenzuron, dimethomorph, diphenamid, ethiofencarb, ethiofencarb sulfone, ethiofencarb sulfoxide, 3-hydroxycarbofuran, metalaxyl, methiocarb, methiocarb sulfone, methiocarb sulfoxide, methomyl, 1-naphthol, oxadixyl, oxamyl, pebulate, piperonyl butoxide, pirimicarb, and propoxur.

With the exception of metalaxyl and butralin, all measured pesticides were below the limit of quantitation. However, based on mouse and rat-specific toxicology data and

exposure assessments, the presence of these two pesticides at such low levels in the tobacco test articles is not expected to contribute in any substantial way to subchronic/chronic toxicity in rats and mice in the feeding studies.

2) Microbiology results

a) RJRT microbial analyses

Figures 1 and 2 indicate the progress of the irradiated test articles with time in terms of microbial endpoints (for the initial samples stored under RJRT conditions at -7°C). Except for total bacterial counts and water activity (which showed changes from the beginning of the study to month 3), there were no other targeted organisms detected at month 3. By month 3, total bacterial counts were slightly increased for the blend and decreased for the extract. However, the water activity for the blend was still below the level where significant growth would be expected, and, although there was a slight increase in the total bacterial counts for the blend, the average values are still within acceptable limits.

Figure 1. Total bacterial counts (RJRT samples)

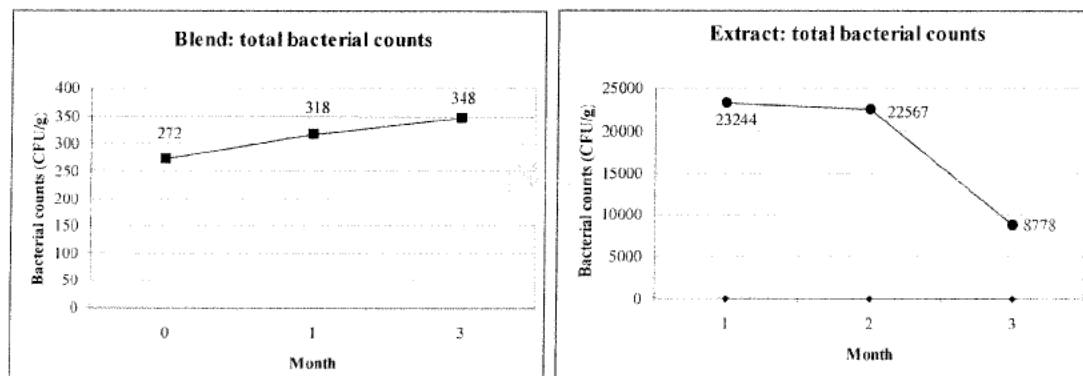
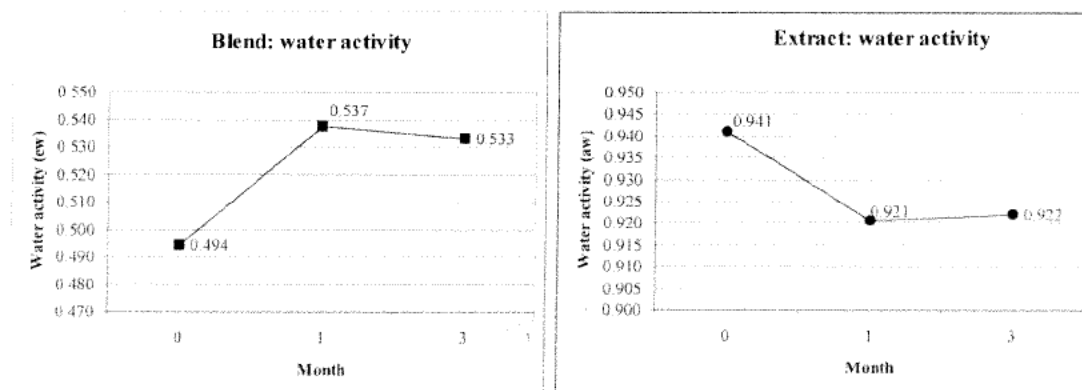


Figure 2. Water activity (RJRT samples)



b) Trilogy toxin analyses

Test articles were tested in April-May 2008 to determine the presence of toxins. No toxins were detected in the blend or extract except for Ochratoxin A. The presence of Ochratoxin A was confirmed by repeating the analysis. However, based on mouse and rat-specific toxicology data and exposure assessments, the presence of Ochratoxin A at such low levels would not be expected to induce Ochratoxin-specific toxicity in the rat and mouse feeding studies.

3) Additional analyses

a) 1-Month stability study: March vs. April 2008 analysis (effects of storage conditions on chemistry endpoints)

A 1-month stability study was conducted using an abbreviated list of compounds to obtain a preliminary read on the stability of key analytes under applicable storage conditions (GN76582). Results from this analysis are presented in Table 5.

Table 5. Test article 1-month stability data (abbreviated analyte list)

Analyte, Measurement unit	2S3			Blend			Extract		
	March	April	April vs. March	March	April	April vs. March	March	April	April vs. March
pH	7.32	7.28	-0.6%	5.45	5.34	-1.9%	5.19	5.45	5.1%
Moisture, %	53.71	54.12	0.8%	10.10	10.06	NS			
Nicotine, mg/g	15.15	15.13	NS	26.28	26.66	NS	22.99	22.80	NS
Nicotine, %	1.51	1.51	NS	2.63	2.67	NS	2.30	2.28	NS
Nornicotine, %	<0.010	0.016	NS	0.068	0.065	NS	0.057	0.055	NS
Myosmine, %	0.001	<0.001	NS	0.002	0.001	NS	0.001	0.001	NS
Anabasine, %	0.003	0.005	NS	0.010	0.011	NS	0.009	0.009	NS
Anatabine, %	0.024	0.026	NS	0.065	0.071	NS	0.056	0.058	NS
Total Alkaloids, %	<1.55	<1.56	NA	2.77	2.81	NS	2.42	2.40	NS
2 nd Total Alkaloids, %	<0.038	<0.049	NA	0.145	0.148	NS	0.123	0.122	NS
Fructose, %	0.16	0.13	NS	1.01	1.29	NS	0.96	1.21	NS
Sucrose, %	<0.10	<0.10	NA	0.19	<0.10	NS	<0.08	<0.09	NA
Glucose, %	<0.10	<0.10	NA	0.29	0.35	NS	0.37	0.38	NS
NNN, µg/g	1.57	1.68	NS	1.02	1.25	NS	1.00	1.02	NS
NNK, µg/g	0.43	0.43	NS	0.40	0.62	NS	0.36	0.39	NS
NAT, µg/g	1.09	1.13	NS	0.68	0.84	NS	0.68	0.69	NS
NAB, µg/g	<0.43	<0.42	NA	<0.43	<0.44	NA	<0.49	<0.49	NA
Total Solids, %							37.76	37.17	-1.6%

NA indicates comparisons of means that include only <LOD values (not applicable)

NS indicates not statistically significant

March and April sample means were compared using z-tests based on test method uncertainty for alkaloids, nitrosamines, and specific sugars. Comparisons of means for pH, moisture, and total solids were made using analysis of variance based on observed variation among replicates which are especially sensitive. P-values were adjusted using Bonferroni's method to control experimental error at a 5% significance level. Total and

secondary alkaloids were calculated by substituting LOD for < LOD quantities. In all other cases, LOD was substituted for < LOD values, with means reported as "<" the calculated mean. Small differences in pH and moisture were statistically significant because little variation was observed among replicates. Otherwise, there were no significant differences for the 2S3, blend, and extract.

For the available values, the analyte levels measured for the 2S3 reference indicated that the methods worked as expected. Overall, these results confirm the stability of the test articles and 2S3 reference during the one month analysis period. This is directly applicable to the formulation regimen employed in the rat and mouse feeding studies.

b) Blend ground vs. non-ground (effects of grinding on chemistry endpoints)

Due to the fact that the blend-diet mixes were not sufficiently homogenous, the blend had to be ground further to achieve a smaller particle size. An additional study was designed to demonstrate that the non-ground and ground blends are equivalent in terms of the analytes selected for analyses (GN77727). Tables 6 and 7 summarize the results from these analyses.

Table 6. Blend ground and non-ground (RJRT data)

Analyte	Mean SD	Test Articles			Comparisons (% Difference)		
		Ground	Non-ground	2S3	Ground vs. Non-Ground	Ground vs. 2S3	Non-Ground vs. 2S3
Ammonia, %	Mean	0.282	0.287	0.253	NS	11.2	13.2
	SD	0.004	0.005	0.005			
pH	Mean	5.37	5.39	7.33	-0.2	-26.7	-26.5
	SD	0.008	0.005	0.008			
Moisture, %	Mean	9.37	9.30	52.50	NS	-82.1	-82.3
	SD	0.038	0.102	0.228			
Total Alkaloids, %	Mean	2.96	2.90	<1.56	2.1	90.3	86.5
	SD	0.038	0.043	0.016			
Secondary Alkaloids, %	Mean	0.163	0.162	<0.053	NS	208.2	206.0
	SD	0.002	0.003	0.001			
Nicotine, %	Mean	2.80	2.74	1.50	NS	86.2	82.3
	SD	0.037	0.042	0.016			
Normicotine, %	Mean	0.074	0.074	0.018	NS	315.2	317.1
	SD	0.001	0.002	0.001			
Myosmine, %	Mean	0.002	0.002	<0.001	NS	NS	NS
	SD	0.000	0.000	0.000			
Anabasine, %	Mean	0.012	0.012	0.006	NS	111.8	107.1
	SD	0.001	0.000	0.000			
Anatabine, %	Mean	0.075	0.074	0.028	NS	164.8	161.4
	SD	0.001	0.002	0.000			
Nicotine, mg/g	Mean	28.0	27.4	15.0	NS	86.2	82.3
	SD	0.37	0.42	0.17			
Chloride, %	Mean	2.53	2.56	5.50	NS	-53.9	-53.4
	SD	0.004	0.035	0.024			
NNN, µg/g	Mean	1.46	1.38	1.61	NS	NS	NS

Analyte	Mean SD	Test Articles			Comparisons (% Difference)		
		Ground	Non-ground	2S3	Ground vs. Non-Ground	Ground vs. 2S3	Non-Ground vs. 2S3
NNK, µg/g	SD	0.046	0.048	0.038			
	Mean	0.60	0.54	0.57	NS	NS	NS
	SD	0.071	0.083	0.067			
NAT, µg/g	Mean	1.13	1.07	1.18	NS	NS	NS
	SD	0.069	0.076	0.052			
NAB, µg/g	Mean	<0.43	<0.43	<0.43	NA	NA	NA
	SD						
Fructose, %	Mean	0.90	0.91	<0.10	NS	798.3	811.7
	SD	0.013	0.020	0.000			
Sucrose, %	Mean	<0.10	<0.10	<0.10	NA	NA	NA
	SD	0.000	0.000	0.000			
Glucose, %	Mean	0.17	0.22	<0.10	NS	66.7	118.3
	SD	0.008	0.045	0.000			
Hydroquinone, mg/g	Mean	BQL	BQL	BQL	NA	NA	NA
	SD						
Catechol, mg/g	Mean	15.36	14.25	12.38	7.8	24.1	15.1
	SD	0.230	0.232	0.163			
Phenol, mg/g	Mean	BQL	BQL	5.46	NA	NA	NA
	SD			0.052			
p,m-Cresol, mg/g	Mean	BQL	BQL	7.14	NA	NA	NA
	SD			0.060			
Arsenic, µg/g	Mean	0.34	0.36	0.48	NS	-27.8	NS
	SD	0.107	0.080	0.029			
Cadmium, µg/g	Mean	0.76	0.73	0.73	4.1	4.1	NS
	SD	0.020	0.010	0.000			
Chromium, µg/g	Mean	0.95	0.81	0.48	NS	97.9	68.8
	SD	0.190	0.190	0.010			
Lead, µg/g	Mean	0.38	0.37	0.30	NS	NS	NS
	SD	0.110	0.040	0.050			
Nickel, µg/g	Mean	1.80	1.60	1.15	12.5	56.5	39.1
	SD	0.060	0.080	0.020			

Table 7. Blend ground and non-ground (Labstat data)

Analyte	Mean SD	Test Articles			Comparisons (% Difference)		
		2S3	Non-Ground	Ground	Non-Ground vs. 2S3	Ground vs. 2S3	Non-Ground vs. Ground
Formaldehyde, µg/g	Mean	0.680	0.351	0.373	-48.4	-45.2	NS
	SD	0.074	0.020	0.053			
Acrolein, µg/g	Mean	* 0.019	<0.008	* 0.010	NA	NA	NA
	SD	0.007	0.000	0.004			
Nitrite, µg/g	Mean	* 1.492	* 0.755	<0.634	NA	NA	NA
	SD	0.592	0.302	0.000			
NDMA, ng/g	Mean	* 6.133	* 3.785	* 4.285	NA	NA	NA
	SD	1.331	1.063	1.138			
NPYR, ng/g	Mean	* 7.930	* 4.010	9.180	NA	NA	128.9
	SD						

Analyte	Mean SD	Test Articles			Comparisons (% Difference)		
		2S3	Non- Ground	Ground	Non- Ground vs. 2S3	Ground vs. 2S3	Non- Ground vs. Ground
NEMA, ng/g	SD	0.000	0.000	0.532			
	Mean	* 3.548	<1.510	* 1.805	NA	NA	NA
NDEA, ng/g	SD	1.417	0.000	0.723			
	Mean	* 3.682	<1.560	* 2.180	NA	NA	NA
NDPA, ng/g	SD	1.474	0.000	0.945			
	Mean	<3.330	<1.690	* 2.086	NA	NA	NA
NDBA, ng/g	SD	0.000	0.000	0.885			
	Mean	<4.640	<2.360	* 2.820	NA	NA	NA
NPIP, ng/g	SD	0.000	0.000	1.127			
	Mean	<5.040	<2.550	<2.560	NA	NA	NA
Naphthalene, ng/g	SD	0.000	0.000	0.000			
	Mean	67.585	38.348	41.454	-43.3	-38.7	NS
Acenaphthylene, ng/g	SD	6.944	4.722	5.994			
	Mean	44.799	2.185	3.715	-95.1	-91.7	NS
Acenaphthene, ng/g	SD	3.872	0.234	0.525			
	Mean	54.838	6.489	7.552	-88.2	-86.2	NS
Fluorene, ng/g	SD	3.585	0.991	1.470			
	Mean	391.164	11.193	14.544	-97.1	-96.3	NS
Phenanthrene, ^{&} ng/g	SD	24.009	1.834	1.197			
	Mean	4762.500	73.180	68.910	-98.5	-98.6	NS
Fluoranthene, ng/g	SD	263.216	8.239	8.367			
	Mean	1845.940	47.190	50.130	-97.4	-97.3	NS
Pyrene, ng/g	SD	61.742	3.214	2.591			
	Mean	1737.980	29.980	31.860	-98.3	-98.2	NS
Benzo(a)anthracene, ^{&} ng/g	SD	54.955	2.835	2.507			
	Mean	348.165	4.128	4.494	-98.8	-98.7	NS
Chrysene, ng/g	SD	7.867	0.620	0.578			
	Mean	492.676	10.482	11.355	-97.9	-97.7	NS
Benzo(b)fluoranthene, ng/g	SD	12.284	0.861	0.980			
	Mean	75.966	2.991	3.892	-96.1	-94.9	NS
Benzo(k)fluoranthene, ng/g	SD	1.222	0.266	0.343			
	Mean	28.940	1.276	1.761	-95.6	-93.9	NS
Benzo(j)fluoranthene, ng/g	SD	1.621	0.104	0.249			
	Mean	39.066	2.064	2.323	-94.7	-94.1	NS
Benzo(e)pyrene, ^{&} ng/g	SD	1.959	0.129	0.204			
	Mean	67.956	2.183	2.837	-96.8	-95.8	NS
Benzo(a)pyrene, ng/g	SD	2.401	0.197	0.231			
	Mean	62.860	1.460	1.970	-97.7	-96.9	NS
Perylene, ^{&} ng/g	SD	1.934	0.218	0.216			
	Mean	8.080	* 0.227	0.416	-97.2	-94.9	83.0*
Indeno(1,2,3-cd)pyrene, ng/g	SD	0.191	0.061	0.054			
	Mean	24.244	1.215	1.825	-95.0	-92.5	NS**

Analyte	Mean SD	Test Articles			Comparisons (% Difference)		
		2S3	Non- Ground	Ground	Non- Ground vs. 2S3	Ground vs. 2S3	Non- Ground vs. Ground
Dibenz(a,h)anthracene, ng/g	SD	1.381	0.158	0.309			
	Mean	5.472	* 0.210	* 0.297	-96.2	-94.6	NA
Benzo(g,h,i)perylene, ^{&} ng/g	SD	1.306	0.056	0.136			
	Mean	25.327	1.447	2.009	-94.3	-92.1	NS
Dry Matter, %	SD	1.799	0.147	0.208			
	Mean	45.475	89.659	89.371	97.2	96.5	-0.3
Moisture, %	SD	0.058	0.088	0.042			
	Mean	54.525	10.341	10.629	-81.0	-80.5	2.8
	SD	0.058	0.088	0.042			

<Indicates all LOD values, *indicates some LOQ values, with midpoint value assigned

*Not statistically significantly different on a dry weight basis

**Statistically significantly different on a dry weight basis

&Indicates additional analytes not requested to be measured but measured and, therefore, reported

Results indicate that, although there were a few statistically significant differences between the ground and non-ground blends, these small statistically significant differences are not expected to translate into biological activity differences in the current set of assays. Therefore, the blends (ground and non-ground) are considered substantially equivalent.

For the available values, the analyte levels measured for the 2S3 reference indicated that the methods worked as expected.

Conclusion

Taking all data into account, results to date 1) indicate that the test articles are appropriately controlled and 2) support the test articles use in the smokeless tobacco and extract rodent feeding toxicology studies.

APPENDIX C: INDIVIDUAL ANIMAL DATA

Table C-1. Individual Animal Clinical Abnormalities – Males

Group	Animal ID	Observation	Observed			Total Number
			First Day	Last Day	Interval	
NT200M	201	Hunched Posture	8	18	11	3
	201	Lethargic	18	18	1	1
	201	Pale	15	18	4	2
	201	Rough Coat	15	18	4	2
	202	Hunched Posture	8	30	23	5
	202	Rough Coat	15	22	8	2
	203	Rough Coat	8	30	23	5
	204	Rough Coat	8	22	15	3
	205	Hunched Posture	15	21	7	2
	205	Lethargic	21	21	1	1
	205	Pale	21	21	1	1
	205	Rough Coat	8	21	14	3
	206	Malocclusion	15	22	8	2
	206	Hunched Posture	8	31	24	5
	206	Lethargic	8	8	1	1
	206	Pale	22	31	10	3
	206	Rough Coat	8	31	24	5
	207	Hunched Posture	8	31	24	5
	207	Pale	15	31	17	4
	207	Rough Coat	8	31	24	5
	208	Hunched Posture	8	8	1	2
	208	Lethargic	8	8	1	2
	208	Thin Appearance	8	8	1	2
	209	Hunched Posture	15	15	1	1
	209	Rough Coat	15	22	8	2

Table C-1. Individual Animal Clinical Abnormalities – Males (Continued)

Group	Animal ID	Observation	Observed			Total Number
			First Day	Last Day	Interval	
NT200M	210	Hunched Posture	8	9	2	2
	210	Pale	9	9	1	1
	210	Rough Coat	8	9	2	2
	210	Labored Respiration	9	9	1	1
B80M	509	Rough Coat	29	29	1	1
	510	Rough Coat	8	8	1	1
B200M	602	Hunched Posture	7	7	1	1
	602	Lethargic	7	7	1	1
	602	Rough Coat	7	7	1	1
	602	Tremors	7	7	1	1
	604	Ataxic (incoordination)	6	6	1	1
	604	Labored Respiration	6	6	1	1
	605	Hunched Posture	7	7	1	1
	605	Lethargic	7	7	1	1
	605	Rough Coat	7	7	1	1
	607	Hunched Posture	8	8	1	2
	607	Rough Coat	8	8	1	2
	608	Hunched Posture	7	7	1	1
	608	Lethargic	7	7	1	1
	608	Rough Coat	7	7	1	1
	609	Hunched Posture	8	8	1	2
	609	Lethargic	8	8	1	2
	609	Thin Appearance	8	8	1	2
	609	Hindlimb Weakness	8	8	1	2
	610	Hunched Posture	7	7	1	1
	610	Lethargic	7	7	1	1
	610	Rough Coat	7	7	1	1

Table C-1. Individual Animal Clinical Abnormalities – Males (Continued)

Group	Animal ID	Observation	Observed			Total Number
			First Day	Last Day	Interval	
E2M	704	Rough Coat	29	30	2	2
E80M	902	Rough Coat	29	29	1	1
	906	Rough Coat	29	29	1	1
E200M	1001	Hunched Posture	7	7	1	1
	1001	Lethargic	7	7	1	1
	1001	Rough Coat	7	7	1	1
	1001	Tremors	7	7	1	1
	1002	Hunched Posture	8	14	7	2
	1002	Pale	14	14	1	1
	1002	Rough Coat	8	14	7	2
	1003	Hunched Posture	14	14	1	1
	1003	Pale	14	14	1	1
	1003	Rough Coat	14	14	1	1
	1005	Hunched Posture	8	14	7	2
	1005	Pale	14	14	1	1
	1005	Rough Coat	14	14	1	1
	1006	Hunched Posture	8	10	3	2
	1006	Lethargic	8	10	3	2
	1006	Rough Coat	8	10	3	2
	1006	Thin Appearance	8	10	3	2
	1006	Labored Respiration	10	10	1	1
	1008	Hunched Posture	8	10	3	2
	1008	Lethargic	8	10	3	2
	1008	Rough Coat	8	10	3	2
	1008	Thin Appearance	8	10	3	2

Table C-2. Individual Animal Clinical Abnormalities – Females

Group	Animal ID	Observation	Observed			Total Number
			First Day	Last Day	Interval	
CF	156	Thin Appearance	31	31	1	1
	252	Hunched Posture	15	30	16	4
	255	Hunched Posture	15	22	8	2
	255	Rough Coat	15	22	8	2
	255	Thin Appearance	15	22	8	2
NT200F	256	Hunched Posture	15	22	8	2
	256	Rough Coat	15	22	8	2
	256	Thin Appearance	15	31	17	4
	257	Hunched Posture	15	31	17	4
	257	Rough Coat	15	31	17	4
	257	Thin Appearance	15	31	17	4
	258	Hunched Posture	15	32	18	4
	258	Pale	22	32	11	3
	258	Rough Coat	15	32	18	4
	258	Thin Appearance	15	32	18	4
	651	Hunched Posture	8	11	4	2
	651	Pale	11	11	1	1
B200F	652	Microphthalmia	10	10	1	1
	652	Hunched Posture	8	10	3	2
	652	Rough Coat	8	10	3	2
	652	Thin Appearance	8	10	3	2
	653	Hunched Posture	8	11	4	2
	653	Rough Coat	8	11	4	2
	653	Thin Appearance	8	11	4	2
	653	Hindlimb Weakness	11	11	1	1

Table C-2. Individual Animal Clinical Abnormalities – Females (Continued)

Group	Animal ID	Observation	Observed			Total Number
			First Day	Last Day	Interval	
B200F	654	Hunched Posture	8	10	3	2
	654	Pale	10	10	1	1
	654	Rough Coat	8	10	3	2
	654	Thin Appearance	8	10	3	2
	655	Hunched Posture	8	10	3	2
	655	Pale	10	10	1	1
	655	Rough Coat	10	10	1	1
	655	Thin Appearance	10	10	1	1
	656	Hunched Posture	8	10	3	2
	656	Pale	10	10	1	1
	656	Rough Coat	8	10	3	2
	656	Thin Appearance	10	10	1	1
	657	Microphthalmia	10	10	1	1
	657	Hunched Posture	8	10	3	2
	657	Rough Coat	8	10	3	2
	657	Thin Appearance	8	10	3	2
	657	Labored Respiration	10	10	1	1
	658	Hunched Posture	8	11	4	2
	658	Rough Coat	8	8	1	1
	658	Thin Appearance	8	11	4	2
	659	Hunched Posture	8	11	4	2
	659	Pale	11	11	1	1
	659	Rough Coat	11	11	1	1
	659	Thin Appearance	8	11	4	2
	660	Hunched Posture	8	10	3	2
	660	Lethargic	10	10	1	1
	660	Rough Coat	8	10	3	2

Table C-2. Individual Animal Clinical Abnormalities – Females (Continued)

Group	Animal ID	Observation	Observed			Total Number
			First Day	Last Day	Interval	
B200F	660	Thin Appearance	8	10	3	2
	660	Labored Respiration	10	10	1	1
E200F	1051	Hunched Posture	8	16	9	3
	1051	Pale	15	16	2	2
	1051	Rough Coat	8	16	9	3
	1051	Tremors	16	16	1	1
	1052	Hunched Posture	8	8	1	1
	1052	Lethargic	12	12	1	1
	1052	Rough Coat	8	8	1	1
	1052	Thin Appearance	8	12	5	2
	1053	Hunched Posture	8	11	4	2
	1053	Pale	11	11	1	1
	1053	Rough Coat	8	11	4	2
	1053	Thin Appearance	8	11	4	2
	1054	Hunched Posture	8	10	3	2
	1054	Pale	10	10	1	1
	1054	Rough Coat	8	10	3	2
	1054	Thin Appearance	8	10	3	2
	1054	Labored Respiration	10	10	1	1
	1056	Hunched Posture	10	10	1	1
	1056	Pale	10	10	1	1
	1056	Rough Coat	10	10	1	1
	1056	Thin Appearance	8	10	3	2
	1056	Tremors	10	10	1	1
	1056	Labored Respiration	10	10	1	1
	1057	Thin Appearance	8	22	15	3

Table C-2. Individual Animal Clinical Abnormalities – Females (Continued)

Group	Animal ID	Observation	Observed			Total Number
			First Day	Last Day	Interval	
E200F	1059	Hunched Posture	8	12	5	2
	1059	Thin Appearance	8	12	5	2
	1060	Hunched Posture	32	32	1	1
	1060	Rough Coat	32	32	1	1

Table C-3. Individual Animal Body Weight (g) Data – Males

Group	Animal	Day								
	ID	1	4	8	11	15	18	22	25	29
CM	101	29.1	29.7	29.4	30.4	29.0	30.7	29.8	30.8	30.6
	102	29.4	29.7	30.9	31.3	31.7	32.5	32.4	33.5	33.3
	103	26.9	27.8	28.3	29.6	30.1	31.6	32.2	33.1	31.6
	104	29.4	31.0	31.1	32.1	33.3	33.4	33.5	34.9	34.0
	105	29.6	31.0	30.7	32.1	32.5	32.7	33.0	33.4	32.5
	106	28.9	29.7	29.8	30.6	31.3	32.8	32.9	33.7	33.6
	107	28.5	29.2	30.0	30.3	28.9	31.4	30.3	32.1	31.1
	108	28.7	29.1	30.4	30.4	31.4	31.8	32.2	32.8	33.3
	109	30.2	31.6	32.9	33.7	34.3	35.6	35.4	36.0	35.9
	110	31.3	32.3	34.1	34.4	36.1	35.8	36.4	37.9	38.2
NT200M	201	28.0	23.4	20.4	20.3	18.9				
	202	29.8	26.2	22.3	22.7	20.8	21.7	21.7	22.3	23.4
	203	27.3	21.8	20.9	24.0	22.9	23.6	24.7	26.8	25.1
	204	30.7	25.6	24.1	24.0	22.9	24.1	27.6	25.7	26.4
	205	29.6	25.2	21.7	20.9	20.6	19.4			
	206	28.3	22.7	21.4	21.0	21.0	20.0	19.7	20.2	21.0
	207	27.0	23.5	20.6	20.7	20.4	19.6	19.7	19.5	19.1
	208	27.8	24.0	19.1						
	209	30.1	24.9	23.6	22.9	21.8	21.3	23.0	23.2	24.5
	210	28.7	23.8	21.7						
B2M	301	28.0	28.8	29.7	29.7	29.8	30.4	30.6	31.8	31.4
	302	29.2	29.6	29.5	30.7	31.8	32.6	32.5	33.4	33.1
	303	27.9	28.1	28.7	29.0	30.2	30.9	31.1	32.9	33.3
	304	29.9	30.2	30.5	31.7	31.2	32.7	32.8	34.0	34.3
	305	28.0	28.5	28.7	29.2	30.3	30.9	30.5	31.5	31.3
	306	28.6	29.1	29.2	29.8	29.6	30.6	31.6	31.9	32.0
	307	29.6	30.6	30.2	31.2	32.7	33.2	33.2	34.1	34.0
	308	28.5	28.6	29.4	30.4	31.3	31.5	32.2	33.0	32.3
	309	29.2	29.7	29.3	30.3	29.2	31.9	32.2	33.7	33.1

Table C-3. Individual Animal Body Weight (g) Data – Males (Continued)

Group	Animal	Day								
	ID	1	4	8	11	15	18	22	25	29
B2M	310	27.6	27.7	26.4	29.3	29.4	30.3	31.4	32.0	30.0
B20M	401	30.6	30.5	31.1	32.4	34.1	35.2	35.9	36.6	36.4
	402	28.1	28.1	27.4	29.1	29.2	31.0	31.1	32.0	33.3
	403	28.8	28.7	29.6	30.2	31.6	32.3	32.8	33.9	33.4
	404	29.1	29.4	29.6	30.8	32.6	33.4	33.1	33.7	34.1
	405	30.9	30.9	30.6	31.1	32.3	32.9	33.0	34.4	33.7
	406	29.0	29.6	29.1	29.7	29.7	30.2	30.7	32.0	30.9
	407	28.5	29.2	29.5	30.7	31.2	32.1	32.8	32.5	31.9
	408	27.9	27.2	27.7	28.6	28.8	29.1	29.6	30.8	31.2
	409	28.6	29.4	30.1	30.1	31.2	31.1	31.8	33.2	32.3
	410	31.1	32.0	31.3	33.2	33.6	34.6	35.0	35.5	34.6
B80M	501	30.1	29.6	28.0	30.2	33.4	32.9	33.2	34.9	35.3
	502	31.8	29.0	27.7	28.0	29.8	30.9	32.0	33.5	33.6
	503	28.7	26.8	23.5	23.7	23.7	24.5	24.9	26.2	26.8
	504	28.2	26.4	24.8	25.2	25.2	26.5	26.9	27.8	28.5
	505	26.3	23.4	22.2	22.2	23.5	25.0	25.0	26.2	25.2
	506	27.4	25.1	23.4	24.4	26.2	27.8	29.2	29.9	30.2
	507	31.0	28.2	25.9	27.9	30.0	30.5	31.5	32.8	34.7
	508	29.1	26.8	25.9	27.8	28.5	31.1	29.6	30.2	30.2
	509	27.5	24.3	22.5	25.6	24.7	24.9	25.5	27.3	27.2
	510	28.9	25.9	24.9	26.2	26.1	27.2	26.9	28.7	28.7
B200M	601	28.8	22.3							
	602	26.2	20.1							
	603	28.2	22.2							
	604	29.8	23.6							
	605	29.9	23.1							
	606	31.5	23.5							
	607	28.7	24.1	21.7						

Table C-3. Individual Animal Body Weight (g) Data – Males (Continued)

Group	Animal	Day								
	ID	1	4	8	11	15	18	22	25	29
B200M	608	29.9	23.9							
	609	30.8	25.3	21.3						
	610	27.1	22.0							
E2M	701	29.1	29.9	30.8	31.6	32.6	33.4	33.2	34.1	34.0
	702	30.2	30.2	31.0	31.9	32.5	33.4	33.3	34.6	34.5
	703	27.1	28.3	29.1	29.5	30.4	30.5	30.5	32.2	31.8
	704	29.6	32.2	32.6	33.1	33.4	36.4	33.8	36.0	33.4
	705	28.0	28.9	30.0	31.0	31.7	33.6	33.2	34.6	34.0
	706	29.7	28.9	30.4	30.7	29.9	31.9	29.7	31.7	29.3
	707	30.8	32.1	33.1	34.0	34.4	34.4	33.7	36.4	36.4
	708	29.2	30.4	30.9	31.5	31.8	32.6	32.8	34.2	34.4
	709	27.2	27.9	28.3	29.2	31.0	31.3	31.6	33.1	32.6
	710	27.8	27.8	28.0	27.8	28.4	28.6	29.1	30.0	31.2
E20M	801	30.3	28.5	29.5	30.5	31.5	32.7	33.1	33.7	34.3
	802	28.6	28.2	28.2	29.5	29.7	29.9	31.0	31.6	32.6
	803	30.9	30.9	29.9	31.4	32.0	33.4	32.9	34.7	34.5
	804	29.6	29.5	29.4	30.1	29.3	32.3	33.0	32.1	33.3
	805	31.9	31.4	33.2	33.3	34.2	34.9	34.7	36.3	36.2
	806	27.6	27.9	26.7	28.2	27.7	29.0	28.9	29.6	29.3
	807	30.7	30.6	30.7	31.6	31.2	32.2	32.1	33.2	33.6
	808	28.2	27.0	25.7	27.5	28.1	28.6	28.7	29.5	30.1
	809	28.6	28.2	28.4	29.8	31.8	32.0	32.8	32.6	33.2
	810	27.5	27.1	26.4	28.1	28.8	29.4	29.8	30.8	30.6
E80M	901	29.8	27.3	25.3	26.7	26.5	26.3	26.7	27.6	26.8
	902	27.2	26.9	24.4	25.2	26.0	26.6	26.9	26.9	27.4
	903	29.6	26.5	24.8	24.6	25.6	26.4	27.9	29.3	30.6
	904	27.4	26.1	23.5	26.7	29.1	29.7	30.2	31.8	32.9
	905	29.4	26.8	24.6	25.5	26.7	28.1	29.3	30.7	31.8

Table C-3. Individual Animal Body Weight (g) Data – Males (Continued)

Group	Animal ID	Day								
		1	4	8	11	15	18	22	25	29
E80M	906	29.8	26.3	26.2	26.5	27.5	29.8	28.8	30.5	30.5
	907	28.2	24.1	24.3	26.3	28.1	29.1	29.3	30.2	29.3
	908	29.9	26.4	26.8	27.0	29.3	29.3	30.2	30.8	31.8
	909	29.7	24.8	24.4	25.9	24.5	26.4	26.6	27.4	27.3
	910	28.7	27.6	26.1	26.5	26.8	27.8	28.8	29.9	30.1
E200M	1001	30.4	22.5							
	1002	27.6	24.1	24.6	22.1					
	1003	29.5	24.1	23.9	22.2					
	1004	29.6	21.2							
	1005	28.3	22.6	21.9	21.8					
	1006	27.2	22.4	18.1						
	1007	28.7	23.5							
	1008	29.8	26.5	21.0						
	1009	27.1	20.9							
	1010	28.2	22.0							

Table C-4. Individual Animal Body Weight (g) Data – Females

Group	Animal	Day								
	ID	1	4	8	11	15	18	22	25	29
CF	151	23.0	23.3	20.8	24.8	22.4	24.3	22.9	25.3	24.4
	152	22.1	23.7	21.7	25.6	22.2	25.4	23.8	25.7	23.7
	153	20.5	21.8	19.9	22.6	21.1	23.9	21.4	24.5	22.6
	154	23.1	24.2	21.3	25.1	22.1	25.8	23.4	25.3	23.7
	155	22.2	23.9	22.5	25.0	25.8	26.8	26.3	27.2	28.3
	156	20.5	22.6	20.9	23.0	22.5	22.8	23.0	23.4	22.8
	157	20.7	21.3	20.9	23.4	23.4	23.0	23.2	25.3	26.1
	158	22.8	24.4	23.0	25.2	24.9	24.6	24.4	26.7	26.4
	159	24.9	26.3	25.9	27.2	29.2	29.7	28.7	32.3	31.7
	160	22.8	24.5	24.3	25.0	25.1	25.6	25.4	26.8	26.9
NT200F	251	21.7	20.8	19.2	20.0	19.1	20.5	19.5	20.5	20.7
	252	22.8	21.2	19.9	19.5	18.2	19.0	19.2	20.1	21.2
	253	22.9	21.7	20.2	21.1	21.1	23.3	22.4	24.0	23.7
	254	24.2	21.2	19.4	20.0	20.3	22.7	21.4	22.9	23.9
	255	21.8	19.2	18.3	18.1	17.1	17.5	18.8	20.2	21.1
	256	21.6	18.3	18.0	17.7	16.9	17.0	17.3	17.5	18.2
	257	23.3	19.6	18.1	17.1	16.5	16.5	16.8	16.3	16.3
	258	21.8	18.5	17.2	15.9	15.0	14.3	14.4	14.6	14.5
	259	24.7	20.1	18.9	19.3	20.0	21.6	23.5	24.9	23.8
	260	23.4	20.4	18.6	21.1	22.0	22.9	21.9	23.9	25.0
B2F	351	23.6	24.6	23.9	25.0	25.5	25.9	26.1	29.3	27.2
	352	21.1	21.1	20.9	22.8	22.8	23.7	22.4	24.0	23.2
	353	21.8	22.1	22.8	23.1	24.0	24.6	24.8	27.2	25.6
	354	25.6	25.0	24.7	25.2	26.2	26.9	26.3	28.4	27.9
	355	24.1	24.1	23.0	24.7	25.6	26.9	27.0	28.0	27.5
	356	24.1	24.0	23.5	26.5	26.6	26.7	27.0	29.4	28.9
	357	24.5	24.2	23.4	26.6	27.0	28.5	28.4	30.0	29.0
	358	24.7	24.3	23.2	26.5	26.9	27.0	27.7	29.7	29.1

Table C-4. Individual Animal Body Weight (g) Data – Females (Continued)

Group	Animal	Day								
	ID	1	4	8	11	15	18	22	25	29
B2F	359	22.0	23.5	23.4	23.7	24.6	24.3	25.0	29.4	27.4
	360	21.9	23.6	23.4	23.6	24.6	24.8	23.6	25.7	26.0
B20F	451	23.2	22.7	21.9	23.9	23.2	24.0	24.2	25.8	24.7
	452	23.2	22.4	22.0	24.4	22.7	24.9	25.6	25.9	25.5
	453	20.2	21.6	21.0	22.4	21.7	24.4	24.3	26.0	25.2
	454	23.2	21.8	21.8	24.2	23.3	26.2	26.0	25.5	24.2
	455	23.8	23.6	23.8	25.2	25.1	24.9	25.1	26.0	25.1
	456	21.9	21.9	21.4	23.1	22.7	22.8	23.4	24.4	23.8
	457	23.1	23.5	22.8	24.0	23.9	25.0	24.7	27.2	25.6
	458	23.7	23.5	22.9	25.9	25.7	26.9	26.4	27.0	26.4
	459	22.7	22.5	23.6	24.9	25.3	25.3	26.3	25.7	25.7
	460	25.0	24.6	25.1	25.2	25.6	26.8	26.3	25.5	26.8
B80F	551	23.7	21.6	21.1	23.3	24.2	25.6	25.2	25.2	25.7
	552	23.0	21.3	20.6	22.6	23.8	23.9	24.4	25.1	25.4
	553	19.7	20.0	20.1	22.3	22.4	23.1	23.3	24.3	24.6
	554	23.0	22.0	20.2	21.6	22.2	23.7	24.0	25.0	24.4
	555	21.6	21.1	21.1	23.2	23.3	24.1	23.4	25.4	24.2
	556	24.4	23.1	23.0	24.2	24.3	25.1	24.1	26.2	25.1
	557	22.2	21.0	21.1	23.9	24.9	24.7	23.9	25.3	25.0
	558	23.2	21.7	21.5	23.3	24.1	25.9	24.4	26.9	25.6
	559	24.4	22.9	22.2	22.3	24.4	24.3	25.1	27.2	27.0
	560	22.9	21.7	22.1	23.1	24.3	24.7	25.3	27.3	26.5
B200F	651	23.6	19.2	18.6						
	652	23.4	19.2	15.1						
	653	23.0	18.8	16.7						
	654	22.8	18.4	15.2						
	655	22.7	18.2	17.1						
	656	23.2	19.1	16.1						

Table C-4. Individual Animal Body Weight (g) Data – Females (Continued)

Group	Animal	Day								
	ID	1	4	8	11	15	18	22	25	29
B200F	657	23.1	18.3	15.8						
	658	22.5	18.4	16.6						
	659	23.5	19.5	18.1						
	660	23.5	17.8	15.0						
E2F	751	21.4	24.5	23.4	24.2	24.3	25.2	23.8	29.6	24.6
	752	22.4	24.7	24.4	24.3	23.6	25.8	23.0	26.2	22.8
	753	21.6	23.4	23.5	24.6	23.9	25.8	23.5	29.7	24.1
	754	21.7	24.0	23.4	23.8	23.3	26.0	25.0	29.0	24.0
	755	21.6	22.3	21.9	22.6	22.7	23.1	23.0	24.4	22.9
	756	21.7	22.9	23.3	25.4	25.8	26.3	26.2	26.5	25.9
	757	22.9	23.2	23.6	24.5	26.6	27.4	26.3	27.1	26.4
	758	23.0	24.4	24.5	25.9	26.4	25.9	27.9	29.9	27.4
	759	24.6	24.9	24.4	25.7	25.6	27.4	26.7	28.1	27.5
	760	22.5	22.7	21.8	23.6	23.2	23.4	23.3	25.2	25.1
E20F	851	21.3	21.9	20.6	22.2	23.0	23.2	23.5	25.2	23.5
	852	22.4	21.4	20.8	22.4	22.8	22.8	22.5	24.5	22.4
	853	22.6	23.4	22.0	22.9	24.1	25.2	25.1	25.6	24.2
	854	21.9	22.8	21.4	22.7	23.2	23.7	24.4	27.5	24.0
	855	22.8	23.2	22.7	24.3	24.7	26.1	25.5	27.0	27.3
	856	22.0	21.7	21.1	22.9	23.0	24.9	24.6	25.6	25.1
	857	21.4	20.9	20.4	22.1	22.4	22.5	23.6	24.3	24.4
	858	21.9	22.9	21.4	23.3	23.3	25.1	24.0	24.9	25.7
	859	21.1	20.3	21.0	21.6	23.4	22.3	23.2	24.9	24.4
	860	23.8	23.0	23.2	24.5	24.4	24.0	24.8	25.2	25.7
E80F	951	23.7	23.3	22.5	24.5	25.6	26.3	25.6	26.9	26.8
	952	22.8	22.2	21.5	23.7	22.2	24.8	22.8	24.5	24.4
	953	22.6	22.0	21.7	23.6	23.8	25.0	23.7	26.7	25.3
	954	23.2	21.1	20.1	21.5	22.2	24.9	23.0	25.2	25.0

Table C-4. Individual Animal Body Weight (g) Data – Females (Continued)

Group	Animal ID	Day								
		1	4	8	11	15	18	22	25	29
E80F	955	21.8	20.1	20.6	23.0	23.8	24.4	23.5	24.8	25.1
	956	22.7	22.1	22.4	24.2	24.6	25.1	23.9	26.4	27.5
	957	22.7	22.0	22.8	24.0	24.5	23.8	23.8	25.8	25.3
	958	24.1	21.3	21.0	22.3	24.4	25.3	24.5	25.4	26.0
	959	21.4	21.1	20.6	22.5	23.0	23.4	23.6	24.0	24.9
	960	23.4	24.5	25.0	25.8	27.4	28.1	27.2	28.3	28.4
E200F	1051	23.6	21.6	18.7	17.8	17.3				
	1052	21.9	18.5	16.3	16.0					
	1053	24.4	19.8	17.5	16.8					
	1054	22.3	16.2	15.5						
	1055	21.7	17.5	16.8	18.2	18.8	20.8	21.5	22.1	22.6
	1056	22.2	18.6	16.0						
	1057	21.0	18.1	16.1	16.4	16.2	16.2	16.6	18.5	18.9
	1058	24.6	21.9	19.9	20.6	20.3	20.8	21.9	23.7	24.6
	1059	20.4	15.9	13.7	13.1					
	1060	22.6	20.6	18.9	18.5	18.0	18.0	18.4	18.9	18.9

Table C-5. Individual TK Animal Body Weight (g) Data – Males

Group	Animal ID	Day					
		1	8	14	15	22	28
CM	111	35.1	35.9		36.5	37.8	39.6
	112	33.0	33.4		35.1	37.0	37.8
	113	35.2	37.2		38.3	40.0	40.4
	114	33.7	34.6		35.0	36.7	37.3
	115	33.8	35.2		35.0	37.0	39.0
NT200M	211	30.7	21.3				
	212	29.0	21.5				
	213	31.0	23.2				
	214	30.5	22.6	19.4			
	215	32.1	22.1	21.8			
	216	32.1	23.7	20.3			
	217	29.1	21.8	20.4			
	218	33.7	24.2				
	219	30.5	22.0	21.6			
	220	32.1	27.7	25.6			
	221	29.5	23.1	24.8			
	222	31.8	23.8	22.4			
	223	28.3	24.2	21.4			
	224	27.0	22.3	22.0			
	225	27.4	24.2	23.4			
	226	28.4	21.2	19.0			
	227	29.8	21.6				
	228	29.4	22.7				
	229	35.3	28.9	26.8			
	230	31.9	25.4	21.5			
	231	31.5	21.4				
	232	31.9	22.8	21.9			
	233	29.5	25.5	26.0			

Table C-5. Individual TK Animal Body Weight (g) Data – Males (Continued)

Group	Animal	Day					
	ID	1	8	14	15	22	28
B2M	311	31.9	32.5	32.4			
	312	29.6	30.6	31.6			
	313	30.5	33.5	28.8			
	314	29.9	33.4	31.3			
	315	29.1	30.3	31.1			
	316	33.7	35.1	34.9			
	317	28.5	31.0	34.0			
	318	32.9	33.7	26.5			
	319	28.0	29.1	31.0			
	320	32.3	33.5	34.5			
	321	30.4	31.4	32.2			
	322	30.9	31.6	32.4			
	323	29.7	30.5	31.6			
	324	28.3	29.6	29.5			
	325	29.1	30.1	30.5			
	326	28.6	33.9	34.8			
	327	30.2	30.6	30.5			
	328	29.0	25.9	30.0			
	329	30.0	31.6		32.9	34.0	34.4
	330	35.5	35.5		37.4	38.4	39.3
	331	29.0	29.6		32.4	33.2	33.6
	332	31.7	32.4		33.6	33.9	34.1
	333	28.7	26.4		30.0	31.7	33.2
B20M	411	32.2	27.0	35.3			
	412	32.3	32.0	32.0			
	413	32.6	30.8	30.5			
	414	31.7	31.5	27.0			
	415	30.5	25.8	31.3			

Table C-5. Individual TK Animal Body Weight (g) Data – Males (Continued)

Group	Animal	Day					
	ID	1	8	14	15	22	28
B20M	416	30.0	24.5	28.5			
	417	31.8	32.4	32.1			
	418	32.4	30.3	33.7			
	419	27.4	25.0	26.5			
	420	27.1	30.8	29.6			
	421	30.2	30.8	30.4			
	422	29.4	27.3	29.2			
	423	30.7	32.0	33.0			
	424	32.4	32.2	31.2			
	425	29.9	29.4	28.6			
	426	29.7	30.0	31.0			
	427	31.2	32.8	32.0			
	428	30.9	32.7	33.0			
	429	27.1	27.4		28.7	29.5	32.1
	430	28.2	28.9		29.3	31.1	31.8
	431	28.5	29.2		30.1	31.4	32.7
	432	33.9	34.5		35.3	36.6	36.9
	433	30.5	32.5		33.2	34.2	34.9
B80M	511	28.4	27.1	29.8			
	512	31.6	27.1	26.6			
	513	30.1	24.8	24.5			
	514	30.3	23.4	24.3			
	515	30.1	26.8	26.5			
	516	30.0	25.1	25.1			
	517	30.3	27.2	28.7			
	518	27.3	24.0	24.7			
	519	31.3	28.1	26.7			
	520	29.6	24.4	23.3			

Table C-5. Individual TK Animal Body Weight (g) Data – Males (Continued)

Group	Animal ID	Day					
		1	8	14	15	22	28
B80M	521	31.9	24.7	23.9			
	522	33.0	26.6	25.5			
	523	31.8	26.1	25.6			
	524	31.9	23.9	22.6			
	525	28.6	25.5	27.0			
	526	32.8	28.6	29.3			
	527	30.6	24.6	24.3			
	528	29.8	23.8	23.5			
	529	28.8	23.8		25.6	27.6	27.7
	530	30.2	23.6		25.4	28.0	30.0
	531	30.8	24.7		23.1		
	532	32.6	25.6		24.0	26.3	29.4
	533	31.9	26.3		27.8	29.5	31.6
E2M	711	29.0	31.0	32.1			
	712	30.9	32.4	34.3			
	713	31.4	32.6	32.6			
	714	29.9	30.6	32.4			
	715	29.8	29.6	30.1			
	716	30.5	32.7	33.8			
	717	33.6	33.7	35.0			
	718	30.3	32.3	32.5			
	719	27.6	29.2	30.0			
	720	28.7	29.7	29.8			
	721	31.4	32.3	32.6			
	722	31.4	32.1	32.7			
	723	31.3	32.3	33.3			
	724	31.3	33.0	34.2			
	725	28.7	30.8	31.0			
	726	26.1	27.8	28.9			

Table C-5. Individual TK Animal Body Weight (g) Data – Males (Continued)

Group	Animal ID	Day					
		1	8	14	15	22	28
E2M	727	33.5	30.9	35.3			
	728	31.9	27.0	34.9			
	729	32.6	34.4		36.0	37.3	37.1
	730	30.9	28.7		32.3	32.3	32.5
	731	32.0	32.4		36.0	35.7	37.0
	732	29.4	29.6		31.4	32.9	35.0
	733	29.2	30.1		31.2	32.5	33.8
E20M	811	28.3	28.8	30.9			
	812	30.6	30.6	30.3			
	813	29.1	29.9	30.6			
	814	30.0	29.9	30.3			
	815	32.0	33.4	33.1			
	816	29.0	30.7	31.5			
	817	27.0	27.3	29.9			
	818	30.0	30.2	31.8			
	819	31.0	32.0	33.1			
	820	30.3	30.9	31.7			
	821	26.2	28.1	27.3			
	822	29.7	29.3	30.9			
	823	31.0	29.7	31.0			
	824	32.9	32.7	33.0			
	825	29.3	29.4	31.0			
	826	29.3	30.0	30.9			
	827	30.9	30.0	30.8			
	828	30.1	30.9	30.1			
	829	28.6	28.1		29.4	29.8	30.8
	830	27.3	27.3		28.3	29.3	30.3
	831	27.6	28.7		30.4	30.6	33.1

Table C-5. Individual TK Animal Body Weight (g) Data – Males (Continued)

Group	Animal ID	Day					
		1	8	14	15	22	28
E20M	832	30.0	29.0		30.2	28.3	30.9
	833	30.5	31.6		34.7	35.4	38.0
E80M	911	30.2	28.6	30.3			
	912	29.3	27.2	29.5			
	913	28.7	23.1	24.2			
	914	27.5	27.3	27.4			
	915	32.5	30.3	29.9			
	916	30.6	29.2	29.4			
	917	28.2	25.3	26.4			
	918	29.3	25.6	27.1			
	919	27.2	25.7	27.3			
	920	30.5	29.1	30.0			
	921	30.9	28.4	28.4			
	922	29.7	29.0	28.0			
	923	26.3	23.9	22.0			
	924	28.5	25.6	28.0			
	925	28.2	25.2	28.2			
	926	29.0	23.9	23.4			
	927	30.2	24.2	26.0			
	928	30.7	25.1	24.7			
	929	32.1	28.2		31.3	33.3	38.1
	930	26.3	24.5		25.9	26.1	27.3
	931	31.1	26.2		27.8	29.6	31.1
	932	29.3	24.5		23.5	27.6	31.3
	933	28.9	25.4		29.1	28.4	31.5
E200M	1011	30.3	21.4				
	1012	30.1					
	1013	30.6					

Table C-5. Individual TK Animal Body Weight (g) Data – Males (Continued)

Group	Animal ID	Day					
		1	8	14	15	22	28
E200M	1014	30.1					
	1015	29.2	21.1				
	1016	29.5					
	1017	30.8	22.4				
	1018	28.9					
	1019	28.9					
	1020	31.3	26.3	22.2			
	1021	30.1					
	1022	29.3	20.6				
	1023	28.0					
	1024	28.6					
	1025	30.1	22.2				
	1026	31.7					
	1027	30.7					
	1028	29.7					
	1029	28.9					
	1030	31.2	23.1				
	1031	30.7	25.0	23.7			
	1032	29.5	23.2	22.0			
	1033	28.8					

Table C-6. Individual TK Animal Body Weight (g) Data – Females

Group	Animal ID	Day					
		1	8	14	15	22	28
CF	161	25.2	27.5		28.0	29.1	30.3
	162	25.7	26.9		28.3	28.2	32.7
	163	26.0	27.5		28.1	28.2	30.6
	164	27.2	27.6		29.5	29.4	29.7
	165	27.7	28.8		31.6	30.8	30.8
NT200F	261	23.4	19.8	19.6			
	262	24.9	21.6	21.6			
	263	24.2	20.4	19.1			
	264	23.1	18.2	17.7			
	265	22.0	23.0	22.6			
	266	25.3	25.6	26.1			
	267	24.7	23.6	22.1			
	268	27.8	26.8	26.1			
	269	23.7	16.3	15.2			
	270	23.2	21.9	21.8			
	271	24.8	22.9	20.8			
	272	24.1	20.3	19.9			
	273	21.6	20.5	20.9			
	274	23.6	19.7	19.6			
	275	25.1	22.9	22.9			
	276	22.9	20.9	21.8			
	277	25.4	19.6	17.1			
	278	27.0	22.6	23.3			
	279	25.3	22.2		22.8	24.8	25.9
	280	21.7	17.3		17.8	20.7	21.4
	281	25.5	20.4		20.0	23.3	24.7
	282	23.5	18.4		18.8	22.8	24.0
	283	23.0	20.1		20.6	22.2	22.9

Table C-6. Individual TK Animal Body Weight (g) Data – Females (Continued)

