R. J. Reynolds Tobacco Company

Research and Development

Study Number: TOX210

Final Report

Two Week Investigational Study of the Palatability of Smokeless Tobacco Blend and Extract Formulated in NTP-2000 Diets for Mice

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I. FACILITIES AND ADMINISTRATION

1. Sponsor

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Research and Development
Product Integrity
Winston-Salem, NC 27102

2. Facility

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3. Contractors

a. Charles River Laboratories: Serology
Wilmington, MA

b. Research Resources of NC, Inc.: Animal Care, Quality Assurance
On-Site

c. Seventh Wave Health Screen Lung
Burlington, NC

4. Study Administration

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5. Study Schedule

Quarantine Start:	April 9, 2008
End of Quarantine:	April 16, 2008
First Day of Dosing:	May 21, 2008
Study Termination*:*	June 3, 2008

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QUALITY ASSURANCE STATEMENT

The following Quality Assurance Statement is limited to the review of the Draft Final Report Data Tables, which were reviewed for completeness and accuracy. The dates of inspection/audit and the submission dates of written reports to the study director and management were as follows:

Study Phase	Dates of Inspection/Audit	Dates Reports Submitted to Study Director/Management
Draft Final Report Data Tables	12/17, 18, 19, /08; 1/7, 13, 14/09	213QAU01 (1/14/09)

Quality Assurance Investigator

1-May-09
Date

II. LIST OF TABLES

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V. LIST OF ABBREVIATIONS USED IN REPORT

bw	body weight
C	centigrade
CARB	ciliated associated respiratory bacillus
CFR	Code of Federal Regulations
ECUN	*Encephalitozoon cuniculi*
EDIM	epizootic diarrhea of infant mice virus
FID	flame ionization detector
g	gram
GC	gas chromatography
GDVII	murine encephalomyelitis virus
HANT	Hantaan virus
IACUC	Institutional Animal Care and Use Committee
kg	kilogram
LCMV	lymphocytic choriomeningitis virus
LD_{50}	lethal dose for 50% of treated animals
MS	mass spectroscopy
MAV	mouse adenovirus
MCMV	mouse cytomegalovirus
mg	milligram
MHV	mouse hepatitis virus
Ml	milliliter
MNV	murine norovirus
MPV	mouse parvovirus
MTD	maximum tolerated dose
MTLV	mouse thymic virus
MVM	minute virus of mice
nic	nicotine
NOAEL	no observable adverse effect level
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
PVM	pneumonia virus of mice
R&D	research and development
RJRT	R. J. Reynolds Tobacco Company

FINAL REPORT

Investigational Study of the Palatability of Smokeless Tobacco Test Articles Formulated in NTP-2000 Diets for Mice

V. EXECUTIVE SUMMARY

The objective of this study was to evaluate the palatability of diets formulated in NTP-2000 rodent feed with either a smokeless tobacco blend, an aqueous tobacco extract of the tobacco blend or nicotine hydrogen tartrate, as positive control, when fed to CFW Swiss Webster mice. A smokeless tobacco blend and an aqueous extract of the tobacco blend will be tested in an upcoming series of toxicology studies. Also, a positive control, nicotine hydrogen tartrate will be used in some of the planned studies. This study will provide information useful in the design of these studies.

Palatability was assessed by comparing the cumulative percent body weight gain and body weight of mice fed NTP-2000 diets formulated to contain increasing concentrations of the smokeless tobacco blend, the tobacco extract and the positive control to the cumulative percent body weight gain and body weight of the negative control mice fed the standard NTP-2000 feed with no additions. The duration of the feeding and data collection period was 14 days. Feed intake and body weight were measured daily during the study. Twice daily mortality and morbidity observations were conducted as well as standard clinical observations conducted twice weekly. No additional data were collected.

Review of the feed formulation nicotine analysis data indicated the formulation methodology developed for this study produced feed containing appropriate concentrations of either the smokeless tobacco blend, aqueous extract of the tobacco blend or nicotine hydrogen tartrate. Utilization of a trial feed formulation provided insight into improved technical changes to the mixing methodology. Investigation of the homogeneity of added materials within the NTP-2000 powdered rodent feed indicated the test articles and positive control were homogenously mixed within the feed to an extent that avoided the occurrence of random areas of either low or higher concentrations that would produce inconsistencies in dosing the mice. Within the limitations of the methodology, analytical determinations of the nicotine content in the feed produced for the various doses and test articles and positive control indicated the formulated feeds provided the appropriate doses of nicotine and tobacco components to the mice. The stability of the test articles and positive control, measured as nicotine, was assessed to determine the frequency of preparation of the formulated feed. This investigation revealed the formulated feed was stable for at least 10-days and confirmed the adequacy of weekly preparation of formulated feed. An additional stability investigation indicated that the formulated feed was stable for at least one-month when stored at room temperature. This indicates that future studies would not require weekly feed formulation.

Overall, these preliminary investigations provided evidence of the adequacy of the dosed feed formulation for this palatability study in respect to homogeneity, confirmation of anticipated dose and the stability of the formulated feed.

The male, CFW Swiss Webster mice used in this study were maintained under protocol specified conditions throughout the study and data related to room temperature, humidity, room air exchanges and water quality demonstrated that there were no excursions that could impact the results of the study. In addition, serological testing of the sentinel mice indicated no antibodies to disease while pathological examination of the lungs indicated no evidence of the presence of contagious disease. These data indicated that the environmental conditions and the health of the animals were appropriate for this study.

The control mice, with the exception of one mouse, in this study demonstrated the expected body weight gains throughout this study, indicating that there were no conditions other than the dosed feed that could unduly affect body weight during the study.

The effect on body weight produced by feeding diets formulated with the test articles and positive control is clearly seen when expressed as cumulative percent body weight gain. Mice provided feed formulated with the tobacco blend at doses of 0.2-20 mg nicotine/kg bw/day did not demonstrate dose related trends in body weight gain that were clearly different from that of the untreated control group. At a dose of 40 mg nicotine/kg bw/day, the tobacco blend produced a consistent trend toward reduced body weight gain that may indicate a test article related effect. However, the final body weights at 40 mg nicotine/kg bw/day were not statistically different from the control; in part, because of the large standard deviation in the control group produced by a single mouse.

Cumulative percent body weight gain in mice fed feed containing the tobacco extract at doses equivalent to those fed the tobacco blend did not demonstrate a definitive change in body weight gain at any dose. The reasons for the lack of concordance between the tobacco blend and the tobacco extract are not evident from the results of this study.

When mice were fed feed formulated with nicotine hydrogen tartrate, the cumulative percent body weight gain at doses of 2 and 8 mg nicotine/kg bw/day were not different from the control. At 20 mg nicotine/kg bw/day, the trend is not as definitive and from day 10 of the study did not differ from that seen at 2 mg/kg/day. This may indicate that an initial aversion to the feed was overcome. In contrast, at 40 mg nicotine/kg bw/day there was a definitive reduction in cumulative percent body weight gain. After day six, cumulative percent body weight gain stabilizes at a range below that of the control and the tobacco blend at an equivalent dose. However, the final body weights at 40 mg nicotine/kg bw/day were not statistically different from the control; again, resulting from the large standard deviation in the control group.

Evaluation of the data from this study indicates that mice fed the tobacco blend or tobacco extract at quantities that yield nicotine as high as 20 mg nicotine/kg bw/day show little or no effect in any of the variables determined in this study, especially cumulative percent body weight gain. At a nicotine dose of 40 mg/kg bw/day, percent body weight gain tended to decrease with the tobacco blend while a more definitive trend was seen with nicotine hydrogen tartrate. No effect on percent body weight gain was seen with the tobacco extract. These data are in sharp contrast to the decreased cumulative body weight gain seen with rats at identical nicotine doses (TOX209). This indicates mice are more resistant to the effects measured in this study than are rats.

The major recommendation based upon the results of this study is that it should be repeated using higher doses of nicotine and that the low dose for a repeat of this study be 40 mg nicotine/kg bw/day. In addition, the number of control animals should be increased from five to ten to insure a determination of statistical differences.

VI. INTRODUCTION

The objective of this study was to evaluate the palatability of NTP-2000 rodent feed formulated with the addition of either a smokeless tobacco blend, an aqueous tobacco extract of the tobacco blend or nicotine hydrogen tartrate, as a positive control, when fed to male, CFW Swiss Webster mice. This study was run in parallel with TOX209, a similar study using male, Wistar Han rats.

Short-term repeated dosing studies and subchronic toxicity studies using both genders of Swiss Webster mice may be conducted using the oral route of administration via feed formulated with a smokeless tobacco blend, an aqueous tobacco extract of the tobacco blend or a positive control (nicotine hydrogen tartrate). It is possible that addition of these materials to the rodent's feed may alter its organoleptic characteristics and thereby decrease its palatability. This could result in unacceptably large decreases in feed intake and consequential unacceptable decreases in body weight gain and body weight that would confound interpretation of the data from these anticipated studies. The generally acceptable decrease in rodent body weight during long term studies is 10% and a dose of test article that produces such a decrease is termed the Maximally Tolerated Dose (MTD). This study should provide valuable insight into the design of longer term, more comprehensive toxicology studies.

A number of variables had to be considered in the design of this palatability study. First, it was necessary to determine the basis upon which the dosing and addition of the test articles to the feed would be accomplished. The simplest method would be to add the test articles on a mg test article/g of feed basis. However, this would not allow an analytical determination of the actual quantity of test article in the diet. Tobacco is a natural plant product that consists of a large number of individual chemicals. Among these chemicals is nicotine, which has received considerable research interest. In addition, analytical methodology for the determination of nicotine in complex mixtures is available in a number of laboratories. The toxicity of nicotine has been investigated in a number of animal species, including rats and mice (HSDB, 2008). These factors support the use of nicotine as the basis for dosing the mice and the formulation of the rodent feed. Therefore, the dosing of the mice and the formulation of the dosed feed was based upon mg nicotine/kg body weight (bw)/day. This basis for dosing and feed formulation requires knowledge of the nicotine content of the smokeless tobacco blend, tobacco extract and the available nicotine from the nicotine salt used in the study. It also allows the determination of nicotine in the dosed feed to confirm the animals received their anticipated doses.