Group	Animal ID	Day					
		1	8	14	15	22	28
B2F	361	24.1	25.0	25.7			
	362	23.5	25.7	26.0			
	363	24.5	25.5	25.3			
	364	26.3	28.4	29.1			
	365	22.6	24.2	26.7			
	366	23.6	26.1	27.1			
	367	22.9	24.8	25.0			
	368	25.1	25.9	27.1			
	369	21.3	22.9	24.2			
	370	21.5	22.1	23.8			
	371	22.1	24.7	24.8			
	372	22.0	23.5	22.8			
	373	23.7	24.5	25.4			
	374	22.3	24.2	26.9			
	375	22.8	23.6	26.3			
	376	22.7	23.6	24.3			
	377	23.8	25.4	26.6			
	378	23.3	25.0	25.0			
	379	25.2	24.7		27.5	26.9	27.8
	380	23.1	24.2		24.0	26.1	27.4
	381	25.5	26.7		26.0	28.8	27.1
B20F	382	22.8	23.2		26.3	25.6	24.7
	383	25.2	24.9		25.6	26.8	26.0
	461	25.7	24.5	27.7			
	462	24.0	23.2	25.5			
	463	23.6	22.1	25.7			
	464	23.9	23.2	25.3			

Table C-6. Individual TK Animal Body Weight (g) Data – Females (Continued)

Group	Animal	Day					
	ID	1	8	14	15	22	28
B20F	465	23.8	23.6	25.5			
	466	24.4	26.3	27.9			
	467	24.6	25.8	26.3			
	468	24.8	25.9	26.4			
	469	23.0	24.3	25.2			
	470	23.9	25.1	25.7			
	471	23.1	23.7	24.9			
	472	22.7	23.0	24.5			
	473	21.8	22.3	23.5			
	474	21.9	23.3	24.2			
	475	21.6	23.6	25.3			
	476	21.7	22.8	24.3			
	477	24.9	24.3	24.0			
	478	24.8	26.0	26.5			
	479	24.3	26.3		26.7	29.4	28.7
	480	25.4	24.2		26.2	27.0	25.8
	481	22.5	22.3		23.5	24.6	24.3
	482	22.8	23.1		24.2	26.9	26.3
	483	23.8	23.4		26.1	25.9	28.3
B80F	561	24.7	22.6	24.8			
	562	22.8	21.2	23.5			
	563	22.9	22.4	24.4			
	564	22.0	21.7	23.0			
	565	23.1	23.5	26.2			
	566	22.3	22.9	24.6			
	567	20.9	21.0	22.8			
	568	22.7	20.7	22.7			
	569	25.8	25.3	26.1			

Table C-6. Individual TK Animal Body Weight (g) Data – Females (Continued)

Group	Animal ID	Day					
		1	8	14	15	22	28
B80F	570	25.4	23.6	24.6			
	571	22.5	22.9	25.2			
	572	23.8	23.2	24.0			
	573	20.3	21.8	23.0			
	574	22.7	24.3	23.9			
	575	22.6	23.9	25.7			
	576	22.5	22.8	23.4			
	577	25.4	26.4	25.8			
	578	24.8	25.1	24.9			
	579	25.3	25.2		25.3	25.9	26.5
	580	24.6	22.9		24.6	25.3	28.2
	581	24.6	23.5		25.7	26.3	27.7
	582	25.7	24.9		26.8	26.6	28.4
	583	23.6	21.9		23.2	23.6	24.2
B200F	661	21.3					
	662	19.2					
	663	18.1					
	664	21.0					
	665	22.7					
	666	23.0					
	667	22.4					
	668	22.4					
	669	23.3					
	670	23.0					
	671	20.1					
	672	21.5					
	673	23.7					
	674	24.5					

Table C-6. Individual TK Animal Body Weight (g) Data – Females (Continued)

Group	Animal ID	Day					
		1	8	14	15	22	28
B200F	675	24.4					
	676	21.4					
	677	25.8					
	678	25.0					
	679	24.8					
	680	27.0					
	681	26.1					
	682	24.9					
	683	22.3					
E2F	761	21.9	21.3	21.2			
	762	23.7	25.6	25.9			
	763	24.6	25.7	26.0			
	764	25.6	26.1	27.6			
	765	23.5	21.9	24.4			
	766	22.6	19.7	21.8			
	767	24.3	20.7	21.9			
	768	20.7	18.3	19.9			
	769	22.8	23.4	25.2			
	770	21.8	21.2	22.6			
	771	23.2	23.3	24.6			
	772	23.6	23.7	24.1			
	773	23.2	22.0	26.5			
	774	24.0	22.8	26.2			
	775	23.6	22.4	26.4			
	776	23.0	21.8	24.9			
	777	20.1	20.5	22.4			
	778	21.9	21.9	24.3			
	779	23.3	23.3		26.3	24.9	29.0

Table C-6. Individual TK Animal Body Weight (g) Data – Females (Continued)

Group	Animal	Day					
	ID	1	8	14	15	22	28
E2F	780	23.7	24.3		26.7	25.6	27.7
	781	24.1	23.9		25.4	25.4	25.2
	782	21.9	21.7		23.3	22.6	22.3
	783	22.2	21.4		24.0	22.4	23.1
E20F	861	23.3	21.5	24.8			
	862	21.8	20.2	23.9			
	863	23.7	21.5	24.8			
	864	22.8	22.5	25.7			
	865	22.4	21.7	22.7			
	866	26.4	25.0	25.9			
	867	21.6	20.3	21.3			
	868	23.6	22.3	22.4			
	869	24.1	21.9	25.3			
	870	26.5	24.9	26.3			
	871	26.1	24.8	26.8			
	872	25.1	23.8	27.6			
	873	24.1	23.4	26.4			
	874	24.6	24.4	28.5			
	875	23.7	23.3	26.4			
	876	21.6	21.4	23.8			
	877	21.0	21.2	22.1			
	878	24.3	26.6	28.8			
	879	24.0	24.8		24.2	26.1	27.7
	880	21.5	21.9		22.8	22.4	23.6
	881	26.5	24.7		26.6	26.1	28.2
	882	24.5	24.0		27.9	27.2	27.8
	883	23.6	22.9		25.9	25.5	26.7

Table C-6. Individual TK Animal Body Weight (g) Data – Females (Continued)

Group	Animal	Day					
	ID	1	8	14	15	22	28
E80F	961	25.9	25.4	26.2			
	962	24.5	22.8	25.1			
	963	23.9	23.3	25.7			
	964	23.6	21.9	25.5			
	965	22.1	23.3	24.4			
	966	21.5	20.4	21.4			
	967	25.9	24.0	24.9			
	968	22.4	20.9	21.6			
	969	24.0	23.6	22.1			
	970	24.1	21.4	23.0			
	971	26.1	25.5	26.7			
	972	24.6	23.6	23.6			
	973	23.3	24.7	26.0			
	974	20.3	21.2	23.4			
	975	22.9	22.8	25.4			
	976	21.7	21.7	23.7			
	977	23.8	22.5	24.4			
	978	21.9	21.5	22.5			
	979	25.7	25.3		24.8	26.3	25.9
	980	22.5	21.1		23.3	23.7	24.7
	981	24.6	23.7		26.2	24.7	25.9
	982	24.2	20.6		25.5	24.4	25.7
	983	27.1	24.2		26.6	25.7	30.7
E200F	1061	25.8	20.1	25.0			
	1062	23.7	18.5	17.6			
	1063	23.9	23.4	19.1			
	1064	22.7	20.0	22.0			
	1065	23.0	18.6	18.5			

Table C-6. Individual TK Animal Body Weight (g) Data – Females (Continued)

Group	Animal ID	Day					
		1	8	14	15	22	28
E200F	1066	24.1	18.6	18.5			
	1067	24.8	21.3	19.0			
	1068	23.8	18.3	17.0			
	1069	22.9	20.0	18.5			
	1070	21.2	19.7	19.6			
	1071	22.6	22.7	25.0			
	1072	20.4	15.5	15.4			
	1073	23.4	20.7	21.8			
	1074	22.8	21.4	22.0			
	1075	23.6	21.6	21.0			
	1076	23.0	16.8	15.0			
	1077	23.0	21.2	19.4			
	1078	21.6	19.0	16.9			
	1079	24.9	20.7		19.5	25.9	29.2
	1080	20.6	17.9		16.3	18.6	20.6
	1081	22.9	17.8		17.3	18.3	20.2
	1082	26.0	20.0		18.7	19.8	22.2
	1083	23.6	20.0		19.1	20.0	21.0

Table C-7. Individual Animal Feed Consumed (g) per Day Data – Males

Group	Animal	Day							
	ID	4	8	11	15	18	22	25	29
CM	101	4.5	4.6	4.1	3.9	4.8	4.4	4.1	5.1
	102	4.6	4.8	4.9	4.6	5.1	4.2	5.9	4.8
	103	4.3	4.6	4.7	4.5	5.3	4.8	5.1	3.9
	104	4.6	4.8	5.3	4.7	5.0	4.9	4.5	5.0
	105	5.1	4.7	5.1	5.1	5.0	4.6	4.7	3.8
	106	4.6	4.5	4.9	4.4	4.5	5.1	4.8	6.0
	107	4.4	4.9	4.9	3.1	5.6	4.0	4.6	4.1
	108	4.4	5.2	4.8	4.7	4.9	4.8	4.8	5.6
	109	5.1	4.6	5.4	5.0	5.3	5.2	5.1	4.8
	110	5.4	5.7	5.2	5.6	5.4	5.4	5.3	5.6
NT200M	201	3.3	1.5	2.5	1.5	1.3			
	202	3.2	1.4	3.3	2.1	3.4	2.4	3.5	3.0
	203	10.3	1.5	5.5	2.5	7.5	3.2	5.2	3.5
	204	2.9	1.5	3.8	2.4	3.3		3.5	3.8
	205	3.1	1.4	2.8	1.4	2.5	0.7		
	206	2.3	1.5	2.6	1.2	2.2	1.7	3.6	2.2
	207	3.3	2.6	3.1	1.6	2.3	1.2	1.4	1.0
	208	5.3	1.1						
	209	4.0	1.1	3.7	1.9	5.3	1.2	3.3	3.4
	210	1.1	1.0	0.9					
B2M	301	4.4	4.3	4.5	3.8	4.7	4.4	4.8	4.4
	302	4.6	3.9	4.6	4.4	4.8	4.5	4.4	4.4
	303	4.7	5.3	4.7	4.7	4.8	4.4	4.9	5.2
	304	6.6	5.5	6.0	5.4	5.0	5.6	5.0	6.0
	305	4.4	5.3	5.0	5.1	5.2	4.1	4.6	4.7
	306	5.0	4.4	5.0	4.0	4.9	4.8	4.5	5.1
	307	4.7	4.0	4.7	4.0	4.4	3.6	4.5	4.5
	308	4.4	4.9	4.9	4.5	3.5	4.4	4.7	3.8

Table C-7. Individual Animal Feed Consumed (g) per Day Data – Males (Continued)

Group	Animal ID	Day							
		4	8	11	15	18	22	25	29
B2M	309	4.4	4.1	4.8	3.9	5.8	4.7	4.5	4.8
	310	4.4	4.4	5.5	4.3	4.2	5.0	5.0	3.8
B20M	401	3.8	4.4	5.2	5.1	5.5	5.3	5.3	5.0
	402	4.0	4.2	5.1	4.0	4.6	4.3	3.9	5.3
	403	4.3	5.0	4.7	4.8	4.8	4.8	4.7	3.9
	404	4.0	4.7	5.0	4.8	5.0	4.7	4.9	4.3
	405	4.8	5.2	5.2	5.1	5.3	4.7	5.2	4.3
	406	3.8	4.6	4.3	4.1	4.4	3.8	3.5	4.2
	407	4.4	5.2	5.6	5.0	5.3	5.3	4.5	5.5
	408	3.1	4.7	4.6	4.4	3.7	4.2	4.3	4.8
	409	5.2	4.6	4.7	4.8	4.4	4.8	5.3	3.9
	410	5.8	4.9	5.7	5.1	5.1	5.0	4.4	5.2
B80M	501	4.9	3.8	5.0	4.5	4.1	4.2	4.2	4.4
	502	3.3	3.2	4.0	4.2	4.6	4.5	4.3	4.5
	503	4.7	2.5	4.0	2.8	3.6	3.1	3.6	3.5
	504	3.2	3.0	3.6	3.2	3.9	3.4	3.5	3.8
	505	5.3	2.3	6.3	3.5	5.1	3.8	4.6	4.0
	506	2.9	2.2	3.8	3.5	4.7	4.0	4.2	4.1
	507	2.8	3.4	4.2	4.4	4.3	4.4	4.3	4.5
	508	5.2	3.6	4.7	4.4	5.6	4.0	4.2	4.9
	509	5.4	3.5	6.1	2.5	3.9	3.4	4.3	3.5
	510	6.3	1.9	4.4	3.0	4.4	3.3	3.7	3.7
B200M	601	1.6	0.3						
	602	5.1	0.9						
	603	7.7	0.5						
	604	3.6	0.3						
	605	1.7	1.1						
	606	2.9	0.4						

Table C-7. Individual Animal Feed Consumed (g) per Day Data – Males (Continued)

Group	Animal ID	Day							
		4	8	11	15	18	22	25	29
B200M	607	2.7	1.1						
	608	3.4							
	609	5.7	1.6						
	610	3.3	1.9						
E2M	701	4.7	4.8	5.5	4.9	4.4	5.4	5.5	5.4
	702	5.3	5.7	5.5	4.4	5.6	4.9	5.5	4.7
	703	5.5	5.4	5.0	4.8	4.8	4.7	6.1	4.3
	704	6.4	6.0	6.6	5.7	6.7	5.8	6.6	6.0
	705	4.8	5.2	5.3	5.0	5.4	5.4	5.5	5.2
	706	4.8	5.8	5.0	4.5	5.0	4.7	4.6	5.4
	707	6.5	5.8	5.9	6.2	4.9	4.8	6.3	5.9
	708	5.6	4.8	5.4	4.6	5.9	4.6	5.4	5.3
	709	5.5	5.0	5.6	5.0	5.8	5.0	5.4	5.7
	710	4.1	4.7	4.2	4.2	4.6	4.3	3.4	5.3
E20M	801	4.5	4.9	5.0	5.0	5.1	4.9	5.0	5.0
	802	4.4	4.5	5.4	4.4	5.0	5.1	4.7	5.2
	803	4.3	5.4	5.5	4.5	6.1	4.2	5.7	4.4
	804	3.9	5.0	5.2	4.0	6.9	5.2	3.6	5.8
	805	5.2	5.7	5.9	5.9	6.4	5.1	6.8	5.6
	806	4.7	4.3	5.3	3.9	4.8	4.8	4.7	4.5
	807	4.7	4.7	6.0	4.5	5.0	4.4	5.2	5.0
	808	3.2	2.8	4.0	4.9	3.8	5.7	4.6	4.4
	809	3.4	4.0	5.1	4.3	5.1	5.1	3.7	5.4
	810	4.6	3.9	5.7	4.8	5.3	5.0	5.4	5.0
E80M	901	5.5	3.1	6.1	3.5	4.6	4.0	4.5	3.8
	902	5.4	3.3	4.8	3.9	5.1	3.7	5.2	3.7
	903	3.8	2.3	3.1	3.3	3.9	4.1	4.4	4.3
	904	6.1	0.7	5.3	4.1	4.1	4.9	4.4	4.8

Table C-7. Individual Animal Feed Consumed (g) per Day Data – Males (Continued)

Group	Animal ID	Day							
		4	8	11	15	18	22	25	29
E80M	905	4.4	3.0	4.5	4.1	5.0	4.0	4.3	4.6
	906	5.3	4.2	6.1	3.7	5.2	3.8	5.4	4.3
	907	6.2	3.9	6.9	3.7	5.3	4.8	6.0	5.3
	908	4.1	3.7	7.1	4.7	5.6	4.2	8.6	4.7
	909	5.8	2.8	4.7	2.5	4.7	3.3	4.1	3.8
	910	7.4	2.3	4.3	3.0	4.6	3.7	4.4	4.1
E200M	1001	2.9	0.4						
	1002	6.8	2.3	7.8	4.3				
	1003	6.6	0.9	6.3	2.7				
	1004	4.5	0.2						
	1005	5.3	0.5	3.2	1.6				
	1006	2.3	0.8	2.4					
	1007	4.4	0.6						
	1008	7.7	2.6	1.5					
	1009	6.8	0.7						
	1010	5.0	1.5						

Table C-8. Individual Animal Feed Consumed (g) per Day Data – Females

Group	Animal	Day							
	ID	4	8	11	15	18	22	25	29
CF	151	4.2	2.8	5.3	2.8	4.3	3.8	4.1	3.6
	152	4.2	2.8	5.3	2.8	4.3	3.8	4.1	3.6
	153	4.2	2.8	5.3	2.8	4.3	3.8	4.1	3.6
	154	4.2	2.8	5.3	2.8	4.3	3.8	4.1	3.6
	155	4.1	3.6	4.8	3.9	4.2	4.3	3.7	4.4
	156	4.1	3.6	4.8	3.9	4.2	4.3	3.7	4.4
	157	4.1	3.6	4.8	3.9	4.2	4.3	3.7	4.4
	158	4.1	3.6	4.8	3.9	4.2	4.3	3.7	4.4
	159	6.8	4.6	4.9	4.9	6.1	3.8	5.5	6.2
	160	6.8	4.6	4.9	4.9	6.1	3.8	5.5	6.2
NT200F	251	3.9	2.5	2.5	2.5	3.4	2.4	3.0	3.1
	252	3.9	2.5	2.5	2.5	3.4	2.4	3.0	3.1
	253	3.9	2.5	2.5	2.5	3.4	2.4	3.0	3.1
	254	3.9	2.5	2.5	2.5	3.4	2.4	3.0	3.1
	255	5.4	1.0	5.4	0.9	2.1	1.4	2.0	2.0
	256	5.4	1.0	5.4	0.9	2.1	1.4	2.0	2.0
	257	5.4	1.0	5.4	0.9	2.1	1.4	2.0	2.0
	258	5.4	1.0	5.4	0.9	2.1	1.4	2.0	2.0
	259	4.8	3.5	3.7	3.0	5.9	2.8	4.1	3.9
	260	4.8	3.5	3.7	3.0	5.9	2.8	4.1	3.9
B2F	351	3.7	3.0	3.9	3.8	3.8	4.1	4.6	3.6
	352	3.7	3.0	3.9	3.8	3.8	4.1	4.6	3.6
	353	3.7	3.0	3.9	3.8	3.8	4.1	4.6	3.6
	354	3.7	3.0	3.9	3.8	3.8	4.1	4.6	3.6
	355	4.1	3.7	5.1	4.3	4.5	3.8	4.3	4.0
	356	4.1	3.7	5.1	4.3	4.5	3.8	4.3	4.0
	357	4.1	3.7	5.1	4.3	4.5	3.8	4.3	4.0
	358	4.1	3.7	5.1	4.3	4.5	3.8	4.3	4.0

Table C-8. Individual Animal Feed Consumed (g) per Day Data – Females (Continued)

Group	Animal ID	Day							
		4	8	11	15	18	22	25	29
B2F	359	4.0	4.5	3.7	4.3	4.7	1.6	5.3	4.1
	360	4.0	4.5	3.7	4.3	4.7	1.6	5.3	4.1
B20F	451	2.9	3.4	4.0	3.8	4.2	4.4	3.8	3.7
	452	2.9	3.4	4.0	3.8	4.2	4.4	3.8	3.7
	453	2.9	3.4	4.0	3.8	4.2	4.4	3.8	3.7
	454	2.9	3.4	4.0	3.8	4.2	4.4	3.8	3.7
	455	4.2	4.1	4.3	4.1	3.3	3.5	3.6	4.2
	456	4.2	4.1	4.3	4.1	3.3	3.5	3.6	4.2
	457	4.2	4.1	4.3	4.1	3.3	3.5	3.6	4.2
	458	4.2	4.1	4.3	4.1	3.3	3.5	3.6	4.2
	459	5.0	4.9	4.3	4.2	5.8	2.9	4.2	4.4
	460	5.0	4.9	4.3	4.2	5.8	2.9	4.2	4.4
B80F	551	4.0	2.7	3.5	3.4	4.3	3.4	3.8	3.3
	552	4.0	2.7	3.5	3.4	4.3	3.4	3.8	3.3
	553	4.0	2.7	3.5	3.4	4.3	3.4	3.8	3.3
	554	4.0	2.7	3.5	3.4	4.3	3.4	3.8	3.3
	555	4.2	2.2	3.0	3.2	4.6	2.1	3.8	2.8
	556	4.2	2.2	3.0	3.2	4.6	2.1	3.8	2.8
	557	4.2	2.2	3.0	3.2	4.6	2.1	3.8	2.8
	558	4.2	2.2	3.0	3.2	4.6	2.1	3.8	2.8
	559	6.0	4.0	3.0	3.9	4.9	3.5	3.8	4.0
	560	6.0	4.0	3.0	3.9	4.9	3.5	3.8	4.0
B200F	651	5.8	1.7	3.4					
	652	5.8	1.7	3.4					
	653	5.8	1.7	3.4					
	654	5.8	1.7	3.4					
	655	6.8	1.2	2.4					
	656	6.8	1.2	2.4					

Table C-8. Individual Animal Feed Consumed (g) per Day Data – Females (Continued)

Group	Animal ID	Day							
		4	8	11	15	18	22	25	29
B200F	657	6.8	1.2	2.4					
	658	6.8	1.2	2.4					
	659	12.9	1.7	3.0					
	660	12.9	1.7	3.0					
E2F	751	4.8	3.7	4.9	4.0	5.4	3.2	5.9	2.0
	752	4.8	3.7	4.9	4.0	5.4	3.2	5.9	2.0
	753	4.8	3.7	4.9	4.0	5.4	3.2	5.9	2.0
	754	4.8	3.7	4.9	4.0	5.4	3.2	5.9	2.0
	755	5.1	3.6	4.8	3.7	5.4	3.6	4.4	3.1
	756	5.1	3.6	4.8	3.7	5.4	3.6	4.4	3.1
	757	5.1	3.6	4.8	3.7	5.4	3.6	4.4	3.1
	758	5.1	3.6	4.8	3.7	5.4	3.6	4.4	3.1
	759	3.7	3.8	4.5	3.2	4.6	3.4	4.3	4.0
	760	3.7	3.8	4.5	3.2	4.6	3.4	4.3	4.0
E20F	851	3.5	4.1	4.1	4.1	4.4	3.4	4.1	3.7
	852	3.5	4.1	4.1	4.1	4.4	3.4	4.1	3.7
	853	3.5	4.1	4.1	4.1	4.4	3.4	4.1	3.7
	854	3.5	4.1	4.1	4.1	4.4	3.4	4.1	3.7
	855	3.8	3.8	4.5	3.9	5.2	3.5	4.8	3.3
	856	3.8	3.8	4.5	3.9	5.2	3.5	4.8	3.3
	857	3.8	3.8	4.5	3.9	5.2	3.5	4.8	3.3
	858	3.8	3.8	4.5	3.9	5.2	3.5	4.8	3.3
	859	4.8	5.3	5.0	4.6	7.5	4.2	5.0	5.4
	860	4.8	5.3	5.0	4.6	7.5	4.2	5.0	5.4
E80F	951	3.9	3.1	3.3	3.2	3.4	3.4	3.8	3.9
	952	3.9	3.1	3.3	3.2	3.4	3.4	3.8	3.9
	953	3.9	3.1	3.3	3.2	3.4	3.4	3.8	3.9
	954	3.9	3.1	3.3	3.2	3.4	3.4	3.8	3.9

Table C-8. Individual Animal Feed Consumed (g) per Day Data – Females (Continued)

Group	Animal ID	Day							
		4	8	11	15	18	22	25	29
E80F	955	3.9	3.9	3.8	3.3	5.0	2.7	3.8	4.1
	956	3.9	3.9	3.8	3.3	5.0	2.7	3.8	4.1
	957	3.9	3.9	3.8	3.3	5.0	2.7	3.8	4.1
	958	3.9	3.9	3.8	3.3	5.0	2.7	3.8	4.1
	959	4.9	3.7	4.5	3.2	6.1	3.1	5.0	3.0
	960	4.9	3.7	4.5	3.2	6.1	3.1	5.0	3.0
E200F	1051	5.6	1.3	2.2	2.8	11.6			
	1052	5.6	1.3	2.2	2.8				
	1053	5.6	1.3	2.2					
	1054	5.6	1.3	2.2					
	1055	4.5	2.2	2.5	2.2	4.9	1.8	2.5	3.5
	1056	4.5	2.2	2.5					
	1057	4.5	2.2	2.5	2.2	4.9	1.8	2.5	3.5
	1058	4.5	2.2	2.5	2.2	4.9	1.8	2.5	3.5
	1059	5.5	1.9	2.1	2.4				
	1060	5.5	1.9	2.1	2.4	3.2	2.6	2.8	2.6

Table C-9. Individual Animal Hematology Data – Males

Group	Animal ID	Day	Red Blood Cell Count (10⁶/μL)	Hemoglobin (g/dL)	Hematocrit (%)
CM	102	30	10.62	15.8	48.3
	104	30	10.40	16.4	49.9
	106	31	10.85	16.0	49.3
	108	32	10.17	15.6	48.4
	110	32	12.32	17.9	54.8
NT200M	202	30	11.19	16.8	50.0
	204	30	10.40	15.9	48.5
	206	31	9.64	13.6	41.7
B2M	302	30	10.56	16.7	49.5
	304	30	10.80	16.4	50.4
	306	31	10.19	15.7	46.4
	308	32	9.93	15.2	45.7
B20M	402	30	10.27	16.6	49.1
	404	30	11.27	17.3	51.1
	406	31	10.65	17.3	49.7
	408	32	10.56	15.4	47.3
	410	32	9.73	16.2	47.4
B80M	502	30	11.12	17.5	53.5
	504	30	11.05	16.9	50.6
	506	31	10.86	16.5	49.4
	508	32	9.78	14.3	45.1
	510	32	11.07	15.8	49.8
E2M	702	30	11.01	17.0	52.3
	704	30	10.02	15.7	48.1
	706	31	10.27	16.9	48.9
	708	32	10.77	15.9	47.5
	710	32	10.73	16.6	50.2

Table C-9. Individual Animal Hematology Data – Males (Continued)

Group	Animal ID	Day	Red Blood Cell Count (10⁶/μL)	Hemoglobin (g/dL)	Hematocrit (%)
E20M	802	30	10.96	17.5	51.6
	804	30	10.39	15.5	48.3
	806	31	10.64	15.8	47.7
	808	32	10.03	15.0	45.3
	810	32	11.54	17.4	52.5
E80M	902	30	11.27	16.7	49.9
	904	30	10.54	15.4	47.7
	906	31	11.82	17.1	53.6
	908	32	9.84	14.7	45.3
	910	32	10.17	15.3	46.5

Table C-9. Individual Animal Hematology Data – Males (Continued)

Group	Animal ID	Day	Mean Corpuscular Volume (fL)	Mean Corpuscular Hemoglobin (pg)	Mean Corpuscular Hemoglobin Concentration (g/dL)
CM	102	30	45.5	14.9	32.7
	104	30	48.0	15.8	32.8
	106	31	45.4	14.7	32.4
	108	32	47.6	15.3	32.2
	110	32	44.5	14.5	32.6
NT200M	202	30	44.7	15.0	33.6
	204	30	46.6	15.3	32.8
	206	31	43.3	14.2	32.7
B2M	302	30	46.8	15.8	33.7
	304	30	46.7	15.2	32.6
	306	31	45.5	15.4	33.9
	308	32	46.0	15.3	33.2
B20M	402	30	47.8	16.2	33.9
	404	30	45.4	15.4	33.8
	406	31	46.6	16.2	34.8
	408	32	44.8	14.6	32.6
	410	32	48.8	16.6	34.1
B80M	502	30	48.1	15.7	32.7
	504	30	45.8	15.3	33.3
	506	31	45.5	15.2	33.4
	508	32	46.1	14.6	31.7
	510	32	45.0	14.3	31.8
E2M	702	30	47.5	15.4	32.5
	704	30	48.0	15.7	32.7
	706	31	47.6	16.5	34.6
	708	32	44.1	14.8	33.4
	710	32	46.8	15.5	33.1

Table C-9. Individual Animal Hematology Data – Males (Continued)

Group	Animal ID	Day	Mean Corpuscular Volume (fL)	Mean Corpuscular Hemoglobin (pg)	Mean Corpuscular Hemoglobin Concentration (g/dL)
E20M	802	30	47.1	16.0	34.0
	804	30	46.5	14.9	32.0
	806	31	44.8	14.8	33.1
	808	32	45.2	14.9	33.0
	810	32	45.5	15.1	33.2
E80M	902	30	44.3	14.8	33.4
	904	30	45.2	14.6	32.3
	906	31	45.4	14.4	31.8
	908	32	46.0	14.9	32.4
	910	32	45.8	15.1	33.0

Table C-9. Individual Animal Hematology Data – Males (Continued)

Group	Animal ID	Day	Platelet Count (10³/μL)	Reticulocytes (10³/μL)
CM	102	30	809	344.0
	104	30	1141	315.4
	106	31	1191	370.6
	108	32	917	381.3
	110	32	352	379.9
NT200M	202	30	660	381.3
	204	30	882	482.1
	206	31	1763	885.1
B2M	302	30	759	366.6
	304	30	1264	302.7
	306	31	1005	334.2
	308	32	991	436.5
B20M	402	30	621	354.5
	404	30	694	325.0
	406	31	720	359.2
	408	32	1148	363.7
	410	32	907	313.2
B80M	502	30	1051	463.8
	504	30	688	413.3
	506	31	708	319.2
	508	32	1157	406.9
	510	32	1081	446.8
E2M	702	30	678	306.4
	704	30	1201	313.1
	706	31	661	329.1
	708	32	1047	395.6
	710	32	1123	341.3

Table C-9. Individual Animal Hematology Data – Males (Continued)

Group	Animal ID	Day	Platelet Count (10³/μL)	Reticulocytes (10³/μL)
E20M	802	30	1015	381.3
	804	30	1147	371.0
	806	31	985	263.3
	808	32	906	415.4
	810	32	945	353.9
E80M	902	30	607	360.9
	904	30	739	471.0
	906	31	596	451.3
	908	32	1078	389.9
	910	32	830	477.9

Table C-10. Individual Animal Hematology Data – Females

Group	Animal ID	Day	Red Blood Cell Count (10⁶/μL)	Hemoglobin (g/dL)	Hematocrit (%)
CF	152	30	10.91	17.0	50.6
	154	31	10.60	15.9	48.8
	156	31	11.33	17.6	52.3
	158	32	10.17	16.3	47.9
	160	32	10.09	15.2	46.7
NT200F	252	30	10.74	16.2	48.1
	254	31	11.37	18.8	56.2
	256	31	11.29	16.8	49.0
	258	32	11.27	16.4	46.4
	260	32	9.72	14.9	44.0
B2F	352	30	11.06	17.3	50.2
	354	31	10.89	17.8	51.1
	356	31	10.04	15.2	47.0
	358	32	9.21	14.9	45.5
	360	32	10.69	16.7	49.0
B20F	452	30	10.35	15.0	47.3
	454	31	10.64	16.2	48.6
	456	31	9.31	14.9	45.4
	458	32	10.81	16.8	50.0
	460	32	9.58	14.7	45.1
B80F	552	30	11.73	18.8	55.5
	554	31	10.37	15.9	47.3
	556	31	11.63	18.7	56.1
	558	32	10.53	16.3	49.2
	560	32	10.38	16.7	50.6
E2F	754	31	11.01	17.5	50.1
	756	31	10.41	17.0	49.3
	758	32	10.84	16.3	48.7
	760	32	11.43	17.3	51.6

Table C-10. Individual Animal Hematology Data – Females (Continued)

Group	Animal ID	Day	Red Blood Cell Count (10⁶/μL)	Hemoglobin (g/dL)	Hematocrit (%)
E20F	852	30	10.34	15.8	47.5
	854	31	11.77	19.2	55.2
	856	31	9.99	16.2	47.2
	858	32	9.79	15.2	47.4
	860	32	11.27	17.9	51.7
E80F	952	30	10.30	16.0	47.3
	954	31	11.10	16.8	51.5
	956	31	11.00	17.4	50.7
	958	32	10.89	17.4	51.8
	960	32	11.29	16.7	50.2
E200F	1058	32	10.42	15.8	49.5
	1060	32	11.59	15.6	48.1

Table C-10. Individual Animal Hematology Data – Females (Continued)

Group	Animal ID	Day	Mean Corpuscular Volume (fL)	Mean Corpuscular Hemoglobin (pg)	Mean Corpuscular Hemoglobin Concentration (g/dL)
CF	152	30	46.4	15.6	33.6
	154	31	46.1	15.0	32.6
	156	31	46.2	15.5	33.6
	158	32	47.1	16.0	34.0
	160	32	46.3	15.1	32.5
NT200F	252	30	44.7	15.0	33.6
	254	31	49.4	16.5	33.5
	256	31	43.4	14.9	34.4
	258	32	41.2	14.5	35.3
	260	32	45.3	15.3	33.8
B2F	352	30	45.4	15.6	34.4
	354	31	46.9	16.4	34.9
	356	31	46.8	15.1	32.2
	358	32	49.4	16.2	32.8
	360	32	45.8	15.6	34.1
B20F	452	30	45.7	14.5	31.8
	454	31	45.6	15.2	33.3
	456	31	48.8	16.0	32.7
	458	32	46.2	15.6	33.7
	460	32	47.1	15.3	32.5
B80F	552	30	47.3	16.0	33.9
	554	31	45.6	15.3	33.6
	556	31	48.3	16.1	33.3
	558	32	46.7	15.5	33.1
	560	32	48.7	16.1	33.1

Table C-10. Individual Animal Hematology Data – Females (Continued)

Group	Animal ID	Day	Mean Corpuscular Volume (fL)	Mean Corpuscular Hemoglobin (pg)	Mean Corpuscular Hemoglobin Concentration (g/dL)
E2F	754	31	45.5	15.9	35.0
	756	31	47.3	16.4	34.6
	758	32	44.9	15.0	33.4
	760	32	45.1	15.2	33.6
E20F	852	30	46.0	15.3	33.2
	854	31	46.9	16.3	34.7
	856	31	47.2	16.2	34.3
	858	32	48.4	15.5	32.1
	860	32	45.8	15.9	34.6
E80F	952	30	45.9	15.6	33.9
	954	31	46.4	15.2	32.7
	956	31	46.1	15.8	34.3
	958	32	47.5	15.9	33.5
	960	32	44.4	14.8	33.3
E200F	1058	32	47.5	15.1	31.9
	1060	32	41.5	13.5	32.5

Table C-10. Individual Animal Hematology Data – Females (Continued)

Group	Animal ID	Day	Platelet Count (10³/μL)	Reticulocytes (10³/μL)
CF	152	30	876	254.7
	154	31	929	291.2
	156	31	592	273.6
	158	32	249	409.4
	160	32	153	344.1
NT200F	252	30	1200	638.6
	254	31	389	500.4
	256	31	638	548.0
	258	32	694	105.6
	260	32	1074	434.1
B2F	352	30	927	253.1
	354	31	624	316.3
	356	31	1323	245.5
	358	32	1239	526.1
	360	32	1024	323.0
B20F	452	30	1051	350.7
	454	31	629	244.3
	456	31	1061	372.9
	458	32	692	385.2
	460	32	1356	342.6
B80F	552	30	555	410.0
	554	31	1053	386.5
	556	31	623	239.0
	558	32	839	214.1
	560	32	639	375.0
E2F	754	31	1019	110.7
	756	31	726	260.5
	758	32	922	344.1
	760	32	592	342.0

Table C-10. Individual Animal Hematology Data – Females (Continued)

Group	Animal ID	Day	Platelet Count (10³/μL)	Reticulocytes (10³/μL)
E20F	852	30	1300	336.3
	854	31	468	213.1
	856	31	1108	312.6
	858	32	1032	559.2
	860	32	540	403.2
E80F	952	30	736	422.1
	954	31	485	388.8
	956	31	850	538.8
	958	32	463	472.4
	960	32	993	340.0
E200F	1058	32	1217	523.6
	1060	32	1167	778.8

Table C-11. Individual Animal Absolute WBC Differential Count Data – Males

Group	White Blood Cell				
	Animal ID	Day	Count (10 ³ /μL)	Neutrophils (10 ³ /μL)	Total Lymphocytes (10 ³ /μL)
CM	102	30	3.47	0.90	2.53
	104	30	6.67	1.60	5.07
	106	31	6.97	1.57	4.97
	108	32	2.72	0.19	2.50
	110	32	4.74	1.04	3.56
NT200M	202	30	0.61	0.17	0.43
	204	30	4.65	0.51	4.09
	206	31	4.74	4.03	0.71
B2M	302	30	4.82	0.43	4.24
	304	30	6.33	1.18	4.87
	306	31	2.91	0.67	2.24
	308	32	3.63	0.69	2.90
B20M	402	30	3.40	0.32	2.94
	404	30	3.07	0.40	2.67
	406	31	4.14	0.29	3.85
	408	32	3.52	0.60	2.85
	410	32	4.63	1.16	3.43
B80M	502	30	2.38	0.25	2.06
	504	30	4.99	0.54	4.18
	506	31	4.45	0.58	3.87
	508	32	1.24	0.21	1.02
	510	32	3.38	0.38	2.85
E2M	702	30	2.70	0.38	2.27
	704	30	2.37	0.38	1.97
	706	31	3.30	0.92	2.38
	708	32	3.26	0.68	2.54
	710	32	1.98	0.36	1.56

Table C-11. Individual Animal Absolute WBC Differential Count Data – Males (Continued)

Group	White Blood Cell				
	Animal ID	Day	Count (10³/μL)	Neutrophils (10³/μL)	Total Lymphocytes (10³/μL)
E20M	802	30	5.85	1.76	4.04
	804	30	3.51	0.95	2.56
	806	31	5.29	1.11	4.07
	808	32	2.65	0.40	2.20
	810	32	4.31	0.84	3.33
E80M	902	30	3.51	0.63	2.74
	904	30	2.25	0.18	2.07
	906	31	2.17	0.48	1.65
	908	32	4.45	1.02	3.03
	910	32	1.64	0.21	1.43

Table C-11. Individual Animal Absolute WBC Differential Count Data – Males (Continued)

Group	Animal ID	Day	Monocytes (10³/μL)	Eosinophils (10³/μL)	Basophils (10³/μL)
CM	102	30	0.03	0.00	0.00
	104	30	0.00	0.00	0.00
	106	31	0.06	0.35	0.03
	108	32	0.00	0.03	0.00
	110	32	0.00	0.14	0.00
NT200M	202	30	0.01	0.00	0.00
	204	30	0.00	0.05	0.00
	206	31	0.00	0.00	0.00
B2M	302	30	0.10	0.05	0.00
	304	30	0.04	0.23	0.02
	306	31	0.00	0.00	0.00
	308	32	0.04	0.00	0.00
B20M	402	30	0.04	0.09	0.01
	404	30	0.00	0.00	0.00
	406	31	0.00	0.00	0.00
	408	32	0.04	0.04	0.00
	410	32	0.00	0.05	0.00
B80M	502	30	0.02	0.03	0.00
	504	30	0.02	0.25	0.00
	506	31	0.00	0.00	0.00
	508	32	0.01	0.00	0.00
	510	32	0.06	0.09	0.01
E2M	702	30	0.00	0.05	0.00
	704	30	0.00	0.02	0.00
	706	31	0.00	0.00	0.00
	708	32	0.03	0.00	0.00
	710	32	0.02	0.04	0.00

Table C-11. Individual Animal Absolute WBC Differential Count Data – Males (Continued)

Group	Animal ID	Day	Monocytes (10³/μL)	Eosinophils (10³/μL)	Basophils (10³/μL)
E20M	802	30	0.00	0.06	0.00
	804	30	0.00	0.00	0.00
	806	31	0.00	0.11	0.00
	808	32	0.05	0.00	0.00
	810	32	0.02	0.12	0.00
E80M	902	30	0.04	0.11	0.00
	904	30	0.00	0.00	0.00
	906	31	0.00	0.04	0.00
	908	32	0.13	0.27	0.00
	910	32	0.00	0.00	0.00

Table C-12. Individual Animal Absolute WBC Differential Count Data – Females

Group	White Blood Cell				
	Animal ID	Day	Count (10 ³ /μL)	Neutrophils (10 ³ /μL)	Total Lymphocytes (10 ³ /μL)
CF	152	30	5.77	0.46	5.19
	154	31	4.96	0.46	4.10
	156	31	3.53	0.42	3.11
	158	32	4.60	0.55	3.77
	160	32	1.71	0.15	1.54
NT200F	252	30	3.70	0.96	2.66
	254	31	3.64	0.25	3.35
	256	31	5.00	1.25	3.60
	258	32	4.93	2.07	2.76
	260	32	1.95	0.25	1.70
B2F	352	30	3.14	0.25	2.83
	354	31	1.19	0.33	0.77
	356	31	10.17	1.08	7.95
	358	32	4.39	1.49	2.37
	360	32	5.69	0.91	4.59
B20F	452	30	4.68	0.75	3.79
	454	31	3.24	0.39	2.82
	456	31	3.36	0.58	2.56
	458	32	5.21	0.94	4.17
	460	32	5.54	0.94	4.41
B80F	552	30	1.67	0.27	1.39
	554	31	5.42	1.25	4.12
	556	31	4.45	0.45	3.96
	558	32	6.23	0.31	5.73
	560	32	6.63	1.39	5.11

Table C-12. Individual Animal Absolute WBC Differential Count Data – Females (Continued)

Group	White Blood Cell				
	Animal ID	Day	Count (10 ³ /μL)	Neutrophils (10 ³ /μL)	Total Lymphocytes (10 ³ /μL)
E2F	754	31	3.46	0.75	2.59
	756	31	5.50	0.83	4.46
	758	32	2.89	0.38	2.31
	760	32	3.62	0.80	2.75
E20F	852	30	2.21	0.46	1.75
	854	31	4.23	1.38	2.69
	856	31	6.83	1.11	5.59
	858	32	3.21	0.48	2.60
	860	32	5.76	0.46	5.30
E80F	952	30	3.41	0.20	3.11
	954	31	4.87	0.29	4.53
	956	31	6.39	0.56	5.57
	958	32	2.77	0.47	2.22
	960	32	5.49	0.60	4.63
E200F	1058	32	2.33	0.24	1.97
	1060	32	2.24	1.16	1.08

Table C-12. Individual Animal Absolute WBC Differential Count Data – Females (Continued)

Group	Animal ID	Day	Monocytes (10³/μL)	Eosinophils (10³/μL)	Basophils (10³/μL)
CF	152	30	0.06	0.06	0.00
	154	31	0.03	0.35	0.02
	156	31	0.00	0.00	0.00
	158	32	0.05	0.23	0.00
	160	32	0.00	0.02	0.00
NT200F	252	30	0.04	0.04	0.00
	254	31	0.04	0.00	0.00
	256	31	0.00	0.15	0.00
	258	32	0.10	0.00	0.00
	260	32	0.00	0.00	0.00
B2F	352	30	0.00	0.06	0.00
	354	31	0.01	0.07	0.01
	356	31	0.12	0.99	0.03
	358	32	0.09	0.44	0.00
	360	32	0.01	0.18	0.01
B20F	452	30	0.05	0.09	0.00
	454	31	0.00	0.03	0.00
	456	31	0.01	0.20	0.00
	458	32	0.05	0.05	0.00
	460	32	0.03	0.17	0.01
B80F	552	30	0.02	0.00	0.00
	554	31	0.00	0.05	0.00
	556	31	0.00	0.04	0.00
	558	32	0.00	0.19	0.00
	560	32	0.00	0.13	0.00
E2F	754	31	0.03	0.09	0.00
	756	31	0.06	0.17	0.00
	758	32	0.03	0.17	0.00
	760	32	0.04	0.04	0.00

Table C-12. Individual Animal Absolute WBC Differential Count Data – Females (Continued)

Group	Animal ID	Day	Monocytes (10³/μL)	Eosinophils (10³/μL)	Basophils (10³/μL)
E20F	852	30	0.00	0.00	0.00
	854	31	0.02	0.13	0.01
	856	31	0.01	0.09	0.02
	858	32	0.00	0.13	0.00
	860	32	0.00	0.00	0.00
E80F	952	30	0.02	0.07	0.00
	954	31	0.00	0.05	0.00
	956	31	0.10	0.13	0.02
	958	32	0.03	0.06	0.00
	960	32	0.06	0.17	0.02
E200F	1058	32	0.03	0.08	0.00
	1060	32	0.00	0.00	0.00

Table C-13. Individual Animal Serum Chemistry Data – Males

Group	Animal ID	Day	Alkaline Phosphatase (U/L)	Aspartate Aminotransferase (U/L)	Gamma Glutamyltransferase (U/L)
CM	101	30	95	79	1
	103	30	58	51	1
	105	31	101	83	1
	107	31	151	93	1
	109	32	99	58	1
NT200M	203	30	157	81	0
	207	31	NT ^a	249	NT
	209	32	84	95	1
B2M	301	30	98	74	1
	303	30	83	68	0
	305	31	88	60	1
	307	31	78	84	1
	309	32	121	59	1
B20M	401	30	87	55	1
	403	30	109	69	1
	405	31	99	143	1
	407	31	82	68	1
	409	32	72	75	1
B80M	501	30	112	69	1
	503	30	284	94	1
	505	31	93	74	1
	507	31	116	60	1
	509	32	99	67	1
E2M	701	30	93	52	1
	703	30	72	50	1
	705	31	85	54	1
	707	31	80	57	0
	709	32	63	57	1

Table C-13. Individual Animal Serum Chemistry Data – Males (Continued)

Group	Animal ID	Day	Alkaline Phosphatase (U/L)	Aspartate Aminotransferase (U/L)	Gamma Glutamyltransferase (U/L)
E20M	801	30	104	76	1
	803	30	70	60	1
	805	31	69	48	0
	807	31	86	52	1
	809	32	84	164	1
E80M	901	30	83	93	1
	903	30	NT	72	0
	905	31	97	49	0
	907	31	119	54	1
	909	32	148	90	1

Table C-13. Individual Animal Serum Chemistry Data – Males (Continued)

Group	Animal ID	Day	Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)	Total Protein (g/dL)
CM	101	30	0.14	0.05	5.8
	103	30	0.18	0.04	6.0
	105	31	0.18	0.05	5.8
	107	31	0.11	0.04	5.8
	109	32	0.15	0.05	5.8
NT200M	203	30	0.18	0.04	6.2
	207	31	0.11	0.04	NT
	209	32	0.17	0.07	5.6
B2M	301	30	0.18	0.06	6.0
	303	30	0.14	0.04	6.1
	305	31	0.15	0.06	5.6
	307	31	0.15	0.04	6.0
	309	32	0.16	0.05	6.2
B20M	401	30	0.18	0.04	6.1
	403	30	0.13	0.04	5.8
	405	31	0.16	0.06	6.0
	407	31	0.17	0.06	6.1
	409	32	0.16	0.05	5.6
B80M	501	30	0.14	0.03	6.5
	503	30	0.18	0.05	5.9
	505	31	0.21	0.06	5.8
	507	31	0.09	0.03	5.8
	509	32	0.15	0.04	5.4
E2M	701	30	0.18	0.04	5.9
	703	30	0.17	0.04	5.7
	705	31	0.17	0.06	5.6
	707	31	0.17	0.05	5.5
	709	32	0.13	0.06	5.8

Table C-13. Individual Animal Serum Chemistry Data – Males (Continued)

Group	Animal ID	Day	Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)	Total Protein (g/dL)
E20M	801	30	0.19	0.05	5.8
	803	30	0.17	0.05	5.5
	805	31	0.10	0.04	5.9
	807	31	0.15	0.05	6.1
	809	32	0.15	0.06	5.9
E80M	901	30	0.18	0.05	5.5
	903	30	0.14	0.02	NT
	905	31	0.15	0.03	5.7
	907	31	0.20	0.06	5.8
	909	32	0.13	0.06	6.0

Table C-13. Individual Animal Serum Chemistry Data – Males (Continued)

Group	Animal ID	Day	Glucose (mg/dL)	Albumin (g/dL)	Globulin (g/dL)
CM	101	30	128	3.8	2.0
	103	30	89	3.9	2.1
	105	31	117	3.7	2.1
	107	31	75	3.8	2.0
	109	32	129	3.7	2.1
NT200M	203	30	134	3.9	2.3
	207	31	NT	NT	NT
	209	32	119	4.2	1.4
B2M	301	30	115	4.0	2.0
	303	30	92	4.1	2.0
	305	31	112	3.8	1.8
	307	31	92	3.8	2.2
	309	32	120	3.9	2.3
B20M	401	30	92	4.1	2.0
	403	30	111	3.9	1.9
	405	31	122	4.1	1.9
	407	31	110	4.0	2.1
	409	32	100	3.7	1.9
B80M	501	30	127	4.2	2.3
	503	30	109	4.1	1.8
	505	31	164	3.9	1.9
	507	31	76	3.9	1.9
	509	32	138	3.6	1.8
E2M	701	30	111	3.8	2.1
	703	30	106	3.8	1.9
	705	31	97	3.7	1.9
	707	31	95	3.7	1.8
	709	32	102	4.0	1.8

Table C-13. Individual Animal Serum Chemistry Data – Males (Continued)

Group	Animal ID	Day	Glucose (mg/dL)	Albumin (g/dL)	Globulin (g/dL)
E20M	801	30	121	3.9	1.9
	803	30	142	3.7	1.8
	805	31	141	3.8	2.1
	807	31	130	4.1	2.0
	809	32	115	3.9	2.0
E80M	901	30	114	3.7	1.8
	903	30	NT	4.2	NT
	905	31	145	4.0	1.7
	907	31	155	3.9	1.9
	909	32	111	4.2	1.8

Table C-13. Individual Animal Serum Chemistry Data – Males (Continued)

Group	Animal ID	Day	Albumin/Globulin Ratio	Blood Urea Nitrogen (mg/dL)	Creatinine (mg/dL)
CM	101	30	1.90	21	0.5
	103	30	1.86	19	0.5
	105	31	1.76	17	0.5
	107	31	1.90	16	0.5
	109	32	1.76	22	0.5
NT200M	203	30	1.70	18	0.5
	207	31	NT	NT	NT
	209	32	3.00	30	0.4
B2M	301	30	2.00	22	0.5
	303	30	2.05	20	0.4
	305	31	2.11	31	0.5
	307	31	1.73	18	0.4
	309	32	1.70	19	0.4
B20M	401	30	2.05	23	0.5
	403	30	2.05	17	0.4
	405	31	2.16	23	0.5
	407	31	1.90	22	0.4
	409	32	1.95	24	0.4
B80M	501	30	1.83	24	0.5
	503	30	2.28	25	0.4
	505	31	2.05	16	0.5
	507	31	2.05	19	0.5
	509	32	2.00	22	0.4
E2M	701	30	1.81	19	0.5
	703	30	2.00	17	0.4
	705	31	1.95	20	0.5
	707	31	2.06	19	0.4
	709	32	2.22	22	0.5

Table C-13. Individual Animal Serum Chemistry Data – Males (Continued)

Group	Animal ID	Day	Albumin/Globulin Ratio	Blood Urea Nitrogen (mg/dL)	Creatinine (mg/dL)
E20M	801	30	2.05	30	0.5
	803	30	2.06	20	0.5
	805	31	1.81	21	0.5
	807	31	2.05	17	0.4
	809	32	1.95	22	0.5
E80M	901	30	2.06	18	0.5
	903	30	NT	NT	NT
	905	31	2.35	19	0.5
	907	31	2.05	18	0.4
	909	32	2.33	27	0.5

Table C-13. Individual Animal Serum Chemistry Data – Males (Continued)

Group	Animal ID	Day	Triglycerides (mg/dL)	Cholesterol (mg/dL)	Calcium (mg/dL)
CM	101	30	69	138	9.8
	103	30	51	161	10.5
	105	31	108	203	10.3
	107	31	51	204	9.9
	109	32	80	176	9.7
NT200M	203	30	150	276	NT
	207	31	NT	NT	NT
	209	32	38	173	10.1
B2M	301	30	50	148	9.9
	303	30	44	150	10.3
	305	31	53	182	10.0
	307	31	101	211	10.4
	309	32	76	168	10.0
B20M	401	30	79	240	10.8
	403	30	40	168	9.8
	405	31	110	145	10.1
	407	31	30	162	10.2
	409	32	47	126	9.9
B80M	501	30	69	237	10.4
	503	30	39	157	10.3
	505	31	62	173	9.9
	507	31	44	160	9.7
	509	32	50	158	9.4
E2M	701	30	66	208	10.2
	703	30	52	161	10.1
	705	31	53	123	9.8
	707	31	58	196	9.8
	709	32	85	143	10.4

Table C-13. Individual Animal Serum Chemistry Data – Males (Continued)

Group	Animal ID	Day	Triglycerides (mg/dL)	Cholesterol (mg/dL)	Calcium (mg/dL)
E20M	801	30	72	213	9.8
	803	30	65	161	10.6
	805	31	71	141	9.8
	807	31	82	170	10.0
	809	32	61	207	10.1
E80M	901	30	300	219	10.0
	903	30	NT	NT	NT
	905	31	37	175	9.8
	907	31	90	181	10.0
	909	32	46	176	9.7

Table C-13. Individual Animal Serum Chemistry Data – Males (Continued)

Group	Animal ID	Day	Phosphorus (mg/dL)	Sodium (mmol/L)	Potassium (mmol/L)
CM	101	30	8.5	150	6.4
	103	30	8.6	154	8.0
	105	31	8.7	154	8.0
	107	31	8.7	153	7.9
	109	32	7.9	158	7.9
NT200M	203	30	11.2	152	9.0
	207	31	NT	NT	NT
	209	32	7.1	156	7.8
B2M	301	30	7.6	152	7.5
	303	30	9.2	153	8.6
	305	31	9.5	157	7.3
	307	31	9.5	154	6.9
	309	32	8.6	158	7.1
B20M	401	30	9.7	153	8.2
	403	30	8.0	152	7.6
	405	31	8.8	157	7.0
	407	31	9.4	152	7.3
	409	32	8.9	159	8.2
B80M	501	30	8.5	154	7.4
	503	30	9.8	154	8.3
	505	31	7.8	151	6.9
	507	31	7.6	154	7.4
	509	32	9.2	157	7.7
E2M	701	30	8.6	154	7.6
	703	30	10.0	151	7.5
	705	31	9.2	155	7.8
	707	31	9.4	153	7.1
	709	32	12.0	158	8.2

Table C-13. Individual Animal Serum Chemistry Data – Males (Continued)

Group	Animal ID	Day	Phosphorus (mg/dL)	Sodium (mmol/L)	Potassium (mmol/L)
E20M	801	30	8.1	152	7.0
	803	30	9.2	154	7.2
	805	31	9.7	156	7.9
	807	31	9.0	154	7.5
	809	32	8.8	159	7.3
E80M	901	30	6.7	155	7.0
	903	30	NT	NT	NT
	905	31	8.8	147	7.6
	907	31	8.3	151	7.5
	909	32	8.7	159	7.2