The second variable was a determination of the quantities of test article that should be added to the diet to determine if they affected the palatability of the feed. Ideally, the doses would range from a dose that had no impact on the palatability of the diet to a dose that demonstrated decreased palatability. Limitations on the high dose to be used in the study included: 1) it should not significantly dilute the dietary nutrients and 2) it should not be high enough to produce acute toxicity in the mice. The generally acceptable rule for dilution of nutrients in rodent feed is the feed should not be diluted more than 10% by the addition of test articles and a lower dilution percentage is preferred. Based upon the acute toxicity of nicotine, this limitation would not be reached. In respect to not inducing acute toxicity from nicotine, the scientific literature associated with nicotine toxicity was reviewed for this study. The oral LD_{50} (a dose that results in death of 50% of the treated animals) of nicotine for mice has been reported to range from 50-60 mg/kg body weight to 188 mg/kg body weight (HSDB, 2008) for a single oral bolus dose. Based upon these and other data, the doses selected for nicotine in this study were 0, 0.2, 2.0, 4.0, 8.0, 20.0 and 40.0 mg nicotine/kg body weight/day. Even though the high dose is close to one of the reported oral LD_{50}'s for nicotine, the mice would not receive their nicotine dose as a single bolus but as a feed component. This would result in smaller exposures each time a mouse feeds. In addition, nicotine is rapidly metabolized by mice to less toxic metabolites resulting in a further reduction in plasma nicotine concentrations and a reduction in toxicity using this route of administration. The chosen dose range should encompass potential nicotine doses to be used in the anticipated toxicology studies and provide information on the palatability of the diets to the mice without causing undue acute toxicity. These identical doses were also used in a parallel study using rats (TOX209) based upon a similar rational. These studies are useful in distinguishing any differences in the responses of these two rodent species commonly used in toxicology studies.

A third variable was whether to use both genders of mice or to use a single gender. To limit the size of this short term investigational study to approximately 100 animals, it was decided to use only males. This was based upon the assumption that there would not be significant gender differences in the palatability of the feed, although it was recognized that there could be gender differences in the neurophysiological responses to nicotine.

A fourth variable was the strain of mouse to be used in the study. It was decided that the most appropriate mouse strain for the planned toxicology studies was the outbred Swiss Webster strain. Supporting this decision was the choice of this mouse strain for longer term toxicology studies by the National Toxicology Program (NTP) based upon the robust nature of this strain.

An additional variable was the type of feed to be used in the study. First, a powdered feed would be required to allow homogeneous incorporation of the test articles and positive control into the feed. Second, a feed that allows the mice to thrive, especially in longer term studies, was required. Evaluation of the available feeds resulted in the choice of the NTP-2000 rodent feed developed and used by the NTP. A major reason for this choice is that this feed is adequate in all essential nutrients for mice but has a lower caloric content compared to other possible feeds. The lower caloric content results in a slightly slower body weight gain and better survival of rodents used in toxicology studies of two year duration. This diet has been chosen by the NTP for all its long term toxicology studies.

The design of this study was based upon the aforementioned considerations and provides important information useful in the design of rodent toxicology studies using the test articles of interest. The study minimized the risk that mice would find the diets unpalatable during longer term feeding studies while using a minimal number of animals.

VII. MATERIALS AND METHODS

A. TEST ARTICLES

Two test articles and a positive control were used for the study.

1. Test Article 1 Tobacco Blend

Test Article 1 was identified as Tobacco Blend Lot#0T162AF and consisted of a blend of natural tobaccos ground to a powder. It contained no preservatives or other additives. It was reported to contain 2.63% nicotine and all diet formulation calculations were based upon this reported nicotine content. [Subsequent analysis of Test Article 1 reported a nicotine content of 2.94%]. The Certificate of Analysis (CofA) for Test Article 1 is on file with the Sponsor. Because tobacco is a complex mixture of natural components, its purity can not be ascertained. Upon arrival at the testing facility, the test article was stored at 4 ^{o}C for no more than three weeks before use for the last feed formulation. The Test Article was mixed to ensure uniformity before aliquots were removed for feed formulation. After the removal of the aliquots required for feed formulation, the test article was stored at 4 ^{o}C for potential additional use.

2. Test Article 2 Tobacco Extract

Test Article 2 was identified as Tobacco Extract Lot#0T162AE and consisted of an aqueous extract of Test Article 1. Its water content was adjusted to result in 1 ml of Test Article 2 being equivalent to 1 g of Test Article 1. It contained no components not contained in the tobacco and the water used for extraction. The water used for extraction of the tobacco was analyzed for a series of components and the results are on file with the sponsor. Because the aqueous extract is a complex mixture of materials extracted from the tobacco, its purity can not be ascertained. Test Article 2 was reported to contain 2.30% nicotine and all dose formulation calculations were based upon this reported value. [Subsequent analysis of Test Article 2 reported a nicotine content of 2.25%]. Preliminary determination of the density of Test Article 2 revealed a density of 1.203 g/ml and is provided in Appendix II. The Certificate of Analysis for Test Article 2 is on file with the Sponsor. Upon arrival at the testing facility, Test Article 2 was maintained frozen at approximately -25 ^{o}C for no more than three weeks before use for the feed formulation. Before each use for feed formulation, the extract was thawed at room temperature, shaken to insure complete mixing and appropriate quantities of extract removed for dosed feed formulation and the extract then refrozen.

3. Positive Control Nicotine Hydrogen Tartrate

The positive control used in this study was nicotine hydrogen tartrate (Lot#077K1810) obtained from Sigma-Aldrich Co., St. Louis, MO. The Certificate of Analysis provided by the manufacturer for the nicotine salt stated it was 98% pure. Preliminary analysis of the salt at RJRT indicated it was at least 98% pure, if not of higher purity than reported (Moldoveanu and Coleman, 2008). Analysis indicated that the positive control test article contained 0.25% nicotyrine (CAS# 487-19-4), less than 0.1% nicotine oxide (CAS# 491-26-9), 0.11% ethyl tartrate (CAS# 87-91-2) and 0.20% hydroxysuccinic acid (CAS# 97-67-6). The nicotine free base is 35.1% of the bulk salt (2.85 g salt contains 1 g of free nicotine). Feed formulation was based upon the free nicotine content and not the bulk salt. The nicotine hydrogen tartrate was stored at room temperature, as recommended by the supplier. After formulation of the first test diet, the nicotine hydrogen tartrate was stored desiccated to minimize potential absorption of water from the atmosphere.

4. Safety

Safety procedures were employed for personal protection because of the use of materials of known and unknown toxicological potential. These procedures adhered to the provisions of the RJRT R&D Chemical Hygiene Plan (developed to comply with the OSHA Laboratory Standard, 29 CFR 1910.1450) and included protective clothing and gloves; use of a dust mask, in situations where a dust could be generated; the use of protective eyewear; use of a ventilated fume hood; room ventilation system and use of a container-within-a-container system for transport of the test articles and positive control dosed feed. Feed formulation operations were confined to Room 78 in Building 630-2, both of which had controlled entry.

During feed formulation and mixing, two people were present in case of any direct exposures of the technical staff to nicotine were to occur. In the event of any mishap (i.e., direct nicotine exposure), the individual would immediately wash the exposed areas with cold water for a period of no less than five minutes. While the exposed person was washing the exposed area, the second person would call 1-911, if it was determined the exposed individual was, in fact, actually exposed.

B. EXPERIMENTAL DESIGN

1. Study Animals

a) Animals

The protocol and the use of animals for this study were reviewed and approved by the RJRT Institutional Animal Care and Use Committee (IACUC) on March 27, 2008, before arrival of the animals into the facility. Ninety one, male, CFW Swiss Webster mice (5-7 weeks of age) from Charles River Laboratories (Portage, Mich.) were received into the facility on April 09, 2008, along with 10 sentinel mice. Sentinel mice were retired breeders and maintained under identical conditions as the study animals, except they were fed Lab Diet, Certified Rodent Diet #5002 feed (PMI Nutrition International), provided as pellets, *ad libitum* throughout the study.

b) Animal Identification

Mice were identified by cage card during the pretest period. After allocation to study groups the mice were identified with their study number by tail marking with an indelible marking pen. Animals were numbered consecutively with a unique identification number (Table 1).

Table 1: **Treatment Groups and Doses**[1]

Group Number	Treatment Group and Nicotine Dose (mg nicotine/kg body weight/day)		Number of Mice	Mouse ID Numbers
	Control			
1	NTP-2000 feed	(0.0)	5	1-5
	Tobacco Blend			
2	Dose 1 Tobacco in NTP-2000 feed	(0.2)	5	6-10
3	Dose 2 Tobacco in NTP-2000 feed	(2.0)	5	11-15
4	Dose 3 Tobacco in NTP-2000 feed	(4.0)	5	16-20
5	Dose 4 Tobacco in NTP-2000 feed	(8.0)	5	21-25
6	Dose 5 Tobacco in NTP-2000 feed	(20.0)	5	26-30
7	Dose 6 Tobacco in NTP-2000 feed	(40.0)	5	31-35
	Tobacco Extract			
8	Dose 1 Tobacco Extract in NTP-2000 feed	(0.2)	5	36-40
9	Dose 2 Tobacco Extract in NTP-2000 feed	(2.0)	5	41-45
10	Dose 3 Tobacco Extract in NTP-2000 feed	(4.0)	5	46-50
11	Dose 4 Tobacco Extract in NTP-2000 feed	(8.0)	5	51-55
12	Dose 5 Tobacco Extract in NTP-2000 feed	(20.0)	5	56-60
13	Dose 6 Tobacco Extract in NTP-2000 feed	(40.0)	5	61-65
	Positive Control			
14	Dose 1 Nicotine Tartrate in NTP-2000 feed	(2.0)	5	66-70
15	Dose 2 Nicotine Tartrate in NTP-2000 feed	(8.0)	5	71-75
16	Dose 3 Nicotine Tartrate in NTP-2000 feed	(20.0)	5	76-80
17	Dose 4 Nicotine Tartrate in NTP-2000 feed	(40.0)	5	81-85
	Sentinels			
	Sentinels (no treatment)		10	86-95

[1] Doses in parenthesis represent the nicotine dose in mg nicotine/kg body weight/day.

Data associated with the use of mice on study were acquired with the aid of the Path/Tox (Xybion Medical Systems, Cedar Knolls, NJ) software version 4.2.2 resident on a VAX operating system under the Path/Tox protocols referred to as TOX210A and TOX210B.

Because of the limitations in the Path/Tox system, two Xybion protocols were created to accommodate all 17 dosed groups. TOX210A contains Dose Groups 1-13. TOX210B contains the four nicotine hydrogen tartrate positive control groups (i.e. TOX210B Group 1 is study Group 14; Group 2 is Group 15; Group 3 is Group 16; and Group 4 is Group 17). The Xybion data collection protocols TOX210A and TOX210B were used for body weights, feed consumption and clinical signs of mice used on this study. Data were input into the Xybion Path/Tox collection protocols under "A" module, "AINPUT'.

Because the start of the exposure phase was delayed five days for both TOX210A and TOX210B studies, Day 7 of the Xybion data output is actually study Day 1 (May 21, 2008), etc.

c) Animal Housing

The mice were housed and cared for in accordance with the Institute of Laboratory Animal Research (ILAR), Commission of Life Sciences, National Research Council document entitled, *Guide for the Care and Use of Laboratory Animals* (1996) in an Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited animal facility in Building 630-2.

The mice were housed in room 40 in the Building 630-2 vivarium with controlled lighting (12 hours of darkness, from 6:00 p.m. to 6:00 a.m. ± 30 minutes. The room temperature was set to maintain 18-26 ºC with a relative humidity of 30-70%. Room airflow was greater than 10 room air changes/hour. Room airflow, temperature, humidity and light cycles were monitored continuously and data recorded every 30 minutes to a computer file via an automated facility data collection system. In addition, seven-day, continuous chart-wheel recordings were kept for room temperature and relative humidity as backup to the automated system. Mice were individually housed in stainless steel, wire bottomed cages whose dimensions were 9 in (L) x 3.75 in. (W) x 5 in. (H) placed on stainless steel racks.

Mice were to be quarantined and acclimated to the facility for a minimum of seven days prior to initiation of the study. The Attending Veterinarian performed a health examination of all mice within four days after delivery. Commencement of dosing the mice was dependent upon a favorable review of the health examination, as well as a written statement from the Attending Veterinarian releasing the mice from quarantine. Mice were released from quarantine on April 16, 2008 but continued under quarantine conditions until initiation of the study.

d) Feed and Water

Mice were fed *ad libitum* Certified Rodent Diet #5002 feed (PMI Nutrition International) pellets during the early period of the quarantine. Starting on April 11, 2008 and continuing for the remainder of the study, all groups, with the exception of the sentinel mice, had *ad libitum* access to NTP-2000 feed (Zeigler Bros., Inc., Gardners, PA) to acclimate the animals to a powdered diet. The sentinel mice were continued on the Certified Rodent Diet #5002 to study termination. Throughout the dosing period, NTP-2000 feed formulated with the appropriate doses of test articles, positive control or as a control diet with no test article were provided *ad libitum* to the mice. Clean feeders were provided weekly. Data for mice that spilled or contaminated their feed could be censored for days when excess spillage was reported or when the data were unreasonable for the specific animal based upon group means and previous and subsequent feed intake for that specific animal. For instance, if an animal's feed intake more than doubled or was reduced by more than half, the data for that animal on that day could be censored.

Feed was provided to the mice in glass feed cups with stainless steel lids that minimized spillage but provided the mice access to the feed. The volume of the feed cups was adequate for several days feed; however, feed consumption was determined daily and fresh feed placed in the feed cups. This resulted in a large waste of feed each day. To minimize loss of feed resulting from determination of daily feed consumption, a Delrin spacer was added to the feed cups to displace a portion of the feed. This minimized feed waste yet provided the mice adequate quantities of feed to insure *ad libitum* feeding. Because the spacer was below the surface of the feed the mice did not have assess to the spacer and there was no evidence of gnawing or biting on the spacers.

Water was provided to the mice on an *ad libitum* basis through an automatic system. The water source originates from the municipal supply of the City of Winston-Salem, and is subsequently filtered through activated carbon and 5-micron particulate filters prior to delivery to the mice. Facility water is chemically analyzed twice each year to ensure it contains no substances at concentrations that could affect the results of the studies. The water analysis from the period closest to the start of the study (March 19, 2008) is provided in the study file. There were no known contaminants found to be present in the feed or water in concentrations that would be anticipated to interfere with the outcome of the study.

f) Allocation of Animals to Study Groups

On April 12, 2008, mice were assigned to dose groups according to body weight using the "A" module of the PATH/TOX software (version 4.2.2; Xybion Medical Systems; Cedar Knolls, NJ). Body weights and detailed clinical signs were recorded before allocation. At the discretion of the Study Director, mice exhibiting positive clinical signs, demonstrating body weight loss, or representing low or high extremes of body weight could be excluded from the allocation process. To ensure groups of similar mean body weight, all groups within the PATH/TOX protocol were compared by analysis of variance (ANOVA) and least significant difference criteria, and demonstrated not to be significantly different at a 5 percent, two-sided probability level. Following allocation into groups, mice were uniquely identified with their permanent identification number by tail marking on April 15, 2008 with their unique animal number using indelible ink and assigned to cages with permanent cage cards attached, recording the study number, Study Director's name, species and gender of the animal, group number, pre-allocation animal number, and the animal's permanent identification number.

2. Study Design

a) Route of Administration

The route of administration of the test articles and positive control used in this study was oral through mixing the test articles or positive control into the feed for the mice.

b) Dose Regimen

A total of 17 groups were used along with a sentinel group (Table 1). Each treatment group contained 5 male mice. Mice in Group 1 served as the untreated control group and were fed NTP-2000 feed without the addition of either of the test articles or the positive control. Groups 2-7 were fed NTP-2000 feed with additions of the tobacco blend to yield the following doses: 0.2, 2.0, 4.0, 8.0, 20.0 and 40.0 mg nicotine/kg bw/day. Nicotine dosing was based upon reported acute short term toxicity data for nicotine (HSDB, 2008) and chosen to be below a dose that would be expected to be acutely toxic but believed to be suitable for the determination of palatibility. Groups 8-13 were fed NTP-2000 feed with additions of the tobacco extract to yield the mg nicotine/kg bw/day equivalent to those mice in Groups 2-7. The positive control Groups 14-17, were fed NTP-2000 feed that contained nicotine hydrogen tartrate to yield the following doses in mg nicotine/kg bw/day: 2.0, 8.0, 20.0 and 40.0, respectively.

Formulation of the feed to yield the required doses of nicotine for the duration of the study is dependent upon two factors. First, the mean body weight range of the mice for the duration of the dosing period must be assumed. An assumption of 30g (0.03kg) was used for the Series 1 feed formulations (first feed formulation for week 1 of the study) based upon published data for the age to weight relationship for this mouse strain. Second, the mean feed intake range of the mice for the duration of the feeding period must also be assumed and is related to the mean body weight. An assumption of 5 g feed consumption/day/mouse was used based upon data provided by the animal supplier for the Series 1 feed formulation. Series 2 feed formulations used during the second week of the study were based upon estimates of body weight and feed consumption (30 g bw/mouse and 6.5 g feed consumption/mouse) derived from the first week of the study. The calculations for the amount of both test articles and the positive control to yield the required nicotine concentrations at each dose are provided in Appendix III.

Sentinel mice were fed pelleted Lab Diet, Certified Rodent Diet #5002 (PMI Feeds, Inc.) *ad libitum* using cage feeders designed for pelleted feed. Sentinel mice were used to detect any disease or other factors that may influence the study and received no treatment.

c) Dosed Feed Formulation

The bulk NTP-2000 unformulated feed was stored at refrigerator temperatures (4ºC) in Lab 95 before being aliquotted to the control group and before it was aliquotted to prepare the formulated feeds.

Formulated feed was prepared weekly because of a lack of stability data for the test articles and positive control when mixed into the NTP-2000 feed. Dosed feed was formulated by the addition of the appropriate quantity of test article to a portion (premix) of the total diet to be formulated (approximately 25% of the total required feed). Mixing was accomplished by the use of KitchenAid 10 speed commercial mixers using 5.7 liter stainless steel mixing bowls and the flat beater. The test articles and positive control were weighed on a Mettler AE 163 analytical balance and the powdered diet was weighed on a Mettler PM2000 balance. Test Article 1 (tobacco blend) was added to the premix as supplied. Test Article 2 (tobacco extract) was added to the premix as supplied avoiding contact with the mixing bowl and beater because of its tendency to adhere to these surfaces. The positive control (nicotine hydrogen tartrate) was placed in a clean porcelain mortar containing approximately five grams of NTP-2000 feed and ground lightly with the pestle to break up any lumps of the tartrate salt before addition to the premix. After addition of each test article or the positive control to the NTP-2000 powdered diet premix, it was mixed by hand by use of a spatula to ensure it was distributed into the diet. The premix was then subjected to mechanical mixing for approximately five minutes to assure apparent homogeneity. The appropriate quantity of NTP-2000 powdered diet was then added to the pre-mix and mechanically mixed for approximately 10 minutes to obtain homogeneity. The sequence of preparation of formulated feed for each test article and the positive control was from the low dose to the high dose to minimize any carryover between doses. All mixing bowls and other apparatus used in feed formulation were cleaned before moving to the next higher dose formulation. A trial feed formulation (as part of the TOX209 study) using each test article and the positive control was produced to assess the adequacy and refine the methodologies to be used in the study. Feed formulations were conducted during the week before initiation of feeding the formulated diets and again approximately at the midpoint of the 14-day study. Formulated feeds and the control NTP-2000 feed were stored at room temperature. The control feed was maintained identical to the formulated feed during each feeding period. Table 2 provides the intended concentration of tobacco, tobacco extract or positive control in the formulated feed on a mg per g of feed basis.

Table 2: **Concentration of Test Articles and Positive Control in NTP-2000 Feed**

Group	**Treatment Group (mg nicotine/kg body weight/day)**		**Concentration of Test Article, Positive Control in NTP-2000 Feed (mg/g feed[1])**	
			Series 1[2]	**Series 2[3]**
	Control			
1	NTP-2000 feed	(0.0)[4]	0.00	0.00
	Tobacco Blend			
2	Dose 1 Tobacco Blend in NTP-2000 feed	(0.2)	0.04	0.03
3	Dose 2 Tobacco Blend in NTP-2000 feed	(2.0)	0.46	0.35
4	Dose 3 Tobacco Blend in NTP-2000 feed	(4.0)	0.91	0.70
5	Dose 4 Tobacco Blend in NTP-2000 feed	(8.0)	1.83	1.41
6	Dose 5 Tobacco Blend in NTP-2000 feed	(20.0)	4.57	3.51
7	Dose 6 Tobacco Blend in NTP-2000 feed	(40.0)	9.13	7.03
	Tobacco Extract			
8	Dose 1 Tobacco Extract in NTP-2000 feed	(0.2)	0.05	0.04
9	Dose 2 Tobacco Extract in NTP-2000 feed	(2.0)	0.52	0.40
10	Dose 3 Tobacco Extract in NTP-2000 feed	(4.0)	1.04	0.80
11	Dose 4 Tobacco Extract in NTP-2000 feed	(8.0)	2.09	1.61
12	Dose 5 Tobacco Extract in NTP-2000 feed	(20.0)	5.22	4.01
13	Dose 6 Tobacco Extract in NTP-2000 feed	(40.0)	10.42	8.03
	Positive Control			
14	Dose 1 Nicotine Tartrate in NTP-2000 feed	(2.0)	0.03	0.026
15	Dose 2 Nicotine Tartrate in NTP-2000 feed	(8.0)	0.14	0.11
16	Dose 3 Nicotine Tartrate in NTP-2000 feed	(20.0)	0.34	0.26
17	Dose 4 Nicotine Tartrate in NTP-2000 feed	(40.0)	0.68	0.53

[1] Concentration is expressed as the amount of test article added/g feed. For instance, 0.04 mg of the tobacco blend added per g of feed and 0.05 mg of the tobacco extract added per g of feed.

[2] Series 1 feed formulations were used during the first week of the study. Required concentrations to yield nicotine doses were based upon an estimated bw of 30 g/mouse and an estimated feed intake of 5 g/mouse.

[3] Series 2 feed formulations were used during the second week of the study. Required concentrations to yield nicotine doses were based upon data from the first week of the study resulting in an estimated bw of 30 g/mouse and an estimated feed intake of 6.5 g/mouse.