Table C-13. Individual Animal Serum Chemistry Data – Males (Continued)

Group	Animal ID	Day	Chloride (mmol/L)
CM	101	30	111
	103	30	112
	105	31	113
	107	31	113
	109	32	120
NT200M	203	30	110
	207	31	NT
	209	32	120
B2M	301	30	110
	303	30	112
	305	31	114
	307	31	110
	309	32	116
B20M	401	30	111
	403	30	112
	405	31	115
	407	31	111
	409	32	118
B80M	501	30	110
	503	30	113
	505	31	110
	507	31	111
	509	32	118
E2M	701	30	109
	703	30	109
	705	31	114
	707	31	113
	709	32	115

Table C-13. Individual Animal Serum Chemistry Data – Males (Continued)

Group	Animal ID	Day	Chloride (mmol/L)
E20M	801	30	111
	803	30	115
	805	31	115
	807	31	110
	809	32	119
E80M	901	30	113
	903	30	NT
	905	31	108
	907	31	110
	909	32	119

a. NT = not tested due to insufficient volume

Table C-14. Individual Animal Serum Chemistry Data – Females

Group	Animal ID	Day	Alkaline Phosphatase (U/L)	Aspartate Aminotransferase (U/L)	Gamma Glutamyltransferase (U/L)
CF	151	30	108	91	1
	153	30	71	74	0
	155	31	108	71	1
	157	31	79	106	1
	159	32	106	90	1
NT200F	251	30	171	106	1
	253	30	NT ^a	NT	1
	255	31	131	188	1
	257	31	96	188	0
	259	32	162	88	NT
B2F	351	30	82	99	1
	353	30	95	83	1
	355	31	88	73	1
	357	31	78	77	1
	359	32	NT	98	NT
B20F	451	30	89	76	1
	453	30	NT	NT	1
	455	31	150	142	1
	457	31	113	115	1
	459	32	172	115	1
B80F	551	30	97	109	1
	553	30	127	85	0
	555	31	101	94	0
	557	31	146	80	0
	559	32	174	98	1
E2F	751	30	88	69	1
	753	30	126	116	1
	755	31	81	121	0

Table C-14. Individual Animal Serum Chemistry Data – Females (Continued)

Group	Animal ID	Day	Alkaline Phosphatase (U/L)	Aspartate Aminotransferase (U/L)	Gamma Glutamyltransferase (U/L)
E2F	757	31	79	76	1
	759	32	76	89	1
E20F	851	30	128	130	1
	853	30	128	79	0
	855	31	128	143	1
	857	31	66	82	1
	859	32	90	86	1
E80F	951	30	117	117	1
	953	30	90	90	1
	955	31	79	122	1
	957	31	97	80	1
	959	32	113	128	1
E200F	1055	31	171	124	1
	1057	31	144	111	1

Table C-14. Individual Animal Serum Chemistry Data – Females (Continued)

Group	Animal ID	Day	Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)	Total Protein (g/dL)
CF	151	30	0.13	0.03	6.1
	153	30	0.12	0.03	NT ^a
	155	31	0.12	0.03	6.6
	157	31	0.11	0.03	5.7
	159	32	0.13	0.04	6.0
NT200F	251	30	0.18	0.06	6.2
	253	30	0.03	NT	NT
	255	31	0.16	0.07	5.7
	257	31	0.14	0.04	4.6
	259	32	0.11	0.04	NT
B2F	351	30	0.11	0.02	5.5
	353	30	0.11	0.03	5.7
	355	31	0.10	0.03	6.2
	357	31	0.09	0.02	6.1
	359	32	0.09	0.03	NT
B20F	451	30	0.10	0.02	5.3
	453	30	NT	NT	NT
	455	31	0.09	0.06	6.3
	457	31	0.12	0.03	5.9
	459	32	0.14	0.04	6.2
B80F	551	30	0.09	0.03	5.3
	553	30	0.12	0.03	5.8
	555	31	0.06	0.03	5.5
	557	31	0.10	0.04	5.8
	559	32	0.11	0.03	5.8
E2F	751	30	0.12	0.02	5.4
	753	30	0.19	0.04	6.4
	755	31	0.13	0.03	5.9

Table C-14. Individual Animal Serum Chemistry Data – Females (Continued)

Group	Animal ID	Day	Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)	Total Protein (g/dL)
E2F	757	31	0.10	0.04	5.9
	759	32	0.08	0.04	5.3
E20F	851	30	0.12	0.03	6.4
	853	30	0.10	0.03	5.5
	855	31	0.09	0.03	6.1
	857	31	0.08	0.04	NT
	859	32	0.15	0.05	5.9
E80F	951	30	0.11	0.03	5.8
	953	30	0.12	0.03	5.7
	955	31	0.11	0.05	5.8
	957	31	0.08	0.05	5.7
	959	32	0.11	0.04	5.8
E200F	1055	31	0.15	0.04	5.2
	1057	31	0.15	0.05	5.9

Table C-14. Individual Animal Serum Chemistry Data – Females (Continued)

Group	Animal ID	Day	Glucose (mg/dL)	Albumin (g/dL)	Globulin (g/dL)
CF	151	30	72	4.4	1.7
	153	30	NT	3.9	NT
	155	31	86	4.3	2.3
	157	31	83	3.9	1.8
	159	32	80	4.3	1.7
NT200F	251	30	89	4.5	1.7
	253	30	NT	NT	NT
	255	31	70	4.2	1.5
	257	31	33	3.1	1.5
	259	32	68	4.1	NT
B2F	351	30	103	3.5	2.0
	353	30	97	4.0	1.7
	355	31	67	4.3	1.9
	357	31	77	4.0	2.1
	359	32	NT	3.8	NT
B20F	451	30	107	3.8	1.5
	453	30	NT	4.1	NT
	455	31	121	4.4	1.9
	457	31	72	4.3	1.6
	459	32	81	4.4	1.8
B80F	551	30	88	3.7	1.6
	553	30	75	4.3	1.5
	555	31	80	3.9	1.6
	557	31	64	4.2	1.6
	559	32	40	3.9	1.9
E2F	751	30	87	4.1	1.3
	753	30	94	4.7	1.7
	755	31	83	4.2	1.7

Table C-14. Individual Animal Serum Chemistry Data – Females (Continued)

Group	Animal ID	Day	Glucose (mg/dL)	Albumin (g/dL)	Globulin (g/dL)
E2F	757	31	81	4.4	1.5
	759	32	67	3.8	1.5
E20F	851	30	94	4.6	1.8
	853	30	75	4.0	1.5
	855	31	66	4.3	1.8
	857	31	57	3.9	NT
	859	32	82	4.3	1.6
	951	30	61	4.1	1.7
E80F	953	30	72	4.2	1.5
	955	31	83	3.9	1.9
	957	31	93	4.1	1.6
	959	32	68	4.2	1.6
E200F	1055	31	59	3.8	1.4
	1057	31	69	4.3	1.6

Table C-14. Individual Animal Serum Chemistry Data – Females (Continued)

Group	Animal ID	Day	Albumin/Globulin Ratio	Blood Urea Nitrogen (mg/dL)	Creatinine (mg/dL)
CF	151	30	2.59	13	0.5
	153	30	NT	NT	NT
	155	31	1.87	10	0.5
	157	31	2.17	10	0.4
	159	32	2.53	15	0.5
NT200F	251	30	2.65	19	0.5
	253	30	NT	NT	NT
	255	31	2.80	22	0.5
	257	31	2.07	56	0.4
	259	32	NT	NT	NT
B2F	351	30	1.75	12	0.4
	353	30	2.35	13	0.5
	355	31	2.26	12	0.4
	357	31	1.90	13	0.5
	359	32	NT	NT	NT
B20F	451	30	2.53	14	0.4
	453	30	NT	NT	NT
	455	31	2.32	21	0.5
	457	31	2.69	11	0.4
	459	32	2.44	13	0.5
B80F	551	30	2.31	19	0.5
	553	30	2.87	15	0.5
	555	31	2.44	13	0.4
	557	31	2.63	10	0.4
	559	32	2.05	17	0.4
E2F	751	30	3.15	11	0.5
	753	30	2.76	18	0.5
	755	31	2.47	13	0.5

Table C-14. Individual Animal Serum Chemistry Data – Females (Continued)

Group	Animal ID	Day	Albumin/Globulin Ratio	Blood Urea Nitrogen (mg/dL)	Creatinine (mg/dL)
E2F	757	31	2.93	13	0.4
	759	32	2.53	10	0.4
E20F	851	30	2.56	13	0.5
	853	30	2.67	12	0.4
	855	31	2.39	14	NT
	857	31	NT	NT	NT
	859	32	2.69	13	0.5
	951	30	2.41	15	0.5
E80F	953	30	2.80	12	0.5
	955	31	2.05	13	NT
	957	31	2.56	11	0.5
	959	32	2.63	15	0.5
	1055	31	2.71	18	0.4
E200F	1057	31	2.69	19	0.4

Table C-14. Individual Animal Serum Chemistry Data – Females (Continued)

Group	Animal ID	Day	Triglycerides (mg/dL)	Cholesterol (mg/dL)	Calcium (mg/dL)
CF	151	30	51	101	10.4
	153	30	NT	NT	NT
	155	31	68	133	10.4
	157	31	51	125	10.1
	159	32	47	100	10.0
NT200F	251	30	53	142	10.2
	253	30	NT	NT	NT
	255	31	51	164	9.9
	257	31	23	184	10.7
	259	32	NT	NT	NT
B2F	351	30	51	82	10.0
	353	30	54	122	10.1
	355	31	50	119	10.2
	357	31	41	119	10.2
	359	32	NT	NT	NT
B20F	451	30	37	156	10.1
	453	30	NT	NT	NT
	455	31	42	120	9.9
	457	31	58	79	9.7
	459	32	62	117	10.1
B80F	551	30	51	96	10.0
	553	30	60	126	10.5
	555	31	40	88	10.1
	557	31	41	94	10.3
	559	32	46	131	10.5
E2F	751	30	39	110	10.4
	753	30	36	134	9.9
	755	31	59	97	10.0

Table C-14. Individual Animal Serum Chemistry Data – Females (Continued)

Group	Animal ID	Day	Triglycerides (mg/dL)	Cholesterol (mg/dL)	Calcium (mg/dL)
E2F	757	31	48	119	10.3
	759	32	41	108	10.2
E20F	851	30	51	145	10.4
	853	30	34	137	10.1
	855	31	74	NT	10.2
	857	31	46	NT	NT
	859	32	45	114	10.0
	951	30	59	150	10.3
E80F	953	30	39	95	10.2
	955	31	52	80	10.2
	957	31	63	133	10.2
	959	32	32	112	10.1
	1055	31	59	141	9.7
E200F	1057	31	67	146	10.2

Table C-14. Individual Animal Serum Chemistry Data – Females (Continued)

Group	Animal ID	Day	Phosphorus (mg/dL)	Sodium (mmol/L)	Potassium (mmol/L)
CF	151	30	7.8	155	6.8
	153	30	NT	NT	NT
	155	31	8.1	154	7.4
	157	31	9.2	152	6.8
	159	32	8.5	157	7.4
NT200F	251	30	8.8	151	6.7
	253	30	NT	NT	NT
	255	31	8.5	156	6.9
	257	31	12.1	154	7.3
	259	32	NT	NT	NT
B2F	351	30	7.5	153	6.5
	353	30	7.4	150	7.2
	355	31	9.0	152	7.3
	357	31	10.4	152	7.8
	359	32	NT	NT	NT
B20F	451	30	9.2	154	7.2
	453	30	NT	NT	NT
	455	31	6.6	154	6.7
	457	31	8.0	156	6.6
	459	32	9.3	156	7.0
B80F	551	30	9.7	153	7.4
	553	30	7.4	154	6.6
	555	31	9.6	154	6.9
	557	31	7.6	155	7.1
	559	32	11.1	158	7.8
E2F	751	30	9.6	153	7.0
	753	30	9.0	153	6.6
	755	31	8.4	154	6.3

Table C-14. Individual Animal Serum Chemistry Data – Females (Continued)

Group	Animal ID	Day	Phosphorus (mg/dL)	Sodium (mmol/L)	Potassium (mmol/L)
E2F	757	31	7.4	152	6.6
	759	32	9.2	157	6.8
E20F	851	30	7.1	156	7.0
	853	30	9.2	154	7.2
	855	31	8.6	151	7.0
	857	31	NT	NT	NT
	859	32	7.6	156	7.1
	951	30	9.6	154	6.7
E80F	953	30	9.3	155	7.4
	955	31	NT	154	7.1
	957	31	7.0	154	6.9
	959	32	8.2	159	6.8
	1055	31	10.6	156	7.6
E200F	1057	31	9.1	157	7.0

Table C-14. Individual Animal Serum Chemistry Data – Females (Continued)

Group	Animal ID	Day	Chloride (mmol/L)
CF	151	30	115
	153	30	NT
	155	31	114
	157	31	109
	159	32	116
NT200F	251	30	116
	253	30	NT
	255	31	116
	257	31	118
	259	32	NT
B2F	351	30	114
	353	30	111
	355	31	111
	357	31	113
	359	32	NT
B20F	451	30	117
	453	30	NT
	455	31	117
	457	31	114
	459	32	115
B80F	551	30	114
	553	30	112
	555	31	116
	557	31	111
	559	32	116
E2F	751	30	112
	753	30	116
	755	31	115

Table C-14. Individual Animal Serum Chemistry Data – Females (Continued)

Group	Animal ID	Day	Chloride (mmol/L)
E2F	757	31	113
	759	32	115
E20F	851	30	114
	853	30	112
	855	31	111
	857	31	NT
	859	32	117
	951	30	115
E80F	953	30	115
	955	31	115
	957	31	113
	959	32	117
	1055	31	117
E200F	1057	31	113

a. NT = not tested due to insufficient volume

APPENDIX D: PATHOLOGY INDIVIDUAL ANIMAL DATA

Table D-1. Individual Animal Absolute Organ Weights (g) – Males

Group	Animal ID	Day	Brain	Epididymides	Heart	Kidneys
CM	101	30	0.489	0.0921	0.170	0.535
	102	30	0.506	0.0832	0.159	0.439
	103	30	0.496	0.0971	0.158	0.384
	104	30	0.495	0.1065	0.202	0.556
	105	31	0.478	0.1133	0.246	0.586
	106	31	0.505	0.1085	0.268	0.482
	107	31	0.495	0.1139	0.158	0.500
	108	32	0.499	0.0906	0.265	0.430
	109	32	0.494	0.1415	0.246	0.522
	110	32	0.472	0.0843	0.178	0.529
NT200M	202	30	0.504	0.0795	0.152	0.344
	203	30	0.467	0.0778	0.149	0.408
	204	30	0.480	0.0846	0.202	0.395
	206	31	0.448	0.0482	0.128	0.276
	207	31	0.444	0.0407	0.124	0.221
	209	32	0.448	0.0718	0.138	0.366
B2M	301	30	0.506	0.0922	0.194	0.475
	302	30	0.505	0.1161	0.212	0.419
	303	30	0.516	0.1037	0.230	0.460
	304	30	0.486	0.0960	0.203	0.521
	305	31	0.485	0.0987	0.207	0.465
	306	31	0.486	0.0953	0.223	0.490
	307	31	0.524	0.1038	0.175	0.506
	308	32	0.559	0.0986	0.175	0.442
	309	32	0.484	0.0897	0.206	0.525
	310	32	0.484	0.0759	0.161	0.451
B20M	401	30	0.497	0.0958	0.182	0.575
	402	30	0.480	0.1204	0.226	0.549

Table D-1. Individual Animal Absolute Organ Weights (g) – Males (Continued)

Group	Animal ID	Day	Brain	Epididymides	Heart	Kidneys
B20M	403	30	0.473	0.0837	0.172	0.494
	404	30	0.466	0.0946	0.189	0.410
	405	31	0.482	0.1038	0.155	0.498
	406	31	0.481	0.1041	0.230	0.462
	407	31	0.473	0.0993	0.175	0.476
	408	32	0.465	0.0683	0.251	0.432
	409	32	0.509	0.0903	0.158	0.498
	410	32	0.483	0.0969	0.180	0.573
B80M	501	30	0.464	0.1179	0.152	0.472
	502	30	0.488	0.1055	0.225	0.556
	503	30	0.446	0.0881	0.185	0.351
	504	30	0.463	0.0642	0.131	0.415
	505	31	0.469	0.1021	0.150	0.458
	506	31	0.488	0.0970	0.149	0.388
	507	31	0.459	0.1175	0.173	0.518
	508	32	0.478	0.1174	0.171	0.507
	509	32	0.480	0.1025	0.156	0.457
	510	32	0.483	0.1134	0.143	0.360
E2M	701	30	0.515	0.0831	0.163	0.474
	702	30	0.474	0.0879	0.219	0.478
	703	30	0.477	0.0963	0.165	0.464
	704	30	0.482	0.0899	0.187	0.526
	705	31	0.499	0.0949	0.209	0.572
	706	31	0.488	0.1094	0.180	0.469
	707	31	0.486	0.1270	0.188	0.556
	708	32	0.501	0.1238	0.183	0.530
	709	32	0.485	0.0936	0.187	0.444
	710	32	0.455	0.0843	0.202	0.424

Table D-1. Individual Animal Absolute Organ Weights (g) – Males (Continued)

Group	Animal		Brain	Epididymides	Heart	Kidneys
	ID	Day				
E20M	801	30	0.506	0.1092	0.167	0.561
	802	30	0.491	0.0811	0.174	0.427
	803	30	0.498	0.0911	0.206	0.554
	804	30	0.528	0.1099	0.182	0.506
	805	31	0.463	0.1218	0.233	0.545
	806	31	0.486	0.0978	0.208	0.401
	807	31	0.472	0.1045	0.239	0.433
	808	32	0.476	0.0749	0.234	0.488
	809	32	0.497	0.0998	0.195	0.431
	810	32	0.523	0.1001	0.149	0.537
E80M	901	30	0.497	0.0938	0.165	0.425
	902	30	0.470	0.0772	0.152	0.357
	903	30	0.457	0.0781	0.192	0.327
	904	30	0.468	0.1049	0.228	0.445
	905	31	0.473	0.0930	0.154	0.474
	906	31	0.477	0.0854	0.151	0.442
	907	31	0.493	0.0895	0.231	0.530
	908	32	0.490	0.0954	0.218	0.453
	909	32	0.452	0.0800	0.132	0.420
	910	32	0.472	0.0914	0.219	0.411

Table D-1. Individual Animal Absolute Organ Weights (g) – Males (Continued)

Group	Animal ID	Day	Liver	Salivary Gland	Spleen	Testes
CM	101	30	1.235	0.257	0.085	0.196
	102	30	1.266	0.211	0.088	0.250
	103	30	0.991	0.172	0.079	0.278
	104	30	1.294	0.245	0.102	0.250
	105	31	1.211	0.191	0.076	0.245
	106	31	1.237	0.252	0.086	0.245
	107	31	1.192	0.235	0.078	0.262
	108	32	1.210	0.244	0.090	0.281
	109	32	1.308	0.227	0.078	0.316
	110	32	1.466	0.230	0.098	0.208
NT200M	202	30	0.970	0.118	0.046	0.210
	203	30	1.248	0.178	0.063	0.180
	204	30	1.166	0.201	0.060	0.187
	206	31	0.731	0.109	0.031	0.117
	207	31	0.671	0.117	0.020	0.103
	209	32	0.996	0.153	0.035	0.179
B2M	301	30	1.151	0.225	0.071	0.218
	302	30	1.208	0.195	0.093	0.221
	303	30	1.226	0.235	0.079	0.272
	304	30	1.312	0.213	0.090	0.187
	305	31	1.137	0.172	0.085	0.226
	306	31	1.303	0.245	0.093	0.270
	307	31	1.318	0.220	0.121	0.262
	308	32	1.162	0.199	0.096	0.219
	309	32	1.242	0.223	0.091	0.295
	310	32	1.061	0.183	0.091	0.216
B20M	401	30	1.401	0.230	0.111	0.268
	402	30	1.479	0.236	0.085	0.261

Table D-1. Individual Animal Absolute Organ Weights (g) – Males (Continued)

Group	Animal ID	Day	Liver	Salivary Gland	Spleen	Testes
B20M	403	30	1.177	0.205	0.084	0.204
	404	30	1.120	0.227	0.070	0.240
	405	31	1.180	0.241	0.083	0.237
	406	31	1.135	0.174	0.081	0.241
	407	31	1.175	0.183	0.081	0.188
	408	32	1.113	0.219	0.079	0.222
	409	32	1.216	0.246	0.085	0.233
	410	32	1.311	0.217	0.091	0.223
B80M	501	30	1.365	0.245	0.095	0.241
	502	30	1.251	0.206	0.094	0.212
	503	30	0.997	0.162	0.049	0.232
	504	30	1.100	0.152	0.077	0.192
	505	31	1.306	0.209	0.067	0.227
	506	31	1.088	0.216	0.082	0.237
	507	31	1.242	0.193	0.086	0.225
	508	32	1.239	0.213	0.071	0.251
	509	32	1.087	0.195	0.070	0.261
	510	32	1.103	0.178	0.069	0.241
E2M	701	30	1.217	0.192	0.093	0.230
	702	30	1.184	0.217	0.087	0.260
	703	30	1.064	0.218	0.078	0.250
	704	30	1.258	0.226	0.075	0.215
	705	31	1.170	0.215	0.077	0.247
	706	31	1.108	0.196	0.073	0.189
	707	31	1.290	0.226	0.097	0.235
	708	32	1.234	0.204	0.092	0.262
	709	32	1.138	0.177	0.081	0.241
	710	32	1.094	0.131	0.077	0.111

Table D-1. Individual Animal Absolute Organ Weights (g) – Males (Continued)

Group	Animal ID	Day	Liver	Salivary Gland	Spleen	Testes
E20M	801	30	1.291	0.209	0.107	0.207
	802	30	1.156	0.187	0.089	0.208
	803	30	1.236	0.218	0.108	0.257
	804	30	1.273	0.247	0.083	0.248
	805	31	1.295	0.188	0.114	0.212
	806	31	1.053	0.199	0.094	0.242
	807	31	1.252	0.228	0.106	0.236
	808	32	1.174	0.184	0.093	0.184
	809	32	1.280	0.226	0.084	0.324
	810	32	1.085	0.244	0.071	0.241
E80M	901	30	1.394	0.170	0.064	0.242
	902	30	1.067	0.188	0.065	0.210
	903	30	1.053	0.163	0.090	0.231
	904	30	1.258	0.200	0.104	0.258
	905	31	1.199	0.205	0.064	0.214
	906	31	1.160	0.207	0.071	0.229
	907	31	1.559	0.217	0.100	0.248
	908	32	1.787	0.198	0.094	0.218
	909	32	1.051	0.185	0.051	0.212
	910	32	1.141	0.147	0.075	0.248

Table D-1. Individual Animal Absolute Organ Weights (g) – Males (Continued)

Group	Animal		Thymus
	ID	Day	
CM	101	30	0.041
	102	30	0.047
	103	30	0.054
	104	30	0.031
	105	31	0.047
	106	31	0.042
	107	31	0.038
	108	32	0.065
	109	32	0.039
	110	32	0.043
NT200M	202	30	0.016
	203	30	0.051
	204	30	0.035
	206	31	0.015
	207	31	0.015
	209	32	0.013
B2M	301	30	0.039
	302	30	0.054
	303	30	0.039
	304	30	0.061
	305	31	0.051
	306	31	0.038
	307	31	0.051
	308	32	0.036
	309	32	0.042
	310	32	0.032
B20M	401	30	0.060
	402	30	0.063

Table D-1. Individual Animal Absolute Organ Weights (g) – Males (Continued)

Group	Animal		Thymus
	ID	Day	
B20M	403	30	0.045
	404	30	0.044
	405	31	0.039
	406	31	0.045
	407	31	0.034
	408	32	0.042
	409	32	0.063
	410	32	0.041
B80M	501	30	0.060
	502	30	0.051
	503	30	0.039
	504	30	0.042
	505	31	0.036
	506	31	0.047
	507	31	0.052
	508	32	0.042
	509	32	0.046
	510	32	0.040
E2M	701	30	0.034
	702	30	0.044
	703	30	0.044
	704	30	0.037
	705	31	0.037
	706	31	0.037
	707	31	0.058
	708	32	0.062
	709	32	0.045
	710	32	0.037

Table D-1. Individual Animal Absolute Organ Weights (g) – Males (Continued)

Group	Animal		Thymus
	ID	Day	
E20M	801	30	0.042
	802	30	0.049
	803	30	0.044
	804	30	0.038
	805	31	0.051
	806	31	0.039
	807	31	0.060
	808	32	0.032
	809	32	0.023
	810	32	0.033
E80M	901	30	0.030
	902	30	0.033
	903	30	0.051
	904	30	0.054
	905	31	0.056
	906	31	0.057
	907	31	0.022
	908	32	0.068
	909	32	0.027
	910	32	0.030

Table D-2. Individual Animal Absolute Organ Weights (g) – Females

Group	Animal		Brain	Heart	Kidneys	Liver
	ID	Day				
CF	151	30	0.464	0.134	0.306	0.917
	152	30	0.487	0.205	0.317	0.914
	153	30	0.483	0.125	0.312	0.798
	154	31	0.480	0.127	0.333	1.121
	155	31	0.516	0.164	0.362	1.028
	156	31	0.482	0.112	0.294	0.853
	157	31	0.488	0.164	0.379	1.109
	158	32	0.454	0.132	0.297	0.932
	159	32	0.523	0.194	0.370	1.123
	160	32	0.532	0.149	0.364	1.017
NT200F	251	30	0.449	0.090	0.247	0.812
	252	30	0.471	0.143	0.238	0.935
	253	30	0.465	0.154	0.261	0.858
	254	31	0.459	0.104	0.276	0.960
	255	31	0.454	0.109	0.235	0.850
	256	31	0.395	0.108	0.210	0.867
	257	31	0.413	0.103	0.200	0.688
	258	32	0.398	0.072	0.184	0.533
	259	32	0.475	0.129	0.264	1.036
	260	32	0.436	0.115	0.323	1.027
B2F	351	30	0.507	0.225	0.300	1.129
	352	30	0.480	0.143	0.261	0.862
	353	30	0.477	0.158	0.310	0.937
	354	31	0.491	0.175	0.368	1.213
	355	31	0.460	0.153	0.393	1.290
	356	31	0.494	0.162	0.380	1.192
	357	31	0.478	0.147	0.344	1.102
	358	32	0.564	0.166	0.331	1.294

Table D-2. Individual Animal Absolute Organ Weights (g) – Females (Continued)

Group	Animal		Brain	Heart	Kidneys	Liver
	ID	Day				
B2F	359	32	0.473	0.145	0.340	1.134
	360	32	0.433	0.136	0.314	1.045
B20F	451	30	0.470	0.152	0.309	0.914
	452	30	0.512	0.171	0.284	0.933
	453	30	0.455	0.166	0.258	0.798
	454	31	0.491	0.146	0.316	0.990
	455	31	0.486	0.132	0.264	0.896
	456	31	0.499	0.135	0.309	0.925
	457	31	0.467	0.177	0.293	0.893
	458	32	0.487	0.192	0.340	1.115
	459	32	0.499	0.133	0.355	0.961
	460	32	0.485	0.137	0.393	1.125
B80F	551	30	0.477	0.117	0.276	0.957
	552	30	0.509	0.134	0.301	0.908
	553	30	0.476	0.172	0.252	0.898
	554	31	0.473	0.124	0.324	0.941
	555	31	0.480	0.123	0.273	0.991
	556	31	0.486	0.169	0.288	0.912
	557	31	0.484	0.131	0.316	0.818
	558	32	0.457	0.176	0.307	0.940
	559	32	0.511	0.143	0.319	1.106
	560	32	0.479	0.133	0.320	1.096
E2F	751	30	0.478	0.159	0.278	0.939
	752	30	0.474	0.170	0.287	0.809
	753	30	0.500	0.197	0.270	0.821
	754	31	0.469	0.206	0.328	0.977
	755	31	0.469	0.154	0.276	0.923
	756	31	0.492	0.191	0.323	0.978

Table D-2. Individual Animal Absolute Organ Weights (g) – Females (Continued)

Group	Animal ID	Day	Brain	Heart	Kidneys	Liver
E2F	757	31	0.467	0.174	0.316	1.014
	758	32	0.525	0.226	0.371	1.146
	759	32	0.513	0.159	0.340	1.100
	760	32	0.504	0.142	0.310	0.972
E20F	851	30	0.479	0.130	0.264	0.790
	852	30	0.481	0.142	0.302	0.844
	853	30	0.513	0.140	0.283	0.901
	854	31	0.464	0.172	0.275	0.848
	855	31	0.503	0.139	0.353	1.112
	856	31	0.492	0.129	0.307	0.890
	857	31	0.500	0.159	0.279	0.972
	858	32	0.489	0.229	0.315	1.165
	859	32	0.498	0.142	0.302	0.934
	860	32	0.464	0.148	0.303	1.002
E80F	951	30	0.463	0.131	0.294	0.997
	952	30	0.503	0.137	0.265	0.805
	953	30	0.478	0.141	0.285	0.884
	954	31	0.528	0.182	0.330	1.046
	955	31	0.450	0.118	0.355	1.131
	956	31	0.524	0.192	0.304	0.987
	957	31	0.462	0.146	0.307	1.011
	958	32	0.484	0.137	0.313	0.982
	959	32	0.508	0.110	0.317	0.973
	960	32	0.467	0.138	0.322	1.101
E200F	1055	31	0.473	0.129	0.273	0.846
	1057	31	0.447	0.104	0.253	0.698
	1058	32	0.448	0.164	0.305	1.035
	1060	32	0.437	0.102	0.245	0.774

Table D-2. Individual Animal Absolute Organ Weights (g) – Females (Continued)

Group	Animal		Salivary Gland	Spleen	Thymus	Uterus
	ID	Day				
CF	151	30	0.145	0.087	0.062	0.114
	152	30	0.145	0.099	0.038	0.108
	153	30	0.164	0.079	0.043	0.135
	154	31	0.183	0.109	0.047	0.175
	155	31	0.135	0.111	0.046	0.178
	156	31	0.089	0.085	0.033	0.133
	157	31	0.140	0.080	0.059	0.097
	158	32	0.173	0.092	0.058	0.107
	159	32	0.134	0.082	0.041	0.169
	160	32	0.112	0.073	0.043	0.203
NT200F	251	30	0.124	0.046	0.056	0.078
	252	30	0.082	0.071	0.043	0.052
	253	30	0.121	0.072	0.060	0.085
	254	31	0.109	0.092	0.056	0.113
	255	31	0.139	0.049	0.037	0.078
	256	31	0.083	0.059	0.025	0.049
	257	31	0.073	0.073	0.019	0.029
	258	32	0.069	0.022	0.005	0.027
	259	32	0.099	0.089	0.058	0.077
	260	32	0.111	0.075	0.054	0.084
B2F	351	30	0.181	0.142	0.039	0.117
	352	30	0.139	0.070	0.066	0.163
	353	30	0.149	0.082	0.047	0.116
	354	31	0.121	0.095	0.047	0.229
	355	31	0.159	0.103	0.067	0.186
	356	31	0.143	0.091	0.070	0.122
	357	31	0.181	0.102	0.080	0.188
	358	32	0.157	0.107	0.051	0.115

Table D-2. Individual Animal Absolute Organ Weights (g) – Females (Continued)

Group	Animal		Salivary Gland	Spleen	Thymus	Uterus
	ID	Day				
B2F	359	32	0.117	0.123	0.057	0.260
	360	32	0.138	0.090	0.051	0.127
B20F	451	30	0.122	0.110	0.056	0.211
	452	30	0.117	0.076	0.034	0.126
	453	30	0.128	0.090	0.061	0.252
	454	31	0.152	0.082	0.038	0.269
	455	31	0.118	0.106	0.033	0.141
	456	31	0.105	0.082	0.065	0.194
	457	31	0.139	0.080	0.029	0.120
	458	32	0.167	0.108	0.058	0.256
	459	32	0.159	0.056	0.039	0.284
	460	32	0.156	0.075	0.045	0.187
B80F	551	30	0.134	0.078	0.041	0.138
	552	30	0.169	0.068	0.067	0.209
	553	30	0.133	0.093	0.052	0.092
	554	31	0.152	0.070	0.056	0.104
	555	31	0.146	0.072	0.032	0.221
	556	31	0.143	0.079	0.050	0.089
	557	31	0.122	0.084	0.050	0.165
	558	32	0.152	0.083	0.051	0.074
	559	32	0.146	0.118	0.057	0.284
	560	32	0.114	0.102	0.064	0.247
E2F	751	30	0.173	0.073	0.059	0.088
	752	30	0.124	0.056	0.025	0.125
	753	30	0.152	0.048	0.028	0.090
	754	31	0.104	0.082	0.039	0.146
	755	31	0.140	0.083	0.034	0.086
	756	31	0.169	0.100	0.054	0.117

Table D-2. Individual Animal Absolute Organ Weights (g) – Females (Continued)

Group	Animal ID	Day	Salivary Gland	Spleen	Thymus	Uterus
E2F	757	31	0.161	0.100	0.066	0.166
	758	32	0.143	0.084	0.050	0.275
	759	32	0.169	0.097	0.052	0.305
	760	32	0.116	0.087	0.055	0.102
E20F	851	30	0.155	0.058	0.042	0.174
	852	30	0.124	0.060	0.053	0.091
	853	30	0.153	0.083	0.058	0.121
	854	31	0.136	0.055	0.043	0.244
	855	31	0.137	0.098	0.062	0.111
	856	31	0.126	0.068	0.043	0.104
	857	31	0.166	0.079	0.048	0.175
	858	32	0.149	0.118	0.054	0.110
	859	32	0.147	0.062	0.042	0.128
	860	32	0.161	0.116	0.059	0.217
E80F	951	30	0.135	0.104	0.062	0.083
	952	30	0.113	0.078	0.044	0.135
	953	30	0.086	0.069	0.043	0.093
	954	31	0.132	0.083	0.078	0.186
	955	31	0.127	0.100	0.029	0.199
	956	31	0.136	0.094	0.037	0.243
	957	31	0.153	0.096	0.062	0.264
	958	32	0.131	0.083	0.059	0.128
	959	32	0.161	0.075	0.032	0.093
	960	32	0.115	0.090	0.049	0.142
E200F	1055	31	0.131	0.058	0.042	0.222
	1057	31	0.116	0.042	0.034	0.051
	1058	32	0.160	0.105	0.049	0.210
	1060	32	0.094	0.043	0.014	0.030

Table D-3. Individual Animal Terminal Body Weights (g) and Percent Organ to Body Weight Ratios – Males

Group	Animal ID	Day	Terminal				
			Body Weight	Brain	Epididymides	Heart	Kidneys
CM	101	30	27.6	1.772	0.3337	0.615	1.937
	102	30	30.4	1.664	0.2737	0.523	1.445
	103	30	28.7	1.729	0.3383	0.552	1.339
	104	30	30.5	1.624	0.3492	0.663	1.821
	105	31	30.2	1.582	0.3752	0.815	1.941
	106	31	30.5	1.655	0.3557	0.878	1.579
	107	31	28.1	1.760	0.4053	0.562	1.780
	108	32	31.1	1.604	0.2913	0.852	1.382
	109	32	32.5	1.521	0.4354	0.756	1.605
	110	32	35.5	1.330	0.2375	0.501	1.490
NT200M	202	30	22.6	2.231	0.3518	0.672	1.524
	203	30	27.2	1.718	0.2860	0.547	1.499
	204	30	26.0	1.847	0.3254	0.776	1.518
	206	31	19.1	2.347	0.2524	0.671	1.443
	207	31	18.4	2.414	0.2212	0.671	1.200
	209	32	23.4	1.912	0.3068	0.588	1.563
B2M	301	30	28.9	1.750	0.3190	0.670	1.643
	302	30	30.2	1.671	0.3844	0.700	1.388
	303	30	29.4	1.756	0.3527	0.782	1.565
	304	30	30.5	1.592	0.3148	0.666	1.708
	305	31	29.4	1.648	0.3357	0.704	1.580
	306	31	28.7	1.692	0.3321	0.777	1.709
	307	31	31.7	1.653	0.3274	0.551	1.597
	308	32	29.8	1.877	0.3309	0.588	1.485
	309	32	30.8	1.572	0.2912	0.668	1.705
	310	32	29.5	1.639	0.2573	0.544	1.530

Table D-3. Individual Animal Terminal Body Weights (g) and Percent Organ to Body Weight Ratios – Males (Continued)

Group	Animal ID	Day	Terminal				Kidneys
			Body Weight	Brain	Epididymides	Heart	
B20M	401	30	33.4	1.488	0.2868	0.545	1.720
	402	30	32.0	1.501	0.3763	0.708	1.714
	403	30	30.1	1.572	0.2781	0.571	1.643
	404	30	30.6	1.522	0.3092	0.618	1.339
	405	31	30.2	1.597	0.3437	0.514	1.647
	406	31	27.9	1.724	0.3731	0.826	1.656
	407	31	28.9	1.636	0.3436	0.605	1.648
	408	32	29.1	1.598	0.2347	0.862	1.484
	409	32	30.1	1.690	0.3000	0.525	1.654
	410	32	32.1	1.506	0.3019	0.559	1.786
B80M	501	30	32.1	1.445	0.3673	0.474	1.469
	502	30	31.4	1.554	0.3360	0.716	1.771
	503	30	25.0	1.782	0.3524	0.741	1.402
	504	30	25.2	1.838	0.2548	0.521	1.646
	505	31	26.0	1.805	0.3927	0.578	1.760
	506	31	27.1	1.800	0.3579	0.548	1.431
	507	31	30.5	1.506	0.3852	0.566	1.697
	508	32	28.3	1.688	0.4148	0.604	1.790
	509	32	26.6	1.805	0.3853	0.586	1.717
	510	32	27.5	1.758	0.4124	0.520	1.308
E2M	701	30	31.3	1.645	0.2655	0.522	1.515
	702	30	30.7	1.545	0.2863	0.712	1.557
	703	30	28.5	1.673	0.3379	0.579	1.627
	704	30	32.3	1.492	0.2783	0.580	1.628
	705	31	30.5	1.636	0.3111	0.686	1.874
	706	31	26.4	1.848	0.4144	0.680	1.775
	707	31	32.6	1.491	0.3896	0.576	1.706

Table D-3. Individual Animal Terminal Body Weights (g) and Percent Organ to Body Weight Ratios – Males (Continued)

Group	Animal ID	Day	Terminal Body Weight		Brain	Epididymides	Heart	Kidneys
E2M	708	32	31.6		1.584	0.3918	0.579	1.677
	709	32	29.4		1.650	0.3184	0.635	1.511
	710	32	28.0		1.625	0.3011	0.720	1.515
E20M	801	30	31.6		1.602	0.3456	0.527	1.776
	802	30	29.5		1.665	0.2749	0.591	1.446
	803	30	32.5		1.532	0.2803	0.634	1.704
	804	30	30.5		1.731	0.3603	0.597	1.658
	805	31	33.1		1.399	0.3680	0.703	1.646
	806	31	26.3		1.848	0.3719	0.792	1.524
	807	31	30.4		1.554	0.3438	0.785	1.426
	808	32	28.1		1.693	0.2665	0.834	1.736
	809	32	29.9		1.663	0.3338	0.652	1.442
	810	32	28.9		1.811	0.3464	0.514	1.860
E80M	901	30	28.4		1.749	0.3303	0.582	1.495
	902	30	25.3		1.857	0.3051	0.601	1.411
	903	30	27.6		1.655	0.2830	0.697	1.184
	904	30	29.9		1.564	0.3508	0.764	1.487
	905	31	28.6		1.655	0.3252	0.540	1.658
	906	31	27.2		1.752	0.3140	0.554	1.624
	907	31	31.6		1.559	0.2832	0.730	1.678
	908	32	32.0		1.530	0.2981	0.681	1.414
	909	32	25.7		1.758	0.3113	0.512	1.633
	910	32	28.4		1.663	0.3218	0.771	1.448

Table D-3. Individual Animal Terminal Body Weights (g) and Percent Organ to Body Weight Ratios – Males (Continued)

Group	Animal ID	Day	Terminal		Liver	Salivary Gland	Spleen	Testes
			Body Weight					
CM	101	30	27.6		4.475	0.932	0.309	0.708
	102	30	30.4		4.163	0.692	0.288	0.822
	103	30	28.7		3.453	0.598	0.277	0.968
	104	30	30.5		4.244	0.803	0.336	0.819
	105	31	30.2		4.009	0.631	0.252	0.811
	106	31	30.5		4.054	0.827	0.283	0.803
	107	31	28.1		4.240	0.836	0.277	0.932
	108	32	31.1		3.892	0.783	0.290	0.903
	109	32	32.5		4.026	0.698	0.240	0.973
	110	32	35.5		4.130	0.648	0.275	0.586
NT200M	202	30	22.6		4.292	0.523	0.202	0.927
	203	30	27.2		4.590	0.654	0.232	0.662
	204	30	26.0		4.485	0.774	0.231	0.720
	206	31	19.1		3.826	0.570	0.160	0.615
	207	31	18.4		3.649	0.636	0.107	0.559
	209	32	23.4		4.256	0.656	0.150	0.765
B2M	301	30	28.9		3.981	0.778	0.246	0.754
	302	30	30.2		4.000	0.646	0.309	0.731
	303	30	29.4		4.170	0.798	0.270	0.923
	304	30	30.5		4.301	0.697	0.294	0.613
	305	31	29.4		3.867	0.584	0.290	0.768
	306	31	28.7		4.540	0.852	0.324	0.941
	307	31	31.7		4.158	0.693	0.382	0.825
	308	32	29.8		3.899	0.668	0.322	0.735
	309	32	30.8		4.033	0.723	0.295	0.957
	310	32	29.5		3.597	0.621	0.309	0.731

Table D-3. Individual Animal Terminal Body Weights (g) and Percent Organ to Body Weight Ratios – Males (Continued)

Group	Animal ID	Day	Terminal		Liver	Salivary Gland	Spleen	Testes
			Body Weight					
B20M	401	30	33.4		4.194	0.687	0.331	0.802
	402	30	32.0		4.622	0.738	0.265	0.816
	403	30	30.1		3.909	0.682	0.280	0.678
	404	30	30.6		3.660	0.740	0.230	0.786
	405	31	30.2		3.908	0.797	0.274	0.785
	406	31	27.9		4.067	0.625	0.292	0.863
	407	31	28.9		4.064	0.632	0.280	0.651
	408	32	29.1		3.824	0.754	0.271	0.764
	409	32	30.1		4.041	0.816	0.282	0.773
	410	32	32.1		4.085	0.676	0.283	0.696
B80M	501	30	32.1		4.252	0.764	0.295	0.750
	502	30	31.4		3.984	0.657	0.300	0.675
	503	30	25.0		3.986	0.646	0.195	0.927
	504	30	25.2		4.364	0.602	0.305	0.762
	505	31	26.0		5.021	0.803	0.258	0.872
	506	31	27.1		4.015	0.797	0.303	0.875
	507	31	30.5		4.071	0.634	0.283	0.738
	508	32	28.3		4.378	0.752	0.251	0.888
	509	32	26.6		4.086	0.733	0.263	0.982
	510	32	27.5		4.012	0.647	0.250	0.878
E2M	701	30	31.3		3.887	0.612	0.296	0.733
	702	30	30.7		3.858	0.708	0.282	0.847
	703	30	28.5		3.732	0.765	0.275	0.876
	704	30	32.3		3.893	0.700	0.232	0.666
	705	31	30.5		3.834	0.704	0.251	0.810
	706	31	26.4		4.198	0.744	0.275	0.717
	707	31	32.6		3.958	0.694	0.298	0.721

Table D-3. Individual Animal Terminal Body Weights (g) and Percent Organ to Body Weight Ratios – Males (Continued)

Group	Animal ID	Day	Terminal		Liver	Salivary Gland	Spleen	Testes
			Body Weight					
E2M	708	32	31.6		3.904	0.644	0.292	0.830
	709	32	29.4		3.870	0.600	0.275	0.820
	710	32	28.0		3.906	0.468	0.276	0.395
E20M	801	30	31.6		4.086	0.660	0.337	0.655
	802	30	29.5		3.918	0.633	0.301	0.706
	803	30	32.5		3.804	0.670	0.332	0.791
	804	30	30.5		4.173	0.810	0.271	0.812
	805	31	33.1		3.912	0.567	0.345	0.641
	806	31	26.3		4.005	0.757	0.356	0.918
	807	31	30.4		4.119	0.751	0.347	0.778
	808	32	28.1		4.177	0.654	0.331	0.654
	809	32	29.9		4.279	0.757	0.282	1.082
	810	32	28.9		3.753	0.845	0.247	0.833
E80M	901	30	28.4		4.908	0.599	0.226	0.851
	902	30	25.3		4.217	0.743	0.258	0.830
	903	30	27.6		3.815	0.591	0.325	0.836
	904	30	29.9		4.206	0.669	0.347	0.863
	905	31	28.6		4.191	0.715	0.224	0.749
	906	31	27.2		4.266	0.761	0.260	0.842
	907	31	31.6		4.934	0.685	0.316	0.785
	908	32	32.0		5.584	0.620	0.294	0.681
	909	32	25.7		4.089	0.720	0.199	0.823
	910	32	28.4		4.019	0.518	0.264	0.873

Table D-3. Individual Animal Terminal Body Weights (g) and Percent Organ to Body Weight Ratios – Males (Continued)

Group	Animal ID	Day	Terminal	
			Body Weight	Thymus
CM	101	30	27.6	0.149
	102	30	30.4	0.156
	103	30	28.7	0.188
	104	30	30.5	0.101
	105	31	30.2	0.154
	106	31	30.5	0.137
	107	31	28.1	0.136
	108	32	31.1	0.209
	109	32	32.5	0.121
	110	32	35.5	0.122
NT200M	202	30	22.6	0.069
	203	30	27.2	0.187
	204	30	26.0	0.133
	206	31	19.1	0.078
	207	31	18.4	0.082
	209	32	23.4	0.055
B2M	301	30	28.9	0.133
	302	30	30.2	0.179
	303	30	29.4	0.131
	304	30	30.5	0.199
	305	31	29.4	0.172
	306	31	28.7	0.134
	307	31	31.7	0.160
	308	32	29.8	0.121
	309	32	30.8	0.137
	310	32	29.5	0.107

Table D-3. Individual Animal Terminal Body Weights (g) and Percent Organ to Body Weight Ratios – Males (Continued)

Group	Animal ID	Day	Terminal	
			Body Weight	Thymus
B20M	401	30	33.4	0.179
	402	30	32.0	0.196
	403	30	30.1	0.148
	404	30	30.6	0.144
	405	31	30.2	0.130
	406	31	27.9	0.161
	407	31	28.9	0.118
	408	32	29.1	0.144
	409	32	30.1	0.211
	410	32	32.1	0.127
B80M	501	30	32.1	0.188
	502	30	31.4	0.164
	503	30	25.0	0.158
	504	30	25.2	0.168
	505	31	26.0	0.139
	506	31	27.1	0.174
	507	31	30.5	0.169
	508	32	28.3	0.150
	509	32	26.6	0.173
	510	32	27.5	0.146
E2M	701	30	31.3	0.108
	702	30	30.7	0.142
	703	30	28.5	0.155
	704	30	32.3	0.115
	705	31	30.5	0.121
	706	31	26.4	0.140
	707	31	32.6	0.179

Table D-3. Individual Animal Terminal Body Weights (g) and Percent Organ to Body Weight Ratios – Males (Continued)

Group	Animal ID	Day	Terminal	
			Body Weight	Thymus
E2M	708	32	31.6	0.196
	709	32	29.4	0.152
	710	32	28.0	0.131
E20M	801	30	31.6	0.132
	802	30	29.5	0.167
	803	30	32.5	0.135
	804	30	30.5	0.124
	805	31	33.1	0.153
	806	31	26.3	0.148
	807	31	30.4	0.198
	808	32	28.1	0.115
	809	32	29.9	0.077
	810	32	28.9	0.113
E80M	901	30	28.4	0.104
	902	30	25.3	0.129
	903	30	27.6	0.186
	904	30	29.9	0.180
	905	31	28.6	0.196
	906	31	27.2	0.211
	907	31	31.6	0.068
	908	32	32.0	0.211
	909	32	25.7	0.105
	910	32	28.4	0.107

Table D-4. Individual Animal Terminal Body Weights (g) and Percent Organ to Body Weight Ratios – Females

Group	Animal ID	Day	Terminal Body Weight				
			Brain	Heart	Kidneys	Liver	
CF	151	30	22.3	2.080	0.600	1.372	4.112
	152	30	22.2	2.195	0.923	1.426	4.115
	153	30	21.5	2.246	0.581	1.452	3.713
	154	31	24.4	1.966	0.521	1.364	4.595
	155	31	24.7	2.089	0.663	1.466	4.162
	156	31	19.9	2.424	0.563	1.476	4.286
	157	31	22.9	2.130	0.716	1.653	4.842
	158	32	24.3	1.869	0.544	1.222	3.835
	159	32	26.3	1.989	0.736	1.406	4.270
	160	32	23.4	2.274	0.637	1.556	4.347
NT200F	251	30	18.3	2.451	0.491	1.352	4.436
	252	30	19.0	2.480	0.754	1.253	4.918
	253	30	20.7	2.244	0.742	1.258	4.145
	254	31	21.7	2.114	0.478	1.272	4.423
	255	31	19.4	2.342	0.563	1.212	4.384
	256	31	16.2	2.441	0.667	1.294	5.354
	257	31	15.3	2.699	0.670	1.309	4.495
	258	32	14.2	2.805	0.506	1.294	3.754
	259	32	22.5	2.112	0.572	1.172	4.604
	260	32	22.1	1.972	0.519	1.463	4.648
B2F	351	30	24.9	2.035	0.902	1.205	4.534
	352	30	20.6	2.329	0.692	1.267	4.186
	353	30	22.8	2.093	0.693	1.357	4.108
	354	31	26.3	1.866	0.665	1.399	4.614
	355	31	25.1	1.834	0.609	1.565	5.141
	356	31	26.7	1.851	0.608	1.425	4.464
	357	31	26.4	1.809	0.555	1.302	4.173

Table D-4. Individual Animal Terminal Body Weights (g) and Percent Organ to Body Weight Ratios – Females (Continued)

Group	Animal ID	Day	Terminal				
			Body Weight	Brain	Heart	Kidneys	Liver
B2F	358	32	26.6	2.120	0.623	1.244	4.865
	359	32	24.5	1.932	0.593	1.389	4.627
	360	32	23.5	1.843	0.580	1.336	4.447
B20F	451	30	22.6	2.078	0.672	1.365	4.045
	452	30	24.1	2.123	0.711	1.179	3.871
	453	30	23.8	1.912	0.699	1.083	3.351
	454	31	23.5	2.089	0.622	1.344	4.214
	455	31	21.5	2.259	0.613	1.229	4.165
	456	31	21.7	2.299	0.621	1.424	4.262
	457	31	22.8	2.049	0.778	1.283	3.915
	458	32	24.3	2.002	0.788	1.401	4.589
	459	32	22.9	2.180	0.580	1.548	4.197
	460	32	23.9	2.030	0.573	1.646	4.707
B80F	551	30	23.1	2.065	0.506	1.194	4.144
	552	30	22.5	2.260	0.594	1.336	4.037
	553	30	21.5	2.215	0.801	1.173	4.178
	554	31	22.1	2.139	0.559	1.467	4.256
	555	31	22.2	2.160	0.555	1.232	4.465
	556	31	22.5	2.158	0.749	1.281	4.053
	557	31	22.8	2.121	0.573	1.387	3.587
	558	32	22.9	1.994	0.767	1.341	4.103
	559	32	24.1	2.119	0.595	1.323	4.587
	560	32	24.5	1.954	0.542	1.304	4.471
E2F	751	30	23.4	2.044	0.679	1.187	4.013
	752	30	21.5	2.203	0.792	1.336	3.760
	753	30	22.3	2.242	0.884	1.210	3.680
	754	31	22.9	2.049	0.898	1.431	4.266

Table D-4. Individual Animal Terminal Body Weights (g) and Percent Organ to Body Weight Ratios – Females (Continued)

Group	Animal ID	Day	Terminal				
			Body Weight	Brain	Heart	Kidneys	Liver
E2F	755	31	22.0	2.131	0.700	1.256	4.197
	756	31	23.8	2.069	0.802	1.359	4.111
	757	31	25.2	1.853	0.690	1.254	4.023
	758	32	25.9	2.027	0.871	1.433	4.424
	759	32	25.4	2.020	0.627	1.340	4.332
	760	32	22.0	2.291	0.644	1.411	4.417
E20F	851	30	21.4	2.240	0.607	1.234	3.693
	852	30	20.9	2.302	0.677	1.443	4.037
	853	30	22.6	2.270	0.618	1.253	3.987
	854	31	22.4	2.073	0.769	1.228	3.783
	855	31	24.4	2.063	0.570	1.445	4.555
	856	31	21.2	2.320	0.606	1.446	4.197
	857	31	22.8	2.193	0.699	1.224	4.262
	858	32	23.5	2.080	0.972	1.339	4.959
	859	32	21.3	2.337	0.667	1.415	4.386
	860	32	22.5	2.061	0.657	1.347	4.451
E80F	951	30	24.4	1.895	0.536	1.204	4.084
	952	30	21.5	2.338	0.639	1.233	3.746
	953	30	22.6	2.115	0.623	1.262	3.912
	954	31	23.4	2.257	0.777	1.409	4.470
	955	31	22.4	2.009	0.525	1.587	5.047
	956	31	24.4	2.148	0.786	1.245	4.045
	957	31	22.7	2.034	0.642	1.350	4.455
	958	32	23.8	2.035	0.577	1.314	4.124
	959	32	20.7	2.453	0.529	1.530	4.702
	960	32	25.2	1.854	0.546	1.277	4.370

Table D-4. Individual Animal Terminal Body Weights (g) and Percent Organ to Body Weight Ratios – Females (Continued)

Group	Animal ID	Day	Terminal				
			Body Weight	Brain	Heart	Kidneys	Liver
E200F	1055	31	20.1	2.353	0.640	1.356	4.210
	1057	31	16.7	2.674	0.624	1.514	4.177
	1058	32	22.7	1.975	0.722	1.342	4.558
	1060	32	16.2	2.698	0.628	1.514	4.778

Table D-4. Individual Animal Terminal Body Weights (g) and Percent Organ to Body Weight Ratios – Females (Continued)