[4] Numbers in parenthesis are the target doses of nicotine in mg nicotine/kg bw/day.

Samples from the top, middle and bottom of the high dose and low dose formulated diets for each test article and the positive control were placed in polypropylene plastic containers for analysis of nicotine content to confirm the homogeneity of each test article in the feed. Samples of each test article and positive control were also removed for analysis of nicotine to confirm the proper dose formulation. Additional samples were removed and stored under conditions similar to those used for the formulated diets to determine the stability of the test articles and positive controls via analysis of nicotine.

d) Analysis of Formulated Feed:

The preliminary test batch of formulated feed at the high dose and low dose was submitted for analysis of nicotine to determine homogeneity and dose confirmation. Analysis was based upon a previously reported method that involved treating the formulated feed with base (NaOH) followed by solvent extraction (tert-butyl methyl ether) and GC-FID quantitation. This method had not previously been used to determine the nicotine concentration of rodent feed. The initial analysis revealed that this analytical method was appropriate for the high dose only. The nicotine concentration in the low dose was below the limit of quantitation for the GC-FID method. Therefore, a new analytical method was developed at RJRT contemporaneous with this study to allow quantitation of the nicotine content in the diets at all dose concentrations. This method used GC-MS for quantitation and is not only more sensitive but decreased the sample analysis time. This method did not undergo complete validation and its accuracy and precision are unknown. Certain data presented in this report were obtained during the initial method development phase. However, it appears adequate to demonstrate that the dose formulations were conducted in a manner adequate for the purpose of this investigational study. The method was subsequently validated and reported (Kilby and Ellisor, 2008). It clearly illustrates the presence of nicotine from the added smokeless tobacco blend, the tobacco extract and nicotine hydrogen tartrate. It also clearly illustrates that as the intended doses increased, so did the nicotine content of the dosed feed.

3. Biological Observations

The following parameters were monitored during the in-life portion of this study.

a) Serology/Health Screens

Sentinel mice were handled identical to the study animals and placed in Room 40 with the study animals. Because of the short term nature of the study, prestudy sentinel mice were not employed in this study. At study termination on May 6, 2008, the ten sentinel mice for health screening were anesthetized with 70% carbon dioxide (CO_2) in air and blood was drawn from either the vena cava or heart, with the exception on one animal that lacked a blood sample. While still under anesthesia, the animals were then sacrificed by exsanguination. The health screen mice provided sera appropriate for routine measurement of the following antibodies to disease using the Charles River Laboratory Mouse Assessment Plus profile that consisted of the following: pneumonia virus of mice (PVM), mouse hepatitis virus (MHV), minute virus of mice (MVM), Sendai virus, murine encephalomyelitis virus (GDVII), REO-3, *Mycoplasma pulmonis*, lymphocytic choriomeningitis virus (LCMV), Ectromelia (mousepox), K virus, polyoma virus, mouse adenovirus (MAV) 1 & 2, epizootic diarrhea of infant mice virus (EDIM), mouse cytomegalovirus (MCMV), Hantaan virus (HANT), *Encephalitozoon cuniculi* (ECUN), ciliated associated respiratory bacillus (CARB), mouse parvovirus (MPV) 1 & 2, mouse thymic virus (MTLV) and murine norovirus (MTLV). Serology was performed by Charles River Research Animal Diagnostic Services, Wilmington, MA. In addition, the lungs were removed during necropsy and provided to Seventh Wave, Burlington, NC for histopathological examination for evidence of contagious disease.

Commencement of animal dosing was dependent upon a favorable review of the health status of the mice and a statement from the Attending Veterinarian releasing the animals from quarantine. The mice were released to the study from quarantine on April 16, 2008 and dosing commenced on April 21, 2008.

b) Moribundity/Mortality Checks

Twice daily observations of all animals, once in the morning and once in the afternoon (at least 6-hours apart), were performed to identify dead or moribund mice. Observations were made five days per week (Monday through Friday, excluding holidays). For weekends and holidays, only one observation per day was performed. Mice whose conditions made it unlikely that they would survive to the next observation period or seemed to be in distress or pain could be euthanized.

c) Clinical Observations

Detailed, scheduled clinical observations were performed the day after delivery, when collecting body weights for allocation to study groups and at twice weekly intervals, throughout the study. All findings were recorded in the "A" module of the PATH/TOX system. Negative findings were recorded as normal/no significant change.

d) Body Weights

Individual non-fasted body weights were determined two days after delivery, again prior to study group allocation (i.e., prior to the initial dosing). Upon initiation of feeding the dosed feed, body weights were recorded daily for the duration of the 14-day study. However, body weights were not recorded on the 15th day of the study. This resulted in a lack of body weight data for the last day of the study. The "A" module of the Xybion PATH/TOX system was used for acquisition of body weight data. Weighing took place at approximately the same time each day (7:00-11:00 AM). Individual body weights were used to calculate the mean body weight for each experimental group and cumulative percent body weight gain. Unscheduled body weight determinations could be made at any time, if deemed necessary by the Attending Veterinarian or Study Director. Mouse weights were acquired using Mettler PM2000 balances (Mettler Instrument Corporation, Highstown, NJ).

e) Feed Consumption

Each day of the study, fresh feed was placed into the feed bowl and its weight determined and recorded. The next day, the feed bowl was weighed and the food consumption calculated. Data were entered into the “A” module of the PATH/TOX computer software. Each mouse’s feed consumption was used to calculate the mean feed consumption for the group. In cases of excessive spillage or other inconsistencies, feed weight was recorded but not used to determine mean feed consumption for the group. After determination of the feed consumed by a mouse, fresh feed was placed in a bowl and provided to the mouse after recording the weight in the PATH/TOX software.

g) Terminal Body Weights

The non-fasted, terminal body weights for the mice in each study group were determined for the 14^{th} day of the feeding period.

4. Statistics

Body Weights

Data were analyzed using statistical tests within the PATH/TOX software. Statistical procedures could include: means and standard deviations, one-way analysis of variance, Bartlett's test of homogeneity of variance, Dunnett's t-test of significance, Cochran and Cox's modified t-test of significance.

5. Records Maintained

Records required to reconstruct the study and to demonstrate adherence to the protocol are maintained in the Study Archives located at RJRT.

VIII. RESULTS

A. Feed Formulation Analysis

During the course of the study three feed formulations were prepared. The first formulation was a trial run to refine the formulation methodology and determine the homogeneity obtained at the high and low doses. The trial run used dose concentrations for the TOX209 rat study; however, these data are directly related to this mouse study because the formulation methodology was identical for TOX209 and TOX210. Feed from the trial run was not fed to animals. This was followed by Series 1 formulation for the first week of the study, then Series 2 formulation for the remainder of the study. Calculations of feed requirements (feed consumption and body weight) for Series 1 were based upon extrapolation of published data for the food consumption and growth of Swiss Webster mice. Calculations for Series 2 formulation were based upon data collected during the initial phase of the study. The formulated feed from each of these preparations was analyzed for nicotine concentration to confirm that the feed contained the anticipated concentration of nicotine.

As noted earlier, there was no existing methodology for determination of the nicotine content of rodent feed. Therefore, existing nicotine analytical methods were modified for this endeavor. The modified methodology uses gas chromatography (GC) with flame ionization detection (FID) for the high dose but not for the lower doses. A methodology using GC/mass spectroscopy (MS) that had the sensitivity to detect nicotine at the lower doses was developed contemporaneous with this study. Because of the large sample load and the need for rapid response, this method was validated subsequent to this study (Kilby and Ellison, 2008a).

Homogeneity data from the trial run feed formulation at the high dose of 40 mg nicotine/kg bw/day are presented in Table 3. Samples were obtained from the top of the formulated feed mixture as well as the middle and bottom of the mixture. While the low dose preparation (0.2 mg nicotine/kg body weight/day) was also analyzed for nicotine content, the nicotine concentration was below the limit of quantitation for the GC-IFD analysis method. This led to the development of the GC/MS quantitation method. Homogeneity data obtained by the GC/MS method for the low dose trial run formulation are presented in Table 4.

Table 3: **Trial Run Feed Formulation Homogeneity Data[1] and Dose Confirmation**
(40 mg nicotine/kg body weight/day)

Target Concentration (mg nic/g feed)	Sample Location **Top** (mg nic/g feed)	**Middle** (mg nic/g feed)	**Bottom** (mg nic/g feed)	Determined Average Concentration (mg nic/g feed)
		Tobacco Blend		
0.50	0.46 ± 0.02 (8%)[1]	0.47 ± 0.01 (6%)	0.45 ± 0.02 (10%)	0.46 ± 0.01[2] (8% ± 2%)[2]
		Tobacco Extract		
0.50	0.40 ± 0.04 (20%)	0.43 ± 0.06 (14%)	0.39 ± 0.02 (22%)	0.41 ± 0.02 (18% ± 4%)
		Nicotine Tartrate		
0.50	0.42 ± 0.2 (16%)	0.41 ± 0.01 (18%)	0.40 ± 0.01 (20%)	0.41 ± 0.01 (18% ± 2%)

[1] Data in parentheses represent the percent difference from the target concentration. Analysis method was GC/FID.
[2] Data represent the mean ± the standard deviation where appropriate.

The feed formulated with the tobacco blend demonstrated good homogeneity with the samples being within ± 10% of each other. The mean concentration of nicotine in the feed indicated that it was within ± 10% of the anticipated concentration, indicating adequate dose confirmation. Visual inspection of the formulation indicated no change in the color of the feed and there were no visible evidence of tobacco particles.

Feed formulated with the aqueous tobacco extract at the high dose demonstrated adequate homogeneity but was below the anticipated nicotine concentration. The problem appears to occur at the pre-mix stage. The extract is viscous and tends to stick to the blade of the mixer and to some extent the mixing bowl. This would result in a lower than expected concentration. Based upon these data, the mixing methodology was altered to decrease the potential for the extract to contact the blending device.

The nicotine hydrogen tartrate also demonstrated adequate homogeneity in feed but the nicotine concentration was lower than anticipated. Based upon these data, the mixing methodology was modified by placing a portion (~5 g) of the diet in a mortar and pestle to which the nicotine salt was added. Lumps of the salt were gently broken and mixed with the feed using the pestle. When there were no longer any visible lumps, the feed was then added to the remaining pre-mix for mechanical mixing. After analysis of the trial run data, the nicotine salt was stored in a desiccator to minimize moisture absorption, which could affect the accuracy of weighing.

Data from the trial run at the low doses of 0.2 mg/kg bw/day for the smokeless tobacco blend and 2.0 mg/kg bw/day for the nicotine hydrogen tartrate are presented in Table 4.

Table 4: **Trial Run Feed Formulation Homogeneity Data[1] and Dose Confirmation For Low Doses**

Anticipated Dose for Tobacco Blend and Extract = (0.2 mg/kg bw/day)
Anticipated Dose for Nicotine Hydrogen Tartrate = (2.0 mg/kg bw/day)

Target Concentration (mg nic/g feed)	Sample Location **Top** (mg nic/g feed)	**Middle** (mg nic/g feed)	**Bottom** (mg nic/g feed)	Determined Average Concentration (mg nic/g feed)
		Tobacco Blend		
0.003	0.003 (0%)	0.003 (0%)	0.003 (0%)	0.003 ± 0.000[2] (0% ± 0%)[2]
		Tobacco Extract		
0.003	NA[3] (%)	NA (%)	NA (%)	NA (%)
		Nicotine Tartrate		
0.025	0.022 (12%)	0.021 (16%)	0.020 (20%)	0.021 ± 0.001 (16% ± 4%)

[1] Data represent the mean of duplicate assays. Data in parentheses represent the percent difference from the target concentration. Analysis method was GC/MS and method was under development.
[2] Data represent the mean ± the standard deviation of the concentration and percent difference.
[3] NA = Data not available.

While the GC/FID analytical methodology did not allow the determination of the nicotine concentration in the feed at these low doses, the GC/MS method under development was able to provide estimates of the nicotine concentration.

The tobacco blend yielded feed nicotine concentrations as anticipated in respect to homogeneity and concentration. Data for the tobacco extract are not available. Feed formulated with nicotine hydrogen tartrate demonstrate adequate homogeneity but was slightly lower than anticipated in concentration. Improvements to the feed formulation methodology should improve the accuracy obtaining nicotine concentrations adequate for use in this investigational study.

Series 1 feed formulations were used during the first seven days of study. Analytical data for homogeneity are not available for the high dose. However, homogeneity data is available for the lower doses and is presented in Table 6.

Table 5: **Series 1 Feed Formulations Homogeneity Data[1] and Low Dose Confirmation**
Anticipated Dose for Tobacco Blend and Extract = (0.2 mg/kg bw/day)
Anticipated Dose for Nicotine Hydrogen Tartrate = (2.0 mg/kg bw/day)

Target Concentration (mg nic/g feed)	Sample Location **Top** (mg nic/g feed)	**Middle** (mg nic/g feed)	**Bottom** (mg nic/g feed)	Determined Average Concentration (mg nic/g feed)
		Tobacco Blend		
0.0011	0.0011 (0%)	0.0016 (31%)	0.0011[3] (0%)	0.0013 ± 0.0003[2] (10% ± 18%)[2]
		Tobacco Extract		
0.0012	0.0011 (8%)	0.0009[3] (25%)	0.0013[3] (8%)	0.0011 ± 0.0002 (14% ± 10%)
		Nicotine Tartrate		
0.0120	0.0116 (3%)	0.0107 (11%)	0.010 (17%)	0.0107 ± 0.0008 (10% ± 7%)

[1] Data represent the mean of duplicate analytical runs unless otherwise noted. Data in parentheses represent the percent differences from the target concentration.
[2] Data represent mean ± standard deviation.
[3] Data represent a single analytical determination.

These homogeneity data for the tobacco blend indicated that its homogeneity was appropriate for use in the study. It also indicates that the concentration of nicotine in the feed was adequate.

The homogeneity data for the tobacco extract demonstrated a somewhat greater variability. However, it is believed that the homogeneity was adequate for this preliminary study and would not have an adverse effect on the outcome of the study. This demonstrates that formulations containing the extract are somewhat more difficult to mix than those containing the tobacco blend.

Feed containing the nicotine tartrate salt demonstrated adequate homogeneity and dose concentration. This indicates that the modifications to the mixing procedure improved homogeneity.

Overall, the tobacco blend, tobacco extract and the nicotine hydrogen tartrate at the low dose demonstrated adequate homogeneity and dose confirmation for the purpose of the study. Although homogeneity for the high dose data are not available, the available data imply that the modified feed formulation methodology provided dosed feed homogeneity that was adequate for the study.

Series 1 Dose Confirmation Data: Dose confirmation data for Series 1 formulations used during the first week of the study are presented in Tables 6–8. Development of an improved analytical methodology allowed dose confirmation in the Series 1 formulations. These data are presented as a general comparison, not as absolute quantitative data. They were obtained during the development of the feed nicotine quantitation methodology. The data are provided to confirm that the diets contained nicotine in increasing quantities of nicotine as the dose increased. These data in combination with the dose responses seen in the study generally indicate that the proper formulated feeds were fed to the mice.

Table 6: **Series 1 Dose Confirmation Data Tobacco Blend**[1]

Target Dose (mg of nicotine/kg of bw/day)	**Target Concentration** (mg of nicotine/g of feed)	**Determined Feed Nicotine Concentration** (mg of nicotine/g of feed)
0.2	0.001	0.002
2.0	0.012	0.011
4.0	0.024	0.016
8.0	0.048	0.033
20.0	0.120	0.089
40.0	0.240	0.135[2]

[1] Data are generally the mean of two analytical determinations, each run in duplicate.
[2] Data from a single analytical determination run in duplicate

The data in Table 6 indicates that as the doses of the tobacco blend increased, the determined feed nicotine concentrations appear to diverge from the target concentration, although they do demonstrate a dose response. The two main sources of error in these data are the formulation of the diets and the analytical methodology. The data from the study can not isolate the sources of error in the data nor what percentage of the error may be contributed by each source.

Table 7: **Series 1 Dose Confirmation Data Tobacco Extract**[1]

Target Dose (mg of nicotine/kg of bw/day)	**Target Concentration** (mg of nicotine/g of feed)	**Determined Feed Nicotine Concentration** (mg of nicotine/g of feed)
0.2	0.001	0.001
2.0	0.012	0.004
4.0	0.024	0.014
8.0	0.048	0.038
20.0	0.120	0.061
40.0	0.240	0.284[2]

[1] Data are the mean of two analytical determinations, each run in duplicate determinations.
[2] Data from a single analytical determination run in duplicate

Data for the tobacco extract (Table 7) generally followed the trends seen with the smokeless tobacco blend. The data indicate the presence of nicotine in the formulated feed in concentrations that increase in a dose dependent manner.

Table 8: **Series 1 Dose Confirmation Data Nicotine Tartrate**[1]

Target Dose (mg of nicotine/kg of bw/day)	**Target Concentration** (mg of nicotine/g of feed)	**Determined Feed Nicotine Concentration** (mg of nicotine/g of feed)
2.0	0.012	0.011
8.0	0.048	0.032
20.0	0.120	0.089[2]
40.0	0.240	0.206[2]

[1] Data are generally the mean of two analytical determinations, each run in duplicate determinations.
[2] Data from a single analytical determination run in duplicate

Data for the nicotine hydrogen tartrate positive control formulated feed (Table 8) follow the general trend seen with tobacco blend and tobacco extract. These indicate that the positive control feed contained nicotine concentrations that increased as the target dose increased.

Series 2 Dose Confirmation Data: Series 2 feed formulations were mixed during the first week of the study for use during the second week of the study. A second feed formulation was used because of the lack of data confirming that the formulated feeds were stable in respect to nicotine content. Dose confirmation data for the Series 2 feed formulations are presented in Tables 9-11.

Table 9: **Series 2 Dose Confirmation Data Tobacco Blend**[1]

Target Dose **(mg of nicotine/kg of bw/day)**	**Target Concentration** **(mg of nicotine/g of feed)**	**Determined Feed Nicotine Concentration** **(mg of nicotine/g of feed)**
0.2	0.001	0.001[2]
2.0	0.009	0.010
4.0	0.019	0.017
8.0	0.037	0.031
20.0	0.092	0.083
40.0	0.185	0.172

[1] Data are generally the mean of two analytical determinations, each run in duplicate determinations.
[2] Data from a single analytical determination run in duplicate.

Analytically determined feed nicotine concentrations in the smokeless tobacco blend Series 2 formulations (Table 9) were closer to the target concentrations than those for the Series 1 feed formulations. It is not known if this resulted from improved feed formulation or if it resulted from improved analytical methodology. The data indicate the presence of nicotine in the dosed feeds in a dose dependent manner that were close to the anticipated concentrations.

Table 10: **Series 2 Dose Confirmation Data Tobacco Extract**

Target Dose (mg of nicotine/kg of bw/day)	**Target Concentration** (mg of nicotine/g of feed)	**Determined Feed Nicotine Concentration**[1] (mg of nicotine/g of feed)
0.2	0.001	0.001
2.0	0.009	0.003
4.0	0.017	0.013[2]
8.0	0.037	0.018
20.0	0.092	0.051
40.0	0.185	0.147

[1] Data are the mean of two analytical determinations, each run in duplicate determinations.
[2] Data from a single analytical determination run in duplicate

Nicotine concentrations in the dosed feed containing the aqueous tobacco extract were not as close to the target concentrations as those formulated with the tobacco blend. However, the data do indicate the presence of nicotine in the feed at concentrations that are dose dependent. Even if the concentrations were somewhat less than anticipated, they are adequate for the purposes of this investigational study.

Table 11: **Series 2 Dose Confirmation Data Nicotine Tartrate**

Target Dose (mg of nicotine/kg of bw/day)	**Target Concentration** (mg of nicotine/g of feed)	**Determined Feed Nicotine Concentration**[1] (mg of nicotine/g of feed)
2.0	0.009	0.008
8.0	0.037	0.029
20.0	0.092	0.066[2]
40.0	0.185	0.181

[1] Data are generally the mean of two analytical determinations, each run in duplicate determinations.
[2] Data from a single analytical determination run in duplicate

As seen with the Series 1 feed formulations, the nicotine concentrations in the dosed feed containing the positive control were generally close to the target concentrations (Table 11). An exception is the 20 mg nicotine/kg bw/day dose, but only one analytical run was available at this dose. Overall, these data indicate that the Series 2 feed formulations were adequate for this investigational study.

Determination of the Stability of the Formulated Feed: Stability data for nicotine from each test article and the positive control are available for the high dose formulation made during the prestudy trial run for TOX209. These data are also appropriate for TOX210 because the test articles and feed were identical. A sample set was allowed to remain at room temperature for 1-month after initial formulation. The 1-month old samples are compared to the fresh samples in

Table 12. These data provide no evidence for a lack of stability of nicotine in the 30 day old feed samples.

Table 12: **Prestudy Trial Formulations 30-Day Stability Data from the TOX209[1] Trial Formulation**

Dose (mg nic/kg bw/day)	Target Concentration (mg nic/g feed)	"0" day[2] (mg nic/g feed)	1-Month (mg nic/g feed)	% Difference
		Tobacco Blend		
40	0.50	0.46	0.47	2.3
		Tobacco Extract		
40	0.50	0.41	0.47	12.8
		Nicotine Tartrate		
40	0.50	0.41	0.43	4.7

[1] Data are the mean of duplicate determinations at 40 mg nic/kg. The FID method was used for one determination and the MS method for the other. At the low dose only a single MS determination was available because of the lower sensitivity of the FID method. Formulated feed stored at room temperature.

[2] Data are the mean $\pm$ standard deviation for the "average concentration" from Table 3.