Group	Animal ID	Day	Terminal				
			Body Weight	Salivary Gland	Spleen	Thymus	Uterus
CF	151	30	22.3	0.652	0.391	0.280	0.512
	152	30	22.2	0.654	0.447	0.171	0.487
	153	30	21.5	0.762	0.365	0.198	0.626
	154	31	24.4	0.748	0.445	0.194	0.716
	155	31	24.7	0.548	0.448	0.187	0.722
	156	31	19.9	0.448	0.426	0.167	0.668
	157	31	22.9	0.611	0.351	0.257	0.424
	158	32	24.3	0.712	0.377	0.238	0.440
	159	32	26.3	0.511	0.311	0.156	0.643
	160	32	23.4	0.476	0.311	0.182	0.866
NT200F	251	30	18.3	0.675	0.253	0.305	0.424
	252	30	19.0	0.431	0.374	0.226	0.273
	253	30	20.7	0.586	0.345	0.288	0.412
	254	31	21.7	0.502	0.426	0.259	0.523
	255	31	19.4	0.714	0.250	0.190	0.402
	256	31	16.2	0.514	0.365	0.152	0.302
	257	31	15.3	0.477	0.478	0.126	0.190
	258	32	14.2	0.483	0.155	0.035	0.189
	259	32	22.5	0.441	0.396	0.259	0.342
	260	32	22.1	0.502	0.341	0.245	0.378
B2F	351	30	24.9	0.729	0.571	0.156	0.469
	352	30	20.6	0.673	0.341	0.319	0.791
	353	30	22.8	0.654	0.360	0.207	0.507
	354	31	26.3	0.462	0.362	0.180	0.869
	355	31	25.1	0.635	0.410	0.266	0.740
	356	31	26.7	0.535	0.339	0.262	0.456
	357	31	26.4	0.684	0.388	0.303	0.713

Table D-4. Individual Animal Terminal Body Weights (g) and Percent Organ to Body Weight Ratios – Females (Continued)

Group	Animal ID	Day	Terminal				
			Body Weight	Salivary Gland	Spleen	Thymus	Uterus
B2F	358	32	26.6	0.589	0.402	0.190	0.434
	359	32	24.5	0.479	0.503	0.232	1.060
	360	32	23.5	0.589	0.384	0.215	0.539
B20F	451	30	22.6	0.539	0.488	0.246	0.931
	452	30	24.1	0.485	0.315	0.142	0.521
	453	30	23.8	0.537	0.378	0.255	1.057
	454	31	23.5	0.645	0.348	0.162	1.143
	455	31	21.5	0.551	0.495	0.153	0.653
	456	31	21.7	0.483	0.378	0.301	0.892
	457	31	22.8	0.608	0.349	0.127	0.525
	458	32	24.3	0.686	0.444	0.237	1.055
	459	32	22.9	0.695	0.246	0.172	1.242
	460	32	23.9	0.651	0.314	0.188	0.781
B80F	551	30	23.1	0.578	0.336	0.178	0.599
	552	30	22.5	0.751	0.304	0.299	0.930
	553	30	21.5	0.620	0.433	0.241	0.428
	554	31	22.1	0.689	0.315	0.252	0.471
	555	31	22.2	0.656	0.324	0.145	0.994
	556	31	22.5	0.634	0.351	0.221	0.395
	557	31	22.8	0.537	0.368	0.218	0.725
	558	32	22.9	0.663	0.364	0.221	0.323
	559	32	24.1	0.606	0.490	0.236	1.180
	560	32	24.5	0.464	0.415	0.262	1.008
E2F	751	30	23.4	0.740	0.311	0.253	0.376
	752	30	21.5	0.576	0.262	0.116	0.580
	753	30	22.3	0.683	0.213	0.124	0.402
	754	31	22.9	0.453	0.359	0.170	0.637

Table D-4. Individual Animal Terminal Body Weights (g) and Percent Organ to Body Weight Ratios – Females (Continued)

Group	Animal ID	Day	Terminal				
			Body Weight	Salivary Gland	Spleen	Thymus	Uterus
E2F	755	31	22.0	0.638	0.379	0.155	0.393
	756	31	23.8	0.711	0.419	0.225	0.491
	757	31	25.2	0.639	0.397	0.260	0.658
	758	32	25.9	0.554	0.324	0.194	1.063
	759	32	25.4	0.665	0.380	0.204	1.200
	760	32	22.0	0.529	0.397	0.249	0.463
E20F	851	30	21.4	0.725	0.272	0.196	0.811
	852	30	20.9	0.595	0.285	0.256	0.436
	853	30	22.6	0.676	0.367	0.258	0.535
	854	31	22.4	0.606	0.244	0.194	1.089
	855	31	24.4	0.561	0.401	0.254	0.454
	856	31	21.2	0.592	0.322	0.201	0.492
	857	31	22.8	0.730	0.346	0.212	0.769
	858	32	23.5	0.632	0.503	0.231	0.469
	859	32	21.3	0.688	0.293	0.196	0.601
	860	32	22.5	0.714	0.516	0.264	0.963
E80F	951	30	24.4	0.553	0.426	0.255	0.342
	952	30	21.5	0.524	0.364	0.203	0.630
	953	30	22.6	0.382	0.305	0.192	0.411
	954	31	23.4	0.566	0.356	0.332	0.793
	955	31	22.4	0.565	0.448	0.127	0.888
	956	31	24.4	0.556	0.385	0.152	0.995
	957	31	22.7	0.674	0.422	0.274	1.165
	958	32	23.8	0.548	0.347	0.248	0.537
	959	32	20.7	0.777	0.363	0.155	0.448
	960	32	25.2	0.458	0.358	0.196	0.564

Table D-4. Individual Animal Terminal Body Weights (g) and Percent Organ to Body Weight Ratios – Females (Continued)

Group	Animal ID	Day	Terminal				
			Body Weight	Salivary Gland	Spleen	Thymus	Uterus
E200F	1055	31	20.1	0.653	0.289	0.208	1.105
	1057	31	16.7	0.694	0.250	0.205	0.305
	1058	32	22.7	0.705	0.464	0.217	0.925
	1060	32	16.2	0.581	0.262	0.089	0.186

Table D-5. Individual Animal Brain Weights (g) and Percent Organ to Brain Weight Ratios – Males

Group	Animal ID	Day	Absolute Brain Weight				
				Epididymides	Heart	Kidneys	Liver
CM	101	30	0.489	18.834	34.72	109.35	252.56
	102	30	0.506	16.446	31.41	86.86	250.19
	103	30	0.496	19.565	31.90	77.41	199.68
	104	30	0.495	21.506	40.83	112.18	261.39
	105	31	0.478	23.718	51.50	122.69	253.42
	106	31	0.505	21.494	53.07	95.38	244.95
	107	31	0.495	23.033	31.95	101.13	240.95
	108	32	0.499	18.167	53.12	86.18	242.69
	109	32	0.494	28.632	49.74	105.56	264.75
	110	32	0.472	17.849	37.62	112.03	310.42
NT200M	202	30	0.504	15.768	30.11	68.31	192.36
	203	30	0.467	16.645	31.84	87.23	267.09
	204	30	0.480	17.614	42.02	82.16	242.76
	206	31	0.448	10.754	28.58	61.51	163.05
	207	31	0.444	9.165	27.81	49.72	151.18
	209	32	0.448	16.045	30.77	81.74	222.57
B2M	301	30	0.506	18.232	38.28	93.91	227.51
	302	30	0.505	23.008	41.91	83.06	239.40
	303	30	0.516	20.089	44.52	89.15	237.50
	304	30	0.486	19.769	41.80	107.27	270.16
	305	31	0.485	20.372	42.72	95.87	234.67
	306	31	0.486	19.621	45.89	100.97	268.25
	307	31	0.524	19.805	33.35	96.60	251.48
	308	32	0.559	17.626	31.30	79.08	207.70
	309	32	0.484	18.525	42.48	108.43	256.57
	310	32	0.484	15.698	33.20	93.34	219.48

Table D-5. Individual Animal Brain Weights (g) and Percent Organ to Brain Weight Ratios – Males (Continued)

Group	Animal ID	Day	Absolute Brain Weight				
				Epididymides	Heart	Kidneys	Liver
B20M	401	30	0.497	19.272	36.61	115.59	281.77
	402	30	0.480	25.062	47.13	114.18	307.87
	403	30	0.473	17.692	36.31	104.50	248.70
	404	30	0.466	20.309	40.60	87.98	240.47
	405	31	0.482	21.522	32.18	103.15	244.68
	406	31	0.481	21.647	47.91	96.09	235.93
	407	31	0.473	20.998	36.98	100.70	248.36
	408	32	0.465	14.688	53.91	92.84	239.33
	409	32	0.509	17.748	31.05	97.84	239.07
	410	32	0.483	20.050	37.14	118.62	271.34
B80M	501	30	0.464	25.409	32.80	101.66	294.18
	502	30	0.488	21.628	46.06	114.00	256.46
	503	30	0.446	19.771	41.56	78.66	223.65
	504	30	0.463	13.860	28.35	89.57	237.41
	505	31	0.469	21.760	32.03	97.51	278.24
	506	31	0.488	19.885	30.46	79.48	223.06
	507	31	0.459	25.588	37.59	112.72	270.38
	508	32	0.478	24.571	35.75	106.03	259.31
	509	32	0.480	21.350	32.49	95.15	226.41
	510	32	0.483	23.459	29.60	74.43	228.24
E2M	701	30	0.515	16.142	31.74	92.11	236.34
	702	30	0.474	18.533	46.11	100.76	249.72
	703	30	0.477	20.193	34.60	97.21	223.00
	704	30	0.482	18.659	38.88	109.11	261.00
	705	31	0.499	19.022	41.93	114.57	234.42
	706	31	0.488	22.418	36.80	96.05	227.11
	707	31	0.486	26.121	38.61	114.36	265.38

Table D-5. Individual Animal Brain Weights (g) and Percent Organ to Brain Weight Ratios – Males (Continued)

Group	Animal ID	Day	Absolute				
			Brain Weight	Epididymides	Heart	Kidneys	Liver
E2M	708	32	0.501	24.735	36.56	105.85	246.51
	709	32	0.485	19.295	38.49	91.55	234.53
	710	32	0.455	18.523	44.30	93.23	240.34
E20M	801	30	0.506	21.577	32.92	110.91	255.11
	802	30	0.491	16.511	35.50	86.87	235.32
	803	30	0.498	18.297	41.39	111.21	248.30
	804	30	0.528	20.810	34.48	95.76	241.01
	805	31	0.463	26.301	50.27	117.64	279.64
	806	31	0.486	20.119	42.83	82.43	216.66
	807	31	0.472	22.126	50.50	91.76	265.13
	808	32	0.476	15.742	49.24	102.50	246.66
	809	32	0.497	20.068	39.19	86.71	257.29
	810	32	0.523	19.129	28.38	102.69	207.28
E80M	901	30	0.497	18.888	33.27	85.52	280.69
	902	30	0.470	16.433	32.35	76.01	227.10
	903	30	0.457	17.101	42.13	71.56	230.57
	904	30	0.468	22.439	48.86	95.10	269.01
	905	31	0.473	19.653	32.63	100.21	253.28
	906	31	0.477	17.922	31.65	92.68	243.53
	907	31	0.493	18.173	46.82	107.68	316.57
	908	32	0.490	19.481	44.48	92.40	364.90
	909	32	0.452	17.703	29.10	92.85	232.55
	910	32	0.472	19.356	46.36	87.08	241.72

Table D-5. Individual Animal Brain Weights (g) and Percent Organ to Brain Weight Ratios – Males (Continued)

Group	Animal ID	Day	Absolute Brain Weight	Salivary Gland	Spleen	Testes	Thymus
CM	101	30	0.489	52.58	17.42	39.98	8.38
	102	30	0.506	41.61	17.30	49.38	9.37
	103	30	0.496	34.60	16.00	55.97	10.88
	104	30	0.495	49.45	20.68	50.42	6.24
	105	31	0.478	39.88	15.95	51.27	9.73
	106	31	0.505	49.98	17.08	48.53	8.28
	107	31	0.495	47.50	15.75	52.96	7.70
	108	32	0.499	48.85	18.11	56.29	13.05
	109	32	0.494	45.93	15.80	64.00	7.93
	110	32	0.472	48.68	20.66	44.08	9.15
NT200M	202	30	0.504	23.44	9.06	41.55	3.09
	203	30	0.467	38.08	13.50	38.53	10.87
	204	30	0.480	41.89	12.51	38.98	7.22
	206	31	0.448	24.30	6.83	26.19	3.32
	207	31	0.444	26.35	4.41	23.17	3.38
	209	32	0.448	34.28	7.82	40.00	2.88
B2M	301	30	0.506	44.47	14.04	43.11	7.61
	302	30	0.505	38.68	18.47	43.76	10.70
	303	30	0.516	45.43	15.38	52.60	7.46
	304	30	0.486	43.80	18.47	38.53	12.52
	305	31	0.485	35.42	17.61	46.63	10.42
	306	31	0.486	50.34	19.13	55.63	7.91
	307	31	0.524	41.92	23.13	49.91	9.69
	308	32	0.559	35.57	17.16	39.17	6.44
	309	32	0.484	45.99	18.79	60.88	8.72
	310	32	0.484	37.89	18.86	44.57	6.56

Table D-5. Individual Animal Brain Weights (g) and Percent Organ to Brain Weight Ratios – Males (Continued)

Group	Animal ID	Day	Absolute				
			Brain Weight	Salivary Gland	Spleen	Testes	Thymus
B20M	401	30	0.497	46.17	22.25	53.91	12.03
	402	30	0.480	49.13	17.63	54.37	13.03
	403	30	0.473	43.42	17.84	43.12	9.43
	404	30	0.466	48.63	15.11	51.61	9.49
	405	31	0.482	49.91	17.17	49.14	8.15
	406	31	0.481	36.27	16.93	50.05	9.36
	407	31	0.473	38.61	17.13	39.78	7.23
	408	32	0.465	47.16	16.99	47.83	9.03
	409	32	0.509	48.29	16.69	45.72	12.46
	410	32	0.483	44.88	18.83	46.22	8.44
B80M	501	30	0.464	52.84	20.43	51.85	12.97
	502	30	0.488	42.29	19.33	43.46	10.54
	503	30	0.446	36.27	10.93	52.00	8.84
	504	30	0.463	32.73	16.58	41.47	9.15
	505	31	0.469	44.52	14.32	48.32	7.69
	506	31	0.488	44.28	16.85	48.59	9.66
	507	31	0.459	42.12	18.77	49.04	11.24
	508	32	0.478	44.56	14.88	52.62	8.87
	509	32	0.480	40.60	14.56	54.41	9.56
	510	32	0.483	36.80	14.21	49.94	8.32
E2M	701	30	0.515	37.22	18.01	44.58	6.55
	702	30	0.474	45.81	18.24	54.80	9.17
	703	30	0.477	45.73	16.42	52.38	9.29
	704	30	0.482	46.91	15.53	44.65	7.72
	705	31	0.499	43.05	15.35	49.55	7.38
	706	31	0.488	40.23	14.88	38.77	7.58
	707	31	0.486	46.57	19.99	48.38	11.99

Table D-5. Individual Animal Brain Weights (g) and Percent Organ to Brain Weight Ratios – Males (Continued)

Group	Animal ID	Day	Absolute Brain Weight	Salivary Gland	Spleen	Testes	Thymus
E2M	708	32	0.501	40.66	18.42	52.39	12.37
	709	32	0.485	36.38	16.68	49.70	9.19
	710	32	0.455	28.78	16.96	24.28	8.06
E20M	801	30	0.506	41.24	21.04	40.88	8.26
	802	30	0.491	37.99	18.10	42.43	10.02
	803	30	0.498	43.74	21.69	51.62	8.84
	804	30	0.528	46.75	15.64	46.90	7.16
	805	31	0.463	40.51	24.66	45.80	10.97
	806	31	0.486	40.98	19.23	49.68	8.02
	807	31	0.472	48.36	22.34	50.05	12.75
	808	32	0.476	38.61	19.55	38.65	6.77
	809	32	0.497	45.51	16.95	65.07	4.62
	810	32	0.523	46.65	13.63	45.98	6.23
E80M	901	30	0.497	34.27	12.91	48.65	5.96
	902	30	0.470	40.04	13.90	44.70	6.96
	903	30	0.457	35.73	19.66	50.49	11.21
	904	30	0.468	42.80	22.22	55.21	11.49
	905	31	0.473	43.22	13.57	45.25	11.83
	906	31	0.477	43.46	14.86	48.06	12.03
	907	31	0.493	43.98	20.30	50.40	4.37
	908	32	0.490	40.51	19.20	44.50	13.78
	909	32	0.452	40.94	11.31	46.80	5.97
	910	32	0.472	31.17	15.90	52.48	6.42

Table D-6. Individual Animal Brain Weights (g) and Percent Organ to Brain Weight Ratios – Females

Group	Animal ID	Day	Absolute				Salivary Gland
			Brain Weight	Heart	Kidneys	Liver	
CF	151	30	0.464	28.84	65.94	197.65	31.32
	152	30	0.487	42.07	64.97	187.48	29.78
	153	30	0.483	25.86	64.65	165.29	33.94
	154	31	0.480	26.52	69.39	233.78	38.07
	155	31	0.516	31.74	70.18	199.17	26.22
	156	31	0.482	23.24	60.92	176.84	18.47
	157	31	0.488	33.61	77.63	227.37	28.69
	158	32	0.454	29.13	65.38	205.24	38.12
	159	32	0.523	37.00	70.71	214.70	25.68
	160	32	0.532	28.01	68.42	191.20	20.96
NT200F	251	30	0.449	20.02	55.16	181.00	27.54
	252	30	0.471	30.41	50.53	198.32	17.38
	253	30	0.465	33.05	56.08	184.71	26.14
	254	31	0.459	22.60	60.16	209.18	23.76
	255	31	0.454	24.03	51.76	187.15	30.48
	256	31	0.395	27.34	53.03	219.35	21.04
	257	31	0.413	24.82	48.51	166.55	17.68
	258	32	0.398	18.05	46.15	133.84	17.22
	259	32	0.475	27.08	55.51	217.97	20.88
	260	32	0.436	26.34	74.19	235.70	25.47
B2F	351	30	0.507	44.34	59.21	222.77	35.79
	352	30	0.480	29.73	54.39	179.78	28.89
	353	30	0.477	33.09	64.86	196.27	31.22
	354	31	0.491	35.62	74.99	247.28	24.74
	355	31	0.460	33.19	85.30	280.26	34.60
	356	31	0.494	32.83	76.96	241.13	28.91
	357	31	0.478	30.67	71.96	230.70	37.81

Table D-6. Individual Animal Brain Weights (g) and Percent Organ to Brain Weight Ratios – Females (Continued)

Group	Animal ID	Day	Absolute Brain Weight	Heart	Kidneys	Liver	Salivary Gland
B2F	358	32	0.564	29.38	58.69	229.45	27.78
	359	32	0.473	30.69	71.86	239.46	24.80
	360	32	0.433	31.46	72.46	241.23	31.95
B20F	451	30	0.470	32.35	65.72	194.66	25.94
	452	30	0.512	33.48	55.53	182.33	22.83
	453	30	0.455	36.56	56.62	175.24	28.08
	454	31	0.491	29.76	64.32	201.71	30.86
	455	31	0.486	27.16	54.41	184.41	24.38
	456	31	0.499	27.02	61.96	185.39	21.01
	457	31	0.467	37.95	62.61	191.07	29.67
	458	32	0.487	39.35	69.95	229.18	34.26
	459	32	0.499	26.62	71.02	192.49	31.86
	460	32	0.485	28.22	81.06	231.86	32.09
B80F	551	30	0.477	24.49	57.84	200.69	27.99
	552	30	0.509	26.29	59.12	178.61	33.23
	553	30	0.476	36.17	52.97	188.58	27.97
	554	31	0.473	26.13	68.58	198.96	32.20
	555	31	0.480	25.71	57.01	206.67	30.38
	556	31	0.486	34.72	59.37	187.81	29.37
	557	31	0.484	27.03	65.41	169.11	25.31
	558	32	0.457	38.46	67.28	205.78	33.25
	559	32	0.511	28.08	62.42	216.47	28.61
	560	32	0.479	27.72	66.76	228.85	23.75
E2F	751	30	0.478	33.19	58.05	196.30	36.18
	752	30	0.474	35.96	60.66	170.71	26.14
	753	30	0.500	39.45	53.97	164.17	30.49
	754	31	0.469	43.82	69.86	208.18	22.12

Table D-6. Individual Animal Brain Weights (g) and Percent Organ to Brain Weight Ratios – Females (Continued)

Group	Animal ID	Day	Absolute Brain Weight		Heart	Kidneys	Liver	Salivary Gland
E2F	755	31	0.469		32.86	58.93	196.93	29.92
	756	31	0.492		38.75	65.68	198.70	34.36
	757	31	0.467		37.24	67.64	217.09	34.50
	758	32	0.525		42.98	70.69	218.19	27.31
	759	32	0.513		31.05	66.34	214.50	32.90
	760	32	0.504		28.09	61.58	192.78	23.09
E20F	851	30	0.479		27.10	55.10	164.89	32.36
	852	30	0.481		29.41	62.66	175.35	25.83
	853	30	0.513		27.23	55.17	175.60	29.78
	854	31	0.464		37.10	59.24	182.49	29.22
	855	31	0.503		27.64	70.06	220.84	27.22
	856	31	0.492		26.13	62.34	180.91	25.52
	857	31	0.500		31.87	55.81	194.40	33.29
	858	32	0.489		46.76	64.37	238.47	30.39
	859	32	0.498		28.55	60.58	187.70	29.46
	860	32	0.464		31.87	65.37	215.98	34.63
E80F	951	30	0.463		28.30	63.50	215.46	29.17
	952	30	0.503		27.34	52.75	160.23	22.42
	953	30	0.478		29.46	59.64	184.96	18.05
	954	31	0.528		34.43	62.43	198.07	25.07
	955	31	0.450		26.11	78.98	251.24	28.13
	956	31	0.524		36.57	57.97	188.31	25.87
	957	31	0.462		31.57	66.37	218.97	33.11
	958	32	0.484		28.35	64.59	202.68	26.95
	959	32	0.508		21.56	62.37	191.67	31.67
	960	32	0.467		29.47	68.86	235.72	24.68

Table D-6. Individual Animal Brain Weights (g) and Percent Organ to Brain Weight Ratios – Females (Continued)

Group	Animal ID	Day	Absolute Brain Weight		Heart	Kidneys	Liver	Salivary Gland
E200F	1055	31	0.473		27.21	57.61	178.90	27.76
	1057	31	0.447		23.34	56.64	156.24	25.96
	1058	32	0.448		36.55	67.95	230.73	35.68
	1060	32	0.437		23.30	56.13	177.14	21.53

Table D-6. Individual Animal Brain Weights (g) and Percent Organ to Brain Weight Ratios – Females (Continued)

Group	Animal ID	Day	Absolute			
			Brain Weight	Spleen	Thymus	Uterus
CF	151	30	0.464	18.80	13.45	24.60
	152	30	0.487	20.36	7.78	22.20
	153	30	0.483	16.26	8.82	27.87
	154	31	0.480	22.66	9.88	36.43
	155	31	0.516	21.45	8.95	34.55
	156	31	0.482	17.56	6.88	27.58
	157	31	0.488	16.47	12.06	19.89
	158	32	0.454	20.19	12.73	23.54
	159	32	0.523	15.64	7.84	32.31
	160	32	0.532	13.67	8.01	38.10
NT200F	251	30	0.449	10.32	12.46	17.30
	252	30	0.471	15.09	9.13	10.99
	253	30	0.465	15.39	12.83	18.34
	254	31	0.459	20.14	12.25	24.72
	255	31	0.454	10.67	8.10	17.17
	256	31	0.395	14.95	6.22	12.39
	257	31	0.413	17.70	4.67	7.05
	258	32	0.398	5.52	1.26	6.73
	259	32	0.475	18.77	12.27	16.18
	260	32	0.436	17.30	12.44	19.16
B2F	351	30	0.507	28.04	7.68	23.07
	352	30	0.480	14.63	13.72	33.96
	353	30	0.477	17.18	9.87	24.25
	354	31	0.491	19.42	9.64	46.59
	355	31	0.460	22.37	14.51	40.36
	356	31	0.494	18.31	14.16	24.62
	357	31	0.478	21.42	16.73	39.41

Table D-6. Individual Animal Brain Weights (g) and Percent Organ to Brain Weight Ratios – Females (Continued)

Group	Animal ID	Day	Absolute			
			Brain Weight	Spleen	Thymus	Uterus
B2F	358	32	0.564	18.95	8.95	20.46
	359	32	0.473	26.02	12.02	54.84
	360	32	0.433	20.82	11.66	29.25
B20F	451	30	0.470	23.49	11.86	44.83
	452	30	0.512	14.82	6.70	24.53
	453	30	0.455	19.75	13.36	55.28
	454	31	0.491	16.66	7.76	54.68
	455	31	0.486	21.91	6.75	28.93
	456	31	0.499	16.46	13.09	38.79
	457	31	0.467	17.02	6.19	25.64
	458	32	0.487	22.17	11.84	52.67
	459	32	0.499	11.28	7.87	56.96
	460	32	0.485	15.46	9.27	38.46
B80F	551	30	0.477	16.27	8.62	29.01
	552	30	0.509	13.45	13.23	41.15
	553	30	0.476	19.57	10.88	19.32
	554	31	0.473	14.72	11.78	22.02
	555	31	0.480	14.99	6.73	46.02
	556	31	0.486	16.25	10.26	18.31
	557	31	0.484	17.35	10.30	34.16
	558	32	0.457	18.24	11.10	16.18
	559	32	0.511	23.14	11.14	55.69
	560	32	0.479	21.25	13.43	51.58
E2F	751	30	0.478	15.22	12.40	18.37
	752	30	0.474	11.89	5.26	26.31
	753	30	0.500	9.52	5.52	17.92
	754	31	0.469	17.54	8.29	31.07

Table D-6. Individual Animal Brain Weights (g) and Percent Organ to Brain Weight Ratios – Females (Continued)

Group	Animal ID	Day	Absolute			
			Brain Weight	Spleen	Thymus	Uterus
E2F	755	31	0.469	17.79	7.25	18.43
	756	31	0.492	20.27	10.87	23.74
	757	31	0.467	21.43	14.05	35.52
	758	32	0.525	15.96	9.58	52.41
	759	32	0.513	18.81	10.10	59.40
	760	32	0.504	17.32	10.87	20.19
E20F	851	30	0.479	12.14	8.74	36.20
	852	30	0.481	12.39	11.10	18.95
	853	30	0.513	16.16	11.36	23.56
	854	31	0.464	11.78	9.35	52.54
	855	31	0.503	19.43	12.32	21.99
	856	31	0.492	13.89	8.66	21.23
	857	31	0.500	15.80	9.66	35.09
	858	32	0.489	24.17	11.13	22.55
	859	32	0.498	12.54	8.38	25.72
	860	32	0.464	25.04	12.79	46.73
E80F	951	30	0.463	22.49	13.45	18.03
	952	30	0.503	15.58	8.67	26.94
	953	30	0.478	14.44	9.08	19.41
	954	31	0.528	15.79	14.69	35.13
	955	31	0.450	22.31	6.33	44.20
	956	31	0.524	17.91	7.10	46.34
	957	31	0.462	20.72	13.45	57.25
	958	32	0.484	17.06	12.20	26.41
	959	32	0.508	14.79	6.30	18.27
	960	32	0.467	19.31	10.55	30.42

Table D-6. Individual Animal Brain Weights (g) and Percent Organ to Brain Weight Ratios – Females (Continued)

Group	Animal ID	Day	Absolute			
			Brain Weight	Spleen	Thymus	Uterus
E200F	1055	31	0.473	12.26	8.84	46.98
	1057	31	0.447	9.36	7.68	11.40
	1058	32	0.448	23.48	10.97	46.83
	1060	32	0.437	9.73	3.30	6.89

**APPENDIX E: CLINICAL PATHOLOGY AND ANATOMIC PATHOLOGY
NARRATIVES**

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28-DAY REPEATED DOSE TOXICITY STUDY OF TOBACCO BLEND AND AQUEOUS TOBACCO EXTRACT IN CD-1 MICE**PATHOLOGY NARRATIVE**

Groups of 10 male and 10 female CD-1 mice were fed various test articles at various food concentrations, as listed in Text Table Path A below, for at least 28 days.

TEXT TABLE PATH A. SUMMARY OF STUDY DESIGN	
Group	Target Dosage of Nicotine (mg/kg/day)
1 - Control	0
2 - Nicotine Tartrate High Dose	200
3 - Tobacco Blend Low Dose	2
4 - Tobacco Blend Intermediate Dose 1	20
5 - Tobacco Blend Intermediate Dose 2	80
6 - Tobacco Blend High dose	200
7 - Tobacco Extract Low Dose	2
8 - Tobacco Extract Intermediate Dose 1	20
9 - Tobacco Extract Intermediate Dose 2	80
10 - Tobacco Extract High Dose	200

A necropsy examination was performed on each study mouse.

NECROPSY RESULTS**Terminal Body Weights**

The group mean terminal body weights of the scheduled termination NT200M and NT200F groups were decreased (by 25 and 19%, respectively) and differed statistically from those of the same-sex controls. Also decreased by 19, 8 and 7%, respectively, in comparison to controls, but not with statistical significance, were the group mean terminal body weights of the E200F group, the B80M group and the E80M group. All these decreases were interpreted to be due to feeding with the test articles. Other instances of decreased terminal body weights in groups fed test articles were interpreted to be consistent with biological variation and not due to the test articles.

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Organ Weights

The following alterations of absolute organ weights in groups exposed to the test articles were interpreted to be due to their test article feeding.

The absolute brain weights of the NT200M and NT200F groups and those of the E200F group were decreased (by 6, 10 and 8%, respectively) and were statistically different from the same-sex controls. Decreased absolute brain weights of 4% that were not statistically significant, compared with control, were found in the B80M group and the E80M group; these decreases were interpreted to be due to the test articles. The absolute epididymis weights of the NT200M group were decreased by 35% and statistically different from those of the control males. Also noted with statistical significance were decreased group mean absolute heart weights, compared with same-sex controls, in the NT200M and NT200F groups, of 27 and 25%, respectively, and in the B80M group of 21%. Though not statistically significant, decreased mean heart weight in the E200F group of 17% was interpreted to be due to test article feeding. The absolute renal weights in the NT200M and NT200F groups were decreased by 32 and 27%, respectively, and the same parameter in the E200F group was decreased by 19%. Decreased mean absolute hepatic weight in the NT200M group of 22% was statistically significant. Though not statistically significant, decreased mean hepatic weight in the NT200F group and the E200F group of 13 and 15%, respectively, were interpreted to be due to test article feeding. Decreased absolute salivary gland weights in the NT200M and NT200F groups and the E80M group of 35, 29 and 17%, respectively, were statistically significant. Though not statistically significant, decreased mean absolute salivary gland weight in the E80F and E200F groups of 9 and 12%, respectively, were interpreted to be due to test article feeding. Decreased group mean absolute splenic weights of 51 and 28%, respectively, compared with control, were statistically significant in the NT200M and NT200F groups, and a 31% decrease in mean absolute splenic weight in the E200F group (not statistically significant) all were interpreted to be due to test article feeding. Statistically significant decreased group mean absolute testicular weight (36%) was noted in the

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NT200M group. Decreased mean absolute thymic weight of 47% was noted with statistical significance in the NT200M group. Decreased absolute uterine weight of 53% in the NT200F group was statistically significant when compared with female controls.

Organ-to-body weight ratios reported as percent body weight included statistically significant increased brain/body weight ratios of 28 and 11%, respectively, in the NT200M and NT200F groups, and a 14% increase (not statistically significant) in the E200F group. The terminal body and absolute brain weights of these three groups were decreased due to test article feeding, when compared with those of control mice, but brain mass was conserved in these mice to a greater degree than that of other organs and body mass generally. When expressed as a portion of body weight, the weights of the epididymides, hearts, kidneys, salivary glands, spleens, testes, thymuses and uteruses of groups listed above as having test article-related decreased absolute weights were for the most part only a little decreased when compared to control, and not statistically different from control. However, these slight decreases, such as decreased heart/terminal body weight ratios in the NT200F group (8%) and the B80M group (13%), decreased group mean salivary gland/terminal body weight ratios in the NT200M and NT200F groups of 15 and 13%, respectively, and in the E80M group of 11%, decreased spleen/terminal body weight ratios in the NT200F group of 13% and in the E200F group of 18%, might indicate organ-specific effects. Also, splenic and thymic weight/terminal body weight ratios in the NT200M group were decreased by 36 and 31%, respectively, with statistical significance, compared with the male controls; and the renal/terminal body weight ratio of the NT200F group was decreased by 10%, with statistical significance, compared with female controls, possibly indicating nicotine-induced effects on these organs over and above generalized failure to grow. The liver/terminal body weight ratios of the groups with test article-induced decreased absolute hepatic weights were slightly increased over same-sex control means, suggesting possibly a conservation of liver weight in conditions of generally low weight gain, but these differences were not statistically significant.

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The organ-to-brain weight results served to highlight absolute organ weight decreases found in the NT200M and NT200F groups, but decreases in organ-to-brain weight ratio for some of these organs were also found in the E200F group, the E80M group and the B80M group.

The toxicologic relevance of statistically significant increased liver/brain weight ratio (15%) in the B2F group was interpreted to be questionable, since similar increases were not seen in the groups fed higher amounts of Tobacco Blend.

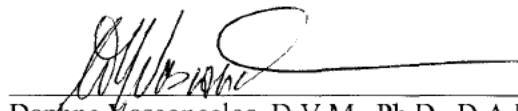
Gross Lesions

The gross findings of this study were all considered to be incidental and unrelated to the test articles, except that the small thymus in NT200F 258 was probably related to its Nicotine feeding.

Gross examination did not reveal findings useful for establishing the cause of death/moribund condition in unscheduled-death mice, but observed clinical signs were consistent with tobacco/nicotine toxicity.


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Study Pathologist

3/10/09
Date


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Date

3/10/09

CN49730D

28-DAY REPEATED DOSE TOXICITY STUDY OF TOBACCO BLEND AND AQUEOUS TOBACCO EXTRACT IN CD-1 MICE

CLINICAL PATHOLOGY

Hematology

Decreased hemoglobin in the NT200M, E80M and the E200F groups of 6, 3, and 4%, respectively, were of uncertain relationship to test article feeding. The decrease in hemoglobin did not appear to be due to reduced red blood cell numbers, because red blood cell counts were not similarly affected, but rather were due to red blood cells being smaller and containing less hemoglobin, as indicated by the decreased mean corpuscular volume and mean corpuscular hemoglobin values found in these three groups, when compared to those of the controls. These three groups also had decreased total white cell counts and lymphocyte counts, when compared with results in same-sex controls. The decreases in total white cell count in the NT200M, B20M, B80M, E2M, E80M and the E200F groups amounted to 32, 34, 33, 45, 43 and 44%, respectively, when compared with same-sex controls; similar comparisons of total lymphocyte count results in these six groups to control-group results showed decreases of 53, 16, 25, 43, 42, and 57%, respectively; mean neutrophil count results were not decreased in the NT200M group, but were decreased in the other four referenced groups of males, relative to same-sex control results, by 48, 43, 49 and 53%, respectively, but were not decreased in the NT200F group. These decreased total white cell, lymphocyte and neutrophil count results were interpreted to be due to test article feeding, and to be of similar severity in all affected groups, and to be stress-mediated. Statistical analysis showed that the mean corpuscular hemoglobin concentration result of the B20M was greater than that of the E20M group. However, both groups had adequate hemoglobin and red cell numbers, and their mean corpuscular hemoglobin concentration results, though different, were both within expected ranges for this parameter in CD-1 mice, so the statistical difference was interpreted to be spurious. It was also not clear that the statistical difference between the neutrophil count results of the B80M group and control males and the lack of statistical difference from controls for the decreases in neutrophil counts in the B20M, E2M and E80M

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indicated any real treatment difference, since all these groups had what were considered to be similar stress-induced altered neutrophil (and other white cell) counts. Similarly, the statistical significance attached to the comparison of the relative decrease in lymphocytes of the E2M group when compared to the B2M group was not interpreted to be definite evidence of a greater effect on lymphocytes by the extract versus the blend.

No other hematology results indicated any effects due to test article feeding.

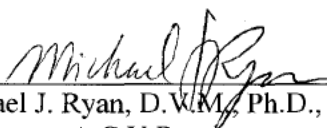
Clinical Chemistry

Increased group mean aspartate aminotransferase and cholesterol levels in the NT200 males and females were of uncertain relationship to test article feeding, since they were so sporadic. Not all animals in these groups had high aspartate aminotransferase levels, but NT200 male 207 and females 255 and 257 had the three highest aspartate aminotransferase results found in the study. Statistical comparisons across groups revealed a greater decrease in aspartate aminotransferase in the E2M group as compared to the B2M group, but overall the results from these groups were similar and did not indicate major release of this enzyme to serum from the liver. Similarly, a statistical indication of higher glucose in the E20M as compared to the B20M group was considered to be spurious because both groups had glucose levels within expected ranges of variation for CD-1 mice. The relatively decreased globulin and increased albumin/globulin ratios of the E20M and E2F groups in comparison to other groups was also considered to be of little biological importance, because of the small size of the differences noted between groups in comparison to variation expected in globulin and albumin/globulin ratio in CD-1 mice. The statistically significant increased alkaline phosphatase results of the NT200 and E200F groups also were not interpreted to be indicative of treatment effects on these groups, because the alkaline phosphatase results of all treated and control mice of this study were within expected ranges of variation for untreated CD-1 mice. The statistical significance of the increased cholesterol in the NT200F group identified this increase as a possible treatment effect of nicotine tartrate.


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No other clinical chemistry results indicated any effects due to test article feeding.

 3/10/09

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APPENDIX F: NICOTINE IN FEED FORMULATION ANALYSIS REPORT

**28-DAY REPEATED DOSE TOXICITY STUDY OF TOBACCO BLEND AND
AQUEOUS TOBACCO EXTRACT IN CD-1 MICE**

NICOTINE IN FEED

FORMULATION ANALYSIS REPORT

Battelle Study Number: CN49730D

March 24, 2009

Prepared By:

Approved By:

Ed A. Psurny 3/24/09
Edward A. Psurny, B.S./Date

Brian L. Burback 3/24/09
Brian L. Burback, Ph.D./Date

EXECUTIVE SUMMARY

Samples prepared for this study were submitted for analysis and successfully analyzed for nicotine concentrations. All pre-dose nicotine formulations that were analyzed for nicotine concentration met acceptance criteria (within 10% of the target concentrations; relative standard deviation [RSD] less than or equal to 10%). Post-dose (animal room) nicotine formulations were also analyzed for nicotine concentration and reported. In general, the post-dose animal room concentrations were lower than the pre-dose concentrations. The tobacco blend formulations were approximately 12% lower on average; the nicotine hydrogen tartrate formulations were approximately 9% lower on average; and the tobacco extract formulations were approximately 3% lower on average.

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I. INTRODUCTION

This report contains a description of the analysis of submitted formulation samples from this study, the results of these analyses, and figures.

This work was performed at Battelle, 505 King Avenue, Columbus, OH 43201.

II. STANDARDS

Nicotine hydrogen tartrate, Lot No. 028K0705, obtained from Sigma-Aldrich Inc., was used as an analytical standard.

This standard was used to perform all work covered in this report.

III. SAMPLES

The formulation samples submitted for analysis are shown in Table 1, (M=Male, F=Female).

Table 1 – Samples Submitted for Formulation Analyses

Type	Group	Formulation ID	Batch	Target Nicotine Concentration ¹ (mg/kg)
Control	1 (CM/CF)	Control Formulation	1-Control-1	0
Tobacco Blend	B2F	571 mg/kg ²	1-Blend-9	15.0
	B2M	666 mg/kg ²	1-Blend-10	17.5
	B20F	5708 mg/kg ²	1-Blend-11	150
	B20M	6659 mg/kg ²	1-Blend-12	175
	B80F	22831 mg/kg ²	1-Blend-13	600
	B80M	26636 mg/kg ²	1-Blend-14	700
	B200F	57077 mg/kg ²	1-Blend-15	1500
	B200M	66589 mg/kg ²	1-Blend-16	1750
Nicotine Hydrogen Tartrate	NT200F	4275 mg/kg ²	1-NT-3	1500
	NT200M	4988 mg/kg ²	1-NT-4	1750
Aqueous Tobacco Extract	E2F	653 mg/kg ²	1-Extract-9	15.0
	E2M	761 mg/kg ²	1-Extract-10	17.5
	E20F	6525 mg/kg ²	1-Extract-11	150
	E20M	7612 mg/kg ²	1-Extract-12	175
	E80F	26098 mg/kg ²	1-Extract-13	600
	E80M	30448 mg/kg ²	1-Extract-14	700
	E200F	65246 mg/kg ²	1-Extract-15	1500
	E200M	76120 mg/kg ²	1-Extract-16	1750

1. Quantity of nicotine (mg) per kg of feed.

2. Quantity of test article (mg) per kg of feed.

IV. FORMULATION ANALYSIS

A. METHOD

The samples were analyzed using the standard operating procedure (SOP) current at the time of analysis. A copy is provided in Appendix A. The data acquisition system used was Atlas, Version 8.2, from Thermo Fisher Scientific.

B. RESULTS

The calibration standards met all acceptance criteria (the correlation coefficient [r] is greater than or equal to 0.99 and percent relative error [%RE] within 10% of nominal for all standards in all runs.

The asymmetry and percent relative standard deviation (RSD) for replicates of the system suitability acceptance criteria was met for each analysis. The efficiency for the system suitability acceptance criteria was met for each analysis. The drifts compared to the system suitability samples met acceptance criteria for each analysis.

Representative overlaid full scale chromatograms from high and low concentration standards, blank with internal standard (IS), and a blank are shown in Figure 1.

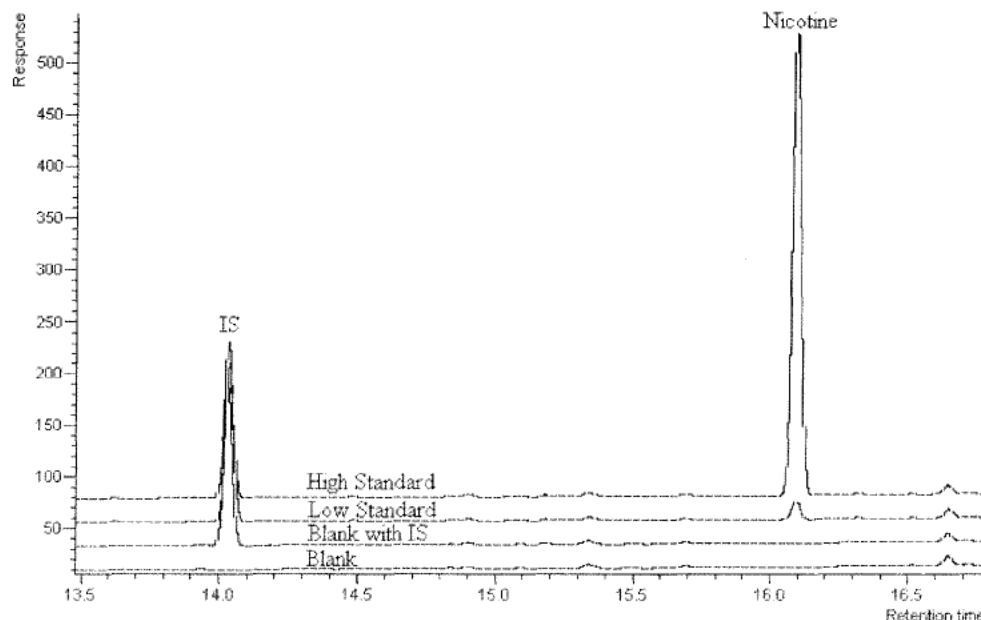


Figure 1 – Representative Overlaid Chromatograms from High and Low Standards, Blank with IS, and a Blank – Full Scale

The results from the formulation analysis for the pre-dose formulations are shown in Table 2 through Table 5. The concentration of all submitted formulations met acceptance criteria (within 10% of target; RSD less than or equal to 10%).

Table 2 – Results for Pre-Dose Control Formulation

Formulation ID (Dose Group)	Target Concentration ¹ (mg/kg)	Corrected Concentration ¹ (mg/kg)	Average Corrected Concentration ¹ (mg/kg)	s (mg/kg)	RSD	%RE	Average %RE
0 mg/kg ² (CM/CF)	0	BLOQ	BLOQ	NA	NA	NA	NA
		BLOQ				NA	
		BLOQ				NA	

BLOQ = Below the limit of quantitation (not detected).

NA = Not applicable.

1. Quantity of nicotine (mg) per kg of feed.

2. Quantity of test article (mg) per kg of feed.

Table 3 – Results for Pre-Dose Tobacco Blend Formulations

Formulation ID (Dose Group)	Target Concentration ¹ (mg/kg)	Corrected Concentration ¹ (mg/kg)	Average Corrected Concentration ¹ (mg/kg)	s (mg/kg)	RSD	%RE	Average %RE
571 mg/kg ² (B2F)	15.0	1.63E+01	1.65E+01	1.0	6.1	8.7	10.0
		1.56E+01				4.0	
		1.76E+01				17.3	
666 mg/kg ² (B2M)	17.5	1.78E+01	1.87E+01	0.9	5.0	1.7	6.8
		1.95E+01				11.4	
		1.88E+01				7.4	
5708 mg/kg ² (B20F)	150	1.56E+02	1.59E+02	3	2.0	4.0	6.0
		1.62E+02				8.0	
		1.59E+02				6.0	
6659 mg/kg ² (B20M)	175	1.85E+02	1.87E+02	5	3.0	5.7	6.7
		1.92E+02				9.7	
		1.83E+02				4.6	
22831 mg/kg ² (B80F)	600	6.28E+02	6.43E+02	14	2.2	4.7	7.1
		6.44E+02				7.3	
		6.56E+02				9.3	
26636 mg/kg ² (B80M)	700	7.51E+02	7.45E+02	8	1.0	7.3	6.4
		7.36E+02				5.1	
		7.47E+02				6.7	
57077 mg/kg ² (B200F)	1500	1.54E+03	1.57E+03	35.1	2.2	2.7	4.9
		1.57E+03				4.7	
		1.61E+03				7.3	
66589 mg/kg ² (B200M)	1750	1.84E+03	1.84E+03	5.77	0.3	5.1	5.3
		1.84E+03				5.1	
		1.85E+03				5.7	

1. Quantity of nicotine (mg) per kg of feed.

2. Quantity of tobacco blend (mg) per kg of feed.

Table 4 – Results for Pre-Dose Nicotine Hydrogen Tartrate Formulations

Formulation ID (Dose Group)	Target Concentration ¹ (mg/kg)	Corrected Concentration ¹ (mg/kg)	Average Corrected Concentration ¹ (mg/kg)	s (mg/kg)	RSD	%RE	Average %RE
4275 mg/kg ² (NT200F)	1500	1.35E+03	1.39E+03	34.6	2.5	-10.0	-7.3
		1.41E+03				-6.0	
		1.41E+03				-6.0	
4988 mg/kg ² (NT200M)	1750	1.64E+03	1.65E+03	17.3	1.0	-6.3	-5.7
		1.67E+03				-4.6	
		1.64E+03				-6.3	

1. Quantity of nicotine (mg) per kg of feed.

2. Quantity of nicotine tartrate (mg) per kg of feed.

Table 5 – Results for Pre-Dose Aqueous Tobacco Extract Formulations

Formulation ID (Dose Group)	Target Concentration ¹ (mg/kg)	Corrected Concentration ¹ (mg/kg)	Average Corrected Concentration ¹ (mg/kg)	s (mg/kg)	RSD	%RE	Average %RE
653 mg/kg ² (E2F)	15.0	1.66E+01	1.62E+01	0.4	2.0	10.7	7.8
		1.58E+01				5.3	
		1.61E+01				7.3	
761 mg/kg ² (E2M)	17.5	1.83E+01	1.85E+01	0.2	1.0	4.6	5.5
		1.86E+01				6.3	
		1.85E+01				5.7	
6525 mg/kg ² (E20F)	150	1.47E+02	1.45E+02	2	1.0	-2.0	-3.1
		1.46E+02				-2.7	
		1.43E+02				-4.7	
7612 mg/kg ² (E20M)	175	1.70E+02	1.67E+02	4	2.0	-2.9	-4.4
		1.69E+02				-3.4	
		1.63E+02				-6.9	
26098 mg/kg ² (E80F)	600	5.86E+02	5.82E+02	5	0.9	-2.3	-3.0
		5.77E+02				-3.8	
		5.82E+02				-3.0	
30448 mg/kg ² (E80M)	700	6.66E+02	6.70E+02	5	0.7	-4.9	-4.3
		6.68E+02				-4.6	
		6.76E+02				-3.4	
65246 mg/kg ² (E200F)	1500	1.40E+03	1.39E+03	6	0.4	-6.7	-7.1
		1.39E+03				-7.3	
		1.39E+03				-7.3	
76120 mg/kg ² (E200M)	1750	1.62E+03	1.61E+03	101	6.3	-7.4	-8.2
		1.50E+03				-14.3	
		1.70E+03				-2.9	

1. Quantity of nicotine (mg) per kg of feed.

2. Quantity of aqueous tobacco extract (mg) per kg of feed.

The results from the formulation analysis for post-dose formulations (animal room) are shown in Table 6 through Table 9. All post-dose samples are from the animal room feeders, except one sample from the 76120 mg/kg aqueous tobacco extract, which was from the bucket in which the feed was contained and stored to fill the feeders. The concentration of all submitted formulations met the following criteria for concentration (%RE within 10% of target; RSD less than or equal to 10%), except for the following:

- The NT200F (4275 mg/kg) formulation had a RE of -16.0%.
- The NT200M (4988 mg/kg) formulation had a RE of -13.9%.
- The E20F (6525 mg/kg) formulation had a RE of -14.7 %.

There was no impact of these failures of the post-dose samples to meet acceptance criteria on the study. Post-dose samples are taken from the animal room feeders. They have been exposed to the animal and are subject to the impact from this exposure on concentration. This may include selective eating of the feed or analyte from the formulation by the animal, contamination of the formulation by urine, feces, bedding, or other materials, and exposure of the formulation to the animal room environment. For these reasons, animal samples should only be used to determine any general trends that may result from exposure of the formulation to the animal room environment.

In general, the post-dose animal room concentrations were lower than the pre-dose concentrations. The tobacco blend formulations were approximately 12% lower on average; the nicotine hydrogen tartrate formulations were approximately 9% lower on average; and the tobacco extract formulations were approximately 3% lower on average.

Table 6 – Results for Post-Dose Control Formulation

Formulation ID (Dose Group)	Target Concentration ¹ (mg/kg)	Corrected Concentration ¹ (mg/kg)	Average Corrected Concentration ¹ (mg/kg)	s (mg/kg)	RSD	%RE	Average %RE
0 mg/kg ² (CM/CF)	0	BLOQ	BLOQ	NA	NA	NA	NA
		BLOQ				NA	
		BLOQ				NA	

BLOQ = Below the limit of quantitation (not detected).

NA = Not applicable.

1. Quantity of nicotine (mg) per kg of feed.

2. Quantity of test article (mg) per kg of feed.

Table 7 – Results for Post-Dose Tobacco Blend Formulations

Formulation ID (Dose Group)	Target Concentration ¹ (mg/kg)	Corrected Concentration ¹ (mg/kg)	Average Corrected Concentration ¹ (mg/kg)	s (mg/kg)	RSD	%RE	Average %RE
571 mg/kg ² (B2F)	15.0	1.40E+01	1.41E+01	0.5	4.0	-6.7	-5.8
		1.37E+01				-8.7	
		1.47E+01				-2.0	
666 mg/kg ² (B2M)	17.5	1.60E+01	1.67E+01	0.7	4.0	-8.6	-4.8
		1.67E+01				-4.6	
		1.73E+01				-1.1	
5708 mg/kg ² (B20F)	150	1.32E+02	1.35E+02	3	2.0	-12.0	-10.0
		1.35E+02				-10.0	
		1.38E+02				-8.0	
6659 mg/kg ² (B20M)	175	1.67E+02	1.66E+02	3	2.0	-4.6	-5.3
		1.62E+02				-7.4	
		1.68E+02				-4.0	
22831 mg/kg ² (B80F)	600	5.46E+02	5.50E+02	5	0.9	-9.0	-8.3
		5.49E+02				-8.5	
		5.56E+02				-7.3	
26636 mg/kg ² (B80M)	700	6.94E+02	7.02E+02	13	1.9	-0.9	0.3
		6.95E+02				-0.7	
		7.17E+02				2.4	
57077 mg/kg ² (B200F)	1500	1.45E+03	1.44E+03	41.6	2.9	-3.3	-4.2
		1.47E+03				-2.0	
		1.39E+03				-7.3	
66589 mg/kg ² (B200M)	1750	1.61E+03	1.65E+03	36.1	2.2	-8.0	-5.7
		1.66E+03				-5.1	
		1.68E+03				-4.0	

1. Quantity of nicotine (mg) per kg of feed.

2. Quantity of tobacco blend (mg) per kg of feed.

Table 8 – Results for Post-Dose Nicotine Hydrogen Tartrate (NT) Formulations

Formulation ID (Dose Group)	Target Concentration ¹ (mg/kg)	Corrected Concentration ¹ (mg/kg)	Average Corrected Concentration ¹ (mg/kg)	s (mg/kg)	RSD	%RE	Average %RE
4275 mg/kg ² (NT200F)	1500	1.25E+03	1.26E+03	17.3	1.4	-16.7	-16.0
		1.28E+03				-14.7	
		1.25E+03				-16.7	
4988 mg/kg ² (NT200M)	1750	1.49E+03	1.51E+03	20.8	1.4	-14.9	-13.9
		1.53E+03				-12.6	
		1.50E+03				-14.3	

1. Quantity of nicotine (mg) per kg of feed.

2. Quantity of nicotine tartrate (mg) per kg of feed.

Table 9 – Results for Post-Dose Aqueous Tobacco Extract Formulations

Formulation ID (Dose Group)	Target Concentration ¹ (mg/kg)	Corrected Concentration ¹ (mg/kg)	Average Corrected Concentration ¹ (mg/kg)	s (mg/kg)	RSD	%RE	Average %RE
653 mg/kg ² (E2F)	15.0	1.67E+01	1.56E+01	1.1	7.1	11.3	3.8
		1.54E+01				2.7	
		1.46E+01				-2.7	
761 mg/kg ² (E2M)	17.5	1.93E+01	1.82E+01	1.1	6.0	10.3	3.8
		1.72E+01				-1.7	
		1.80E+01				2.9	
6525 mg/kg ² (E20F)	150	1.28E+02	1.28E+02	2	2.0	-14.7	-14.7
		1.30E+02				-13.3	
		1.26E+02				-16.0	
7612 mg/kg ² (E20M)	175	1.63E+02	1.61E+02	2	1.0	-6.9	-7.8
		1.60E+02				-8.6	
		1.61E+02				-8.0	
26098 mg/kg ² (E80F)	600	5.34E+02	5.43E+02	14	2.6	-11.0	-9.4
		5.37E+02				-10.5	
		5.59E+02				-6.8	
30448 mg/kg ² (E80M)	700	6.27E+02	6.43E+02	17	2.6	-10.4	-8.1
		6.60E+02				-5.7	
		6.42E+02				-8.3	
65246 mg/kg ² (E200F)	1500	1.36E+03	1.35E+03	36.1	2.7	-9.3	-10.0
		1.38E+03				-8.0	
		1.31E+03				-12.7	
76120 mg/kg ² Repeat (E200M)	1750	1.67E+03	1.68E+03	17.3	1.0	-4.6	-4.0
		1.67E+03				-4.6	
		1.70E+03				-2.9	
76120 mg/kg ² Bucket sample (E200M)	1750	1.59E+03	1.60E+03	32.1	2.0	-9.1	-8.4
		1.64E+03				-6.3	
		1.58E+03				-9.7	

1. Quantity of nicotine (mg) per kg of feed.

2. Quantity of aqueous tobacco extract (mg) per kg of feed.

V. ACKNOWLEDGMENTS

Hans Whittenburg and Dan Burnham performed the analytical work. Ed Psumy wrote this report. Maria Evascu reviewed the data and report for completeness and accuracy.

APPENDIX A**STANDARD OPERATING PROCEDURE (SOP) FOR THE ANALYSIS OF
NICOTINE IN NTP-2000 FEED**

JUN 12 2008

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STANDARD OPERATING PROCEDURE (SOP) FOR THE ANALYSIS OF
NICOTINE IN NTP-2000 FEED

Originator: EL 3 Date 6/12/08

Approved by: Brian Bush Date 6/12/08
Technical Reviewer

Approved by: [Signature] Date 6/12/08
Study Director

Approved by: [Signature] Date 6/12/08
Management

Reviewed and Registered by QAU:
Nathaniel E. Reed Date 6-12-08

Battelle
505 King Avenue
Columbus, Ohio 43201

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I. SCOPE

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II. PURPOSE

The purpose of this SOP is to provide instructions for conducting the analysis of nicotine in feed.

III. REFERENCES

Current SOP for Labeling Reagents, Solutions, Test and Control Articles, and Specimens

Current SOP for Using Electronic Balances

Current SOP for Recording, Reviewing, and Correcting Raw Data

Current SOP for Operation, Calibration, and Maintenance of Fixed and Adjustable Volume Pipettors.

Current SOP for Operation and Maintenance of Gas Chromatographs

Current SOP for Numeric Data and Calculations

Current SOP for Use and Training of the Atlas Chromatography Data System

IV. DEFINITIONS

None.

V. GENERAL INSTRUCTIONS

Calibrate all required balances according to the SOP on balance usage.

Make equivalent dilutions when the volume needed varies from the volume stated in the method.

Label all standard and reagent solutions as specified in the appropriate SOP.

Sign or initial on each page of this document to signify that you have followed the method as written, all materials and reagents are current, and all equipment has been properly calibrated.

Initial and date all data entries on the page on which they were made. If only one person enters all data on a page on a single day, then the documentation may be made in a single location on that page by that person. If multiple staff make entries or one

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person makes entries on different days, all must be initialed and dated by the person making the entry.

The method is written in general chronological order. However, it is not essential that all sections be performed sequentially. The analyst may determine the order for conducting the task in the most efficient manner, unless the order for certain activities is specified.

VI. PROCEDURE

A. SAMPLES

See Chain of Custody for samples.

B. MATERIALS

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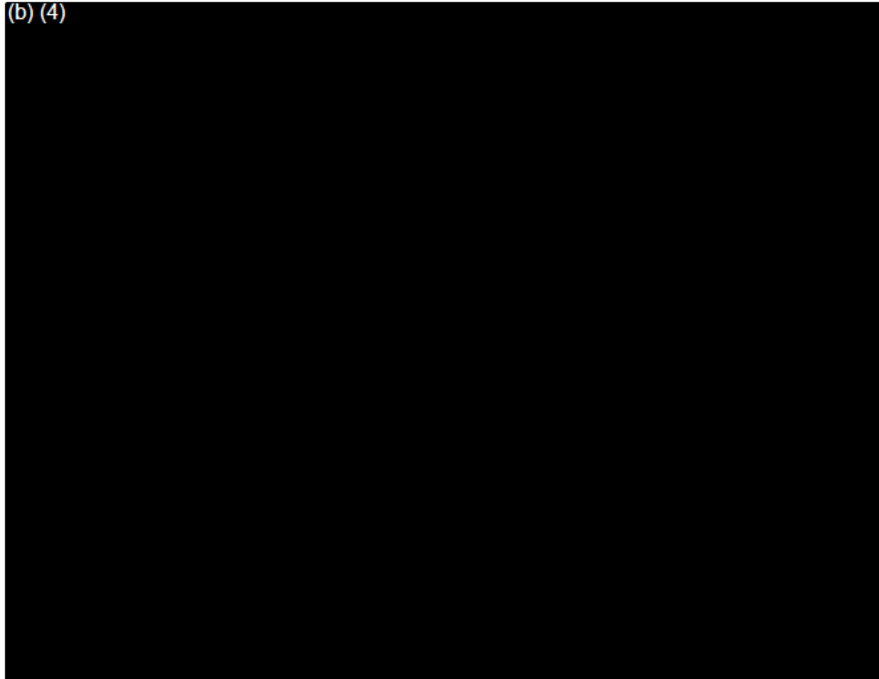
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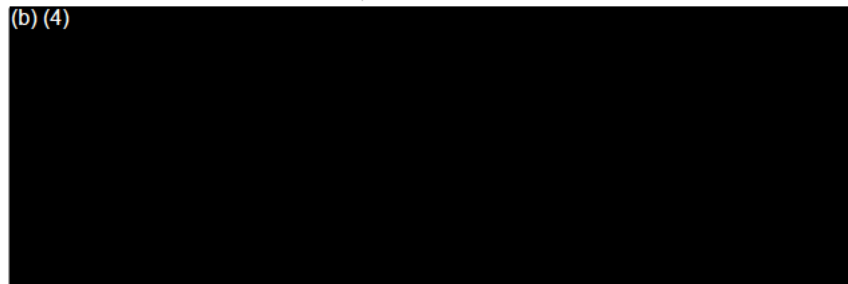
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C. EQUIPMENT

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**D. EXTRACTION SOLUTION**

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**E. PREPARATION OF BLANK FEED EXTRACTED EXTRACTION
SOLUTION**

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Table 4 – Preparation of Solutions C - F

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G. PREPARATION OF BLANKS

1. Feed Blanks

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H. FEED SAMPLE ANALYSIS

To prepare each sample in triplicate, weigh triplicate 10 ± 1 g of sample into individual 120 mL amber glass bottles and record the weight in Table 6, Table 7, Table 8, and Table 9. Add 25 mL of 2N sodium hydroxide into each sample, cap and mix well to wet all of feed, and let stand for ~15 minutes.

Table 6 –Control Sample Weights

Species	Target Formulation Concentration (mg/kg)	Nicotine Concentration (mg/kg)	Aliquot A (g)	Aliquot B (g)	Aliquot C (g)
Rat and/or Mouse	0	0			

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Table 7 – Tobacco Blend Sample Weights

Species	Target Formulation Concentration (mg/kg)	Nicotine Concentration (mg/kg)	Aliquot A (g)	Aliquot B (g)	Aliquot C (g)
Rat	114	3.0			
	137	3.6			
	1142	30			
	1370	36			
	4566	120			
	5479	144			
	11415	300			
	13698	360			
Mouse	571	15.0			
	666	17.5			
	5708	150			
	6659	175			
	22831	600			
	26636	700			
	57077	1500			
	66589	1750			

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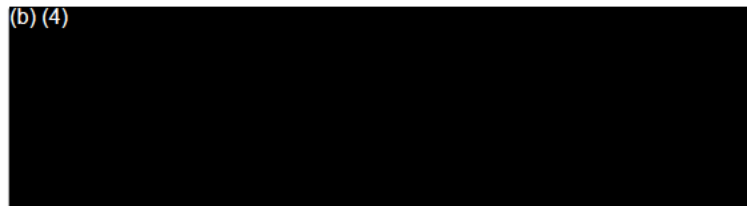
Table 8– Nicotine Hydrogen Tartrate Sample Weights

Species	Target Formulation Concentration (mg/kg)	Nicotine Concentration (mg/kg)	Aliquot A (g)	Aliquot B (g)	Aliquot C (g)
Rat	855	300			
	1026	360			
Mouse	4275	1500			
	4988	1750			

Table 9– Tobacco Extract Sample Weights

Species	Target Formulation Concentration (mg/kg)	Nicotine Concentration (mg/kg)	Aliquot A (g)	Aliquot B (g)	Aliquot C (g)
Rat	130	3.0			
	157	3.6			
	1305	30			
	1566	36			
	5220	120			
	6264	144			
	13049	300			
	15659	360			
Mouse	653	15.0			
	761	17.5			
	6525	150			
	7612	175			
	26098	600			
	30448	700			
	65246	1500			
	76120	1750			

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Follow Table 10 for the dilution of the feed samples with **blank feed extracted extraction solution** to dilute the samples into the standard curve.

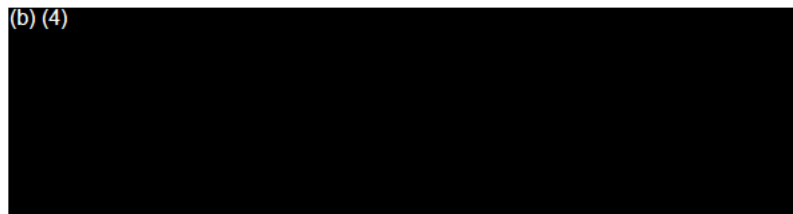
Table 10 – Dilution of Feed Samples

Nicotine Concentration (mg/kg)	Volume of Extract (mL)	Final Dilution Volume (mL)
50-360	1	10
600-1200	1	25
1200-1750	0.5	25

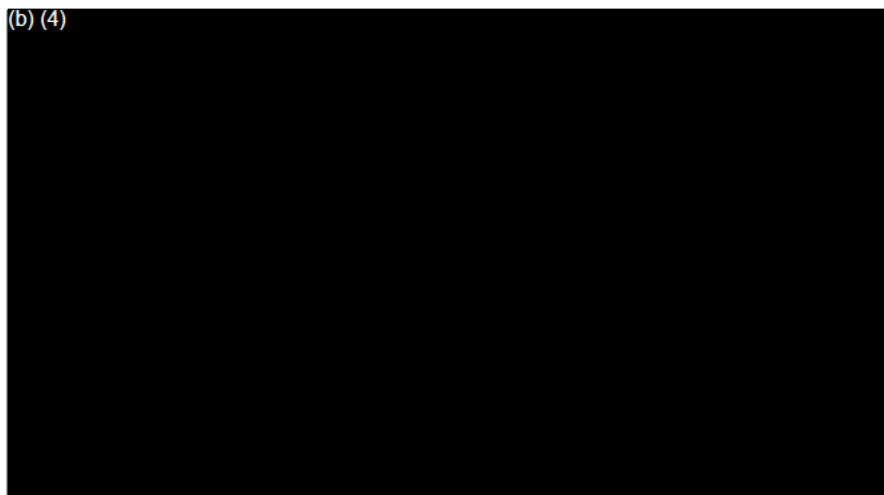
Transfer an appropriate amount of the extraction solution portion of the extract into a GC vial.

I. ANALYSIS

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**Table 11 – GC Conditions**

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*Parameters which may be modified by the analyst.

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Table 12 – Additional GC Conditions

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VII. CALCULATIONS

Examine the integration of the peaks. Modify, as necessary, to obtain consistent integration. Ensure that the response of the standards bracket the response for all filter samples.

Calculate the exact concentration of each standard and enter these into the chromatography data system.

Use the parameters in Table 13 to calculate the regression equation.

Table 13 – Regression Parameters

Model	Linear
Weighting	1/x
y-intercept	Calculate, Do Not Force through Origin
y-values	Nicotine/IS Peak Area Ratio
x-values	Nicotine Standard Concentrations

Calculate the % Relative Error (RE) for each standard. If the RE of any standard is not within 10% of the nominal concentration, evaluate the impact of omitting that calibration standard from the curve. One standard may be omitted from the curve, if deemed technically necessary.

Calculate the chromatography acceptance criteria parameters specified in Table 14 for the system suitabilities.

Calculate the concentration, the average concentration, the standard deviation (s), and the percent relative standard deviation (RSD) for the system suitabilities. Calculate the concentration for each "drift" and compare it to the average of the system suitabilities.

Calculate the amount of nicotine in each formulation sample using its peak ratio response, the regression equation, and dilution factor.

Calculate the average concentration, individual and average RE, s, and RSD for the triplicates. Examine any potential outliers using the Q-test with a 90% confidence interval.

The concentration units in Atlas are mg/kg.

VIII. RESULTS

Place the spreadsheet in the data package. Report all values with concentrations below the specified limit of quantitation as "BLOQ". The Limit of Quantitation (LOQ) is 2.11 mg/kg.

Produce the Atlas Report "Run Reference" and include it the data package.

Include the chain of custody forms in the data package.

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IX. ACCEPTANCE CRITERIA

See Table 14 for acceptance criteria.

Table 14– Acceptance Criteria

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* in System suitability samples only.

X. TASK LEADER RESPONSE TO FAILURE TO MEET ACCEPTANCE CRITERIA

A. ASYMMETRY, EFFICIENCY, RESOLUTION

Verify that the proper instrument system (column, gas, flow rates, etc.) was used for the analysis. If not, samples need to be re-injected using the correct instrument system.

If the correct instrument system was used, compare the current chromatograms to a past analysis. If the chromatography has changed substantially, determine and correct the problem with the instrument system and then re-inject the samples. If the chromatography has not changed substantially the run may be accepted.

B. DRIFTS

Verify that all calculations are correct.

Calculate the drift based on the standard that is used for the drift samples. If the drifts pass based on this calculation notify the CTC management.

If the drifts still do not pass, re-inject the entire analysis.

If the drifts fail after re-injection, repeat the entire analysis from the beginning on a new instrument system if possible.

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C. RSD

Verify that all calculations are correct.

D. CORRELATION COEFFICIENT/RE OF STANDARDS

Verify that all concentrations, the regression model, integration, and calculations are correct.

If the standards still fail after correcting any calculation errors, repeat the analysis from the beginning if possible. The Study Director may choose to accept data with standards outside the normal acceptance range.

XI. COMMENTS/CONCLUSIONS

XII. DATA REVIEW**A. TECHNICAL REVIEW**

Review at least the following to assure they are acceptable: rejection of calibration standards, integration of chromatograms, chromatography data processing and acquisition parameters, calibration standard concentrations, regression model, and compliance of data with acceptance criteria.

B. DATA ACCURACY REVIEW

Review at least the following: completeness and correctness of data entry, formulas used to calculate all values, and accuracy of calculations.

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XIII. SIGNATURES

Technical Review Signature/Date:

Signature of the technical reviewer will be considered documentation that all modifications and/or changes to this SOP (documented during the course of conducting this task) are technically acceptable and have no adverse technical impact unless otherwise noted. Changes or deviations to the acceptance criteria section require independent assessment by the technical reviewer.

Data Accuracy Review Signature/Date:

APPENDIX G: NEUROBEHAVIORAL TOXICITY REPORT

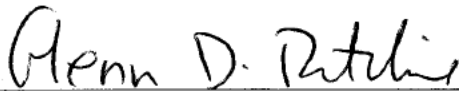
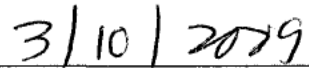
Battelle Study Number CN49730D

**28-DAY REPEATED DOSE TOXICITY STUDY OF TOBACCO
BLEND AND AQUEOUS TOBACCO EXTRACT IN CD-1 MICE**

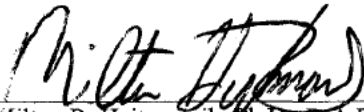
Neurobehavioral Effects Report

**Prepared For:
R.J. Reynolds Tobacco Company**

Battelle
The Business of Innovation

Battelle Study Number CN49730D**28-DAY REPEATED DOSE TOXICITY STUDY OF TOBACCO
BLEND AND AQUEOUS TOBACCO EXTRACT IN CD-1 MICE****Approved, Battelle**Glenn D. Ritchie, Ph.D.
Neurobehavioral Scientist

Date

Milton R. Hejtmancik, Ph.D., D.A.B.T.
Study Director

Date

Battelle Study Number CN49730D**INTRODUCTION AND STUDY DATA**

In this study, five CD-1 mice of each sex were randomly selected from each core study group (see section 10.6 of the study protocol) for the functional observational battery (FOB). Vehicle control group animals (Group 1) were fed powdered NTP-2000 rodent diet without the test article. The nicotine tartrate high dose positive control group (Group 2) was fed powdered NTP-2000 rodent diet containing a target dosage of 200 mg/kg/day nicotine tartrate. Treated mice were fed the diet with the specified quantity of tobacco blend or (aqueous) tobacco extract with target dosages as follows:

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

To evaluate the toxicity of the tobacco blend and the (aqueous) tobacco extract on CNS/neurobehavioral activity in these animals, data were collected for the selected neurobehavioral parameters through use of a 35-test FOB as shown below.

Test	Parameter	Type of Measurement ¹
	HOME CAGE	
1	Posture	Score
2	Tremor Activity	Score
3	Convulsive Activity	Score
4	Lethargy/Arousal	Score
5	Eyelid Closure	Score
	HANDLING RODENTS	
6	Ease of Removing	Score
7	Ease of Handling	Score
9	General Condition/Appearance	Score
10	Urine Stain	Score
11	Fecal Stain	Score
12	Salivation	Score
13	Piloerection	Score
14	Fur Appearance	Score
15	Lacrimation	Score

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16	Palpebral Reflex Responsivity	Score
19	Vocalizations	Yes/No
	OPEN FIELD	
20	# Lines Crossed	Continuous
21	# Rearings	Continuous
22	# Urine Pools	Continuous
23	# Fecal Boluses	Continuous
24	Posture	Score
25	Tremor Activity	Score
26	Convulsive Activity	Score
27	Gait	Score
28	Gait Score	Score
29	Stereotypy	Score
30	Bizarre Behavior	Score
31	Vocalizations – Spontaneous	Yes/No
	REFLEXES	
32	Approach Response	Score
33	Touch Response	Score
34	Startle Response	Score
35	Tail Pinch Response	Score
36	Placing of Paws on Grid	Score
39	Rectal Temperature (°F)	Continuous
46	Grip Strength	Score

Score variables: response represented by a categorical score

Yes/No variables: response given by "Yes" or "No"

Continuous variables: response represents a numeric reading on a continuous scale.

Only data for the CM/CF group, the NT200M/NT200F group, the three tobacco blend dose groups, and four tobacco extract dose groups were considered in this data summary and statistical analysis. All male and female animals in the B200M/B200F group and all male animals in the E200M group died prior to FOB testing and were not considered in the neurobehavioral study.

This report describes the statistical methods applied to these FOB data, summarizes the statistical results, and interprets the neurobehavioral data. All statistical summaries and data analyses in this study were conducted using SAS 9.1.3 (SAS Institute, 2004).

STATISTICAL METHODS

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Battelle Study Number CN49730D**RESULTS**

Data summarization and statistical analyses were conducted independently for each parameter, and for males and females. Unless specified otherwise, the $p \leq 0.05$ level was used to identify statistically significant dose effects.

Categorical Variables (Score and Yes/No Variables)

Significant differences among the dose groups were observed at the 0.05 level for the following FOB parameters:

Male Mice

- **Home Cage Posture** (FOB test 1), where the E80M group was significantly different from the B80M group, the E80M group was significantly different from the CM group, and the NT200M group was significantly different from CM group. In these comparisons, 5 out of 5 male mice in the B80M group and the CM group received negative scores (sleeping or crouching), while 3 out of 5 male mice in the E80M group and 2 out of 3 surviving mice in the NT200M group received score of 1 (rearing and ambulating). These data suggest increased postural activity (arousal or decreased sleeping) in male mice in the E80M group and the NT200M group.
- **Home Cage Lethargy/Arousal** (FOB test 4), where the E80M group was significantly different from the B80M group. All five male mice in the B80M group received scores of -1 (slight lethargy), while 3 out of 5 mice in E80M group received zero scores (alert). It should be noted that 4 out of 5 male mice assigned to the CM group exhibited slight lethargy in the homecage observations.
- **Ease of Removing** (FOB test 6), where the E20M group was significantly different from the B20M group. In this comparison, 3 out of 5 mice in the B20M group received scores of 1 (rodent running around), while 5 out of 5 male mice in the E20M group received zero scores (little resistance). As there were no significant differences between the treated mice and either the CM group or the NT200M group, the statistical difference on the Ease of Removing test is not considered to be toxicologically meaningful.

Female Mice

- **Home Cage Tremor Activity** (FOB test 2), where the B20F group and the E2F group were significantly different from the CF group. In this comparison 5 out of 5 female mice in the B20F group and 5 out of 5 female mice in the E2F group received zero scores (no tremor), while 3 out of 5 female mice in the CF group received scores of 1 (slight tremor). As it is highly atypical for untreated mice (CF) to exhibit clinical tremors and because this effect was not observed in untreated male mice, the effect is not considered to be toxicologically significant.
- **Home Cage Lethargy/Arousal** (FOB test 4), where there was an overall statistically significant treatment effect, but none of the pairwise comparisons of interest were significantly different from each other. Additionally, there were no pairwise comparisons that suggested toxicologically meaningful differences between treated and control mice.

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- **Home Cage Eyelid Closure** (FOB test 5), where there was an overall statistically significant treatment effect, but none of the pairwise comparisons of interest were significantly different from each other. It should be noted that 3 out of 5 female CF mice (with completely closed eyes) may have been sleeping at the time of homecage observation, while a number of the non-sleeping, female treated and NT200F group mice exhibited slightly drooping or “drooping approximately half-way” eyelids. These observations preclude interpretation of the data for the Home Cage Eyelid Closure test.
- **Ease of Removing** (FOB test 6), where the E200F group was significantly different from the CF group. In this comparison, 3 out of 3 surviving female mice in the E200F group received scores of 1 (rodent running around), while 4 out of 5 female mice in the CF group received zero scores (little resistance).
- **Palpebral Reflex Responsivity** (FOB test 16), where the E2F group was significantly different from the B2F group. In this comparison, 5 out of 5 female mice in the B2F group received zero scores (normal), while 3 out of 5 female mice in the E2F group received -1 scores (sluggish closing of eyelid).

Continuous Variables

Means and standard errors of the reported measurements for the continuous variables were calculated for each dose group. Based upon the outcome of the one-way ANOVA applied to these continuous measurements, significant differences were observed at the 0.05 level among the dose groups for the following variables:

Male Mice

- **Rectal Temperature** (FOB test 39), where mean rectal temperature for the NT200M group (mean = 96.3° F) was significantly lower than the CM group (mean = 101.8° F). It should be noted that the mean rectal temperatures of the B80M group, the E20M group, and the E80M group were also numerically lower than the CM group, suggesting that the effect was possibly attributable to the nicotine content of the test articles.

Female Mice

- No overall significant differences were observed for the results of any of the FOB tests identified as continuous variables.

DISCUSSION AND CONCLUSIONS

Treatment of male and female mice with tobacco blend or tobacco extract in the feed resulted in minimal changes in neurobehavior, as evaluated by the FOB. For thirty-five FOB tests, there were toxicologically meaningful differences between the test article treated groups and control groups on three FOB tests for male mice and 2 FOB tests for female mice. Frequently these effects were similar to those observed in mice in the NT200M/NT200F group.

Male mice in the E80M group (the highest dose tobacco extract group of surviving male mice) exhibited significantly increased home cage (FOB Posture test) arousal similar to that exhibited by male

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mice in the NT200M group. The increased arousal observed was significantly greater than the arousal activity observed in the CM group or in the B80M group. Similarly, male mice in the E80M group exhibited significantly more arousal (less sleeping) than did mice assigned to the B80M group, as measured by the FOB Home Cage Lethargy/Arousal test. For female mice, the E200F group exhibited increased resistance to removal from the home cage, as compared to the CF group. It seems probable that these "increased arousal/emotionality" results in male and female mice can be explained by the nicotine content in the tobacco extract and nicotine tartrate treatments. Nicotine has been shown in numerous rodent studies to induce increased arousal/emotionality and to alter sleeping patterns (Ikemoto, 2007 Review). This fact was most evident in the FOB homecage observation tests, during which many of the CM/CF animals were observed to be supine or sleeping, while many of the treated mice were posturally erect.

For female mice there was a significant increase in palpebral reflex responsivity in the B2F group, as compared to the E2F group. Indeed, all three of the tobacco extract groups exhibited numerically decreased palpebral reflex responsivity as compared to the comparable tobacco blend groups. This result would appear counterintuitive, as nicotine is known to generally increase reflexive responses (Ikemoto, 2007 Review). However, Hall and Reit (1966) demonstrated that in unanesthetized cats intraventricular injections of nicotine induced persistent narrowing of the palpebral fissures (distance between the upper and lower eyelids), possibly interfering with the integrity of the palpebral reflex response.

Finally, in male mice the mean rectal temperature for the NT200M group (mean = 96.3° F) was significantly lower than the CM group (mean = 101.8° F). It should be noted that the mean rectal temperatures of the B80M group, the E20M group, and the E80M group were also numerically lower than the CM group suggesting that the hypothermia effects observed may have been attributable to the nicotine content of the test article and positive control treatments (Ruskin et al., 2007).

In summary, following the targeted 28-day ingestion of tobacco blend or tobacco extract in the feed induced very minimal neurobehavioral effects in male and female mice. It would appear that the observed neurobehavioral effects can generally be explained by the effects of the nicotine content in the test articles.

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CN49730D: 00163

Battelle Study Number CN49730D**STATISTICAL METHODS AND RESULTS****INTRODUCTION AND STUDY DATA**

In this study, five CD-1 mice of each sex were randomly assigned to 10 dose groups (see section 10.6 of the study protocol). To evaluate the toxicity of tobacco blend and aqueous tobacco extract on CNS/neurobehavioral activity in these animals, data were collected for the selected parameters through a functional observational battery (FOB).

Test	Parameter	Type of Measurement ¹
	HOME CAGE	
1	Posture	Score
2	Tremor Activity	Score
3	Convulsive Activity	Score
4	Lethargy/Arousal	Score
5	Eyelid Closure	Score
	HANDLING RODENTS	
6	Ease of Removing	Score
7	Ease of Handling	Score
9	General Condition/Appearance	Score
10	Urine Stain	Score
11	Fecal Stain	Score
12	Salivation	Score
13	Piloerection	Score
14	Fur Appearance	Score
15	Lacrimation	Score
16	Palpebral Reflex Responsivity	Score
19	Vocalizations	Yes/No
	OPEN FIELD	
20	# Lines Crossed	Continuous
21	# Rearings	Continuous
22	# Urine Pools	Continuous
23	# Fecal Boluses	Continuous
24	Posture	Score
25	Tremor Activity	Score
26	Convulsive Activity	Score
27	Gait	Score
28	Gait Score	Score
29	Stereotypy	Score
30	Bizarre Behavior	Score
31	Vocalizations – Spontaneous	Yes/No
	REFLEXES	
32	Approach Response	Score
33	Touch Response	Score
34	Startle Response	Score
35	Tail Pinch Response	Score
36	Placing of Paws on Grid	Score

CN49730D: 00164

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39	Rectal Temperature (°F)	Continuous
46	Grip Strength	Score

¹ Score variables: response represented by a categorical score

Yes/No variables: response given by "Yes" or "No"

Continuous variables: response represents a numeric reading on a continuous scale.

Data for the following groups were considered in this data summary and statistical analysis:

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

Note that all animals in the B200M and B200F groups and all male animals in the E200M group did not survive to the FOB. Therefore no data were available for these dose groups for males and females respectively and consequently these doses were not considered in the analysis. Also two male mice in the NT200M group and two females in the E200F group did not survive to the FOB. The absence of FOB information for these animals may cause bias when evaluating the toxicity of the study chemicals.

This report describes the statistical methods applied to these FOB data and summarizes the statistical analysis results. All statistical summaries and analyses of data in this study were conducted using SAS 9.1.3 (SAS Institute, 2004).

STATISTICAL METHODS

(b) (4)



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(b) (4)

**RESULTS**

Data summarization and statistical analyses were conducted independently for each parameter and for males and females. Unless specified otherwise, the significant level is at the 0.05 level for the dose effect.

Categorical Variables (Score and Yes/No Variables)

Table 1A (for males) and Table 1B (for females) present frequency tables that represent the number of animals with responses within each response category, presented by dose group. The results of statistical analyses performed on these variables are summarized in Table 2A (for males) and Table 2B (for

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females). These tables show that significant differences among the dose groups were observed at the 0.05 level in the following parameters:

Male mice (Tables 1A and 2A)

- Posture (home cage), where the E80M group was significantly different from CM and the B80M groups, and the NT200M group was significantly different from the CM group. 3 out of 5 male mice in the E80M group and 2 out of 3 survival mice in the NT200M group received score of 1 (rearing and ambulating), and all five male mice in the B80M and the CM groups received negative scores (sleeping or crouching).
- Lethargy/Arousal, where the E80M group was significantly different from the B80M group. All five male mice in the B80M group received score of -1 (slightly lethargic), and 3 out of 5 mice in E80M group received zero score (alert).
- Ease of removing, where the E20M group was significantly different from the B20M group. All five male mice in E20M group received zero score (little resistance), and 3 out of 5 mice in the B20M group received score of 1 (rodent running around).

Female mice (Tables 1B and 2B)

- Tremor activity, the B20F group and the E2F group were significantly different from the CF. All five female mice in the E2F group and the B20F group received zero score (none), and 3 out of 5 female mice in the CF received score of 1 (slight).
- Lethargy/Arousal, where none of the pairwise comparisons of interest were significantly different from each other.
- Eyelid closure, where none of the pairwise comparisons of interest were significantly different from each other.
- Ease of removing, where the E200F group was significantly different from the CF. All three survival mice in E200F group received score of 1 (rodent running around), and 4 out of 5 mice in the CF dose group received zero score (little resistance).
- Palpebral reflex responsivity, where the E2F group was significantly different from the B2F group. All 5 mice in the B2F group received zero score (normal), and 3 out of 5 mice in the E2F group received -1 score (sluggish closing of eyelid).

Continuous Variables

Means and standard errors of the reported measurements for the continuous variables were calculated for each dose group and are presented in Table 3A (for males) and Table 3B (for females). Based upon the outcome of the one-way ANOVA applied to these continuous measurements, significant differences were observed at the 0.05 level among the dose groups for the following variables:

Male Mice (Table 3A)

- Rectal temperature, where the NT200M was significantly different (and lower than) from the CM group.

Female Mice (Table 3B)

- No significant differences among dose groups were observed for any continuous variables.

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Battelle Study Number CN49730D**REFERENCES**


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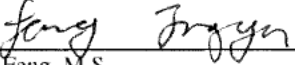
SIGNATURES



James Ma, Ph.D.
Study Statistician



Date



Jinyu Feng, M.S.
Study Statistician



Date

Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T1, HomeCage Parameter=posture

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
-5	Animal found dead	0	0	0	0	0	0	0	0	0	
-4	Lying flat limbs splayed out	0	0	0	0	0	0	0	0	0	
-3	Lying on side limbs in the air	0	0	0	0	0	0	0	0	0	
-2	Hunched posture (crouching over)	0	0	0	0	2	0	0	0	0	
-1	Sleeping, resting, recumbent	5	1	2	2	3	3	4	2		
0	Awake, alert, without rearing	0	0	2	2	0	1	0	0		
1	Rearing, ambulating	0	2	1	1	0	1	1	3		
2	Jumping, running	0	0	0	0	0	0	0	0		
Mean Score		-1.00	0.33	-0.20	-0.20	-1.40	-0.40	-0.60	0.20		

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T2, HomeCage Parameter=tremor activity

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0 None		5	3	5	5	4		5	5	5	
1 Slight		0	0	0	0	1		0	0	0	
2 Moderate		0	0	0	0	0		0	0	0	
3 Severe		0	0	0	0	0		0	0	0	
Mean Score		0.00	0.00	0.00	0.00	0.20		0.00	0.00	0.00	

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T3, HomeCage Parameter=convulsive activity

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0	None	5	2	5	5	5		5	5	5	
1	Excessive stereotype behavior (grooming, fixed staring, etc.)	0	1	0	0	0		0	0	0	
2	Forebody myoclonus (clonic chewing)	0	0	0	0	0		0	0	0	
3	Popcorn seizures (bouncing or popping behavior)	0	0	0	0	0		0	0	0	
4	Clonic seizures (slight/mild tremors or jerkings, contractions followed by relaxations)	0	0	0	0	0		0	0	0	
5	Tonic seizures (repetitive moderate/severe whole body tremors, body is rigid and arched)	0	0	0	0	0		0	0	0	
6	Tonic/clonic seizures (loss of righting)	0	0	0	0	0		0	0	0	
7	Post-ictal depression	0	0	0	0	0		0	0	0	
8	Seizures followed by death	0	0	0	0	0		0	0	0	
Mean Score		0.00	0.33	0.00	0.00	0.00		0.00	0.00	0.00	

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T4, HomeCage Parameter=lethargy/arousal

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
-4	Completely unresponsive/unconscious	0	0	0	0	0	0	0	0	0	0
-3	Severe lethargy, lying on side, immobile but conscious	0	0	0	0	0	0	0	0	0	0
-2	Moderate lethargy, immobile, may be prostrate	0	0	0	0	0	0	0	0	0	0
-1	Slight lethargy, little exploration, but not prostrate	4	1	1	2	5	3	4	2		
0	Alert, responsive, exploratory movements	1	2	4	3	0	2	1	3		
1	Slight arousal, slight excitement, tense	0	0	0	0	0	0	0	0	0	0
2	Moderate arousal, moderate excitement, sudden darting and freezing	0	0	0	0	0	0	0	0	0	0
3	Severe arousal, severe excitement, hyperalert, overaggressive	0	0	0	0	0	0	0	0	0	0
Mean Score		-0.80	-0.33	-0.20	-0.40	-1.00		-0.60	-0.80	-0.40	

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T5, HomeCage Parameter=eyelid closure

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0	Normal	1	1	4	3	2		3	1	3	
1	Slightly drooping	0	0	0	1	0		0	0	0	
2	Drooping approximately half-way	1	1	0	0	1		1	0	1	
3	Completely shut	3	1	1	1	2		1	4	1	
Mean Score		2.20	1.67	0.60	0.80	1.60		1.00	2.40	1.00	

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T6, HomeCage Parameter=ease of removing

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
2	No resistance, body limp	0	0	0	0	0	0	0	0	0	0
1	Very little resistance, body relaxed	0	0	0	0	0	0	0	0	0	0
0	Little resistance, but body tense, may rear slightly, with or without vocalization	4	2	2	2	5	2	5	4		
1	Slight resistance, rodent runs around cage, is difficult to grab, with or without vocalization	1	1	3	3	0	3	0	1		
2	Moderate resistance, rodent may jump from cage, may attempt to bite defensively or initially, with or without vocalization	0	0	0	0	0	0	0	0	0	0
3	Extreme resistance, rodent makes repetitive attempts to bite/attack technician's hand and to escape, with or without vocalizations	0	0	0	0	0	0	0	0	0	0
4	Rodent freezes (with or without vocalizations)	0	0	0	0	0	0	0	0	0	0
Mean Score		0.20	0.33	0.60	0.60	0.00		0.60	0.00	0.20	

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T7, HomeCage Parameter=ease of handling

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
-2	Rodent is limp, lethargic, or even unresponsive	0	0	0	0	0	0	0	0	0	0
-1	Rodent is relaxed and may have limbs pulled up against body	0	0	0	0	0	0	0	0	0	0
0	Rodent is alert, but with little or no resistance	5	2	5	5	5	4	5	5		
1	Moderate resistance, rodent is tense (may squirm, twist, with or without vocalization)	0	1	0	0	0	1	0	0		
2	Extreme resistance, animal attempts to bite, escape	0	0	0	0	0	0	0	0		
Mean Score		0.00	0.33	0.00	0.00	0.00	0.20	0.00	0.00		

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T9, HomeCage Parameter=general condition/appearance

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0	Normal	5	2	5	5	5	5	5	5	5	5
1	Red or crusty deposits around eyes, nose, or mouth	0	0	0	0	0	0	0	0	0	0
2	Thin appearance	0	1	0	0	0	0	0	0	0	0
3	Dehydrated appearance (decreased skin elasticity, thin appearance, hunched posture)	0	0	0	0	0	0	0	0	0	0
4	Hunched posture (without signs of dehydrated appearance)	0	0	0	0	0	0	0	0	0	0
5	Diminished body tone (limp)	0	0	0	0	0	0	0	0	0	0
6	Excessive body tone (rigid)	0	0	0	0	0	0	0	0	0	0
Mean Score		0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T10, HomeCage Parameter=urine stain

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0	None	5	3	5	5	5	5	5	5	5	5
1	Slight	0	0	0	0	0	0	0	0	0	0
2	Moderate	0	0	0	0	0	0	0	0	0	0
3	Severe	0	0	0	0	0	0	0	0	0	0
Mean Score		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T11, HomeCage Parameter=fecal stain

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0	None	5	3	5	5	5	5	5	5	5	5
1	Slight	0	0	0	0	0	0	0	0	0	0
2	Moderate	0	0	0	0	0	0	0	0	0	0
3	Severe	0	0	0	0	0	0	0	0	0	0
Mean Score		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T12, HomeCage Parameter=salivation

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0 None		5	3	5	5	5	5	5	5		
1 Slight		0	0	0	0	0	0	0	0		
2 Moderate		0	0	0	0	0	0	0	0		
3 Severe		0	0	0	0	0	0	0	0		
Mean Score		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T13, HomeCage Parameter=piloerection

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0 None		5	3	5	5	5		5	5	5	
1 Slight		0	0	0	0	0		0	0	0	
2 Moderate		0	0	0	0	0		0	0	0	
3 Severe		0	0	0	0	0		0	0	0	
Mean Score		0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T14, HomeCage Parameter=fur appearance

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0	Normal	5	2	5	5	5		5	5	5	
1	Slightly rough coat	0	1	0	0	0		0	0	0	
2	Moderately rough coat	0	0	0	0	0		0	0	0	
3	Severely rough coat	0	0	0	0	0		0	0	0	
Mean Score		0.00	0.33	0.00	0.00	0.00		0.00	0.00	0.00	

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T15, HomeCage Parameter=lacrimation

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0	None	5	3	5	5	5	5	5	5		
1	Slight	0	0	0	0	0	0	0	0		
2	Moderate	0	0	0	0	0	0	0	0		
3	Severe	0	0	0	0	0	0	0	0		
Mean Score		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T16, HomeCage Parameter=palpebral reflex responsivity

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
-2	No response	1	0	1	1	0		0	0	1	
-1	S sluggish closing of eyelid	1	1	0	0	2		2	1	2	
0	Normal, immediate, brisk closing of eyelid	3	2	4	4	3		3	4	2	
1	Escape or mild vocalization	0	0	0	0	0		0	0	0	
2	Extreme response, vocalization, fleeing, attacking, and biting	0	0	0	0	0		0	0	0	
Mean Score		-0.60	-0.33	-0.40	-0.40	-0.40		-0.40	-0.20	-0.80	

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T19, HomeCage Parameter=vocalizations (1=yes; 0=no)

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0	No	4	2	4	4	4		4	5	2	
1	Yes	1	1	1	1	1		1	0	3	

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T24, OpenField Parameter=posture

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
-4	Lying flat limbs splayed out	0	0	0	0	0	0	0	0	0	0
-3	Lying on side limbs in the air	0	0	0	0	0	0	0	0	0	0
-2	Hunched posture (crouching over)	1	2	0	0	0	0	0	0	0	0
-1	Lying on side (curled up sleeping)	0	0	0	0	0	0	0	0	0	0
0	Normal (sitting or standing normally)	4	1	5	5	5	5	5	5	5	5
1	Rearing	0	0	0	0	0	0	0	0	0	0
2	Hyperactive (jumping, running, etc.)	0	0	0	0	0	0	0	0	0	0
Mean Score		-0.40	-1.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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 DATE: [illegible]

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Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T25, OpenField Parameter=tremor activity

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0 None		5	3	5	5	4		4	5	5	
1 Slight		0	0	0	0	1		1	0	0	
2 Moderate		0	0	0	0	0		0	0	0	
3 Severe		0	0	0	0	0		0	0	0	
Mean Score		0.00	0.00	0.00	0.00	0.20		0.20	0.00	0.00	

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T26, OpenField Parameter=convulsive activity

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0	None	5	3	5	5	5	5	5	5	5	5
1	Excessive stereotype behavior (grooming, fixed staring, etc.)	0	0	0	0	0	0	0	0	0	0
2	Forebody myoclonus (clonic chewing)	0	0	0	0	0	0	0	0	0	0
3	Popcorn seizures (bouncing or popping behavior)	0	0	0	0	0	0	0	0	0	0
4	Clonic seizures (slight/mild tremors or jerkings, contractions followed by relaxations)	0	0	0	0	0	0	0	0	0	0
5	Tonic seizures (repetitive moderate/severe whole body tremors, body is rigid and arched)	0	0	0	0	0	0	0	0	0	0
6	Tonic/clonic seizures (loss of righting)	0	0	0	0	0	0	0	0	0	0
7	Post-ictal depression	0	0	0	0	0	0	0	0	0	0
8	Seizures followed by death	0	0	0	0	0	0	0	0	0	0
Mean Score		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T27, OpenField Parameter=gait

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0	Normal	5	2	5	5	5		5	5	5	
1	Walking on tip toes	0	0	0	0	0		0	0	0	
2	Ataxia (excessive swaying, rocking, or lurching during walk)	0	1	0	0	0		0	0	0	
3	Feet pointing outward from body	0	0	0	0	0		0	0	0	
4	Hindlimbs splayed	0	0	0	0	0		0	0	0	
5	Front limbs dragging (unable to support weight)	0	0	0	0	0		0	0	0	
6	Hindlimbs dragging	0	0	0	0	0		0	0	0	
7	Inability to use all limbs	0	0	0	0	0		0	0	0	
Mean Score		0.00	0.67	0.00	0.00	0.00		0.00	0.00	0.00	

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T28, OpenField Parameter=gait score

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0	Normal	5	2	5	5	5		5	5	5	
1	Slightly abnormal	0	1	0	0	0		0	0	0	
2	Moderately abnormal	0	0	0	0	0		0	0	0	
3	Severely abnormal	0	0	0	0	0		0	0	0	
4	No movement	0	0	0	0	0		0	0	0	
Mean Score		0.00	0.33	0.00	0.00	0.00		0.00	0.00	0.00	

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
	0None	5	3	5	5	5		5	5	5	
	1Repetitive grooming	0	0	0	0	0		0	0	0	
	2Pacing	0	0	0	0	0		0	0	0	
	3Repetitive sniffing	0	0	0	0	0		0	0	0	
	4Head weaving	0	0	0	0	0		0	0	0	
	5Licking	0	0	0	0	0		0	0	0	
Mean Score		0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T30, OpenField Parameter=bizarre behavior

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0	None	5	3	5	5	5	5	5	5		
1	Self-mutilation	0	0	0	0	0	0	0	0	0	0
2	Retropulsion	0	0	0	0	0	0	0	0	0	0
3	Writhing	0	0	0	0	0	0	0	0	0	0
4	Flopping	0	0	0	0	0	0	0	0	0	0
Mean Score		0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T31, OpenField Parameter=vocal.-spontaneous (1=yes; 0=no)

Score Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0No	5	3	5	5	5	5	5	5	5	5
1Yes	0	0	0	0	0	0	0	0	0	0

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T32, OpenField Parameter=approach response

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Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T33, OpenField Parameter=touch response

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
-1	No reaction (i.e., due to stupor, coma, convulsions)	0	0	1	0	0	0	0	0	1	
0	Rodent may slowly turn or walk away	5	3	4	5	5		3	5	4	
1	More energetic response than 0), may include vocalizations	0	0	0	0	0		2	0	0	
2	Exaggerated reaction (e.g., jumps, bites, attacks with or without vocalizations)	0	0	0	0	0		0	0	0	
3	Rodent freezes, actual muscle contractions	0	0	0	0	0		0	0	0	
Mean Score		0.00	0.00	-0.20	0.00	0.00		0.40	0.00	-0.20	

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Q. N. 2009-01-01
Q. O. 2009-01-01

Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T34, OpenField Parameter=startle response

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
-1	No reaction (i.e., due to stupor, coma, convulsions)	0	0	0	0	0		0	0	0	
0	Slight reaction, some evidence that noise was heard, normal flinching or flicking of ears	5	3	5	4	5		5	5	5	
1	More energetic response than 0, may include vocalizations	0	0	0	1	0		0	0	0	
2	Exaggerated reaction (e.g., jumps, bites, attacks with or without vocalizations)	0	0	0	0	0		0	0	0	
3	Rodent freezes, actual muscle contractions	0	0	0	0	0		0	0	0	
Mean Score		0.00	0.00	0.00	0.20	0.00		0.00	0.00	0.00	

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

[illegible]

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Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T35, OpenField Parameter=tail pinch response

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
-1	No reaction (i.e., due to stupor, coma, convulsions)	0	0	0	0	1		0	0	0	
0	Rodent may turn or walk forward	5	3	5	4	4		3	5	4	
1	More energetic response than 0, may include vocalizations	0	0	0	1	0		2	0	1	
2	Exaggerated reaction (e.g., jumps, bites, attacks with or without vocalizations)	0	0	0	0	0		0	0	0	
3	Rodent freezes, actual muscle contractions	0	0	0	0	0		0	0	0	
Mean Score		0.00	0.00	0.00	0.20	-0.20		0.40	0.00	0.20	

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T36, OpenField Parameter=placing of paws on grid

[illegible]

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1A. Data Summary for Categorical Variables (Score and Yes/No Variables)
Male Animals, Test=T46, OpenField Parameter=grip strength

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
-2	No gripping	0	0	0	0	0	0	0	0	0	0
-1	Weak gripping	0	0	0	0	0	0	0	0	0	0
0	Normal gripping	5	3	5	5	5	5	5	5	5	5
1	Excessive gripping	0	0	0	0	0	0	0	0	0	0
Mean Score		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 2A. Male Animals Test Result Summary for Categorical Variables (Score and Yes/No Variables)

Parameter	Dose Effect+ P-value (Chi-Square)	Group 2 vs 1 #S	Group 3 vs 1 #++	Group 4 vs 1 #++	Group 5 vs 1 #++	Group 7 vs 1 #++	Group 8 vs 1 #++	Group 9 vs 1 #++	Group 7 vs 3 #++	Group 8 vs 4 #++	Group 9 vs 5 #++
Posture (home cage)	<0.0001(32.32)*	Yes	No	No	No	No	No	Yes	No	No	Yes
tremor activity	0.9897(1.25)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
convulsive activity	0.9823(1.50)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
lethargy/arousal	<0.0001(46.83)*	No	No	No	No	No	No	No	No	No	Yes
eyelid closure	0.1562(10.62)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
ease of removing	0.0004(26.50)*	No	No	No	No	No	No	No	No	Yes	No
ease of handling	0.9071(2.75)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
general condition/appearance	0.9823(1.50)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
urine stain	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
fecal stain	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
salivation	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
piloerection	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
fur appearance	0.9823(1.50)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
lacrimation	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
palpebral reflex responsivity	0.8834(3.02)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
vocalizations (1=yes; 0=no)	0.7727(4.06)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Posture (open field)	0.4033(7.25)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
tremor activity	0.9271(2.50)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
convulsive activity	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
gait	0.9823(1.50)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
gait score	0.9823(1.50)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
stereotype	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
bizarre behavior	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
vocal -spontaneous (1=yes; 0=no)	0.9950(0.99)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
approach response	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
touch response	0.5594(5.83)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
startle response	0.9897(1.25)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
tail pinch response	0.4203(7.08)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
placing of paws on grid	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
grip strength	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT

Note: Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB, therefore these two dose groups were not included in this analysis.

+: Significant overall dose effects at the 0.05 level (two-sided) were marked by +.

#: Comparisons to the control or blend were performed if the overall dose effect was significant.

++: Significantly different from the control or blend at the 0.05/3=0.0167 level (two-sided) were marked with a "yes".

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S: Significantly different from the control at the 0.05 level (two-sided) were marked with a "yes".

NT: Not tested

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 3A. Male Animals Test Result Summary -- Continuous Variables

Parameter	Dose Effect+ P-value	Gp 1 Mean (SE)	Gp 2 Mean (SE) #S	Gp 3 Mean (SE) #++	Gp 4 Mean (SE) #++	Gp 5 Mean (SE) #++	Gp 7 Mean (SE) #++	Gp 8 Mean (SE) #++	Gp 9 Mean (SE) #++
lines crossed	0.1418	51.80(5.22)	22.33(5.78)	47.20(13.48)	63.80(6.41)	33.20(7.35)	52.00(12.16)	31.60(13.03)	49.60(7.36)
rearings	0.3784	16.60(3.61)	4.00(1.15)	14.80(4.02)	17.20(4.52)	7.40(1.63)	14.00(4.55)	9.40(5.76)	12.40(3.70)
urine pools	0.4977	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.20(0.20)	0.00(0.00)	0.00(0.00)
fecal boluses	0.4073	0.80(0.49)	0.00(0.00)	0.60(0.24)	1.60(0.75)	1.20(0.49)	1.00(0.63)	1.80(0.49)	0.80(0.37)
rectal temperature <0.0001*		101.76(0.16)	96.27(1.13)*	102.14(0.60)	101.84(0.35)	100.60(0.38)	101.84(0.33)	100.58(1.12)	100.80(0.52)

Note: Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB, therefore these two dose groups were not included in this analysis.

+: Significant overall dose effects at the 0.05 level (two-sided) were marked by *.

#: Comparisons to the control were performed if the overall dose effect was significant.

++: Significantly different from the control at the 0.05/3=0.0167 level (two-sided) were marked by *.

\$: Significantly different from the control at the 0.05 level (two-sided) were marked by *.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 3A. Male Animals Test Result Summary --- Continuous Variables

Parameter	Dose Effect+ P-value	Gp 7 vs. Gp 3 #++	Gp 8 vs. Gp 4 #++	Gp 9 vs. Gp 5 #++
lines crossed	0.1418	NT	NT	NT
rearings	0.3784	NT	NT	NT
urine pools	0.4977	NT	NT	NT
fecal boluses	0.4073	NT	NT	NT
rectal temperature	<0.0001*	No	No	No

Note: Animals in the B200M (Group 6) and E200M (Group 10) groups did not survive to the FOB, therefore these two dose groups were not included in this analysis.

+: Significant overall dose effects at the 0.05 level (two-sided) were marked by *.

#: Comparisons to the blend were performed if the overall dose effect was significant.

++: Significantly different from the corresponding blend level at the 0.05/3=0.0167 level (two-sided) were marked with a "yes".

NT: Not tested.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T1, HomeCage Parameter=posture

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
	-5Animal found dead	0	0	0	0	0		0	0	0	0
	-4Lying flat limbs splayed out	0	0	0	0	0		0	0	0	0
	-3Lying on side limbs in the air	0	0	0	0	0		0	0	0	0
	-2Hunched posture (crouching over)	0	2	0	0	0		0	0	0	0
	-1Sleeping, resting, recumbent	3	1	3	4	4		4	1	2	2
	0Awake, alert, without rearing	1	0	0	0	1		1	3	1	1
	1Rearing, ambulating	1	2	2	1	0		0	1	2	0
	2Jumping, running	0	0	0	0	0		0	0	0	0
Mean Score		-0.40	-0.60	-0.20	-0.60	-0.80		-0.80	0.00	0.00	-0.67

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

Figure 1. The effect of the concentration of the *Agrobacterium* suspension on the transformation efficiency of *Agrobacterium* strains.