Ten-day stability data are available for nicotine from low and high dose of each test article and the positive control for the Series 2 feed formulations for TOX209, as seen in Table 13. Because the mouse formulated feed was identical to that prepared for the rat study, except for the exact nicotine concentrations, these data should apply for the mouse and rat feed formulations. These data compare the nicotine content of freshly prepared feed with that of feed maintained at room temperature for 10-days. There is no indication of a lack of stability of nicotine in these samples. The variability of low dose tobacco blend is believed to be due to the analytical variability and not to a loss of nicotine because no differences were seen with the extract and positive control.

Table 13: **Ten-Day Stability Data[1] TOX209 Series 2 Feed Formulation**

Dose (mg nic/kg bw/day)	Target Concentration (mg nic/g feed)	"0" day (mg nic/g feed)	"10th " day (mg nic/g feed)	% Difference
		Tobacco Blend		
40.0	0.433	0.347	0.409	15
0.2	0.002	0.007	0.002	350
		Tobacco Extract		
40.0	0.433	0.342	0.367	6.8
0.2	0.002	0.001	0.001	0.0
		Nicotine Tartrate		
40.0	0.433	0.331	0.354	6.5
2.0	0.022	0.018	0.019	5.3

[1] Data are the mean of duplicates from a single analytical determination. Formulated feed at room temperature for 10-days.

B. BIOLOGICAL EVALUATIONS

1. Study Animals

A total of 101 male CFW Swiss Webster mice, age 5-7 weeks, were received on April 9, 2008 from Charles River Laboratories, Portage, MI that included 10 male Swiss Webster retired breeders to be used as sentinel animals. The mice were placed in Room 40 and individually housed in stainless steel, wire bottomed cages on stainless steel racks. Mice were quarantined for 8 days before being released to the study by the Attending Veterinarian on April 16, 2008. Throughout the period when the mice were in residence in Room 40 the environmental controls of the animal room maintained a mean daily temperature of 71.0 $\pm$ 0.1 ºF (mean $\pm$ standard deviation) and a mean daily relative humidity of 54.2 $\pm$ 2.7%. Filtered (HEPA and charcoal) air was provided with a mean of 124.7 ± 0.1 partial air changes per hour (> 12 room air changes per hour). The light cycle was maintained at 12 hours light/dark. All these data were within the protocol specified ranges. There was one occasion where the automated data collection system missed one of the 30 min. data acquisition points (April 22, 2008). This deviation had no effect on the outcome of the study.

On April 14, 2008, 95 mice were assigned to dose groups, by body weight, using the "A" module of the PATH/TOX software. At the discretion of the Study Director, mice exhibiting positive clinical signs, demonstrating body weight loss (since the initial weighing), or representing low or high extremes of body weight were excluded from the allocation process. After allocation, all group mean body weights were compared by ANOVA and least significant difference criteria and demonstrated to be not significantly different at a $p \leq 0.05$ two-sided significance level. Start of dosing was delayed one week during which time the technique for use of the feeding cups was modified to reduce spillage and minimize waste of feed. Study Day 1 was defined as the first day of dosing, April 14, 2008 and study termination was May 4, 2008. The mice were euthanized on May 5, 2008. Because of the delay of the start of feeding the formulated feeds, study day one is denoted as study day seven in the Xybion data outputs from this study.

Mice were transferred to clean housing at least once per week. Comprehensive records of these activities are maintained as part of the study file.

Prior to initiation of dosing, the mice were fed NTP-2000 powdered feed for one week to acclimate them to the powdered feed. After the initiation of dosing, the animals were provided *ad libitum* access to NTP-2000 powered feed containing the appropriate dose of Test Article, positive control or non-dosed feed. Feed bowls were weighed, refilled and reweighed daily to determine feed intake during the dosing period. Clean feed bowls were provided weekly.

Water was provided to the mice *ad libitum* by an automatic system. Samples of animal drinking water were obtained on March 19, 2008 and submitted for analysis. The results of the March analysis indicated there were no detected analytes that were outside the U.S. Environmental Protection Agency (EPA) compliance range and would affect the outcome of the study. The data for the water analysis is maintained in the study file.

2. Serology/Health Screens

At the end of the in-life phase of the study, 10 sentinel mice were euthanized for serology and necropsy to detect any signs of disease on May 6, 2008. Samples from nine of the mice were suitable for serology. There was no evidence of significant lesions, pathogenic microorganisms or antibodies to disease. Microscopic examination was performed on each of the five lung lobes from the 10 sentinel mice (retired breeders). Findings included congestion, hemorrhage, perivascular lymphocytic infiltrations, nonpigmented macrophages, chronic inflammation and a malignant lymphoma. The chronic inflammation was associated with one lung lobe each from two mice. The malignant lymphoma was present in only one mouse and was considered sporadic. The pathologist considered the occurrences of these changes random and nonspecific and not indicative of the presence of contagious disease. The congestion and hemorrhage reflect the mode of anesthesia/euthanasia while the nonpigmented macrophages and lymphocytic infiltrations were considered backgrounds changes typically seen in mice of this age and strain. Other than the findings indicating no evidence of contagious disease, the lung histopathology is not relevant to the study animals because the sentinel mice were retired breeders that were older than the study animals. Appendix IV provides the data from the serology and histopathology screening.

3. Survival

Survival was 100% during the study. This indicates the doses chosen were below those that could have produced acute toxicity, as anticipated.

4. Clinical Observations of Animals

Clinical observations reported throughout the study are provided in Table 15. There were no clinical observations indicating altered behavior or any other evidence of nicotine toxicity during the study. This indicates that the doses used in the study were below those that may elicit nicotinic effects in the animals detectable by routine clinical observations.

Table 14 **Group Incidences and Durations of Clinical Observations**

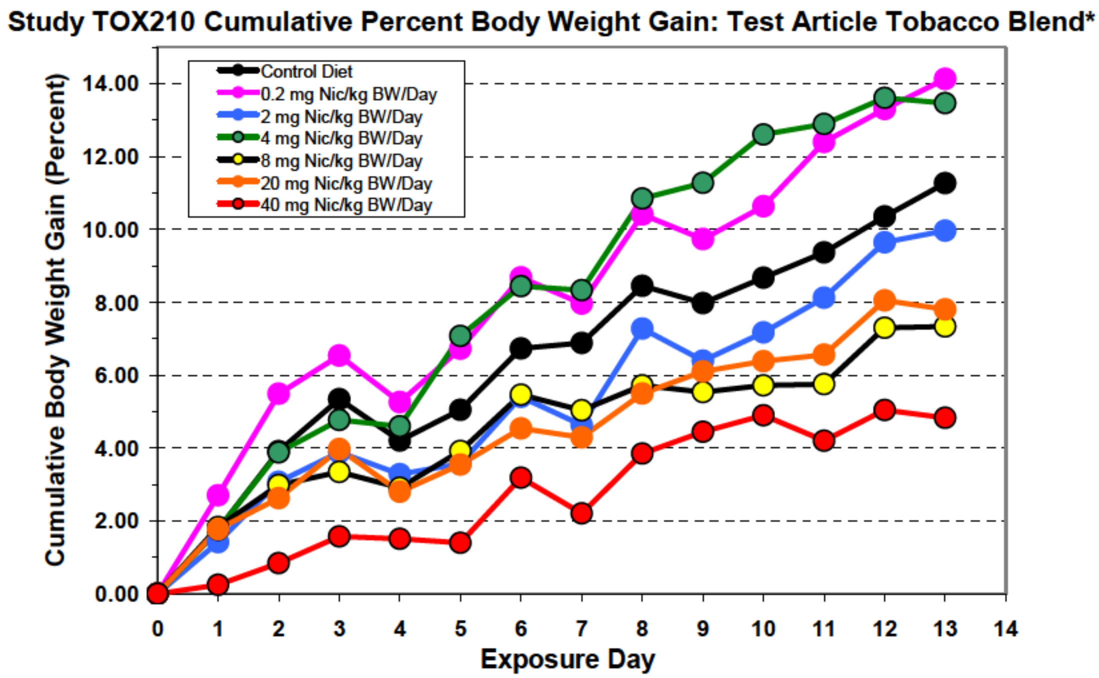
Group	Treatment Group		Observation
	(Doses based on nicotine) (mg nicotine/kg body weight/day)		Normal No visible abnormalities
			Control
1	NTP-2000 Feed		5/5 [100%, 14]
	Tobacco Blend		
2	Dose 1 Tobacco in NTP-2000 Feed	(0.2)	5/5 [100%, 14]
3	Dose 2 Tobacco in NTP-2000 Feed	(2.0)	5/5 [100%, 14]
4	Dose 3 Tobacco in NTP-2000 Feed	(4.0)	5/5 [100%, 14]
5	Dose 4 Tobacco in NTP-2000 Feed	(8.0)	5/5 [100%, 14]
6	Dose 5 Tobacco in NTP-2000 Feed	(20.0)	5/5 [100%, 14]
7	Dose 6 Tobacco in NTP-2000 Feed	(40.0)	5/5 [100%, 14]
	Tobacco Extract		
8	Dose 1 Tobacco Extract in NTP-2000 Feed	(0.2)	5/5 [100%, 14]
9	Dose 2 Tobacco Extract in NTP-2000 Feed	(2.0)	5/5 [100%, 14]
10	Dose 3 Tobacco Extract in NTP-2000 Feed	(4.0)	5/5 [100%, 14]
11	Dose 4 Tobacco Extract in NTP-2000 Feed	(8.0)	5/5 [100%, 14]
12	Dose 5 Tobacco Extract in NTP-2000 Feed	(20.0)	5/5 [100%,14]
13	Dose 6 Tobacco Extract in NTP-2000 Feed	(40.0)	5/5 [100%, 14]
	Positive Control		
14	Dose 1 Nicotine Tartrate in NTP-2000 Feed	(2.0)	5/5 [100%, 14]
15	Dose 2 Nicotine Tartrate in NTP-2000 Feed	(8.0)	5/5 [100%, 14]
16	Dose 3 Nicotine Tartrate in NTP-2000 Feed	(20.0)	5/5 [100%, 14]
17	Dose 4 Nicotine Tartrate in NTP-2000 Feed	(40.0)	5/5 [100%, 14]

[1] Data represent the ratio of the number of animals demonstrating the effect to the initial number of animals in each group. Data in brackets represent the group incidence and number of animal days with the clinical finding.

5. Body Weights

Group mean body weights and body weight gains were recorded daily throughout the study as Individual and Group Mean Animal Body Weights for each weighing period and are presented in Appendix VII. As noted earlier, body weights were not collected on day 15 of the study. This resulted in a lack of data for the 14th day of feeding the formulated feed. The lack of data for the last feeding period is believed to have no impact on the results of the study because the trends in body weight had been established before the last day of the study.