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T2, HomeCage Parameter=tremor activity

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0 None		2	4	4	5	3		5	4	4	2
1 Slight		3	1	1	0	2		0	1	1	1
2 Moderate		0	0	0	0	0		0	0	0	0
3 Severe		0	0	0	0	0		0	0	0	0
Mean Score		0.60	0.20	0.20	0.00	0.40		0.00	0.20	0.20	0.33

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T3, HomeCage Parameter=convulsive activity

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0	None	5	5	5	5	5	5	5	5	5	3
1	Excessive stereotype behavior (grooming, fixed staring, etc.)	0	0	0	0	0	0	0	0	0	0
2	Forebody myoclonus (clonic chewing)	0	0	0	0	0	0	0	0	0	0
3	Popcorn seizures (bouncing or popping behavior)	0	0	0	0	0	0	0	0	0	0
4	Clonic seizures (slight/mild tremors or jerkings, contractions followed by relaxations)	0	0	0	0	0	0	0	0	0	0
5	Tonic seizures (repetitive moderate/severe whole body tremors, body is rigid and arched)	0	0	0	0	0	0	0	0	0	0
6	Tonic/clonic seizures (loss of righting)	0	0	0	0	0	0	0	0	0	0
7	Post-ictal depression	0	0	0	0	0	0	0	0	0	0
8	Seizures followed by death	0	0	0	0	0	0	0	0	0	0
Mean Score		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T4, HomeCage Parameter=lethargy/arousal

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
-4	Completely unresponsive/unconscious	0	0	0	0	0	0	0	0	0	0
-3	Severe lethargy, lying on side, immobile but conscious	0	0	0	0	0	0	0	0	0	0
-2	Moderate lethargy, immobile, may be prostrate	0	0	0	0	0	0	0	0	0	0
-1	Slight lethargy, little exploration, but not prostrate	3	1	3	4	4	4	1	2	3	3
0	Alert, responsive, exploratory movements	2	4	2	1	1	1	3	3	0	0
1	Slight arousal, slight excitement, tense	0	0	0	0	0	0	1	0	0	0
2	Moderate arousal, moderate excitement, sudden darting and freezing	0	0	0	0	0	0	0	0	0	0
3	Severe arousal, severe excitement, hyperalert, overaggressive	0	0	0	0	0	0	0	0	0	0
Mean Score		-0.60	-0.20	-0.60	-0.80	-0.80	-0.80	0.00	-0.40	-1.00	

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T5, HomeCage Parameter=eyelid closure

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0	Normal	2	1	2	1	2	0	3	3	0	
1	Slightly drooping	0	0	0	0	0	0	0	0	0	
2	Drooping approximately half-way	0	3	1	0	1	1	1	0	1	
3	Completely shut	3	1	2	4	2	4	1	2	2	
Mean Score		1.80	1.80	1.60	2.40	1.60	2.80	1.00	1.20	2.67	

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

Q. 1. What is the difference between a *de novo* mutation and a *de novo* disease?

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T6, HomeCage Parameter=ease of removing

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
-2	No resistance, body limp	0	0	0	0	0	0	0	0	0	0
-1	Very little resistance, body relaxed	0	0	0	0	0	0	0	0	0	0
0	Little resistance, but body tense, may rear slightly, with or without vocalization	4	5	5	5	5	5	5	5	4	0
1	Slight resistance, rodent runs around cage, is difficult to grab, with or without vocalization	1	0	0	0	0	0	0	0	1	3
2	Moderate resistance, rodent may jump from cage, may attempt to bite defensively or initially, with or without vocalization	0	0	0	0	0	0	0	0	0	0
3	Extreme resistance, rodent makes repetitive attempts to bite/attack technician's hand and to escape, with or without vocalizations	0	0	0	0	0	0	0	0	0	0
4	Rodent freezes (with or without vocalizations)	0	0	0	0	0	0	0	0	0	0
Mean Score		0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	1.00

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T7, HomeCage Parameter=ease of handling

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
	2 Rodent is limp, lethargic, or even unresponsive	0	0	0	0	0		0	0	0	0
	1 Rodent is relaxed and may have limbs pulled up against body	0	0	0	0	0		0	0	0	0
	0 Rodent is alert, but with little or no resistance	5	5	5	5	5		5	5	5	3
	1 Moderate resistance, rodent is tense (may squirm, twist, with or without vocalization)	0	0	0	0	0		0	0	0	0
	2 Extreme resistance, animal attempts to bite, escape	0	0	0	0	0		0	0	0	0
Mean Score		0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T9, HomeCage Parameter=general condition/appearance

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0	Normal	5	5	5	5	5		5	5	5	3
1	Red or crusty deposits around eyes, nose, or mouth	0	0	0	0	0		0	0	0	0
2	Thin appearance	0	0	0	0	0		0	0	0	0
3	Dehydrated appearance (decreased skin elasticity, thin appearance, hunched posture)	0	0	0	0	0		0	0	0	0
4	Hunched posture (without signs of dehydrated appearance)	0	0	0	0	0		0	0	0	0
5	Diminished body tone (limp)	0	0	0	0	0		0	0	0	0
6	Excessive body tone (rigid)	0	0	0	0	0		0	0	0	0
Mean Score		0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T10, HomeCage Parameter=urine stain

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0 None		5	5	5	5	5	5	5	5	5	3
1 Slight		0	0	0	0	0	0	0	0	0	0
2 Moderate		0	0	0	0	0	0	0	0	0	0
3 Severe		0	0	0	0	0	0	0	0	0	0
Mean Score		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T11, HomeCage Parameter=fecal stain

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0 None		5	5	5	5	5		5	5	5	3
1 Slight		0	0	0	0	0		0	0	0	0
2 Moderate		0	0	0	0	0		0	0	0	0
3 Severe		0	0	0	0	0		0	0	0	0
Mean Score		0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T12, HomeCage Parameter=salivation

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0 None		5	5	5	5	5		5	5	5	3
1 Slight		0	0	0	0	0		0	0	0	0
2 Moderate		0	0	0	0	0		0	0	0	0
3 Severe		0	0	0	0	0		0	0	0	0
Mean Score		0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T13, HomeCage Parameter=piloerection

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0 None		5	5	5	5	5		5	5	5	3
1 Slight		0	0	0	0	0		0	0	0	0
2 Moderate		0	0	0	0	0		0	0	0	0
3 Severe		0	0	0	0	0		0	0	0	0
Mean Score		0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T14, HomeCage Parameter=fur appearance

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0	Normal	5	5	5	5	5		5	5	5	3
1	Slightly rough coat	0	0	0	0	0		0	0	0	0
2	Moderately rough coat	0	0	0	0	0		0	0	0	0
3	Severely rough coat	0	0	0	0	0		0	0	0	0
Mean Score		0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T15, HomeCage Parameter=lacrimation

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0	None	5	5	5	5	5	5	5	5	5	3
1	Slight	0	0	0	0	0	0	0	0	0	0
2	Moderate	0	0	0	0	0	0	0	0	0	0
3	Severe	0	0	0	0	0	0	0	0	0	0
Mean Score		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T16, HomeCage Parameter=palpebral reflex responsivity

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
-2	No response	0	0	0	1	0	0	0	0	0	0
-1	Sluggish closing of eyelid	1	0	0	1	0	3	2	2	1	
0	Normal, immediate, brisk closing of eyelid	4	5	5	3	5	2	3	3	2	
1	Escape or mild vocalization	0	0	0	0	0	0	0	0	0	
2	Extreme response, vocalization, fleeing, attacking, and biting	0	0	0	0	0	0	0	0	0	
Mean Score		-0.20	0.00	0.00	-0.60	0.00	-0.60	-0.40	-0.40	-0.33	

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T19, HomeCage Parameter=vocalizations (1=yes; 0=no)

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0	No	3	5	3	4	2	3	5	3	1	
1	Yes	2	0	2	1	3	2	0	2	2	

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T24, OpenField Parameter=posture

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
-4	Lying flat limbs splayed out	0	0	0	0	0	0	0	0	0	0
-3	Lying on side limbs in the air	0	0	0	0	0	0	0	0	0	0
-2	Hunched posture (crouching over)	0	2	0	0	0	0	0	0	0	0
-1	Lying on side (curled up sleeping)	0	0	0	0	0	0	0	0	0	0
0	Normal (sitting or standing normally)	5	3	5	5	5	5	5	5	5	3
1	Rearing	0	0	0	0	0	0	0	0	0	0
2	Hyperactive (jumping, running, etc.)	0	0	0	0	0	0	0	0	0	0
Mean Score		0.00	-0.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T25, OpenField Parameter=tremor activity

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Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T26, OpenField Parameter=convulsive activity

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0	None	5	4	5	5	5	5	5	5	5	3
1	Excessive stereotype behavior (grooming, fixed staring, etc.)	0	0	0	0	0	0	0	0	0	0
2	Forebody myoclonus (clonic chewing)	0	0	0	0	0	0	0	0	0	0
3	Popcorn seizures (bouncing or popping behavior)	0	0	0	0	0	0	0	0	0	0
4	Clonic seizures (slight/mild tremors or jerkings, contractions followed by relaxations)	0	1	0	0	0	0	0	0	0	0
5	Tonic seizures (repetitive moderate/severe whole body tremors, body is rigid and arched)	0	0	0	0	0	0	0	0	0	0
6	Tonic/clonic seizures (loss of righting)	0	0	0	0	0	0	0	0	0	0
7	Post-ictal depression	0	0	0	0	0	0	0	0	0	0
8	Seizures followed by death	0	0	0	0	0	0	0	0	0	0
Mean Score		0.00	0.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T27, OpenField Parameter=gait

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0	Normal	5	5	5	5	5	5	5	5	5	3
1	Walking on tip toes	0	0	0	0	0	0	0	0	0	0
2	Ataxia (excessive swaying, rocking, or lurching during walk)	0	0	0	0	0	0	0	0	0	0
3	Feet pointing outward from body	0	0	0	0	0	0	0	0	0	0
4	Hindlimbs splayed	0	0	0	0	0	0	0	0	0	0
5	Front limbs dragging (unable to support weight)	0	0	0	0	0	0	0	0	0	0
6	Hindlimbs dragging	0	0	0	0	0	0	0	0	0	0
7	Inability to use all limbs	0	0	0	0	0	0	0	0	0	0
Mean Score		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T28, OpenField Parameter=gait score

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0	Normal	5	5	5	5	5		5	5	5	3
1	Slightly abnormal	0	0	0	0	0		0	0	0	0
2	Moderately abnormal	0	0	0	0	0		0	0	0	0
3	Severely abnormal	0	0	0	0	0		0	0	0	0
4	No movement	0	0	0	0	0		0	0	0	0
Mean Score		0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

TABLE 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
 Female Animals, Test=T28, OpenField Parameter=gait score

Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T29, OpenField Parameter=stereotype

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
	0None	5	5	4	5	5		5	5	5	3
	1Repetitive grooming	0	0	0	0	0		0	0	0	0
	2Pacing	0	0	1	0	0		0	0	0	0
	3Repetitive sniffing	0	0	0	0	0		0	0	0	0
	4Head weaving	0	0	0	0	0		0	0	0	0
	5Licking	0	0	0	0	0		0	0	0	0
Mean Score		0.00	0.00	0.40	0.00	0.00		0.00	0.00	0.00	0.00

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T30, OpenField Parameter=bizarre behavior

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0	None	5	5	5	5	5		5	5	5	3
1	Self-mutilation	0	0	0	0	0		0	0	0	0
2	Retropulsion	0	0	0	0	0		0	0	0	0
3	Writhing	0	0	0	0	0		0	0	0	0
4	Flopping	0	0	0	0	0		0	0	0	0
Mean Score		0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T31, OpenField Parameter=vocal-spontaneous (1=yes; 0=no)

Score Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
0 No	5	5	5	5	5		5	5	5	3
1 Yes	0	0	0	0	0		0	0	0	0

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T32, OpenField Parameter=approach response

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
-2	No reaction	0	0	0	0	0	0	0	0	0	0
-1	Animal senses object but not interested and does not approach	1	0	0	0	1	0	0	0	0	0
0	Animal slowly approaches object, sniffs, and then turns away	4	4	5	5	4	5	5	5	3	
1	Animal shows more energetic response with or without vocalization, rodent sways from side to side	0	0	0	0	0	0	0	0	0	0
2	Animal shows bizarre behavior, may include jumping, biting at object, attacking object with or without vocalization	1	0	0	0	0	0	0	0	0	0
3	Rodent freezes, animal may exhibit muscle contractions	0	0	0	0	0	0	0	0	0	0
Mean Score		-0.20	0.40	0.00	0.00	-0.20	0.00	0.00	0.00	0.00	0.00

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T33, OpenField Parameter=touch response

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
-1	No reaction (i.e., due to stupor, coma, convulsions)	0	0	0	0	1		0	0	0	0
0	Rodent may slowly turn or walk away	5	4	5	5	4		5	5	5	3
1	More energetic response than 0, may include vocalizations	0	0	0	0	0		0	0	0	0
2	Exaggerated reaction (e.g., jumps, bites, attacks with or without vocalizations)	0	1	0	0	0		0	0	0	0
3	Rodent freezes, actual muscle contractions	0	0	0	0	0		0	0	0	0
Mean Score		0.00	0.40	0.00	0.00	-0.20		0.00	0.00	0.00	0.00

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T34, OpenField Parameter=startle response

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
-1	No reaction (i.e., due to stupor, coma, convulsions)	1	0	0	0	0		0	0	1	0
0	Slight reaction, some evidence that noise was heard, normal flinching or flicking of ears	4	5	5	5	5		5	5	4	3
1	More energetic response than 0), may include vocalizations	0	0	0	0	0		0	0	0	0
2	Exaggerated reaction (e.g., jumps, bites, attacks with or without vocalizations)	0	0	0	0	0		0	0	0	0
3	Rodent freezes, actual muscle contractions	0	0	0	0	0		0	0	0	0
Mean Score		-0.20	0.00	0.00	0.00	0.00		0.00	0.00	-0.20	0.00

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T35, OpenField Parameter=tail pinch response

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
-1	No reaction (i.e., due to stupor, coma, convulsions)	0	0	1	0	2	2	1	0	0	0
0	Rodent may turn or walk forward	5	5	4	5	3	3	4	5	3	5
1	More energetic response than 0; may include vocalizations	0	0	0	0	0	0	0	0	0	0
2	Exaggerated reaction (e.g., jumps, bites, attacks with or without vocalizations)	0	0	0	0	0	0	0	0	0	0
3	Rodent freezes, actual muscle contractions	0	0	0	0	0	0	0	0	0	0
Mean Score		0.00	0.00	-0.20	0.00	-0.40	-0.40	-0.20	0.00	0.00	0.00

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

$$\frac{d}{dt} \left(\frac{\partial L}{\partial \dot{x}} \right) = \frac{\partial L}{\partial x}, \quad \frac{d}{dt} \left(\frac{\partial L}{\partial \dot{y}} \right) = \frac{\partial L}{\partial y}$$

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T36, OpenField Parameter=placing of paws on grid

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
-3	Severe, no placing, even when nose makes contact with grid	0	0	0	0	0	0	0	0	0	0
-2	Moderate, places paws on grid after nose touches grid	0	0	0	0	0	0	0	0	0	0
-1	Slight, places paws on grid after vibrissae touch grid	0	1	0	0	0	0	0	0	0	0
0	Normal, rodent reaches immediately, places two paws on grid	5	4	5	5	5	5	5	5	5	3
1	Vigorous visual placing of both paws on grid	0	0	0	0	0	0	0	0	0	0
Mean Score		0.00	-0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 1B. Data Summary for Categorical Variables (Score and Yes/No Variables)
Female Animals, Test=T46, OpenField Parameter=grip strength

Score	Description	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10
-2	No gripping	0	0	0	0	0	0	0	0	0	0
-1	Weak gripping	0	0	0	0	0	0	0	0	0	0
0	Normal gripping	5	5	5	5	5	5	5	5	5	3
1	Excessive gripping	0	0	0	0	0	0	0	0	0	0
Mean Score		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Animals in the B200F (Group 6) group did not survive to the FOB.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 2B. Female Animals Test Result Summary for Categorical Variables (Score and Yes/No Variables)

Parameter	Dose Effect- P-value (Chi-Square) #S	Group 2 vs 1	Group 3 vs 1	Group 4 vs 1	Group 5 vs 1	Group 7 vs 1	Group 8 vs 1	Group 9 vs 1	Group 10 vs 1	Group 7 vs 3	Group 8 vs 4	Group 9 vs 5
		#S	##	##	##	##	##	##	##	##	##	##
Posture (home cage)	0.2357(10.44)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
tremor activity	0.0268(17.33)*	No	No	Yes	No	Yes	No	No	No	No	No	No
convulsive activity	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
lethargy/arousal	<0.0001(50.37)*	No	No	No	No	No	No	No	No	No	No	No
eyelid closure	0.0057(21.60)*	No	No	No	No	No	No	No	No	No	No	No
ease of removing	<0.0001(849063)*	No	No	No	No	No	No	No	Yes	No	No	No
ease of handling	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
general condition/appearance	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
urine stain	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
fecal stain	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
salivation	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
piloerection	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
fur appearance	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
lacrimation	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
palpebral reflex	0.0114(19.73)*	No	No	No	No	No	No	No	No	Yes	No	No
responsivity												
vocalizations (1=yes; 0=no)	0.5441(6.93)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Posture (open field)	0.9117(3.33)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
tremor activity	0.9117(3.33)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
convulsive activity	0.9961(1.25)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
gait	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
gait score	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
stereotype	0.9961(1.25)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
bizarre behavior	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
vocal -spontaneous (1=yes; 0=no)	0.9982(1.01)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
approach response	0.8790(3.75)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
touch response	0.9617(2.50)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
startle response	0.9617(2.50)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
tail pinch response	0.3284(9.17)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
placing of paws on grid	0.9961(1.25)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
grip strength	1.0000(0.00)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT

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Note: Animals in the B200F (Group 6) group did not survive to the FOB, therefore this dose group was not included in this analysis.

+: Significant overall dose effects at the 0.05 level (two-sided) were marked by *.

#: Comparisons to the control or blend were performed if the overall dose effect was significant.

++: Significantly different from the control or blend at the 0.05/3=0.0167 level (two-sided) were marked with a "yes".

–: Significantly different from the control at the 0.05/4=0.0125 level (two-sided) were marked with a "yes"

\$: Significantly different from the control at the 0.05 level (two-sided) were marked with a "yes".

NT: Not tested

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

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Table 3B. Female Animals Test Result Summary --- Continuous Variables

Parameter	Dose Effect+ P-value	Gp 1 Mean (SE)	Gp 2 Mean (SE) #S	Gp 3 Mean (SE) #++	Gp 4 Mean (SE) #++	Gp 5 Mean (SE) #++	Gp 7 Mean (SE) #--	Gp 8 Mean (SE) #--	Gp 9 Mean (SE) #--	Gp 10 Mean (SE) #--
lines crossed	0.8631	34.40(4.49)	40.60(14.13)	27.60(11.66)	23.80(8.13)	39.60(5.37)	20.40(9.28)	39.60(16.82)	36.00(11.34)	29.33(7.69)
rearings	0.1714	5.80(2.22)	16.60(6.65)	5.60(2.25)	2.40(1.60)	8.20(1.93)	6.40(2.50)	6.60(2.01)	7.20(3.26)	4.33(2.40)
urine pools	0.6959	0.20(0.20)	0.00(0.00)	0.00(0.00)	0.20(0.20)	0.00(0.00)	0.00(0.00)	0.20(0.20)	0.00(0.00)	0.00(0.00)
fecal boluses	0.7513	0.00(0.00)	0.80(0.58)	0.80(0.37)	0.80(0.37)	0.80(0.49)	0.80(0.49)	0.60(0.24)	0.60(0.40)	0.00(0.00)
rectal temperature	0.1318	101.64(0.49)	98.46(1.69)	101.02(0.60)	100.32(0.88)	100.98(0.36)	101.32(0.46)	101.72(0.58)	100.52(0.86)	98.83(1.16)

Note: Animals in the B200F (Group 6) group did not survive to the FOB, therefore this dose group was not included in this analysis.

+: Significant overall dose effects at the 0.05 level (two-sided) were marked by *.

#: Comparisons to the control were performed if the overall dose effect was significant.

++: Significantly different from the control at the 0.05/3=0.0167 level (two-sided) were marked by *.

--: Significantly different from the control at the 0.05/4=0.0125 level (two-sided) were marked by *.

S: Significantly different from the control at the 0.05 level (two-sided) were marked by *.

Group #	Group Description	Group Coding		Target Dosage (mg/kg/day)
		Males	Females	
1	Control	CM	CF	0
2	Nicotine Tartrate High Dose	NT200M	NT200F	200
3	Tobacco Blend Low Dose	B2M	B2F	2
4	Tobacco Blend Intermediate Dose 1	B20M	B20F	20
5	Tobacco Blend Intermediate Dose 2	B80M	B80F	80
6	Tobacco Blend High Dose	B200M	B200F	200
7	Tobacco Extract Low Dose	E2M	E2F	2
8	Tobacco Extract Intermediate Dose 1	E20M	E20F	20
9	Tobacco Extract Intermediate Dose 2	E80M	E80F	80
10	Tobacco Extract High Dose	E200M	E200F	200

APPENDIX H: TOXICOKINETIC REPORT

**28-DAY REPEATED DOSE TOXICITY STUDY OF TOBACCO BLEND AND
AQUEOUS TOBACCO EXTRACT IN CD-1 MICE**

SAMPLE ANALYSIS AND KINETICS REPORT

**DETERMINATION OF NICOTINE AND COTININE IN MOUSE PLASMA BY
LIQUID CHROMATOGRAPHY WITH MASS SPECTROMETRY (LC-MS)**

Battelle Study Number: CN49730D

March 11, 2009

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EXECUTIVE SUMMARY

Mouse plasma samples were received frozen from Battelle Memorial Institute's animal facility from the "28-Day Repeated Dose Toxicity Study of Tobacco Blend and Aqueous Tobacco Extract in CD-1 Mice" study for analysis of nicotine and cotinine plasma concentration levels.

(b) (4)



The samples were successfully analyzed.

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APPENDIX A – ANALYSIS DOCUMENTATION FORM

Documentation for the Analysis of Nicotine and Cotinine in Mouse Plasma by LC-MS	A-1
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I. INTRODUCTION

This report contains a description of the analysis of the mouse plasma samples from this study, the results of these analyses, and figures.

This work was performed at Battelle, 505 King Avenue, Columbus, OH 43201.

II. STANDARDS

Nicotine, Lot No. 127K4111, was obtained from Sigma Aldrich, Inc., and was used to prepare the calibration standards and quality control (QC) samples for the analyses.

Nicotine-d₃ salicylate salt, Lot No. 037K4062, was obtained from Sigma-Aldrich, Inc., and used as the internal standard (IS) for nicotine.

Cotinine, Lot No. 048K4031, was obtained from Sigma Aldrich, Inc., and was used to prepare the calibration standards and QC samples for the analyses.

Cotinine-d₃, Lot No. IS1107, was obtained from Sigma-Aldrich, Inc., and used as the IS for cotinine.

All four standards were used to perform the work covered in this report.

III. MATRIX/SPECIES

Plasma, CD-1 mouse with potassium ethylene diamine tetraacetic acid (EDTA), received from Bioreclamation.

IV. PLASMA SAMPLE ANALYSIS**A. METHOD**

(b) (4)

**B. RESULTS**

A summary of each sample analysis, including any discrepancies, is shown in Table 1.

Table 1 – Summary of Sample Analyses

Analysis Date	Analysis Set	Discrepancies and Acceptance Criteria	Data Reported
7/25/08	Set 1	The nicotine blanks were an average of 35.4% of the low standard. All other acceptance criteria met.	Yes
7/28/08	Set 2	All acceptance criteria met.	Yes
7/29/08	Set 3	One nicotine standard was excluded from the nicotine standard curve. The nicotine blanks were an average of 41.0% of the low standard. All other acceptance criteria met.	Yes
7/31/08	Set 4	One nicotine standard was excluded from the nicotine standard curve. The nicotine blanks were an average of 41.9% of the low standard. All other acceptance criteria met.	Yes
8/1/08	Set 5	The nicotine blanks were an average of 38.8% of the low standard. All other acceptance criteria met.	Yes
8/12/08	Set 6	The nicotine blanks were an average of 35.7% of the low standard. All other acceptance criteria met.	Yes
8/15/08	Set 7	One nicotine standard was excluded from the nicotine standard curve. The nicotine blanks were an average of 39.5% of the low standard. All other acceptance criteria met.	Yes

The calibration standards used to form the calibration curve from all reported runs met acceptance criteria (r , the correlation coefficient, greater than or equal to 0.99; average relative error [RE] within 15% of nominal for all standards except the lowest standard which should have an average RE within 20%) in all runs.

The nicotine blanks did not meet acceptance criteria (average response no greater than 33% of the average response of the lowest acceptable standard) in all runs, except for the July 28, 2008 analysis where it was an average of 29.4% of the lowest standard. The other analyses ranged from 35.4% to 41.9% of the lowest standard.

The cotinine blanks met acceptance criteria (average response no greater than 33% of the average response of the lowest acceptable standard) in all runs.

The QC samples met all acceptance criteria (average concentration within 15% of the nominal concentration and relative standard deviation [RSD] less than or equal to 15%) in seven runs. The results of the QC samples are presented in Table 2, Table 3, and Table 4 for nicotine and Table 5, Table 6, and Table 7 for cotinine.

Table 2 – Nicotine Low QC Level Results

Analysis Date	Analysis Set	Nominal Concentration (ng/mL)	Average Determined Concentration (ng/mL)	% RSD	Average % RE
7/25/08	Set 1	2.95E+00	2.61E+00	6.7	-11.7
7/28/08	Set 2	2.95E+00	2.75E+00	5.5	-6.9
7/29/08	Set 3	2.95E+00	2.77E+00	6.9	-6.3
7/31/08	Set 4	2.95E+00	3.00E+00	4.6	1.6
8/1/08	Set 5	2.95E+00	2.61E+00	5.9	-11.7
8/12/08	Set 6	2.95E+00	2.77E+00	11.8	-6.2
8/15/08	Set 7	2.95E+00	2.53E+00	2.1	-14.5

Table 3 – Nicotine Mid QC Level Results

Analysis Date	Analysis Set	Nominal Concentration (ng/mL)	Average Determined Concentration (ng/mL)	% RSD	Average % RE
7/25/08	Set 1	2.95E+01	2.77E+01	3.6	-6.1
7/28/08	Set 2	2.95E+01	2.91E+01	3.4	-1.5
7/29/08	Set 3	2.95E+01	2.77E+01	3.2	-6.0
7/31/08	Set 4	2.95E+01	2.89E+01	2.9	-2.1
8/1/08	Set 5	2.95E+01	2.77E+01	1.7	-6.2
8/12/08	Set 6	2.95E+01	2.80E+01	2.6	-5.3
8/15/08	Set 7	2.95E+01	2.81E+01	1.7	-4.8

Table 4 – Nicotine High QC Level Results

Analysis Date	Analysis Set	Nominal Concentration (ng/mL)	Average Determined Concentration (ng/mL)	% RSD	Average % RE
7/25/08	Set 1	1.48E+02	1.46E+02	3.1	-1.5
7/28/08	Set 2	1.48E+02	1.40E+02	2.5	-5.3
7/29/08	Set 3	1.48E+02	1.40E+02	0.9	-5.2
7/31/08	Set 4	1.48E+02	1.42E+02	2.3	-3.6
8/1/08	Set 5	1.48E+02	1.42E+02	2.7	-4.1
8/12/08	Set 6	1.48E+02	1.43E+02	2.7	-3.0
8/15/08	Set 7	1.48E+02	1.44E+02	1.8	-2.6

Table 5 – Cotinine Low QC Level Results

Analysis Date	Analysis Set	Nominal Concentration (ng/mL)	Average Determined Concentration (ng/mL)	% RSD	Average % RE
7/25/08	Set 1	2.96E+01	2.70E+01	5.4	-8.9
7/28/08	Set 2	2.96E+01	2.72E+01	9.6	-8.2
7/29/08	Set 3	2.96E+01	2.62E+01	3.5	-11.8
7/31/08	Set 4	2.96E+01	2.58E+01	3.2	-12.9
8/1/08	Set 5	2.96E+01	2.72E+01	6.7	-8.3
8/12/08	Set 6	2.96E+01	2.61E+01	2.8	-12.0
8/15/08	Set 7	2.96E+01	2.58E+01	5.7	-13.1

Table 6 – Cotinine Mid QC Level Results

Analysis Date	Analysis Set	Nominal Concentration (ng/mL)	Average Determined Concentration (ng/mL)	% RSD	Average % RE
7/25/08	Set 1	2.96E+02	2.81E+02	4.7	-5.2
7/28/08	Set 2	2.96E+02	2.93E+02	1.6	-1.1
7/29/08	Set 3	2.96E+02	2.71E+02	4.2	-8.5
7/31/08	Set 4	2.96E+02	2.64E+02	10.4	-11.1
8/1/08	Set 5	2.96E+02	2.70E+02	2.8	-9.1
8/12/08	Set 6	2.96E+02	2.81E+02	1.3	-5.2
8/15/08	Set 7	2.96E+02	2.79E+02	3.0	-6.0

Table 7 – Cotinine High QC Level Results

Analysis Date	Analysis Set	Nominal Concentration (ng/mL)	Average Determined Concentration (ng/mL)	% RSD	Average % RE
7/25/08	Set 1	1.48E+03	1.44E+03	5.2	-2.7
7/28/08	Set 2	1.48E+03	1.44E+03	4.2	-3.1
7/29/08	Set 3	1.48E+03	1.39E+03	3.8	-6.1
7/31/08	Set 4	1.48E+03	1.39E+03	5.3	-6.3
8/1/08	Set 5	1.48E+03	1.40E+03	3.7	-5.4
8/12/08	Set 6	1.48E+03	1.42E+03	0.6	-4.4
8/15/08	Set 7	1.48E+03	1.36E+03	5.4	-8.3

Representative overlaid nicotine full and reduced scale chromatograms of high and low concentration standards, a blank with IS, and a blank are shown in Figure 1 and Figure 2. Representative overlaid IS for nicotine full scale chromatograms of high and low concentration standards, a blank with IS, and a blank are shown in Figure 3. Representative overlaid cotinine full and reduced scale chromatograms of high and low concentration standards, a blank with IS, and a blank are shown in Figure 4 and Figure 5. Representative overlaid IS for cotinine full scale chromatograms of high and low concentration standards, a blank with IS, and a blank are shown in Figure 6.

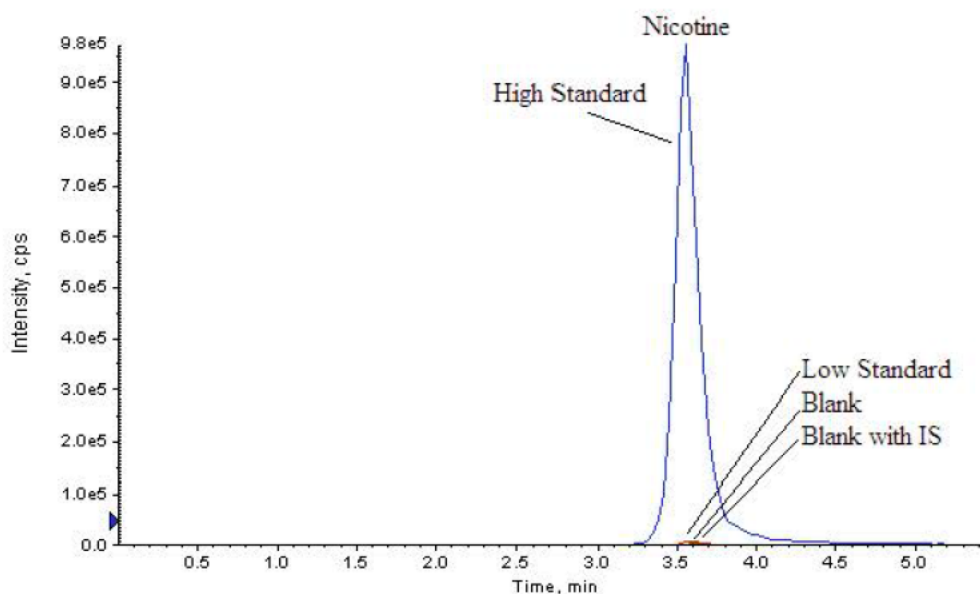


Figure 1 – Representative Overlaid Nicotine Chromatograms from High and Low Standards, a Blank with IS, and a Blank – Full Scale

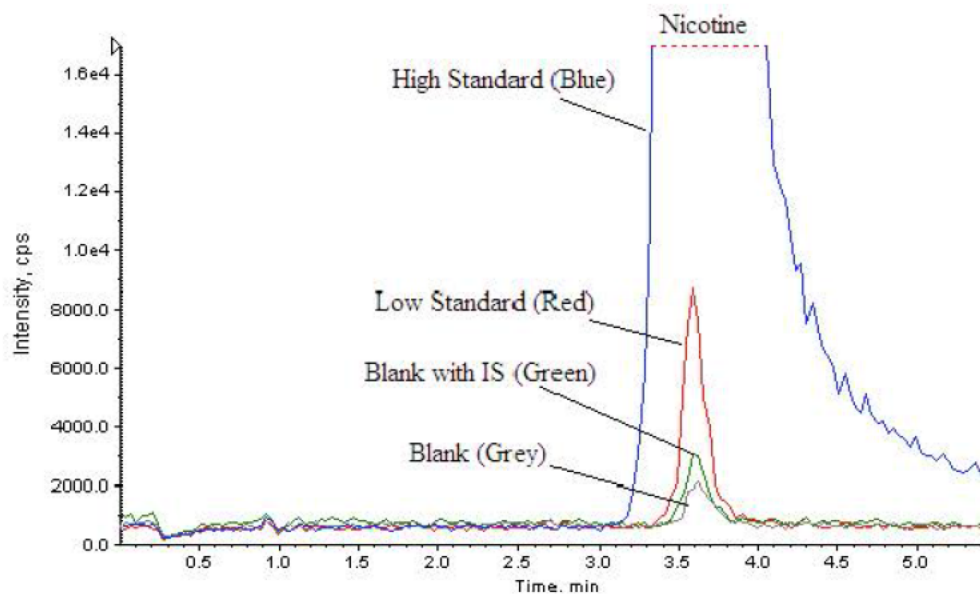


Figure 2 – Representative Overlaid Nicotine Chromatograms from High and Low Standards, a Blank with IS, and a Blank – Reduced Scale

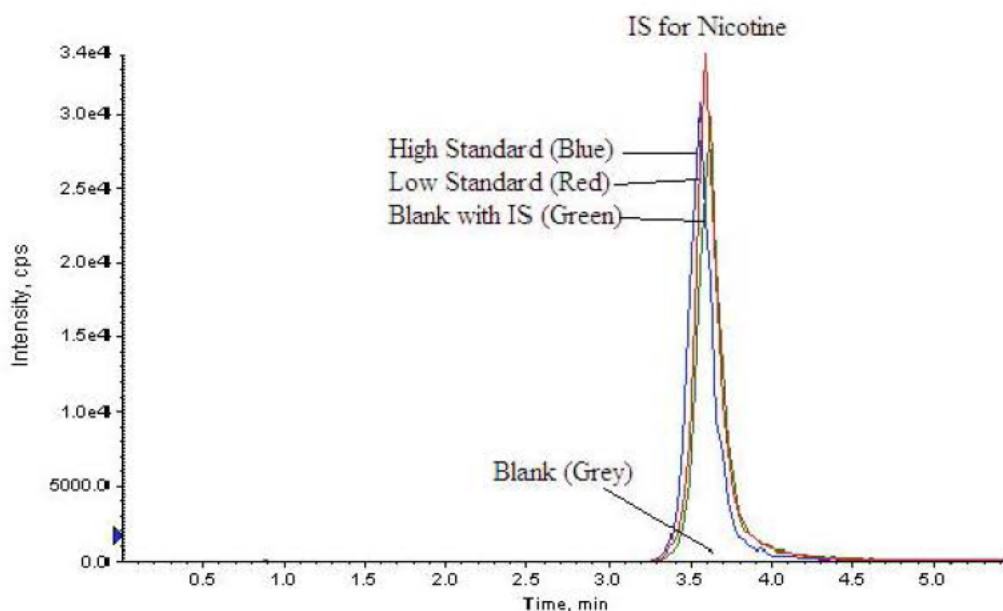


Figure 3 – Representative Overlaid IS for Nicotine Chromatograms from High and Low Standards, a Blank with IS, and a Blank – Full Scale

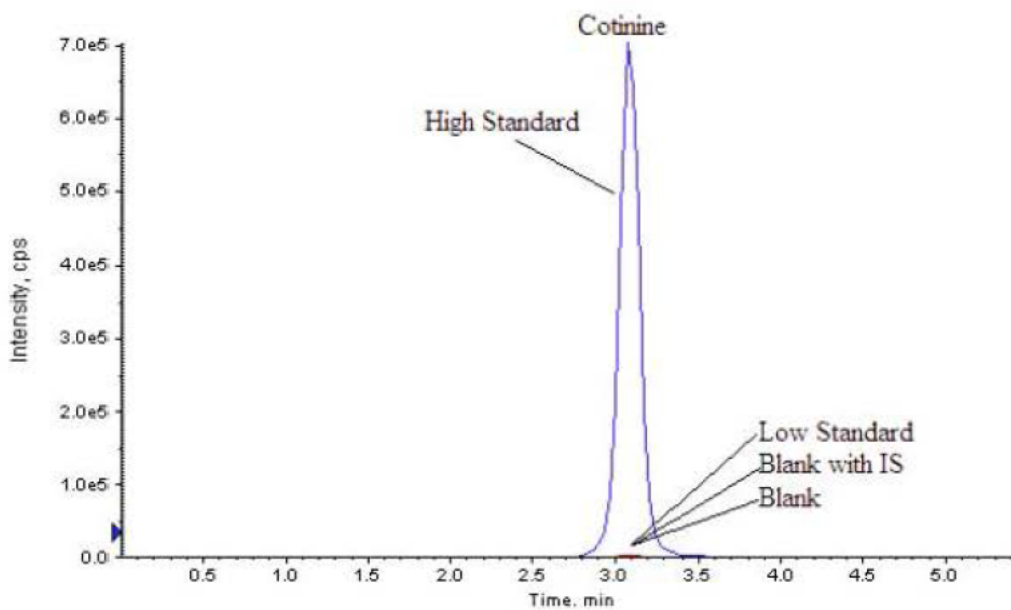


Figure 4 – Representative Overlaid Cotinine Chromatograms from High and Low Standards, a Blank with IS, and a Blank – Full Scale

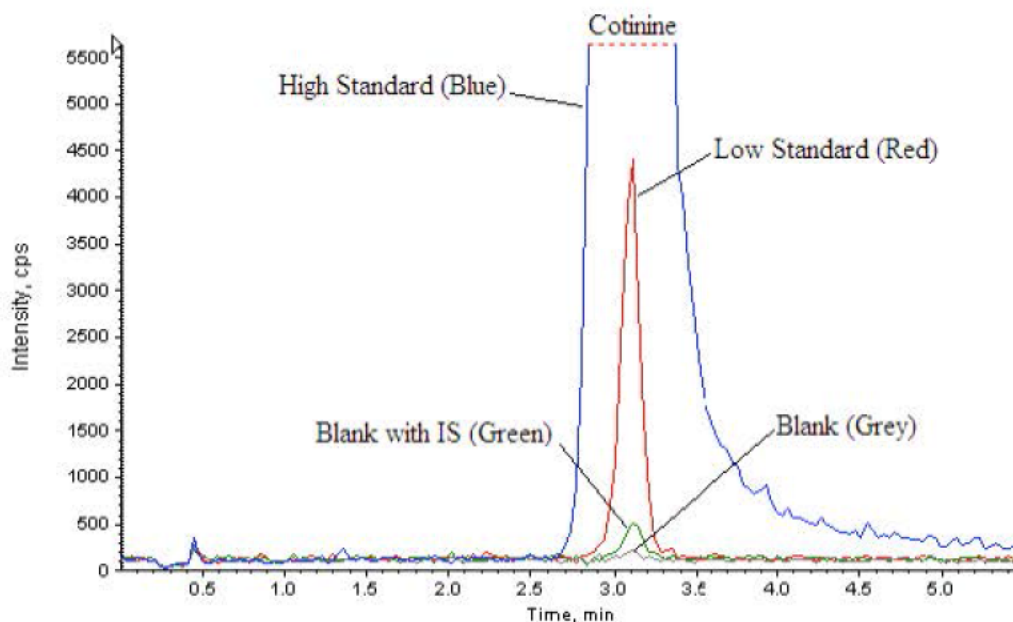


Figure 5 – Representative Overlaid Cotinine Chromatograms from High and Low Standards, a Blank with IS, and a Blank – Reduced Scale

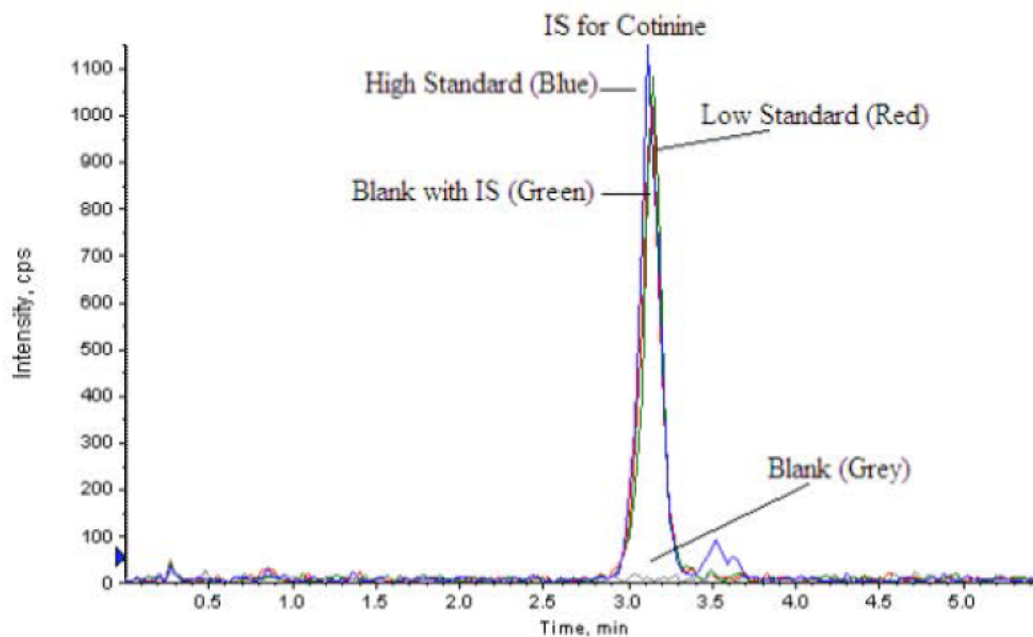


Figure 6 – Representative Overlaid IS for Cotinine Chromatograms from High and Low Standards, a Blank with IS, and a Blank – Full Scale

The results from the analyses for nicotine and cotinine for Days 14 and 15 are shown in Table 8 through Table 16. The results from the analyses for nicotine and cotinine for Day 28 are shown in Table 17 through Table 26. Any samples with calculated concentrations below the limit of quantitation are listed as BLOQ.

Table 8 – NT200M and NT200F (200 mg/kg/day) Results

Animal ID	Sex	Target Time Point	Nicotine Concentration (ng/mL)	Cotinine Concentration (ng/mL)
214	Male	10:00 PM	2.57E+02	2.95E+03
215			1.46E+03	8.07E+03
216			8.34E+02	4.50E+03
217		2:00 AM	4.21E+02	4.40E+03
219			1.29E+03	8.22E+03
220			6.06E+02	8.22E+03
221		6:00 AM	4.77E+02	8.00E+03
222			1.07E+03	5.95E+03
223			1.08E+03	5.62E+03
224		10:00 AM	2.14E+02	4.26E+03
225			7.48E+02	8.18E+03
226			1.55E+03	5.26E+03
229		2:00 PM	8.86E+02	1.25E+04
230		6:00 PM	7.08E+02	5.23E+03
233			1.06E+03	1.01E+04
261	Female	10:00 PM	4.49E+02	8.30E+03
262			8.13E+02	6.99E+03
263			9.23E+02	5.55E+03
264		2:00 AM	4.34E+02	6.52E+03
265			4.77E+02	5.26E+03
266			1.14E+02	3.74E+03
267		6:00 AM	2.68E+02	4.11E+03
268			2.73E+02	4.72E+03
269			6.92E+02	5.25E+03
270		10:00 AM	8.35E+02	7.13E+03
271			5.94E+02	5.58E+03
272			6.99E+02	8.03E+03
273		2:00 PM	8.42E+02	5.17E+03
274			1.10E+03	1.03E+04
275			7.09E+02	6.38E+03
276		6:00 PM	1.23E+03	9.35E+03
277			6.43E+02	3.45E+03
278			1.22E+03	1.31E+04

Table 9 – B2M and B2F (2 mg/kg/day) Results

Animal ID	Sex	Target Time Point	Nicotine Concentration (ng/mL)	Cotinine Concentration (ng/mL)
311	Male	10:00 PM	1.55E+00	4.29E+01*
312			BLOQ	4.16E+01*
313			BLOQ	BLOQ
314		2:00 AM	BLOQ	BLOQ
315			BLOQ	2.41E+01*
316			BLOQ	2.14E+01
317		6:00 AM	BLOQ	1.76E+01
318			BLOQ	BLOQ
319			BLOQ	2.26E+01
320		10:00 AM	1.04E+00	2.43E+01
321			7.96E+00	4.19E+01
322			7.51E+00	4.85E+01
323		2:00 PM	1.39E+00	2.08E+01
324			1.24E+00	1.89E+01
325			6.66E+00	5.10E+01
326		6:00 PM	9.54E-01	3.61E+01
327			2.75E+00	3.88E+01
328			4.41E+00	5.23E+01
361	Female	10:00 PM	BLOQ	3.23E+01
362			BLOQ	2.05E+01
363			1.13E+00	1.52E+01
364		2:00 AM	BLOQ	2.10E+01
365			1.52E+00	1.74E+01
366			BLOQ	BLOQ
367		6:00 AM	1.62E+00	3.40E+01
368			BLOQ	1.02E+01
369			BLOQ	BLOQ
370		10:00 AM	5.94E+00	4.83E+01
371			2.90E+00	6.56E+01
372			5.15E+00	5.32E+01
373		2:00 PM	1.90E+00	4.15E+01
374			1.52E+00	3.56E+01
375			2.39E+00	3.47E+01
376		6:00 PM	2.49E+00	3.64E+01
377			1.40E+00	3.31E+01
378			1.78E+00	2.92E+01

* Average of two results.

Table 10 – B20M and B20F (20 mg/kg/day) Results

Animal ID	Sex	Target Time Point	Nicotine Concentration (ng/mL)	Cotinine Concentration (ng/mL)
411	Male	10:00 PM	2.81E+02	2.47E+03
412			7.75E+00	4.49E+02
413			2.93E+02	2.04E+03
414		2:00 AM	1.59E+02	1.22E+03
415			9.72E+01	1.76E+03
416			9.38E+01	1.79E+03
417		6:00 AM	1.54E+02	1.67E+03
418			9.42E+01	1.28E+03
419			8.20E+00	7.64E+02
420		10:00 AM	2.52E+02	1.04E+03
421			3.68E+02	1.78E+03
423		2:00 PM	1.26E+02	1.01E+03
424			1.25E+02	1.38E+03
425			6.37E+01	9.86E+02
426		6:00 PM	BLOQ	7.33E+01*
427			1.22E+02	8.59E+02
428			2.15E+01	3.23E+02
461	Female	10:00 PM	5.16E+00	2.67E+02
462			BLOQ	1.39E+02*
463			4.23E+00	2.17E+02
464		2:00 AM	8.20E+01	9.11E+02
465			BLOQ	2.40E+01
466			1.63E+01	4.24E+02
467		6:00 AM	BLOQ	BLOQ
468			BLOQ	1.95E+01
469			1.79E+01	3.24E+02
470		10:00 AM	6.55E+01	5.09E+02
471			1.86E+02	1.24E+03
472			1.76E+02	1.64E+03
473		2:00 PM	1.86E+01	3.51E+02
474			1.09E+02	1.64E+03
475			1.85E+02	1.41E+03
476		6:00 PM	2.22E+01	4.19E+02
477			5.59E+00	2.61E+02
478			9.49E+01	8.56E+02

* Average of two results.

Table 11 – B80M and B80F (80 mg/kg/day) Results

Animal ID	Sex	Target Time Point	Nicotine Concentration (ng/mL)	Cotinine Concentration (ng/mL)
511	Male	10:00 PM	3.42E+02	8.30E+03
512			4.40E+02	6.03E+03
513			6.74E+02	6.40E+03
514		2:00 AM	4.34E+02	6.58E+03
515			5.17E+02	6.24E+03
516			3.37E+02	4.23E+03
517		6:00 AM	4.70E+02	6.53E+03
518			4.35E+02	5.18E+03
519			3.96E+02	4.70E+03
520		10:00 AM	6.25E+02	7.53E+03
521			6.82E+02	5.92E+03
522			4.70E+02	4.86E+03
523		2:00 PM	4.76E+02	5.17E+03
524			4.77E+02	5.87E+03
525			1.83E+02	3.55E+03
526		6:00 PM	5.31E+02	6.36E+03
527			4.91E+02	4.96E+03
528			2.94E+02	4.71E+03
561	Female	10:00 PM	1.27E+02	3.63E+03
562			3.48E+02	5.92E+03
563			2.80E+02	4.28E+03
564		2:00 AM	2.82E+02	5.12E+03
565			3.05E+02	5.07E+03
566			BLOQ	1.38E+02
567		6:00 AM	2.02E+02	3.94E+03
568			1.30E+02	3.32E+03
569			1.11E+02	1.45E+03
570		10:00 AM	3.01E+02	4.09E+03
571			3.43E+02	3.98E+03
572			4.30E+02	4.82E+03
573		2:00 PM	2.38E+02	3.41E+03
574			3.84E+02	5.13E+03
575			3.49E+02	4.21E+03
576		6:00 PM	1.84E+02	3.15E+03
577			1.94E+01	7.87E+02
578			2.52E+02	2.71E+03

Table 12 – B200M and B200F (200 mg/kg/day) Results

Animal ID	Sex	Target Time Point	Nicotine Concentration (ng/mL)	Cotinine Concentration (ng/mL)
---	Male	No Samples Received		
---	Female	No Samples Received		

Table 13 – E2M and E2F (2 mg/kg/day) Results

Animal ID	Sex	Target Time Point	Nicotine Concentration (ng/mL)	Cotinine Concentration (ng/mL)
711	Male	10:00 PM	BLOQ	1.94E+01
712			8.04E+00	5.55E+01
713			BLOQ	1.54E+01
714		2:00 AM	1.86E+00	4.20E+01*
715			1.70E+00	1.76E+01
716			2.91E+00	5.08E+01
717		6:00 AM	2.85E+00	3.80E+01
718			4.82E+00	2.94E+01
719			2.72E+00	4.01E+01
720		10:00 AM	2.81E+00	4.48E+01
721			BLOQ	1.46E+01
722			BLOQ	3.12E+01
723		2:00 PM	BLOQ	BLOQ
724			BLOQ	1.75E+01
725			6.14E+00	2.42E+01
726		6:00 PM	2.52E+00	5.96E+01
727			6.70E+00	4.38E+01
728			BLOQ	1.96E+01
761	Female	10:00 PM	BLOQ	1.06E+01
762			BLOQ	2.37E+01
763			BLOQ	BLOQ
764		2:00 AM	1.39E+00	1.53E+01
765			BLOQ	BLOQ
766			BLOQ	BLOQ
767		6:00 AM	BLOQ	BLOQ
768			BLOQ	BLOQ
769			BLOQ	BLOQ
770		10:00 AM	BLOQ	2.34E+01
771			2.78E+00	4.77E+01
772			3.33E+00	3.85E+01
773		2:00 PM	1.93E+00	3.49E+01
774			BLOQ	1.41E+01
775			7.23E+00	4.77E+01
776		6:00 PM	4.54E+00	6.17E+01
777			3.74E+00	7.02E+01
778			7.99E+00	7.56E+01

* Average of two results.

Table 14 – E20M and E20F (20 mg/kg/day) Results

Animal ID	Sex	Target Time Point	Nicotine Concentration (ng/mL)	Cotinine Concentration (ng/mL)
811	Male	10:00 PM	1.04E+02	1.63E+03
812			2.00E+02	1.55E+03
813			2.63E+02	1.59E+03
814		2:00 AM	7.65E+01	1.19E+03
815			3.12E+01	1.21E+03
816			3.47E+02	2.04E+03
817		6:00 AM	2.59E+01	7.16E+02
818			3.55E+01	1.11E+03
819			4.68E+01	1.05E+03
820		10:00 AM	2.31E+02	1.50E+03*
821			1.34E+02	1.34E+03
822			3.55E+02	1.48E+03
823		2:00 PM	2.88E+01	4.71E+02
824			3.29E+01	5.59E+02
825			1.66E+02	1.49E+03
826		6:00 PM	7.84E+01	1.07E+03
827			1.97E+01	5.89E+02
828			4.22E+00	2.09E+02*
861	Female	10:00 PM	3.54E+00	2.18E+02*
862			8.98E+00	3.28E+02
863			7.53E+00	3.31E+02
864		2:00 AM	6.17E+00	2.35E+02*
865			1.82E+02	4.30E+03
866			BLOQ	6.54E+01
868		6:00 AM	1.90E+00	4.64E+01
869			2.95E+00	1.66E+02*
870		10:00 AM	1.42E+02	1.21E+03
871			1.44E+02	1.63E+03
872			1.43E+02	1.41E+03
873		2:00 PM	9.72E+00	2.10E+02
874			1.28E+02	1.61E+03
875			5.55E+01	1.01E+03
876		6:00 PM	9.31E+01	9.34E+02
877			4.08E+01	5.79E+02
878			7.43E+01	7.43E+02

* Average of two results.

Table 15 – E80M and E80F (80 mg/kg/day) Results

Animal ID	Sex	Target Time Point	Nicotine Concentration (ng/mL)	Cotinine Concentration (ng/mL)
911	Male	10:00 PM	4.25E+02	6.24E+03
912			4.41E+02	7.09E+03
913			5.52E+02	4.79E+03
914		2:00 AM	6.52E+02	8.79E+03
915			5.41E+02	5.90E+03
916			3.68E+02	4.80E+03
917		6:00 AM	8.08E+01	2.80E+03
918			4.24E+02	6.32E+03
919			3.01E+02	4.95E+03
920		10:00 AM	2.50E+02	5.25E+03
921			4.64E+02	6.04E+03
922			3.17E+02	3.65E+03
924		2:00 PM	1.10E+02	3.54E+03
925			3.46E+02	5.86E+03
926		6:00 PM	7.47E+02	6.82E+03
927			5.48E+02	5.75E+03
928			3.16E+02	4.72E+03
961	Female	10:00 PM	2.59E+02	4.48E+03
962			2.40E+02	3.86E+03
963			2.68E+02	4.00E+03
964		2:00 AM	2.30E+02	4.22E+03
965			9.72E+01	3.00E+03
966			4.03E+02	4.46E+03
967		6:00 AM	2.64E+02	4.00E+03
968			3.69E+02	4.59E+03
969			5.15E+01	1.83E+03
970		10:00 AM	4.39E+02	3.88E+03
971			3.48E+02	3.49E+03
972			4.47E+02	3.83E+03
973		2:00 PM	2.69E+02	3.49E+03
974			3.59E+02	3.85E+03
975			4.39E+02	5.29E+03
976		6:00 PM	2.95E+02	3.82E+03
977			2.11E+02	2.59E+03
978			1.34E+02	1.72E+03

Table 16 – E200M and E200F (200 mg/kg/day) Results

Animal ID	Sex	Target Time Point	Nicotine Concentration (ng/mL)	Cotinine Concentration (ng/mL)
1020	Male	10:00 PM	6.34E+02	6.91E+03
1031		2:00 AM	7.97E+02	8.40E+03
1032		6:00 AM	9.07E+02	1.02E+04
1061	Female	10:00 PM	4.44E+02	6.72E+03
1062			3.58E+02	4.67E+03
1063			7.69E+02	4.89E+03
1064		2:00 AM	3.95E+02	6.96E+03
1065			2.88E+02	6.84E+03
1066			9.58E+02	8.81E+03
1067		6:00 AM	2.00E+02	4.24E+03
1068			1.68E+02	3.05E+03
1069			2.50E+02	3.64E+03
1070		10:00 AM	1.26E+03	8.03E+03
1071			1.01E+03	1.14E+04
1072			1.26E+03	5.79E+03
1073		2:00 PM	9.86E+02	7.75E+03
1074			6.81E+02	7.94E+03
1075			6.56E+02	6.80E+03
1076		6:00 PM	1.21E+03	5.18E+03
1077			1.33E+03	8.52E+03
1078			1.18E+03	5.47E+03

Table 17 – CM and CF (0 mg/kg/day) Results

Animal ID	Sex	Target Time Point	Nicotine Concentration (ng/mL)	Cotinine Concentration (ng/mL)
111	Male	10:00 AM	BLOQ	BLOQ
112			BLOQ	BLOQ
113			No Sample Taken	
114			BLOQ	BLOQ
115			BLOQ	BLOQ
161	Female	10:00 AM	BLOQ	BLOQ
162			BLOQ	BLOQ
163			BLOQ	BLOQ
164			BLOQ	BLOQ
165			BLOQ	BLOQ

Table 18 – NT200M and NT200F (200 mg/kg/day) Results

Animal ID	Sex	Target Time Point	Nicotine Concentration (ng/mL)	Cotinine Concentration (ng/mL)
---	Male	10:00 AM	No Samples Received	
279	Female	10:00 AM	5.34E+02	5.70E+03
280			4.96E+02	5.63E+03
281			1.49E+02	3.62E+03
282			1.77E+01	1.50E+03
283			1.51E+02	6.08E+03

Table 19 – B2M and B2F (2 mg/kg/day) Results

Animal ID	Sex	Target Time Point	Nicotine Concentration (ng/mL)	Cotinine Concentration (ng/mL)
329	Male	10:00 AM	BLOQ	2.17E+01
330			BLOQ	1.89E+01
331			BLOQ	BLOQ
332			BLOQ	2.08E+01
333			BLOQ	BLOQ
379	Female	10:00 AM	BLOQ	BLOQ
380			BLOQ	BLOQ
381			BLOQ	BLOQ
382			BLOQ	2.09E+01
383			BLOQ	BLOQ

Table 20 – B20M and B20F (20 mg/kg/day) Results

Animal ID	Sex	Target Time Point	Nicotine Concentration (ng/mL)	Cotinine Concentration (ng/mL)
429	Male	10:00 AM	5.99E+00	3.05E+02
430			BLOQ	4.08E+01*
431			1.10E+00	2.20E+02*
432			9.94E+00	7.34E+02
433			BLOQ	2.50E+01*
479	Female	10:00 AM	1.50E+01	6.68E+02
480			1.88E+01	6.94E+02
481			BLOQ	7.33E+01*
482			2.20E+00	1.16E+02
483			BLOQ	2.36E+01*

* Average of two results.