Figure 1



*Data represent the mean cumulative body weight gain expressed as a percent of the initial body weight. Exposure day zero represents the body weight of the mice before being exposed to the dosed feed. Exposure day one represents data acquired after one day of exposure to the untreated control feed or feed formulated with the tobacco blend. Body weights were not determined on day 15 after 14 days of exposure resulting in a lack of data for day 14.

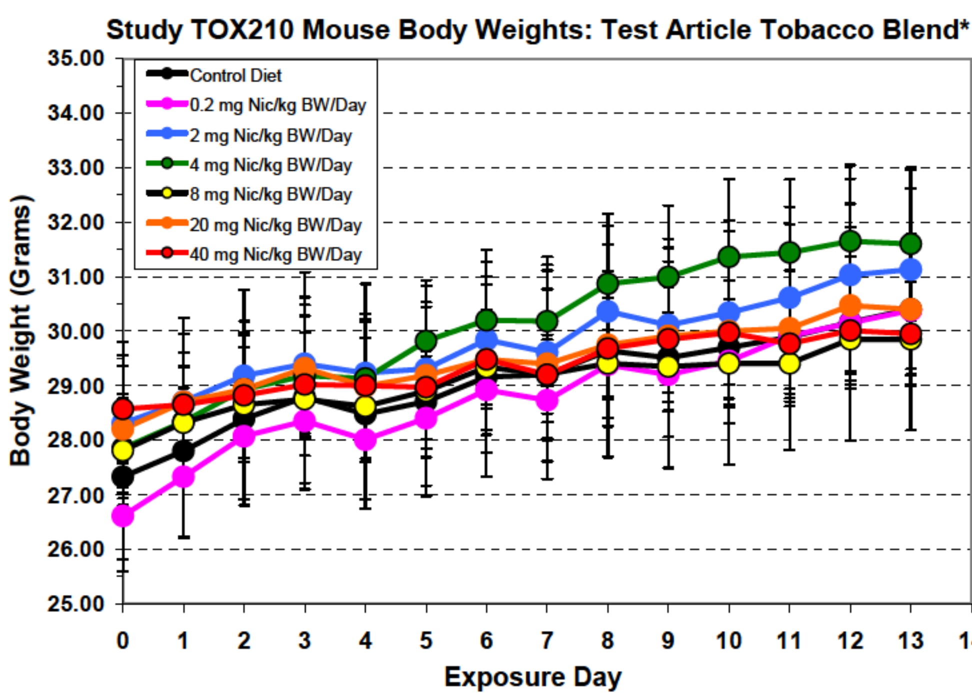
Figure 1 provides the body weight data for mice provided feed formulated with the tobacco blend normalized to cumulative percent body weight gain relative to the body weight on the day prior to the onset of treatment. This normalization removes any influence of the differences in body weight between groups at the initiation of the study produced by the one week delay in the start of the study. It thus provides the clearest picture of the effects of the different feed formulations on changes in body weight. The delay resulted from redesign of the feeding cups to minimize feed waste.

The control group fed the NTP-2000 feed with no additions demonstrated a growth curve with the expected increases in cumulative percent body weight gain for male mice of this age. However, the quantitative mean cumulative body weight gain data may be somewhat less than expected. There were five mice in the control group. One of these mice (mouse no. five) did not demonstrate the expected body weight gain. The mean increase in body weight during the study for the other four mice was 3.76 $\pm$ 0.83g while the increase in body weight for mouse no. five was 0.35 g. This single mouse decreased the group mean body weight and increased the standard deviation for the control group. The clinical observations associated with this mouse provided no evidence as to the reason for its lack of normal body weight increase.

Addition of the tobacco blend at 0.2-20 mg nicotine/kg bw/day produced body weight gain data similar to that of the control group in a non-dose dependent manner. The quantitative differences seen between these dose groups are believed to be within the normal variation associated with body weight gain measured using a small number of animals. However, the data from the group fed feed containing the tobacco blend at 40 mg nicotine/kg bw/day are indicative of a trend toward a diminished cumulative body weight gain compared to the control group. Each day of the study body weight gain in this group was approximately 50% or less of that seen in the control group. Even though diminished, body weight gain increased throughout the study indicating that the mice at this dose were consuming their feed at a rate that allowed increased body weight gain, although at a slower rate than the control group. This may indicate that the mice could detect the presence of the tobacco blend in the feed but the palatability of the feed was not adverse enough to reduce feed intake to an extend that resulted in a loss of body weight.

Group mean body weights in grams and their associated standard deviations are presented in Figure 2. Individual body weights, group mean body weights and body weight gains and their standard deviations are presented in Appendix VII of this report.

Figure 2



*Data represent the group mean daily body weight ± standard deviation. Exposure day zero represents the body weights before exposure to the dosed feed. Exposure day one represents data acquired after one day of exposure to the untreated control feed or feed formulated with the tobacco blend. Body weights were not determined on day 15, resulting in a lack of data for day 14.

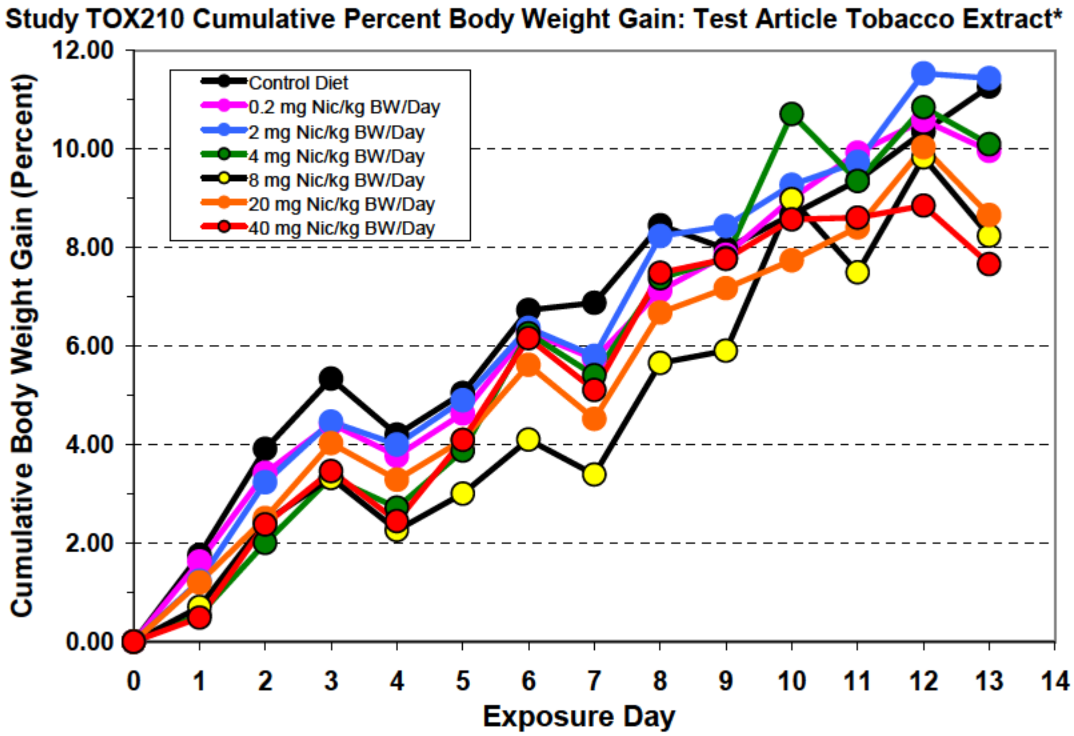
As seen in Figure 2, the control group demonstrated anticipated body weight gains for male mice of this age with the exception of one mouse in the control group, as previously noted. These data also illustrate the drift in body weight seen at the initiation of feeding the formulated diets resulting from the delay in feeding the formulated diets. This resulted in a number of the dose groups having higher mean body weights than the control group during the period of study. This confounds the comparison of the different treatment groups when the data are expressed as body weight in grams, as previously noted.

These data indicate that the mice could detect, either at an organoleptic level or at a neurophysiological level, the presence of the tobacco blend at the high dose used in this study, as seen with the cumulative percent body weight gain data. While these data do not provide evidence for a dose that would produce unwarranted reductions of body weight, they do indicate that a dose of 40 mg nicotine/kg bw/day could be a potential lower dose for additional studies.

At least two possibilities should be considered in respect to understanding the diminished cumulative percent body weight gain seen at the 40 mg/kg bw/day dose. First, at an organoleptic level, the mice may consider diets containing the tobacco to lack palatability and consume them at a lower rate and amount than the control diet. As the dose increased the palatability of the feed became lower resulting in less feed consumption with the resulting decrease in body weight gain. Second, at the neurophysiological level, it is possible that the nicotine in the tobacco blend produced nicotinic effects in the peripheral or central nervous systems that were undetected in this palatability study. These effects could have produced an appetite depression or other effect that may have altered feed intake and resulting body weight gain.

Cumulative percent body weight gain data for mice fed the feed formulated with the tobacco extract are presented in Figure 3.

Figure 3

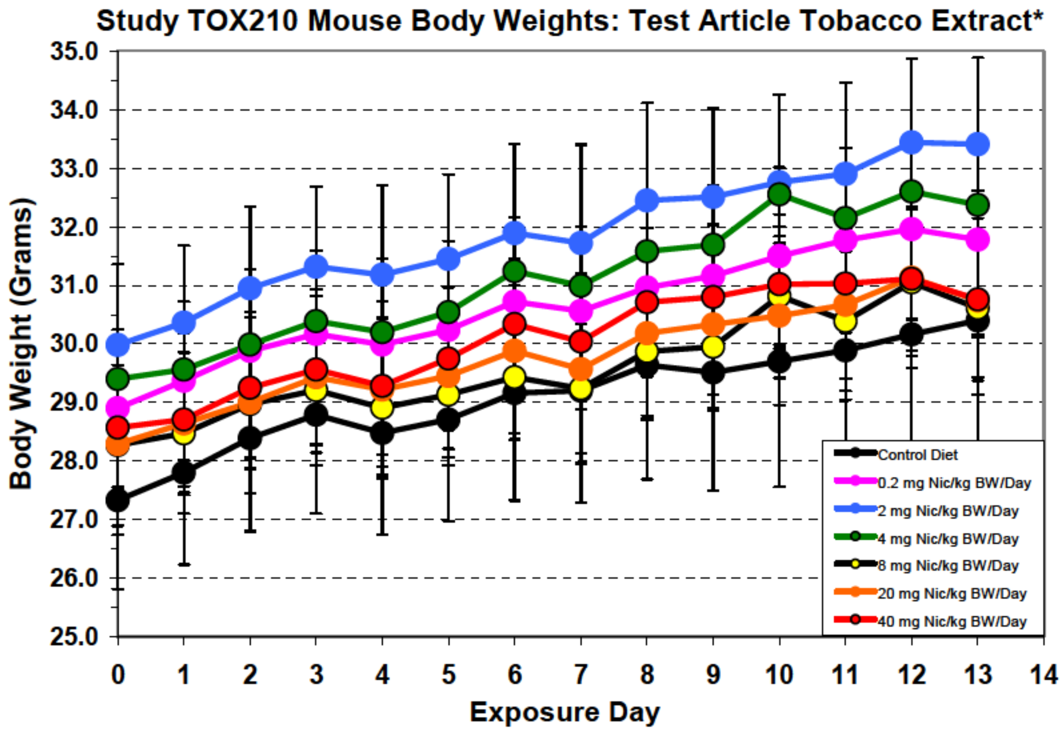


*Data represent the mean cumulative body weight gain expressed as a percent of the initial body weight. Exposure day zero represents the body weight of the mice before being exposed to the dosed feed. Exposure day one represents data acquired after one day of exposure to the untreated control feed or feed formulated with the tobacco extract. Body weights were not determined on day 15 after 14 days of exposure resulting in a lack of data for day 14.