Table 21 – B80M and B80F (80 mg/kg/day) Results

Animal ID	Sex	Target Time Point	Nicotine Concentration (ng/mL)	Cotinine Concentration (ng/mL)
529	Male	10:00 AM	3.73E+02	5.70E+03
530			6.24E+01	3.19E+03
532			1.91E+02	5.25E+03
533			4.53E+02	6.79E+03
579	Female	10:00 AM	5.21E+00	1.63E+02
580			5.63E+01	2.33E+03
581			2.02E+00	1.04E+02*
582			4.83E+00	2.58E+02*
583			1.86E+00	4.09E+02*

* Average of two results.

Table 22 – B200M and B200F (200 mg/kg/day) Results

Animal ID	Sex	Target Time Point	Nicotine Concentration (ng/mL)	Cotinine Concentration (ng/mL)
---	Male	10:00 AM	No Samples Received	
---	Female	10:00 AM	No Samples Received	

Table 23 – E2M and E2F (2 mg/kg/day) Results

Animal ID	Sex	Target Time Point	Nicotine Concentration (ng/mL)	Cotinine Concentration (ng/mL)
729	Male	10:00 AM	BLOQ	BLOQ
730			BLOQ	BLOQ
731			BLOQ	BLOQ
732			BLOQ	BLOQ
733			BLOQ	BLOQ
779	Female	10:00 AM	BLOQ	BLOQ
780			BLOQ	BLOQ
781			BLOQ	BLOQ
782			BLOQ	9.44E+00
783			BLOQ	BLOQ

Table 24 – E20M and E20F (20 mg/kg/day) Results

Animal ID	Sex	Target Time Point	Nicotine Concentration (ng/mL)	Cotinine Concentration (ng/mL)
829	Male	10:00 AM	BLOQ	BLOQ
830			BLOQ	8.30E+01*
831			2.28E+00	3.47E+02
832			BLOQ	4.67E+01*
833			6.20E+00	9.41E+02
879	Female	10:00 AM	BLOQ	1.47E+01
880			BLOQ	3.38E+01*
881			BLOQ	9.34E+01*
882			BLOQ	2.30E+01*
883			BLOQ	4.61E+01*

* Average of two results.

Table 25 – E80M and E80F (80 mg/kg/day) Results

Animal ID	Sex	Target Time Point	Nicotine Concentration (ng/mL)	Cotinine Concentration (ng/mL)
929	Male	10:00 AM	5.18E+01	3.35E+03
930			3.60E+02	6.21E+03
931			4.34E+02	6.07E+03
932			2.62E+02	4.42E+03
933			3.99E+01	2.75E+03
979	Female	10:00 AM	1.62E+02	2.32E+03
980			7.50E+00	3.34E+02*
981			7.16E+01	1.32E+03
982			4.53E+01	2.00E+03
983			2.15E+01	1.27E+03

* Average of two results.

Table 26 – E200M and E200F (200 mg/kg/day) Results

Animal ID	Sex	Target Time Point	Nicotine Concentration (ng/mL)	Cotinine Concentration (ng/mL)
---	Male	10:00 AM	No Samples Received	
1079	Female	10:00 AM	4.52E+02	6.80E+03
1080			4.06E+02	5.54E+03
1081			8.05E+02	6.52E+03
1082			6.69E+02	5.80E+03
1083			7.46E+02	7.51E+03

V. KINETICS

A. METHODS/RESULTS

The test system used was male and female CD-1 mice. Animals were exposed by the dosed feed route of administration to nicotine tartrate, tobacco blend, or tobacco extract for 28 consecutive days at nicotine target doses of 200 mg/kg/day for nicotine tartrate, 2, 20, 80, and 200 mg/kg/day for tobacco blend and tobacco extract. Blood samples were collected from three mice/sex/group/time point on Days 14 and 15 at target times of 10:00 PM, 2:00 AM, 6:00 AM, 10:00 AM, 2:00 PM, and 6:00 PM, and on Day 28 at 10:00 AM. Based on the Days 14 and 15 concentration time profiles, a single sampling time point was selected on Day 28 to be at 10:00 AM. Several of the animals on Day 28 did not have samples collected within the target time criteria of 10:00 AM. However, the samples taken outside the criteria were believed to be sufficiently close to be used in the analysis. TK analysis was performed using the target dose (mg/kg/day), target sample collection time (clock time), and the measured concentrations of nicotine and cotinine (ng/mL).

Figure 7 through Figure 14 show the group mean plasma concentration-time profiles for male and female mice on Days 14 and 15. Figure 15 through Figure 18 illustrate the group mean plasma concentration-time overlay profiles for male and female mice on Days 14 and 15. Observed T_{max} values were varied and often with no discernable peak concentration, i.e., multiple potential times to reach C_{max} . For nicotine, five of the eight male dose groups and six of the eight female dose groups had 10:00 AM as the T_{max} or one of multiple possible T_{max} values. For cotinine, none of the eight male dose groups and three of the eight female dose groups had 10:00 AM as the T_{max} or one of multiple possible T_{max} values. There was no data from the tobacco blend high dose group (Table 27).

Table 27 – Summary of Peak Concentration Time Points

Group	Target Dosage (mg/kg/day)	Analyte	Sex	Time Point(s)
NT200	200	Nicotine	M	10:00 PM, 2:00 AM, 6:00 AM, 10:00 AM ^a
			F	6:00 PM
		Cotinine	M	2:00 AM
			F	6:00 PM
B2	2	Nicotine	M	10:00 AM
			F	10:00 AM
		Cotinine	M	6:00 PM
			F	10:00 AM
B20	20	Nicotine	M	10:00 PM, 10:00 AM ^a
			F	10:00 AM
		Cotinine	M	10:00 PM
			F	10:00 AM
B80	80	Nicotine	M	10:00 AM
			F	10:00 AM
		Cotinine	M	10:00 PM
			F	10:00 PM
B200	200	Nicotine	M	No data
			F	No data
		Cotinine	M	No data
			F	No data
E2	2	Nicotine	M	2:00 PM
			F	6:00 PM
		Cotinine	M	6:00 PM
			F	6:00 PM
E20	20	Nicotine	M	10:00 PM, 10:00 AM ^a
			F	10:00 AM
		Cotinine	M	10:00 PM
			F	2:00 AM

Table 27 – Summary of Peak Concentration Time Points (Continued)

Group	Target Dosage (mg/kg/day)	Analyte	Sex	Time Point(s)
E80	80	Nicotine	M	2:00 AM, 6:00 PM ^a
			F	10:00 AM
		Cotinine	M	2:00 AM
			F	10:00 PM, 2:00 PM ^a
E200	200	Nicotine	M	Unclear ^b
			F	10:00 AM, 6:00 PM ^a
		Cotinine	M	Unclear ^b
			F	2:00 AM, 10:00 AM ^a

a. Variability allows more than one time point to be considered.

b. Not enough data points for evaluation.

Group mean C_{\max} values determined at 10:00 AM on Day 28 are reported in Table 28. The data were difficult to analyze due to the variability and absence of data, e.g., no samples or the samples were BLOQ. Both the tobacco blend and tobacco extract had increasing C_{\max} values for nicotine and cotinine with increasing dose; however, the number of groups available for evaluating the change in concentration with dosage were limited and, for the data available, the results were equivocal. For example, nicotine C_{\max} values were only available for the two intermediate tobacco blend groups. Although there was a four-fold increase in dose between the two groups, there was a 48- (males) and one-(females) fold increase in C_{\max} . The nicotine results were equally limited and equivocal for the tobacco extract results. For example, nicotine C_{\max} values for the males were only available for the two intermediate groups. Although there was a four-fold increase in dose, there was a 54-fold increase in C_{\max} . For the only two female groups that could be compared (e.g., intermediate dose 2 and high dose groups), a 2.5-fold increase in dose resulted in a ten-fold increase in C_{\max} .

An evaluation of the cotinine results was made slightly more possible by a few more groups with data; however, the results were also equivocal. For the tobacco blend groups, the low and two intermediate group doses increased ten- and four-fold, which corresponded to an increase in C_{\max} for the males of 13- and 20-fold, and for the females, 15- and two-fold, respectively. For the tobacco extract groups, the low, two intermediate, and high dose group doses increased ten-, four-, and 2.5-fold, which corresponded to an increase in C_{\max} for the males of 13-fold (two intermediate groups only), and for the females, four-, 34-, and four-fold, respectively.

The tobacco blend and tobacco extract groups had mean C_{\max} values that were similar when comparing the same dose (within three-fold) except for the C_{\max} for nicotine for the female intermediate dose 2 groups which was 4.4-fold higher in

the extract and cotinine for the females intermediate dose group 1 which was 7.5-fold higher in the blend.

Cotinine C_{\max} values were between ten- to 83-fold greater than nicotine for the males and females (Figure 19 and Figure 20).

Male and female mice had similar nicotine C_{\max} values for the intermediate dose 1 tobacco blend groups (within two-fold). Male and female mice had similar cotinine C_{\max} values for the low and intermediate dose 1 tobacco blend group. Females had lower nicotine C_{\max} values for the intermediate dose 2 tobacco blend and tobacco extract groups. Females had lower cotinine C_{\max} values for the intermediate dose 2 tobacco blend and the intermediate dose 1 and 2 tobacco extract groups.

Table 28 – Day 28 C_{\max} Values^{a,b}

Group/Target Dose	Gender	Nicotine C_{\max} (ng/mL)	Cotinine C_{\max} (ng/mL)
NT200 – 200 mg/kg/day	Male	No Samples	No Samples
	Female	269 ± 103	4510 ± 870
B2 – 2 mg/kg/day	Male	BLOQ	20.5 ± 0.6
	Female	BLOQ	20.9
B20 – 20 mg/kg/day	Male	5.67 ± 1.98	265 ± 129
	Female	12.0 ± 3.9	315 ± 150
B80 – 80 mg/kg/day	Male	270 ± 79	5230 ± 670
	Female	14.0 ± 10.6	650 ± 420
B200 – 200 mg/kg/day	Male	No Samples	No Samples
	Female	No Samples	No Samples
E2 – 2 mg/kg/day	Male	BLOQ	BLOQ
	Female	BLOQ	9.44
E20 – 20 mg/kg/day	Male	4.24 ± 1.24	354 ± 185
	Female	BLOQ	42.2 ± 13.8
E80 – 80 mg/kg/day	Male	230 ± 80	4560 ± 700
	Female	61.6 ± 27.4	1450 ± 340
E200 – 200 mg/kg/day	Male	No Samples	No Samples
	Female	616 ± 79	6430 ± 350

a. Sample collection time point at 10:00 AM.

b. Tabled data are the mean ± SEM (n = at least three and up to six animals/group/sex).

B. CONCLUSIONS

Days 14 and 15 concentration time data were used to determine a common Day 14 T_{max} among male and female mice of all dose groups. There was high variability in the group mean C_{max} determinations resulting in many dose groups with multiple time points that could be considered as potential T_{max} values. The 10:00 AM time point was chosen as it occurred with the highest frequency among the dose groups, both male and female. Among dose groups that had only one discernable T_{max} , 10:00 AM again occurred with the highest frequency.

On Day 28, an evaluation of C_{max} values was difficult due to the variability and absence of data for many groups. However, there were gender- and dose-dependent effects but not formulation-dependent effects for the tobacco blend and extract groups. For the intermediate 2 and high dose groups, the males had higher C_{max} values than females. For the low and intermediate 1 dose groups, there was no gender effect on nicotine or cotinine with either formulation. The C_{max} values increased proportionally with an increase in dose for both the tobacco extract and tobacco blends from low dose to intermediate dose 1 for nicotine and cotinine. Male mice had a greater than proportional increase in nicotine and cotinine C_{max} when increasing from the intermediate dose 1 to intermediate dose 2 (20 to 80 mg/kg/day) of both the tobacco blend and extract. Female mice had a greater than dose proportional increase in nicotine and cotinine C_{max} for the intermediate and high dose tobacco extract groups, but the change from intermediate dose 1 to intermediate dose 2 for the tobacco blend was slightly less than dose proportional. Among groups that could be compared, there were no formulation effects as tobacco extract and tobacco blends with the same nicotine dosage had similar C_{max} values for both males and females. There was some variability in the females but it was not considered biologically relevant.

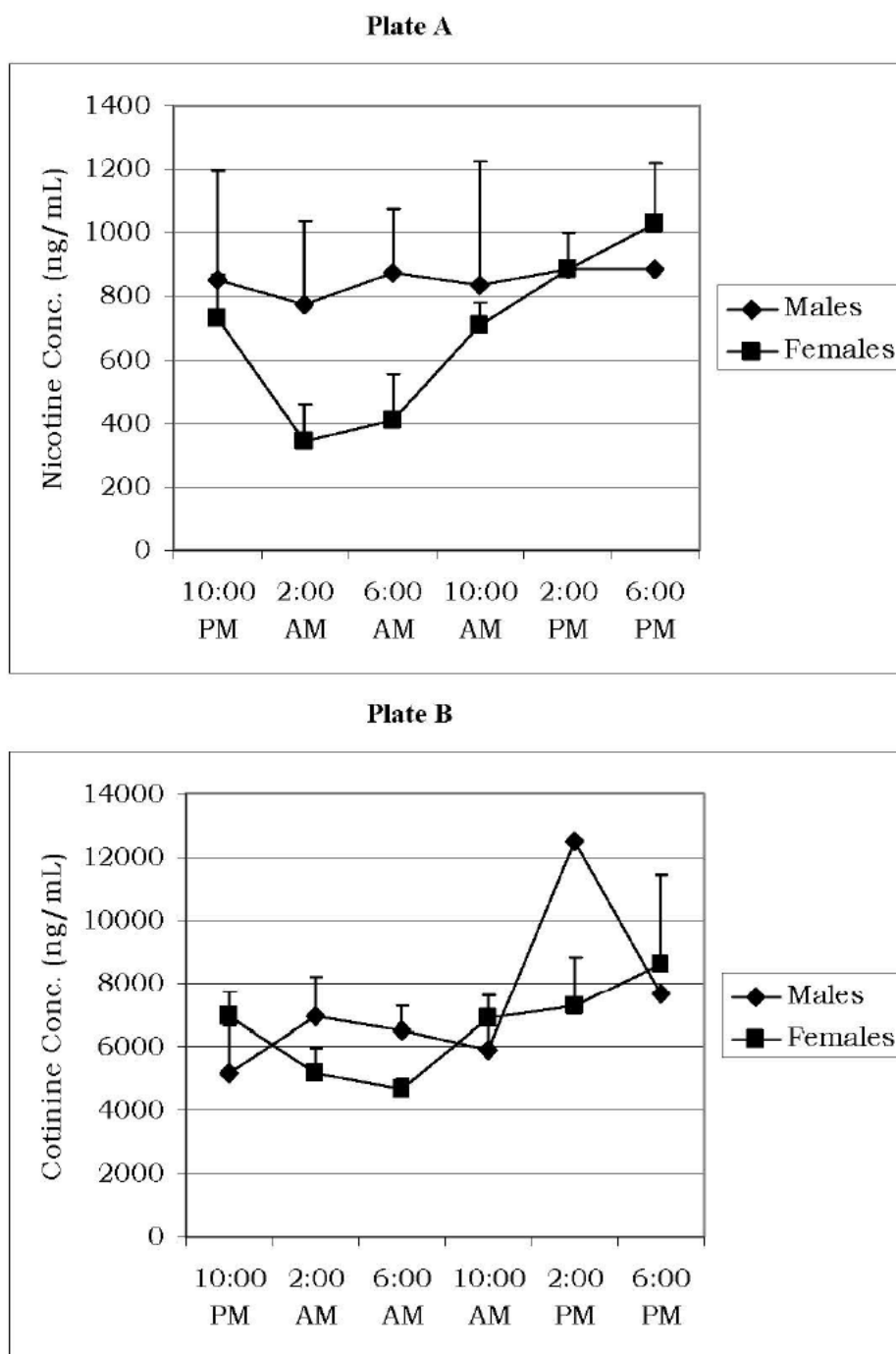


Figure 7 – Plasma Concentration (Mean + SEM)-Time Profile for Male and Female Mice on Days 14 and 15 After Daily Oral Exposure of NT200 (200 mg/kg/day) – Nicotine (Plate A) and Cotinine (Plate B)

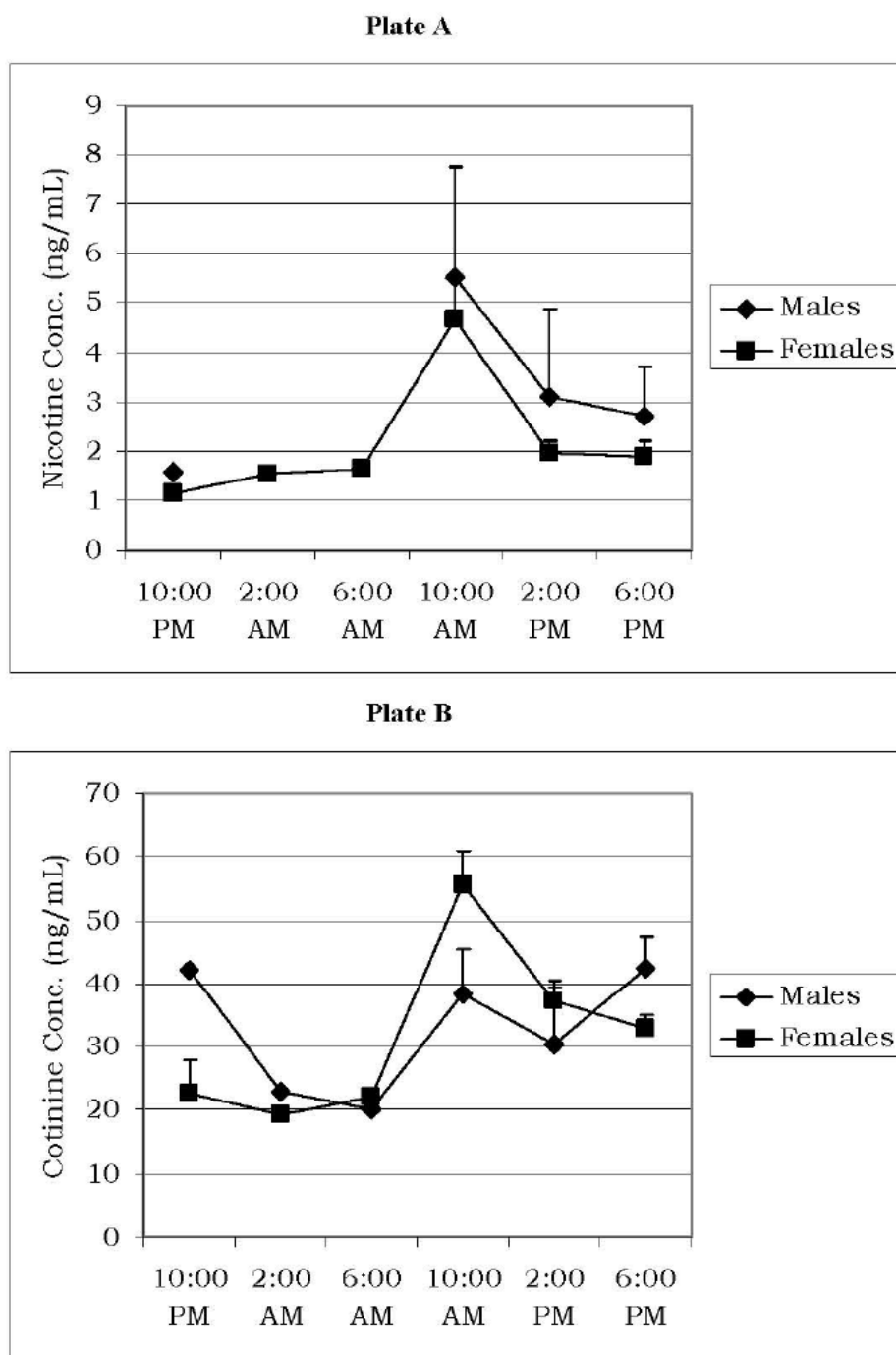


Figure 8 – Plasma Concentration (Mean + SEM)-Time Profile for Male and Female Mice on Days 14 and 15 After Daily Oral Exposure of B2 (2.0 mg/kg/day) – Nicotine (Plate A) and Cotinine (Plate B)

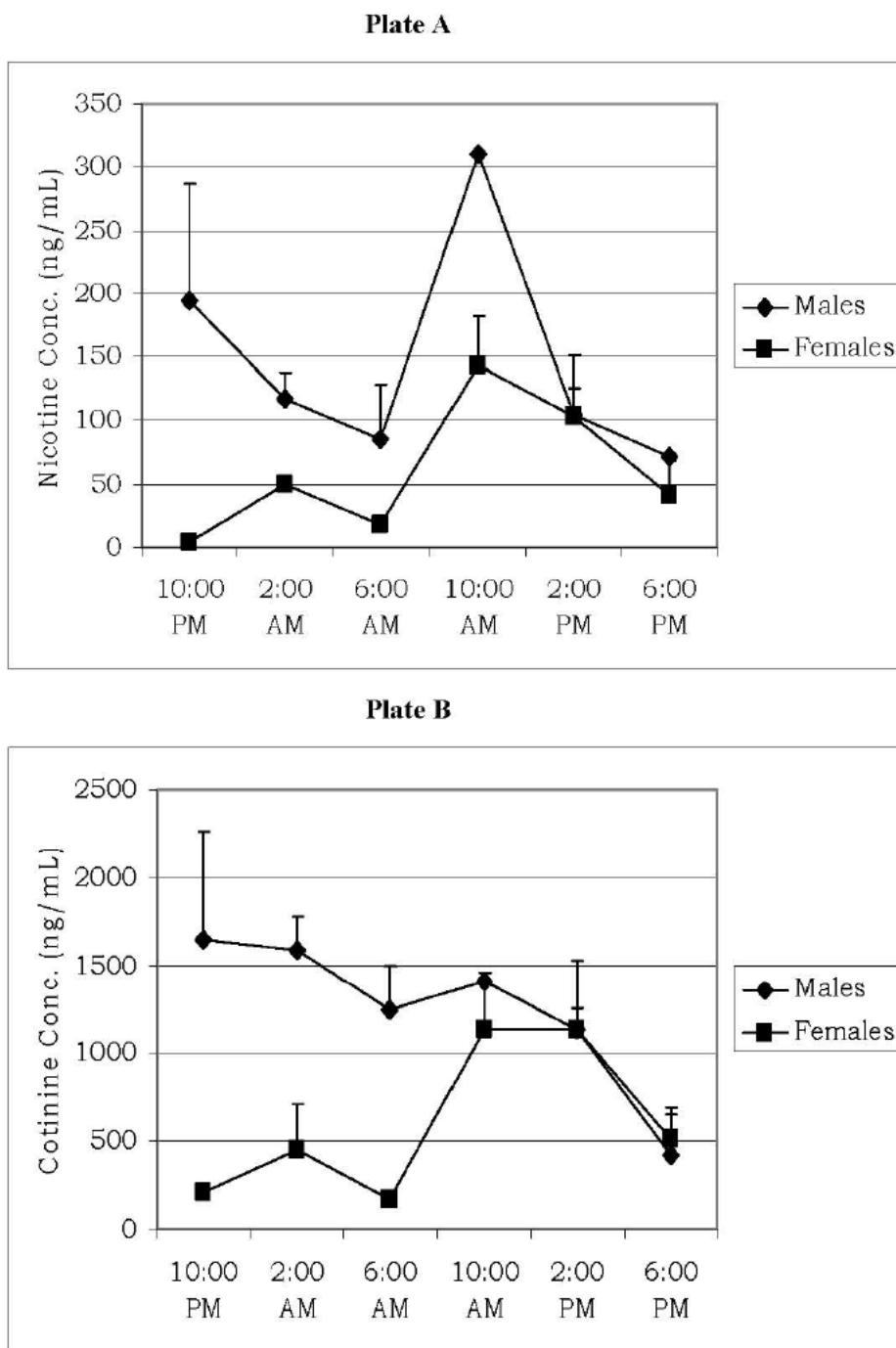


Figure 9 – Plasma Concentration (Mean + SEM)-Time Profile for Male and Female Mice on Days 14 and 15 After Daily Oral Exposure of B20 (20 mg/kg/day) – Nicotine (Plate A) and Cotinine (Plate B)

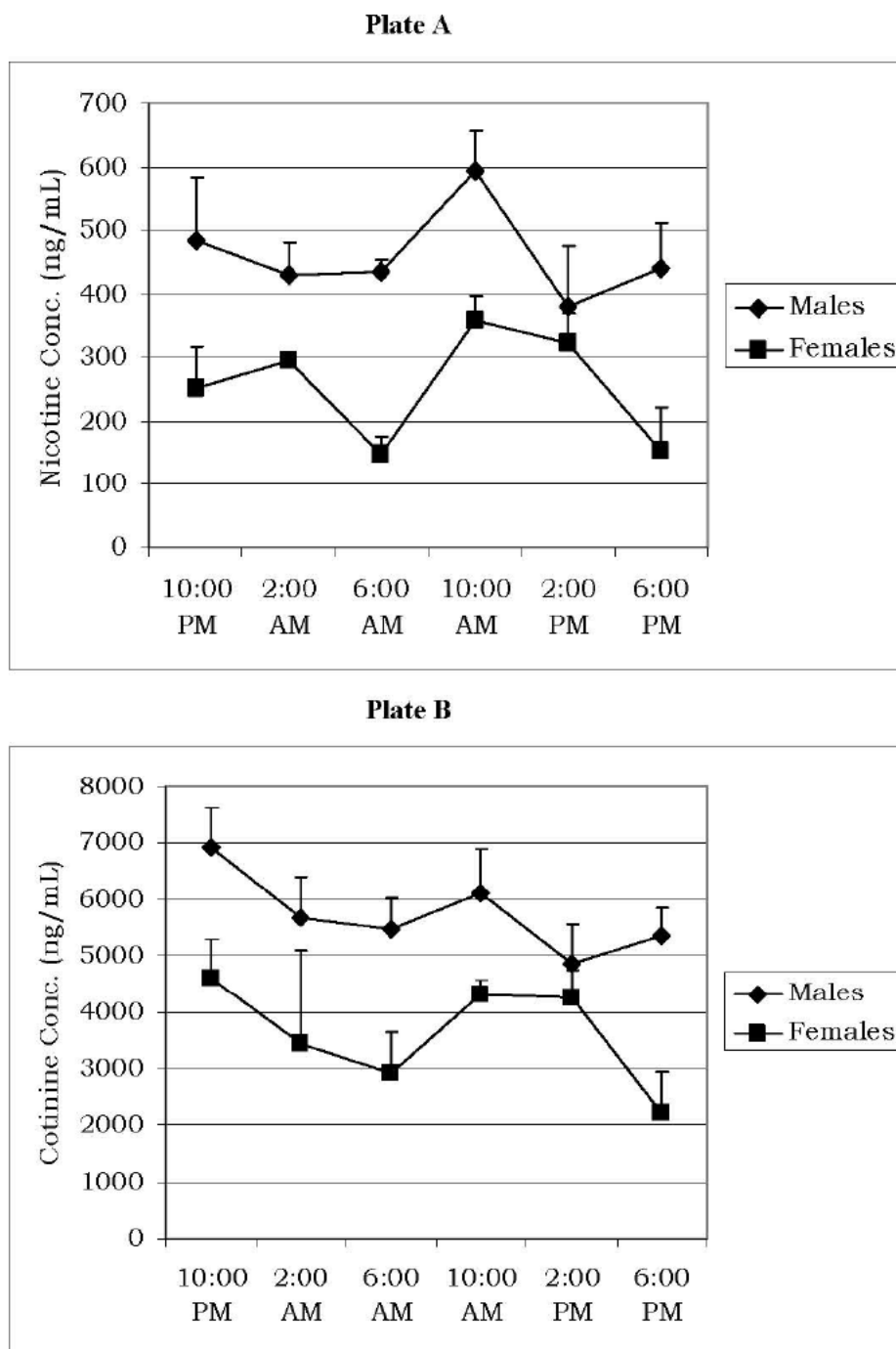


Figure 10 – Plasma Concentration (Mean + SEM)-Time Profile for Male and Female Mice on Days 14 and 15 After Daily Oral Exposure of B80 (80 mg/kg/day) – Nicotine (Plate A) and Cotinine (Plate B)

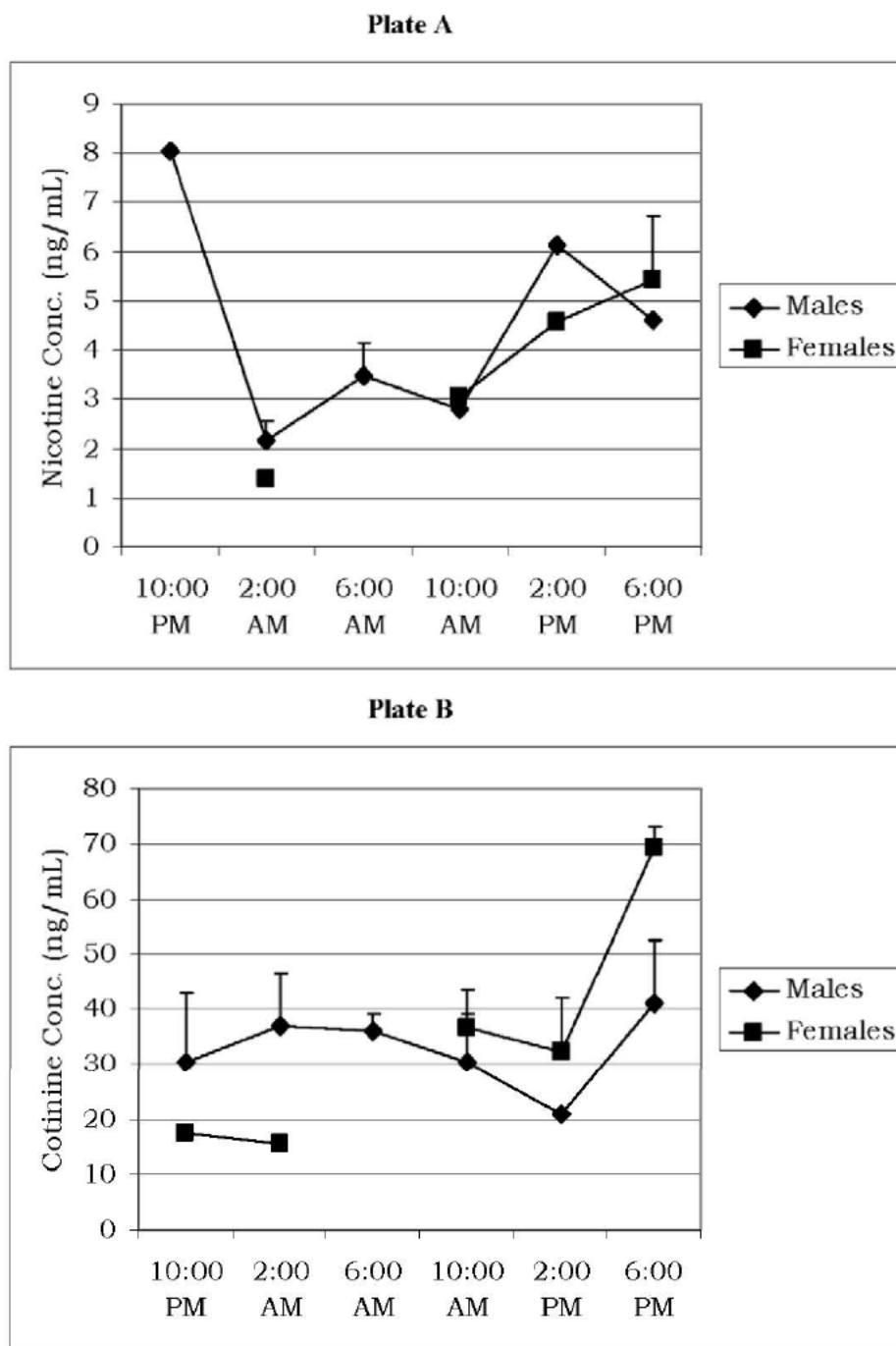


Figure 11 – Plasma Concentration (Mean + SEM)-Time Profile for Male and Female Mice on Days 14 and 15 After Daily Oral Exposure of E2 (2 mg/kg/day) – Nicotine (Plate A) and Cotinine (Plate B)

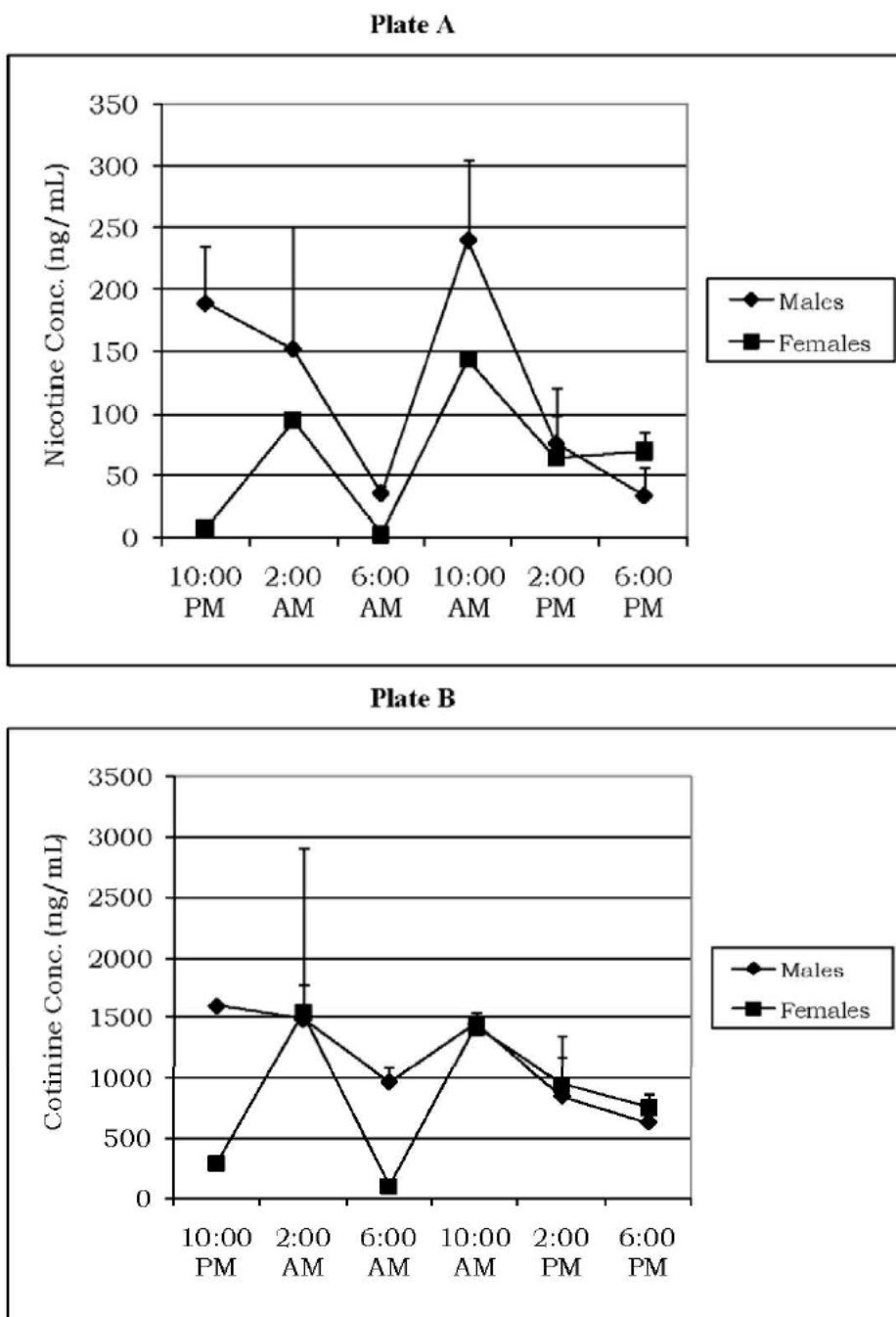


Figure 12 – Plasma Concentration (Mean + SEM)-Time Profile for Male and Female Mice on Days 14 and 15 After Daily Oral Exposure of E20 (20 mg/kg/day) – Nicotine (Plate A) and Cotinine (Plate B)

Plate A

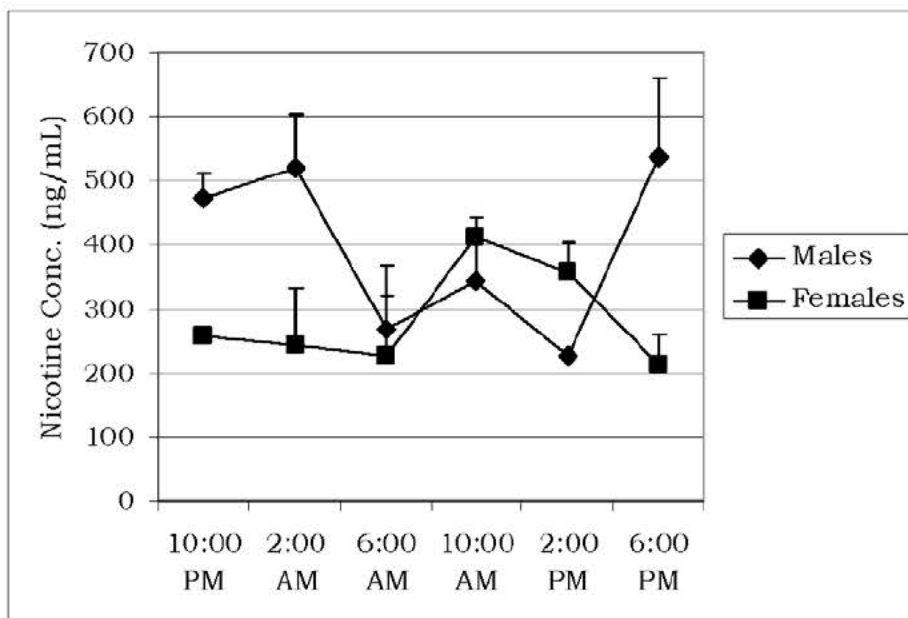


Plate B

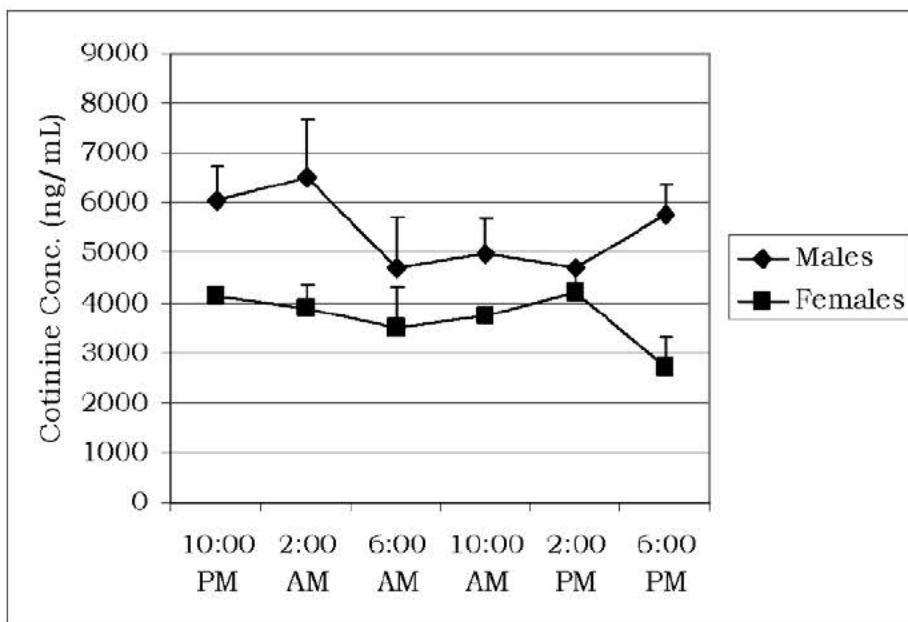


Figure 13 – Plasma Concentration (Mean + SEM)-Time Profile for Male and Female Mice on Days 14 and 15 After Daily Oral Exposure of E80 (80 mg/kg/day) – Nicotine (Plate A) and Cotinine (Plate B)

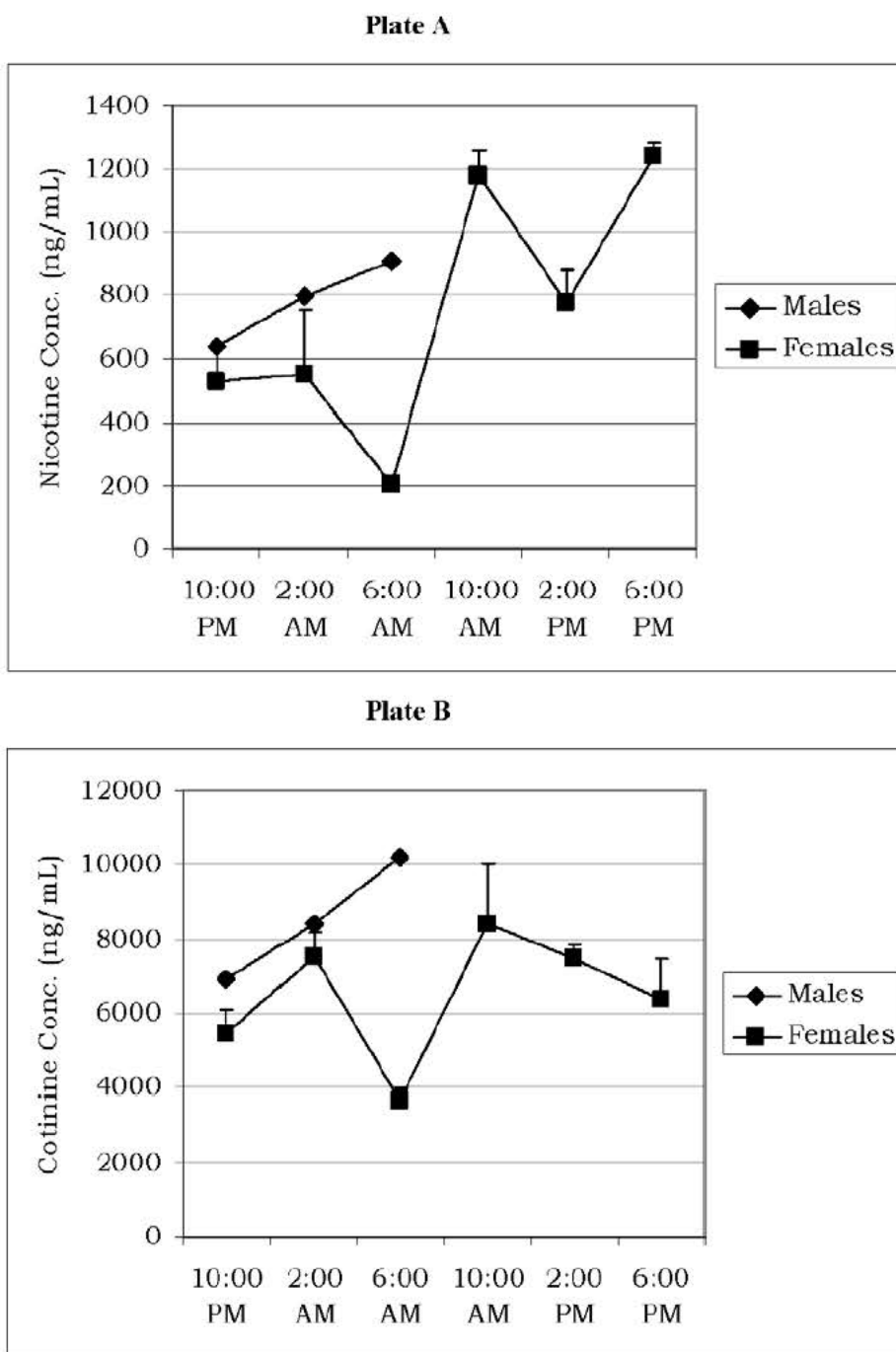


Figure 14 – Plasma Concentration (Mean + SEM)-Time Profile for Male and Female Mice on Days 14 and 15 After Daily Oral Exposure of E200 (200 mg/kg/day) – Nicotine (Plate A) and Cotinine (Plate B)

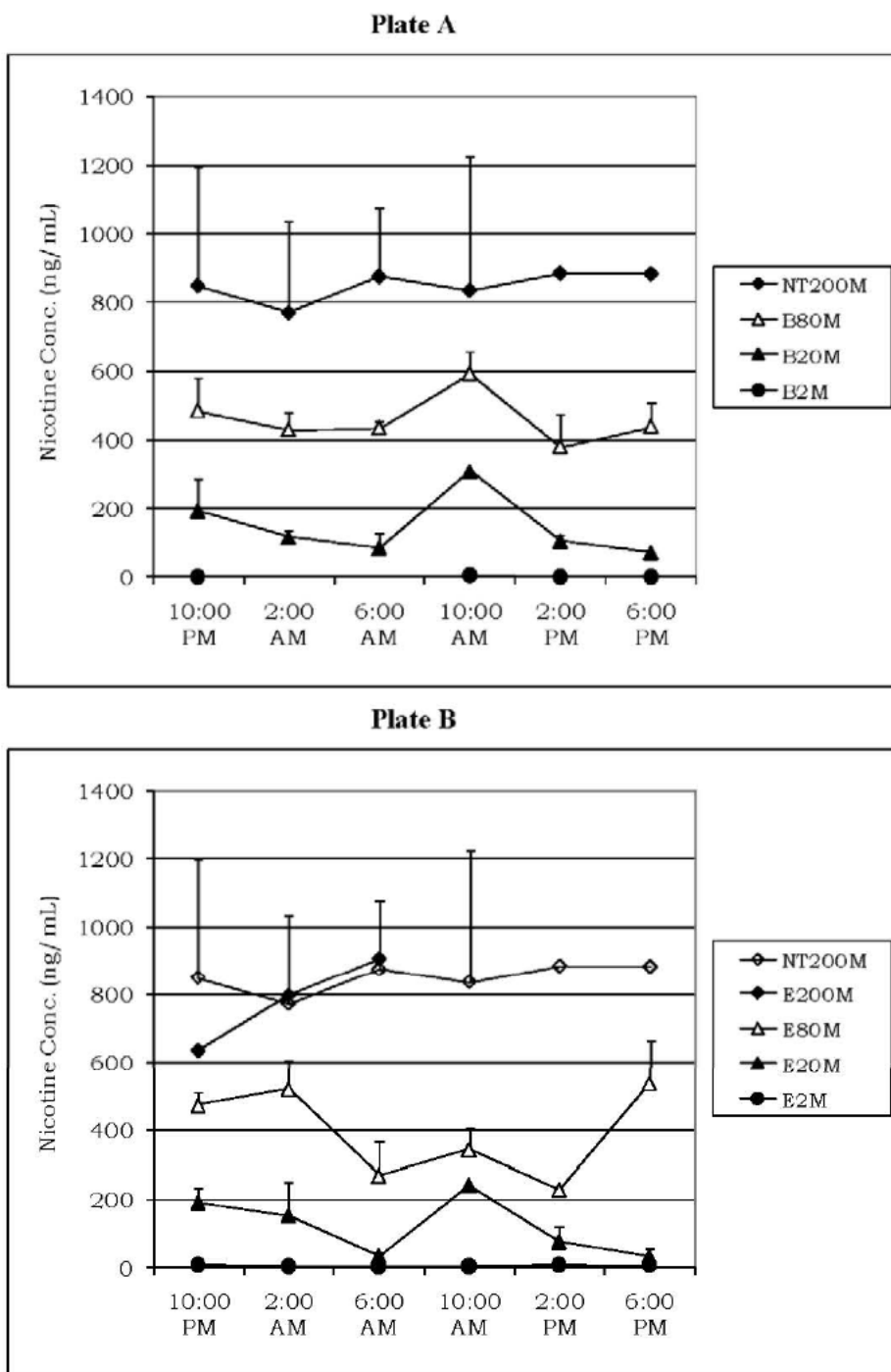


Figure 15 – Nicotine Plasma Concentration (Mean + SEM)-Time Profiles for Male Mice on Days 14 and 15 After Daily Oral Exposure of Nicotine Hydrogen Tartrate and Tobacco Blend (Plate A) and Tobacco Extract (Plate B)

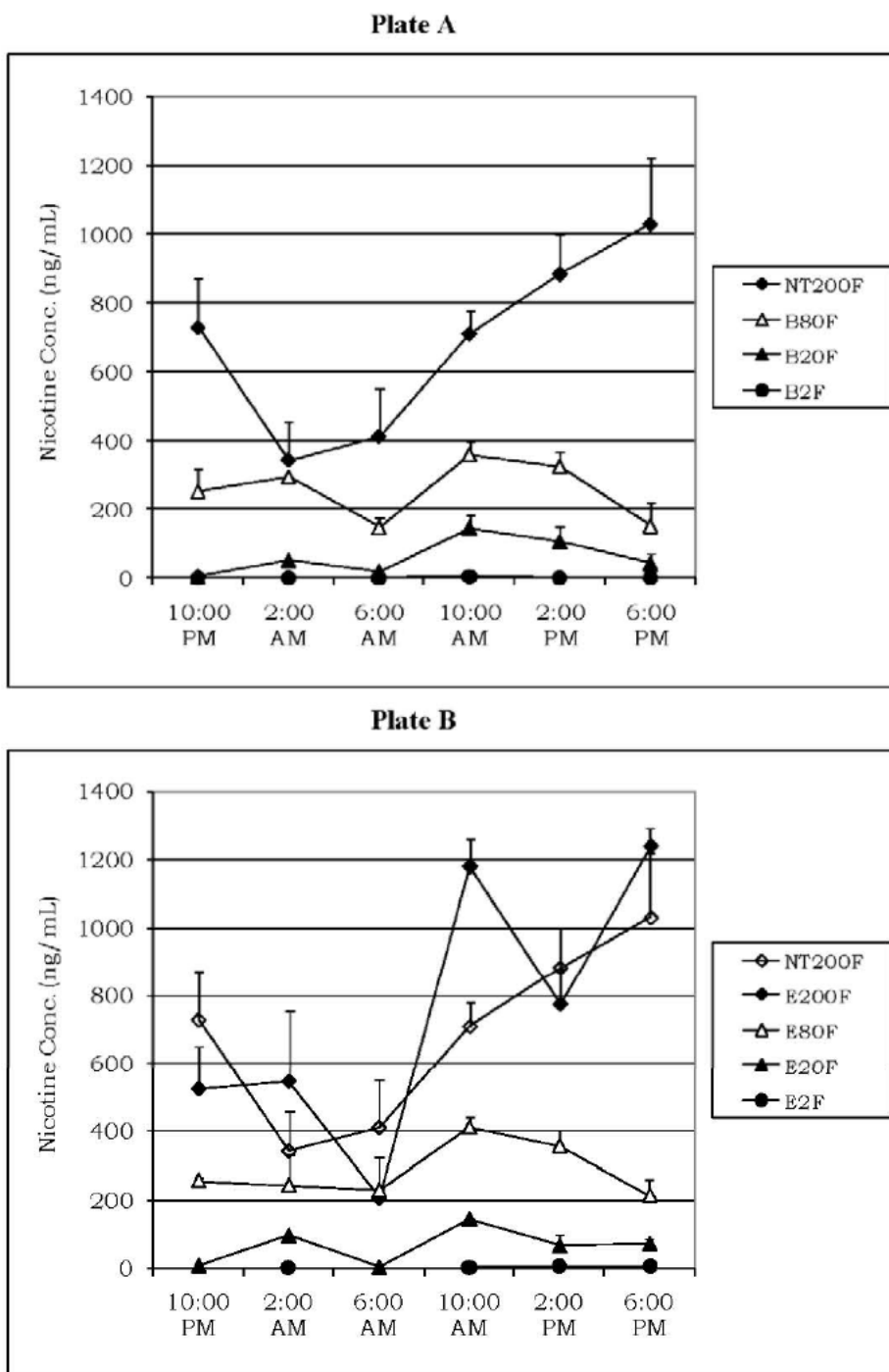


Figure 16 – Nicotine Plasma Concentration (Mean + SEM)-Time Profiles for Female Mice on Days 14 and 15 After Daily Oral Exposure of Nicotine Hydrogen Tartrate and Tobacco Blend (Plate A) and Tobacco Extract (Plate B)

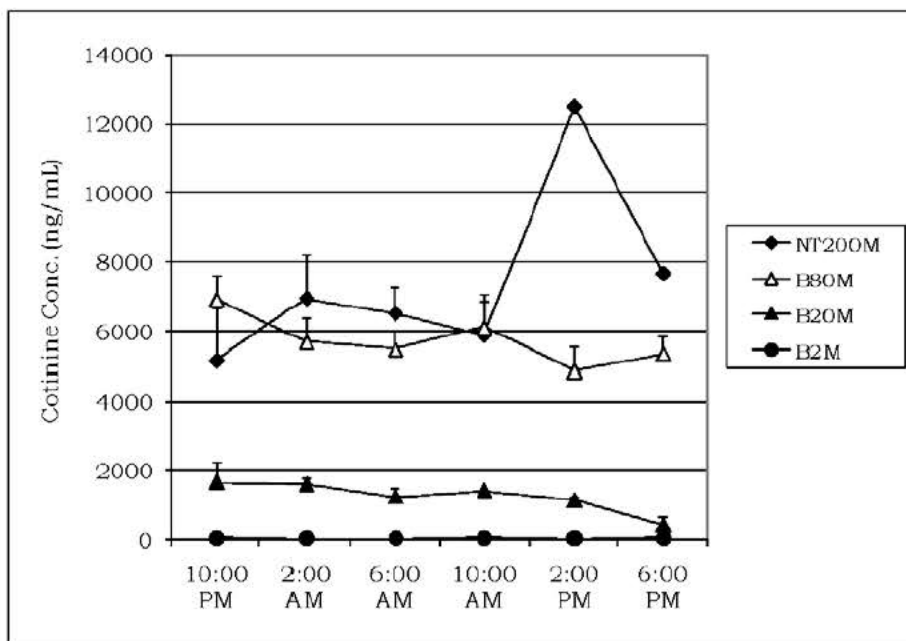
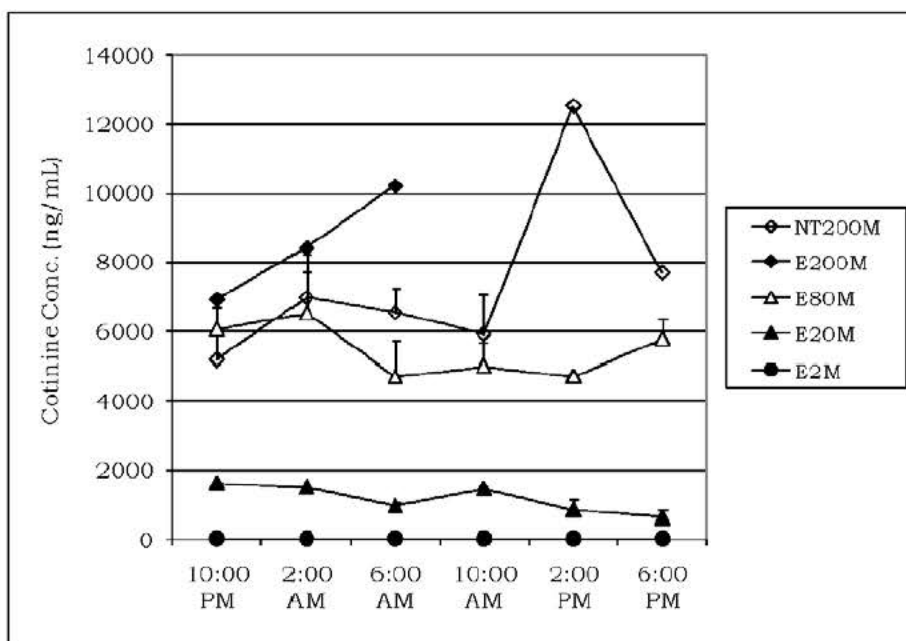
Plate A**Plate B**

Figure 17 – Cotinine Plasma Concentration (Mean + SEM)-Time Profiles for Male Mice on Days 14 and 15 After Daily Oral Exposure of Nicotine Hydrogen Tartrate and Tobacco Blend (Plate A) and Tobacco Extract (Plate B)

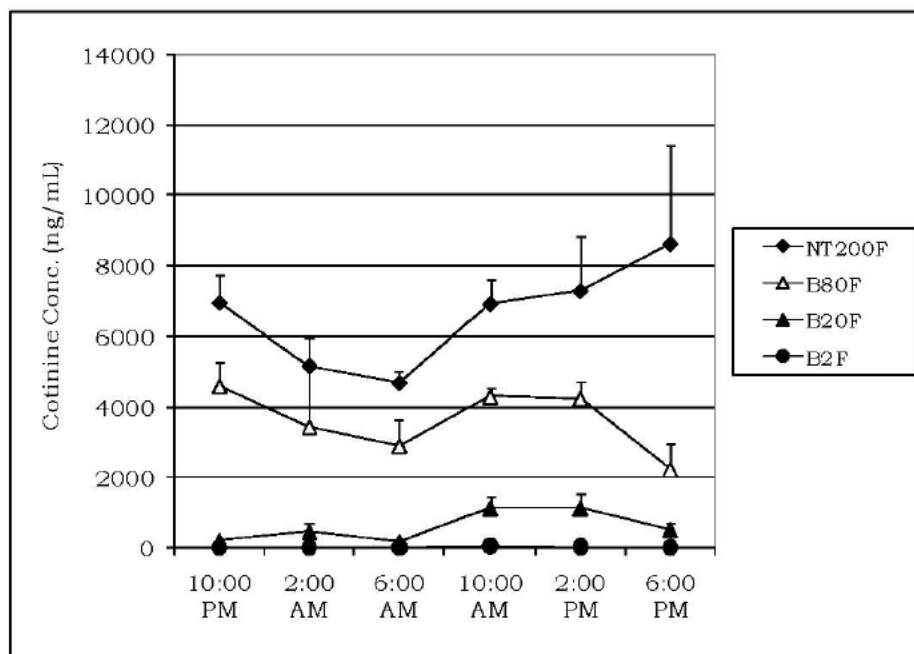
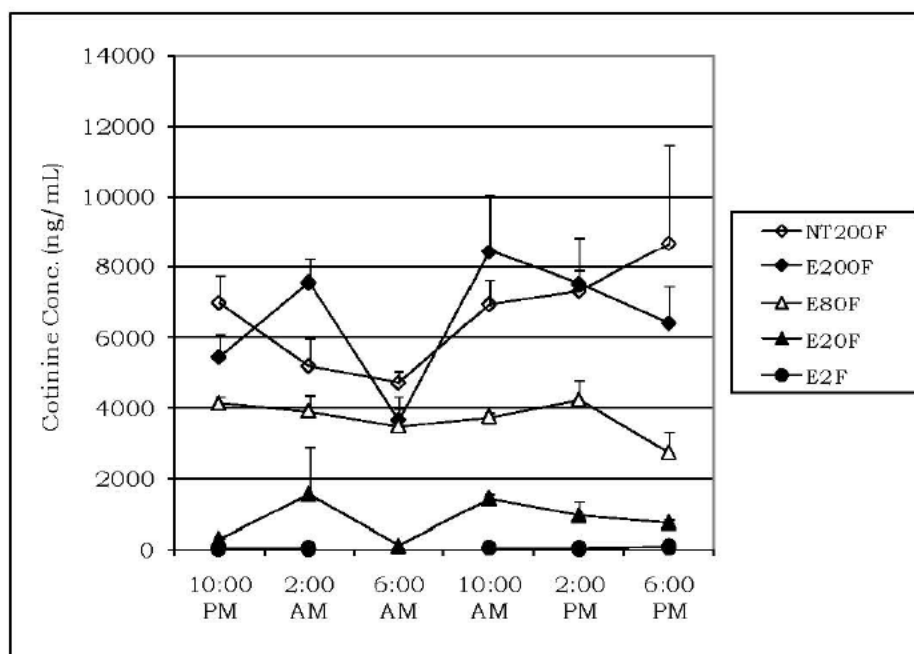
Plate A**Plate B**

Figure 18 – Cotinine Plasma Concentration (Mean + SEM)-Time Profiles for Female Mice on Days 14 and 15 After Daily Oral Exposure of Nicotine Hydrogen Tartrate and Tobacco Blend (Plate A) and Tobacco Extract (Plate B)

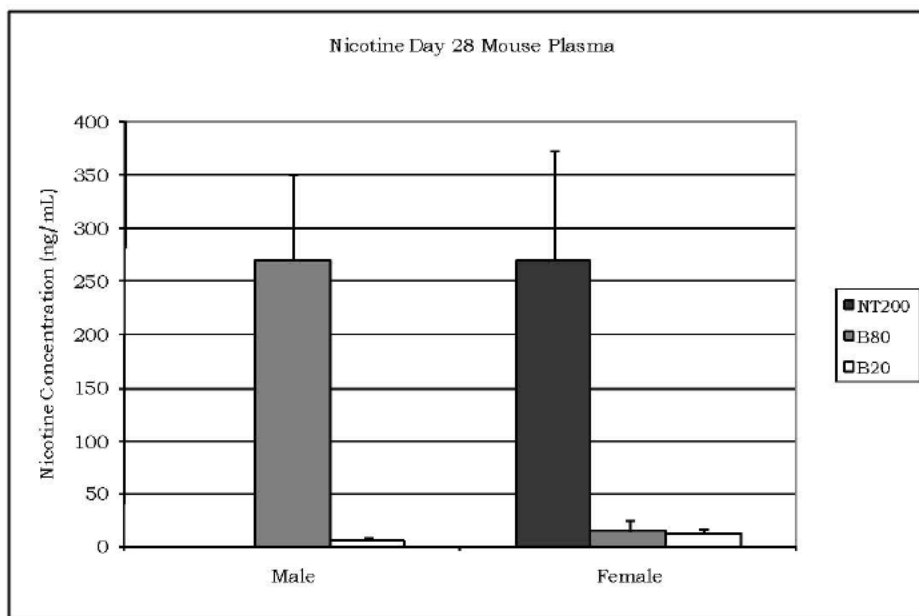
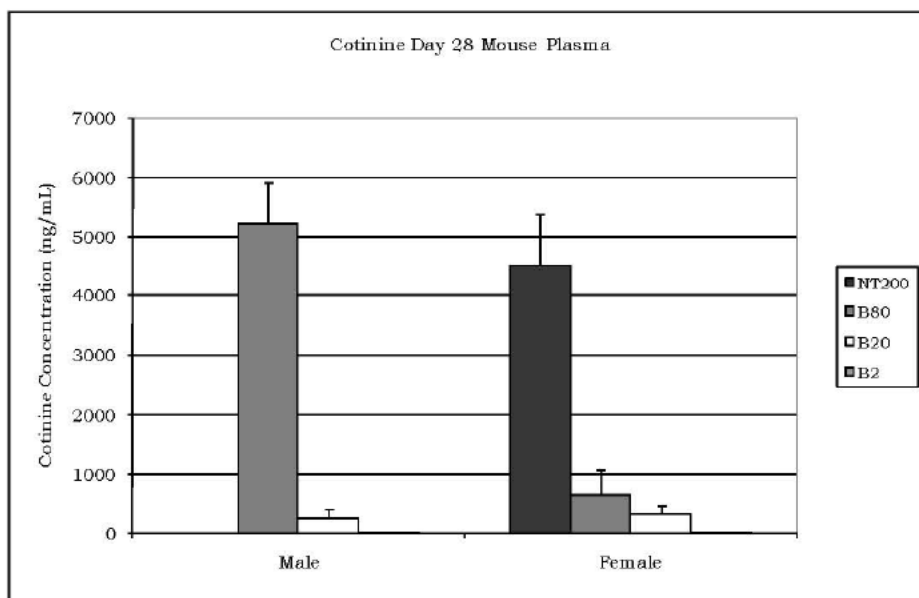
Plate A**Plate B**

Figure 19 – C_{max} (Mean + SEM) for Male and Female Mice on Day 28 After Daily Oral Exposure of Nicotine Hydrogen Tartrate or Tobacco Blend – Nicotine (Plate A) and Cotinine (Plate B)

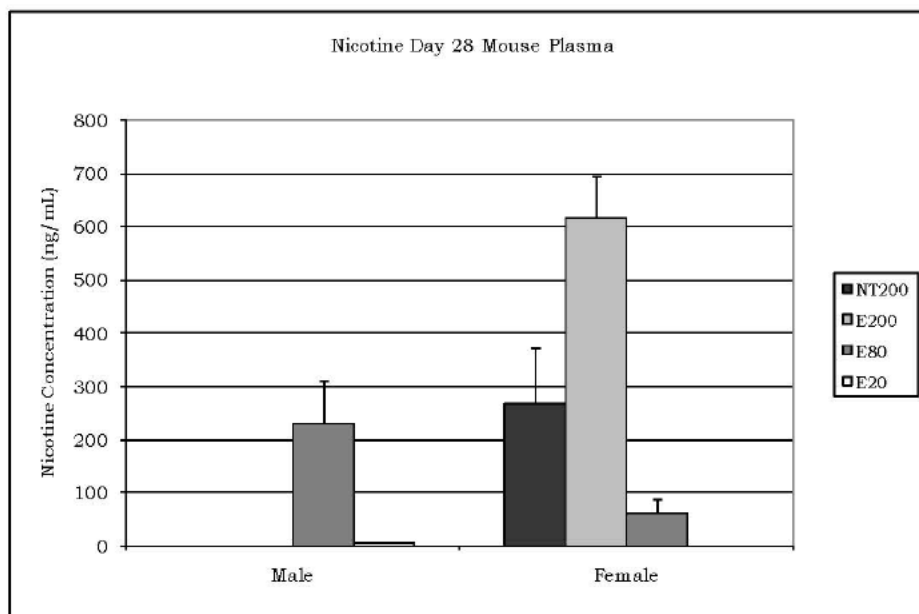
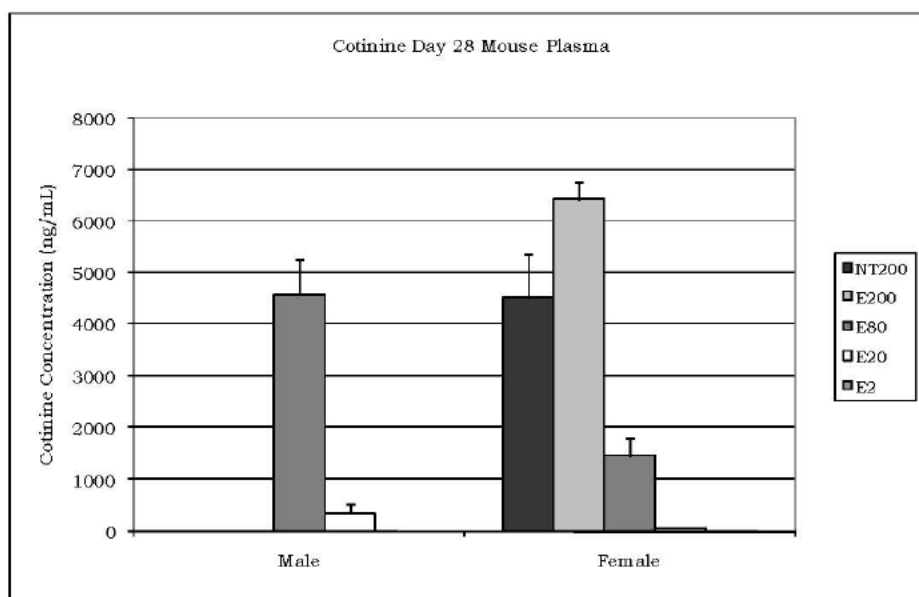
Plate A**Plate B**

Figure 20 – C_{max} (Mean + SEM) for Male and Female Mice on Day 28 After Daily Oral Exposure of Nicotine Hydrogen Tartrate or Tobacco Extract – Nicotine (Plate A) and Cotinine (Plate B)

VI. ACKNOWLEDGEMENTS

Jim Hoskinson, Pat South, and Dan Burnham performed the analytical work. Ed Psurny wrote this report. Drs. Seth Gibbs and Jerry D. Johnson provided the kinetic analysis and report section. Maria Evascu reviewed the data and report for completeness and accuracy.

APPENDIX A – ANALYSIS DOCUMENTATION FORM

Study Number: CN49730D

Date: _____

Analyst: _____

Page 1 of 14

**DOCUMENTATION FORM FOR THE ANALYSIS OF NICOTINE AND COTININE
IN MOUSE PLASMA BY LC-MS****I. SCOPE**

The scope of this work is to determine the concentration of nicotine and cotinine in mouse plasma samples. Plasma calibration standards are prepared from two independently prepared stock solutions. The calibration standards, blanks, and Quality Control (QC) samples are processed by liquid-liquid extraction followed by analysis using liquid chromatography with mass spectrometry (LC-MS). Nicotine and cotinine concentrations are calculated using area response ratios and a regression line constructed from the concentrations and peak area response ratios of the calibrations standards.

II. PURPOSE

The purpose of this documentation form is to provide instructions for conducting the analysis of nicotine and cotinine in mouse plasma.

III. REFERENCES

- Current SOP for Labeling Reagents, Solutions, Test and Control Articles, and Specimens
- Current SOP for Using Electronic Balances
- Current SOP for Recording, Reviewing, and Correcting Raw Data
- Current SOP for Using Pipettors
- Current SOP for Using HPLCs
- Current SOP for Using Mass Spectrometers
- Current SOP for Numeric Data and Calculations
- Current SOP for Using Refrigerators and Freezers
- Current SOP for the Use and Training for Analyst Software

IV. DEFINITIONS

None

Study Number: CN49730D

Date: _____

Analyst: _____

Page 2 of 14

V. PROCEDURE**A. GENERAL INSTRUCTIONS**

USE TWO PAIR OF DISSIMILAR GLOVES DURING NEAT CHEMICAL HANDLING.

Calibrate all required balances according to the SOP on balance usage.

Make equivalent dilutions when the volume needed varies from the volume stated in the method.

Label all standard and reagent solutions as specified in the appropriate SOP.

Document all materials, equipment, and the chromatographic parameters. Initial on the top of each page of this document to signify that you have followed the instructions as written, all materials and reagents are current, and all equipment has been properly calibrated.

Initial and date the top of the page on the day that the work for that page was begun. Other entries made by the analyst on a later date or entries made by another person will be initialed and dated near the data entry.

The procedures are written in general chronological order. However, it is not essential that all sections be performed sequentially. The analyst may determine the order for conducting the task in the most efficient manner, unless the order for certain activities is specified.

Line through any section that is not needed for a specific task.

B. SAMPLES

See attached form for sample list and dilution of samples.

Study Number: CN49730D

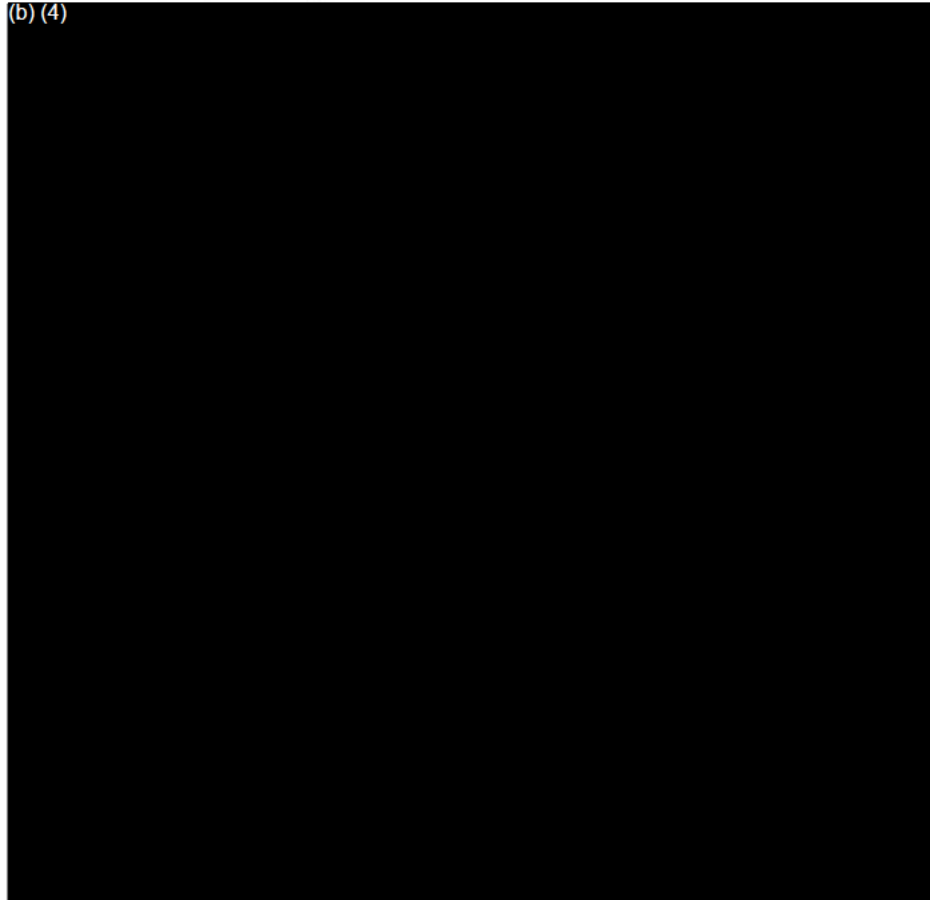
Date: _____

Analyst: _____

Page 3 of 14

C. MATERIALS

(b) (4)



Study Number: CN49730D

Date: _____

Analyst: _____

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D. EQUIPMENT

(b) (4)



Study Number: CN49730D

Date: _____

Analyst: _____

Page 5 of 14

E. PREPARATION OF SOLUTIONS

(b) (4)



Study Number: CN49730D

Date: _____

Analyst: _____

Page 6 of 14

(b) (4)


Study Number: CN49730D

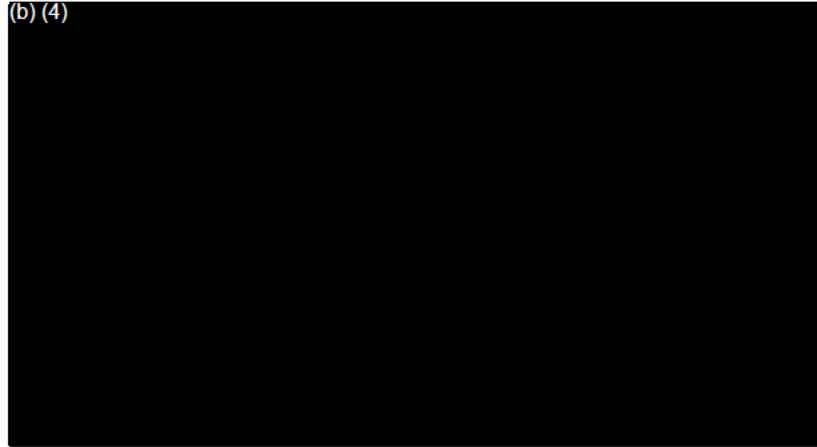
Date: _____

Analyst: _____

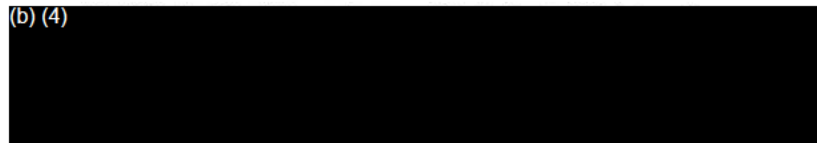
Page 7 of 14

G. PREPARATION OF PLASMA CALIBRATION STANDARDS

(b) (4)

**H. PREPARATION OF BLANKS**

(b) (4)



Study Number: CN49730D

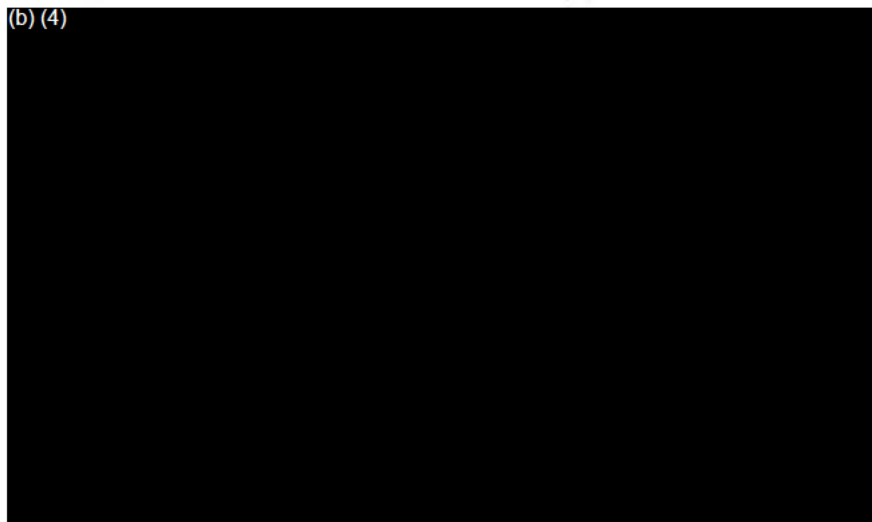
Date: _____

Analyst: _____

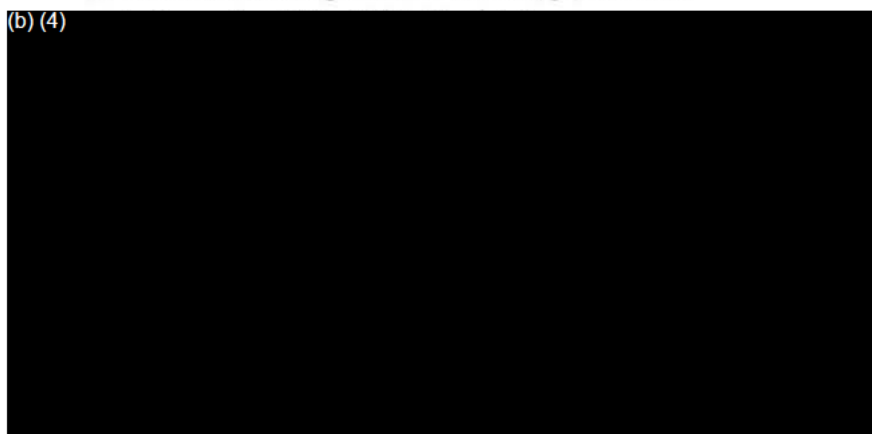
Page 8 of 14

I. PREPARATION OF INTERNAL STANDARD (IS) SOLUTIONS

(b) (4)

**J. PREPARATION OF QUALITY CONTROL (QC) SAMPLES**

(b) (4)



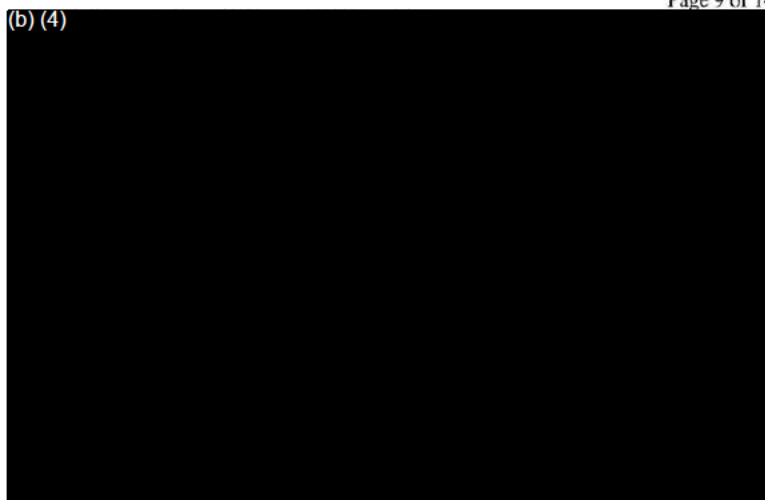
Study Number: CN49730D

Date: _____

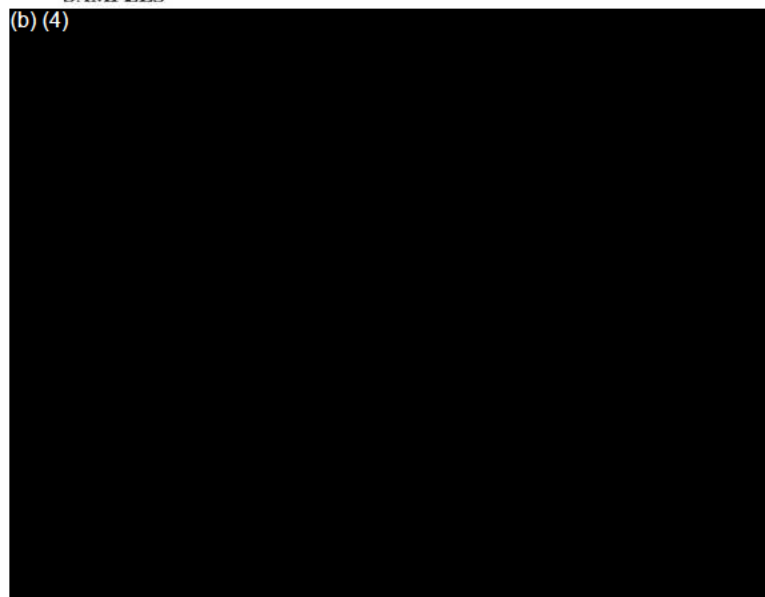
Analyst: _____

Page 9 of 14

(b) (4)

**K. PREPARATION OF PLASMA STANDARDS, BLANKS, QCS, AND
SAMPLES**

(b) (4)



Study Number: CN49730D

Date: _____

Analyst: _____

Page 10 of 14

(b) (4)


Study Number: CN49730D

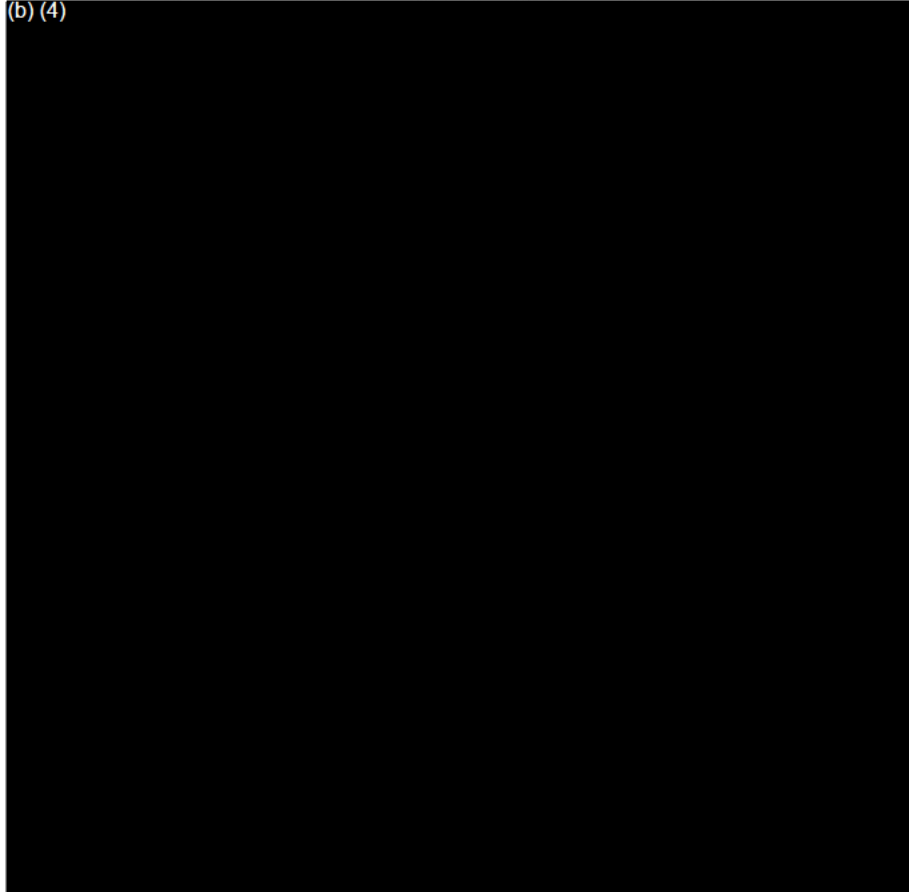
Date: _____

Analyst: _____

Page 11 of 14

L. ANALYSIS OF STANDARDS, BLANKS, SAMPLES, AND QCS

(b) (4)



Study Number: CN49730D

Date: _____

Analyst: _____

Page 12 of 14

VI. CALCULATIONS

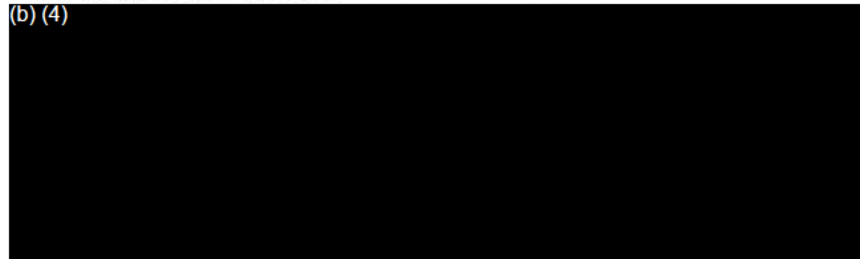
(b) (4)

**VII. RESULTS**

Include printouts of the acquisition method, HPLC method, calibration curve, chromatograms, summary report, data processing parameters, and spreadsheets in the data packet.

VIII. ACCEPTANCE CRITERIA

(b) (4)



Study Number: CN49730D

Date: _____

Analyst: _____

Page 13 of 14

(b) (4)

IX. COMMENTS/CONCLUSIONS

X. DATA REVIEW**Technical Review**

Review at least the following to assure they are acceptable: rejection of calibration standards, integration of chromatograms, chromatography data processing and acquisition parameters, calibration standard concentrations, and regression model

Data Accuracy Review

Review at least the following: completeness and correctness of data entry, formulas used to calculate all values, accuracy of calculations, and compliance of data with acceptance criteria.

Study Number: CN49730D

Date: _____

Analyst: _____

Page 14 of 14

XI. SIGNATURES

Technical Review Signature/Date:

Signature of the technical reviewer will be considered documentation that all modifications and/or changes to this SOP (documented during the course of conducting this task) are technically acceptable and have no adverse technical impact unless otherwise noted. Changes or deviations to the acceptance criteria section require independent assessment by the technical reviewer.

Data Accuracy Review Signature/Date:

APPENDIX I: SEROLOGY REPORTS



FINAL REPORT OF LABORATORY EXAMINATION
MU Research Animal Diagnostic Laboratory
1600 East Rollins, Columbia MO 65211 1-800-669-0825 1-573-882-5983
radil@missouri.edu www.radil.missouri.edu

CASE NUMBER: 14295-2008

RECEIVED ON: 6/25/2008

COMPLETED ON: 6/26/2008

SUBMITTED BY:

Katherine M. Hardin
Battelle Memorial Institute
505 King Ave. Room 7-1-20
Columbus, OH 43201
(614) 424-6328
[614] 458-6328 (fax)

SPECIMEN DESCRIPTION:

SPECIES: mouse

PURCHASE ORDER #: v103399000635

DESCRIPTION: serum samples, diluted

NUMBER OF SPECIMENS: 10

ID	Client ID	Investigator	Room #	Sex
1	CN49730D-1101	D. Fallacara	7C-068	M
2	CN49730D-1102	D. Fallacara	7C-068	M
3	CN49730D-1103	D. Fallacara	7C-068	M
40	CN49730D-1104	D. Fallacara	7C-068	M
5	CN49730D-1105	D. Fallacara	7C-068	M
6	CN49730D-1151	D. Fallacara	7C-068	F
7	CN49730D-1152	D. Fallacara	7C-068	F
8	CN49730D-1153	D. Fallacara	7C-068	F
9	CN49730D-1154	D. Fallacara	7C-068	F
10	CN49730D-1155	D. Fallacara	7C-068	F

TESTS PERFORMED: Basic Serology Profile - mouse

Serologic evaluation for antibodies to: Ectromelia, EDIM, TMEV GDVII, LCM, MHV, MVM, MNV, *M. pulmonis*, MPV, Parvo NS-1, PVM, REO3, Sendai

SUMMARY: All test results were negative.

If you have questions, please call our toll free number at 1-800-669-0825 or e-mail us at radil@missouri.edu.

Technical Review TAP 7/7/08

NOT USED
BB 7-14-08 TR QC: BAE 7-14-08

Re-written because the original entry was destroyed by the hole punch. BAE 7-14-08

Case Number: 14295-2008
Page 2

SEROLOGY:

		1	2	3	4	5	6	7	8	9	10
Ectromelia	MFI (> 1,200)	-	-	-	-	-	-	-	-	-	-
EDIM	MFI (> 5,570)	-	-	-	-	-	-	-	-	-	-
LCM	MFI (> 555)	-	-	-	-	-	-	-	-	-	-
<i>M. pulmonis</i>	MFI (> 2,800)	-	-	-	-	-	-	-	-	-	-
MHV	MFI (> 1,605)	-	-	-	-	-	-	-	-	-	-
MNV	MFI (> 2,450)	-	-	-	-	-	-	-	-	-	-
Parvo NS-1	MFI (> 3,995)	-	-	-	-	-	-	-	-	-	-
MPV	MFI (> 1,000)	-	-	-	-	-	-	-	-	-	-
MVM	MFI (> 960)	-	-	-	-	-	-	-	-	-	-
PVM	MFI (> 995)	-	-	-	-	-	-	-	-	-	-
REO3	MFI (> 3,520)	-	-	-	-	-	-	-	-	-	-
TMEV GDVII	MFI (> 1,380)	-	-	-	-	-	-	-	-	-	-
Sendai	MFI (> 2,265)	-	-	-	-	-	-	-	-	-	-

NOTE: Serum IgG concentrations were determined for all samples. Unless otherwise stated in the summary, these values were within the normal range.

(LEGEND: * = borderline + = positive - = negative blank = test not performed C = cell antigen
 reactor EQ = equivocal HE = hemolysis precluded testing I = insufficient INC = inconclusive
 finding NA = non-specific adherence NF = non-specific fluorescence NH = non-specific
 hemagglutination NR = sample not received NT = not tested S = suspect TC = tissue culture
 reactive W = weak positive WB = Western Blot confirmatory analysis pending)

Positive MFI results are reported as "+" followed by a number from 1 to 33 in thousands rounded off to the nearest thousand.

Comments: Please refrain from using any kind of transparent tape or Parafilm to secure labels or caps to vials; neither will detach under shipping conditions (unless vials and caps are mismatched). We must remove all such materials from vials before we can process your samples using our robotic serum dispenser.

@hole punch destroyed original entries - they are reactor and finding, successively. BIA 7.14.08



FINAL REPORT OF LABORATORY EXAMINATION
MU Research Animal Diagnostic Laboratory
1600 East Rollins, Columbia MO 65211 1-800-669-0825 1-573-882-5983
radil@missouri.edu www.radil.missouri.edu

CASE NUMBER: 14295-2008

RECEIVED ON: 6/25/2008

COMPLETED ON: 6/26/2008

SUBMITTED BY:

Katherine M. Hardin
Battelle Memorial Institute
505 King Ave. Room 7120
Columbus, OH 43201
(614) 424-6328
[614] 458-6328 (fax)

SPECIMEN DESCRIPTION:

SPECIES: mouse

DESCRIPTION: serum samples, diluted

NUMBER OF SPECIMENS: 10

FACILITY CODE: COM

PURCHASE ORDER #: v103399000635

ID	Client ID	Investigator	Room #	Sex
1	CN49730D-1101	D. Fallacara	7C-068	M
2	CN49730D-1102	D. Fallacara	7C-068	M
3	CN49730D-1103	D. Fallacara	7C-068	M
4	CN49730D-1104	D. Fallacara	7C-068	M
5	CN49730D-1105	D. Fallacara	7C-068	M
6	CN49730D-1151	D. Fallacara	7C-068	F
7	CN49730D-1152	D. Fallacara	7C-068	F
8	CN49730D-1153	D. Fallacara	7C-068	F
9	CN49730D-1154	D. Fallacara	7C-068	F
10	CN49730D-1155	D. Fallacara	7C-068	F

TESTS PERFORMED: Basic Serology Profile - mouse

Serologic evaluation for antibodies to: Ectromelia, EDIM, TMEV GDVII, LCM, MHV, MVM, MNV, *M. pulmonis*, MPV, Parvo NS-1, PVM, REO3, Sendai

SUMMARY: All test results were negative.

If you have questions, please call our toll free number at 1-800-669-0825 or e-mail us at
radil@missouri.edu.

Technical Review. TAP 11/6/08
QC Review. BBS 11-11-08

CN49730D

Case Number: 14295-2008
Page 2

SEROLOGY:

		1	2	3	4	5	6	7	8	9	10
Ectromelia	MFI (> 1,200)	-	-	-	-	-	-	-	-	-	-
EDIM	MFI (> 5,570)	-	-	-	-	-	-	-	-	-	-
LCM	MFI (> 555)	-	-	-	-	-	-	-	-	-	-
<i>M. pulmonis</i>	MFI (> 2,800)	-	-	-	-	-	-	-	-	-	-
MHV	MFI (> 1,605)	-	-	-	-	-	-	-	-	-	-
MNV	MFI (> 2,450)	-	-	-	-	-	-	-	-	-	-
Parvo NS-1	MFI (> 3,995)	-	-	-	-	-	-	-	-	-	-
MPV	MFI (> 1,000)	-	-	-	-	-	-	-	-	-	-
MVM	MFI (> 960)	-	-	-	-	-	-	-	-	-	-
PVM	MFI (> 995)	-	-	-	-	-	-	-	-	-	-
REO3	MFI (> 3,520)	-	-	-	-	-	-	-	-	-	-
TMEV GDVII	MFI (> 1,380)	-	-	-	-	-	-	-	-	-	-
Sendai	MFI (> 2,265)	-	-	-	-	-	-	-	-	-	-

NOTE: Serum IgG concentrations were determined for all samples. Unless otherwise stated in the summary, these values were within the normal range.

(LEGEND: * = borderline + = positive - = negative blank = test not performed C = cell antigen reactor EQ = equivocal HE = hemolysis precluded testing I = insufficient INC = inconclusive finding NA = non-specific adherence NF = non-specific fluorescence NH = non-specific hemagglutination NR = sample not received NT = not tested S = suspect TC = tissue culture reactive W = weak positive WB = Western Blot confirmatory analysis pending)

Positive MFI results are reported as "+" followed by a number from 1 to 33 in thousands rounded off to the nearest thousand.

Comments: Please refrain from using any kind of transparent tape or Parafilm to secure labels or caps to vials; neither will detach under shipping conditions (unless vials and caps are mismatched). We must remove all such materials from vials before we can process your samples using our robotic serum dispenser.



**ADDENDUM to
FINAL REPORT OF LABORATORY EXAMINATION
MU Research Animal Diagnostic Laboratory
1600 East Rollins, Columbia MO 65211 1-800-669-0825 1-573-882-5983
radil@missouri.edu www.radil.missouri.edu**

CASE NUMBER: 14295-2008

RECEIVED ON: 6/25/2008

COMPLETED ON: 6/26/2008

ADDENDUM DATED: 9/11/2008

SUBMITTED BY:

Katherine M. Hardin
Battelle Memorial Institute
505 King Ave. Room 7120
Columbus, OH 43201
(614) 424-6328
[614] 458-6328 (fax)

SEROLOGY:

		1	2	3	4	5	6	7	8	9	10
CAR bacillus	MFI (> 4,200)	-	-	-	-	-	-	-	-	-	-
<i>E. cuniculi</i>	MFI (> 1,200)	-	-	-	-	-	-	-	-	-	-
Hantaan	MFI (> 750)	-	-	-	-	-	-	-	-	-	-
K virus	MFI (> 1,450)	-	-	-	-	-	-	-	-	-	-
MAD 1	MFI (> 2,500)	-	-	-	-	-	-	-	-	-	-
MAD 2	MFI (> 4,500)	-	-	-	-	-	-	-	-	-	-
MCMV	MFI (> 645)	-	-	-	-	-	-	-	-	-	-
MTV	IFA	-	-	-	-	-	-	-	-	-	-
Polyoma	MFI (> 1,635)	-	-	-	-	-	-	-	-	-	-

NOTE: Serum IgG concentrations were determined for all samples. Unless otherwise stated in the summary, these values were within the normal range.

(LEGEND: * = borderline + = positive - = negative blank = test not performed C = cell antigen reactor EQ = equivocal HE = hemolysis precluded testing I = insufficient INC = inconclusive finding NA = non-specific adherence NF = non-specific fluorescence NH = non-specific hemagglutination NR = sample not received NT = not tested S = suspect TC = tissue culture reactive W = weak positive WB = Western Blot confirmatory analysis pending)

Positive MFI results are reported as "+" followed by a number from 1 to 33 in thousands rounded off to the nearest thousand.

**If you have questions, please call our toll free number at 1-800-669-0825 or e-mail us at
radil@missouri.edu.**



FINAL REPORT OF LABORATORY EXAMINATION
MU Research Animal Diagnostic Laboratory
1600 East Rollins, Columbia MO 65211 1-800-669-0825 1-573-882-5983
radil@missouri.edu www.radil.missouri.edu

CASE NUMBER: 16479-2008

RECEIVED ON: 8/7/2008

COMPLETED ON: 8/8/2008

SUBMITTED BY:

Katherine M. Hardin
Battelle Memorial Institute
505 King Ave. Room 7-1-20
Columbus, OH 43201
(614) 424-6328
[614] 458-6328 (fax)

CN49730D

SPECIMEN DESCRIPTION:

SPECIES: mouse
DESCRIPTION: serum samples, diluted
NUMBER OF SPECIMENS: 10
FACILITY CODE: COM

PURCHASE ORDER #: V103399000688

ID	Client ID	Investigator	Room #	Sex
1	CN49730D-1106	M. Hejtmancik	7C-068	M
2	CN49730D-1107	M. Hejtmancik	7C-068	M
3	CN49730D-1108	M. Hejtmancik	7C-068	M
4	CN49730D-1109	M. Hejtmancik	7C-068	M
5	CN49730D-1110	M. Hejtmancik	7C-068	M
6	CN49730D-1156	M. Hejtmancik	7C-068	F
7	CN49730D-1157	M. Hejtmancik	7C-068	F
8	CN49730D-1158	M. Hejtmancik	7C-068	F
9	CN49730D-1159	M. Hejtmancik	7C-068	F
10	CN49730D-1160	M. Hejtmancik	7C-068	F

TESTS PERFORMED: Basic Serology Profile - mouse

Serologic evaluation for antibodies to: Ectromelia, EDIM, TMEV GDVII, LCM, MHV, MVM, MNV, M. pulmonis, MPV, Parvo NS-1, PVM, REO3, Sendai

GENERAL COMMENTS: If any positive or reactive in any assay appears, even if the data is preliminary, please notify Dr. Tracy Peace with a verbal immediately. Alternate contact for information is Katie Hardin.

SUMMARY: All test results were negative.

If you have questions, please call our toll free number at 1-800-669-0825 or e-mail us at radil@missouri.edu.

Technical Review *TRP* 9/8/08
QC: 1546 9-26-09

Case Number: 16479-2008

Page 2

SEROLOGY:

		1	2	3	4	5	6	7	8	9	10
Ectromelia	MFI (> 1,200)	-	-	-	-	-	-	-	-	-	-
EDIM	MFI (> 5,570)	-	-	-	-	-	-	-	-	-	-
LCM	MFI (> 555)	-	-	-	-	-	-	-	-	-	-
<i>M. pulmonis</i>	MFI (> 2,800)	-	-	-	-	-	-	-	-	-	-
MHV	MFI (> 1,605)	-	-	-	-	-	-	-	-	-	-
MNV	MFI (> 2,450)	-	-	-	-	-	-	-	-	-	-
Parvo NS-1	MFI (> 3,995)	-	-	-	-	-	-	-	-	-	-
MPV	MFI (> 1,000)	-	-	-	-	-	-	-	-	-	-
MVM	MFI (> 960)	-	-	-	-	-	-	-	-	-	-
PVM	MFI (> 995)	-	-	-	-	-	-	-	-	-	-
REO3	MFI (> 3,520)	-	-	-	-	-	-	-	-	-	-
TMEV GDVII	MFI (> 1,380)	-	-	-	-	-	-	-	-	-	-
Sendai	MFI (> 2,265)	-	-	-	-	-	-	-	-	-	-

NOTE: Serum IgG concentrations were determined for all samples. Unless otherwise stated in the summary, these values were within the normal range.

(LEGEND: * = borderline + = positive - = negative blank = test not performed C = cell antigen reactor EQ = equivocal HE = hemolysis precluded testing I = insufficient INC = inconclusive finding NA = non-specific adherence NF = non-specific fluorescence NH = non-specific hemagglutination NR = sample not received NT = not tested S = suspect TC = tissue culture reactive W = weak positive WB = Western Blot confirmatory analysis pending)

Positive MFI results are reported as "+" followed by a number from 1 to 33 in thousands rounded off to the nearest thousand.

CN49730D



FINAL REPORT OF LABORATORY EXAMINATION
MU Research Animal Diagnostic Laboratory
1600 East Rollins, Columbia MO 65211 1-800-669-0825 1-573-882-5983
radil@missouri.edu www.radil.missouri.edu

CASE NUMBER: 16479-2008

RECEIVED ON: 8/7/2008

COMPLETED ON: 8/8/2008

SUBMITTED BY:

Katherine M. Hardin
Battelle Memorial Institute
505 King Ave. Room 7120
Columbus, OH 43201
(614) 424-6328
[614] 458-6328 (fax)

SPECIMEN DESCRIPTION:

SPECIES: mouse

PURCHASE ORDER #: V103399000688

DESCRIPTION: serum samples, diluted

NUMBER OF SPECIMENS: 10

FACILITY CODE: COM

ID	Client ID	Investigator	Room #	Sex
1	CN49730D-1106	M. Hejtmancik	7C-068	M
2	CN49730D-1107	M. Hejtmancik	7C-068	M
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4	CN49730D-1109	M. Hejtmancik	7C-068	M
5	CN49730D-1110	M. Hejtmancik	7C-068	M
6	CN49730D-1156	M. Hejtmancik	7C-068	F
7	CN49730D-1157	M. Hejtmancik	7C-068	F
8	CN49730D-1158	M. Hejtmancik	7C-068	F
9	CN49730D-1159	M. Hejtmancik	7C-068	F
10	CN49730D-1160	M. Hejtmancik	7C-068	F

TESTS PERFORMED: Basic Serology Profile - mouse

Serologic evaluation for antibodies to: Ectromelia, EDIM, TMEV GDVII, LCM, MHV, MVM, MNV, M. pulmonis, MPV, Parvo NS-1, PVM, REO3, Sendai

GENERAL COMMENTS: If any positive or reactive in any assay appears, even if the data is preliminary, please notify Dr. Tracy Peace with a verbal immediately. Alternate contact for information is Katie Hardin.

SUMMARY: All test results were negative.

If you have questions, please call our toll free number at 1-800-669-0825 or e-mail us at radil@missouri.edu.

QC Review: 6/6 11/1/08

Technical Review Top 11/6/08
CN49730D

Case Number: 16479-2008

Page 2

SEROLOGY:

		1	2	3	4	5	6	7	8	9	10
Ectromelia	MFI (> 1,200)	-	-	-	-	-	-	-	-	-	-
EDIM	MFI (> 5,570)	-	-	-	-	-	-	-	-	-	-
LCM	MFI (> 555)	-	-	-	-	-	-	-	-	-	-
<i>M. pulmonis</i>	MFI (> 2,800)	-	-	-	-	-	-	-	-	-	-
MHV	MFI (> 1,605)	-	-	-	-	-	-	-	-	-	-
MNV	MFI (> 2,450)	-	-	-	-	-	-	-	-	-	-
Parvo NS-1	MFI (> 3,995)	-	-	-	-	-	-	-	-	-	-
MPV	MFI (> 1,000)	-	-	-	-	-	-	-	-	-	-
MVM	MFI (> 960)	-	-	-	-	-	-	-	-	-	-
PVM	MFI (> 995)	-	-	-	-	-	-	-	-	-	-
REO3	MFI (> 3,520)	-	-	-	-	-	-	-	-	-	-
TMEV GDVII	MFI (> 1,380)	-	-	-	-	-	-	-	-	-	-
Sendai	MFI (> 2,265)	-	-	-	-	-	-	-	-	-	-

NOTE: Serum IgG concentrations were determined for all samples. Unless otherwise stated in the summary, these values were within the normal range.

(LEGEND: * = borderline + = positive - = negative blank = test not performed C = cell antigen reactor EQ = equivocal HE = hemolysis precluded testing I = insufficient INC = inconclusive finding NA = non-specific adherence NF = non-specific fluorescence NH = non-specific hemagglutination NR = sample not received NT = not tested S = suspect TC = tissue culture reactive W = weak positive WB = Western Blot confirmatory analysis pending)

Positive MFI results are reported as "+" followed by a number from 1 to 33 in thousands rounded off to the nearest thousand.



ADDENDUM to
FINAL REPORT OF LABORATORY EXAMINATION
MU Research Animal Diagnostic Laboratory
1600 East Rollins, Columbia MO 65211 1-800-669-0825 1-573-882-5983
radil@missouri.edu www.radil.missouri.edu

CASE NUMBER: 16479-2008

RECEIVED ON: 8/7/2008

COMPLETED ON: 8/8/2008

ADDENDUM DATED: 9/10/2008

SUBMITTED BY:

Katherine M. Hardin
 Battelle Memorial Institute
 505 King Ave. Room 7120
 Columbus, OH 43201
 (614) 424-6328
 [614] 458-6328 (fax)

SEROLOGY:

		1	2	3	4	5	6	7	8	9	10
CAR bacillus	MFI (> 4,200)	-	-	-	-	-	-	-	-	-	-
<i>E. cuniculi</i>	MFI (> 1,200)	-	-	-	-	-	-	-	-	-	-
Hantaan	MFI (> 750)	-	-	-	-	-	-	-	-	-	-
K virus	MFI (> 1,450)	-	-	-	-	-	-	-	-	-	-
MAD 1	MFI (> 2,500)	-	-	-	-	-	-	-	-	-	-
MAD 2	MFI (> 4,500)	-	-	-	-	-	-	-	-	-	-
MCMV	MFI (> 645)	-	-	-	-	-	-	-	-	-	-
MTV	IFA	-	-	-	-	-	-	-	-	-	-
Polyoma	MFI (> 1,635)	-	-	-	-	-	-	-	-	-	-

NOTE: Serum IgG concentrations were determined for all samples. Unless otherwise stated in the summary, these values were within the normal range.

(LEGEND: * = borderline + = positive - = negative blank = test not performed C = cell antigen reactor EQ = equivocal HE = hemolysis precluded testing I = insufficient INC = inconclusive finding NA = non-specific adherence NF = non-specific fluorescence NH = non-specific hemagglutination NR = sample not received NT = not tested S = suspect TC = tissue culture reactive W = weak positive WB = Western Blot confirmatory analysis pending)

Positive MFI results are reported as "+" followed by a number from 1 to 33 in thousands rounded off to the nearest thousand.

If you have questions, please call our toll free number at 1-800-669-0825 or e-mail us at radil@missouri.edu.