Unlike the tobacco blend there is no dose that shows a definitive trend toward reduced percent body weight gain. The reasons for the different response compared to the tobacco blend are not apparent from the results of this study.

Figure 4 provides the data for body weights in grams throughout the study. The trends in body weight follow those seen in the percent body weight gain data; however, they are shifted because the differences seen in group mean body weights differed on the first day of the study. By the end of the study, there were no statistical differences in terminal body weight among the control and the treatment groups.

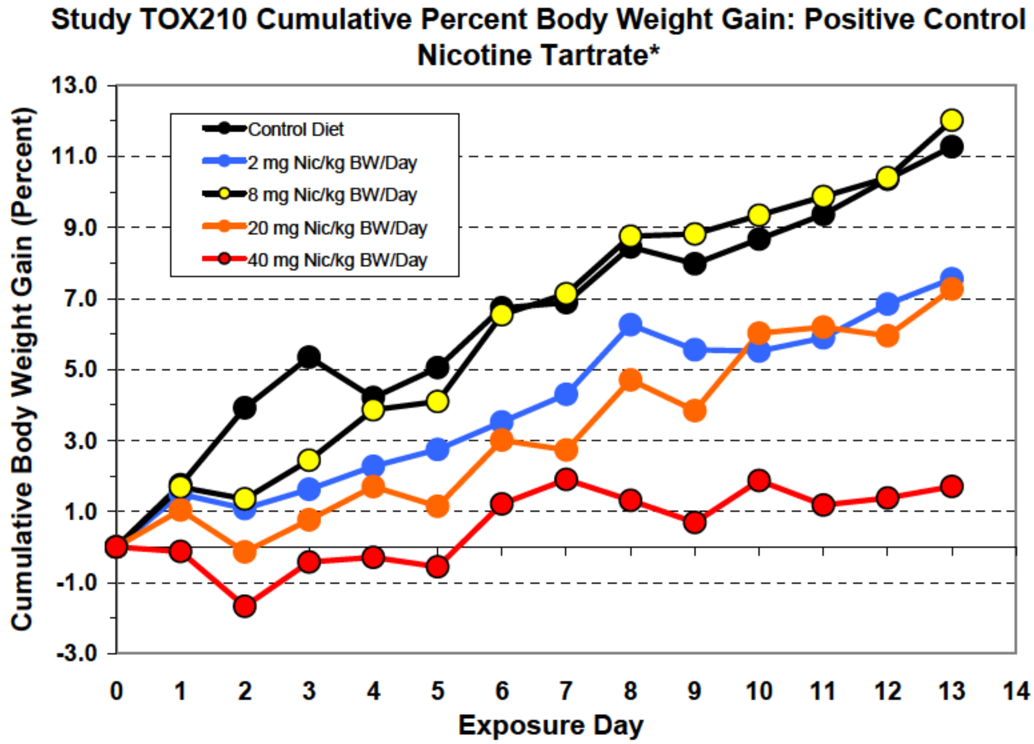
Figure 4



*Data represent the group mean daily body weight ± standard deviation. Exposure day zero represents the body weights before exposure to the dosed feed. Exposure day one represents data acquired after one day of exposure to the untreated control feed or feed formulated with the tobacco extract. Body weights were not determined on day 15, resulting in a lack of data for day 14.

Figure 5 provides the cumulative percent body weight gain data for mice fed diets containing the positive control, nicotine hydrogen tartrate.

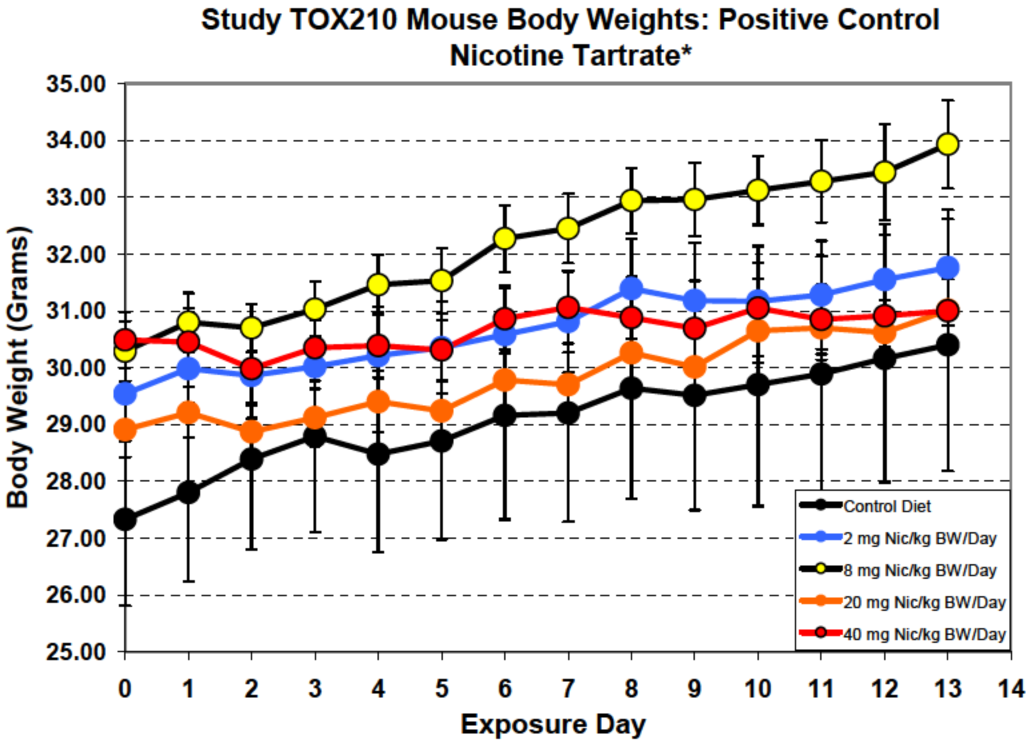
Figure 5



*Data represent the mean cumulative body weight gain expressed as a percent of the initial body weight. Exposure day zero represents the body weight of the mice before being exposed to the dosed feed. Exposure day one represents data acquired after one day of exposure to the untreated control feed or feed formulated with nicotine tartrate. Body weights were not determined on day 15 after 14 days of exposure resulting in a lack of data for day 14.

Mice fed feed containing nicotine tartrate demonstrated a definitive dose response in cumulative percent body weight gain at the 40 mg nicotine/kg bw/day doses. At the high dose there was no increase in percent body weight gain until day six of the study and from day six to study termination there was little change in percent body weight gain at this dose, while the control demonstrated an anticipated increase in percent body weight gain. At 20 mg nicotine/kg bw/day, there was a lag of 2-3 days before body weight gain began to increase. At this dose, body weight gain continued to increase at a rate similar to the control throughout the remainder of the study but remained quantitatively below that of the control. The 2 mg nicotine/kg body weight/day dose followed a trend similar to the 20 mg nicotine dose but remained quantitatively below that of the control. At 8 mg nicotine/kg body weight/day, there was a depression in body weight on days 2 and 3 compared to the control while on day 4 body weight was equivalent to the control. During the second week of the study, this dose group's mean body weight was similar, if not slightly higher, than that of the control group. Nicotine hydrogen tartrate produced a somewhat greater effect at 40 mg nicotine/kg body weight that did the tobacco blend. The overall trends in the data follow those seen with the tobacco blend as opposed to the tobacco extract. Whether or not this is due to a lack of palatability of the feed or to a physiological effect of nicotine can not be ascertained from this study.

Figure 6



*Data represent the group mean daily body weight ± standard deviation. Exposure day zero represents the body weights before exposure to the dosed feed. Exposure day one represents data acquired after one day of exposure to the untreated control feed or feed formulated with the tobacco extract. Body weights were not determined on day 15, resulting in a lack of data for day 14.

Because the nicotine tartrate dosed group contained no tobacco, these data may indicate that the decreased percent body weight gains seen in this study in the various treatment groups may be more associated with their nicotine content than with the presence of other tobacco components.

6. Terminal Body Weights

Group mean terminal body weights at study termination are provided in Table 15. Individual animal terminal body weights are presented in Appendix IX.

Table 15: **Terminal Body Weights**

Group	Treatment		Terminal Body Weight (g) ± SD
1	NTP-2000 Feed		30.40 ± 4.96
	Smokeless Tobacco Blend		
2	Dose 1 Tobacco in NTP-2000 Feed	(0.2)[1]	30.38 ± 2.63
3	Dose 2 Tobacco in NTP-2000 Feed	(2.0)	31.13 ± 4.08
4	Dose 3 Tobacco in NTP-2000 Feed	(4.0)	31.60 ± 3.12
5	Dose 4 Tobacco in NTP-2000 Feed	(8.0)	29.85 ± 1.46
6	Dose 5 Tobacco in NTP-2000 Feed	(20.0)	30.40 ± 3.07
7	Dose 6 Tobacco in NTP-2000 Feed	(40.0)	29.95 ± 2.12
	Tobacco Extract		
8	Dose 1 Tobacco Extract in NTP-2000 Feed	(0.2)	31.78 ± 3.63
9	Dose 2 Tobacco Extract in NTP-2000 Feed	(2.0)	33.41 ± 3.30
10	Dose 3 Tobacco Extract in NTP-2000 Feed	(4.0)	32.37 ± 2.79
11	Dose 4 Tobacco Extract in NTP-2000 Feed	(8.0)	30.61 ± 2.65
12	Dose 5 Tobacco Extract in NTP-2000 Feed	(20.0)	30.74 ± 3.58
13	Dose 6 Tobacco Extract in NTP-2000 Feed	(40.0)	30.76 ± 3.10
	Positive Control		
14	Dose 1 Nicotine Tartrate in NTP-2000 Feed	(2.0)	31.76 ± 2.26
15	Dose 2 Nicotine Tartrate in NTP-2000 Feed	(8.0)	33.93 ± 1.73
16	Dose 3 Nicotine Tartrate in NTP-2000 Feed	(20.0)	31.01 ± 1.39
17	Dose 4 Nicotine Tartrate in NTP-2000 Feed	(40.0)	31.00 ± 1.25

[1]Nicotine doses in mg nicotine/kg body weight/day are provided in parentheses.
*Statistically significant from Group 1 NTP-2000 Feed ($p \leq 0.05$).

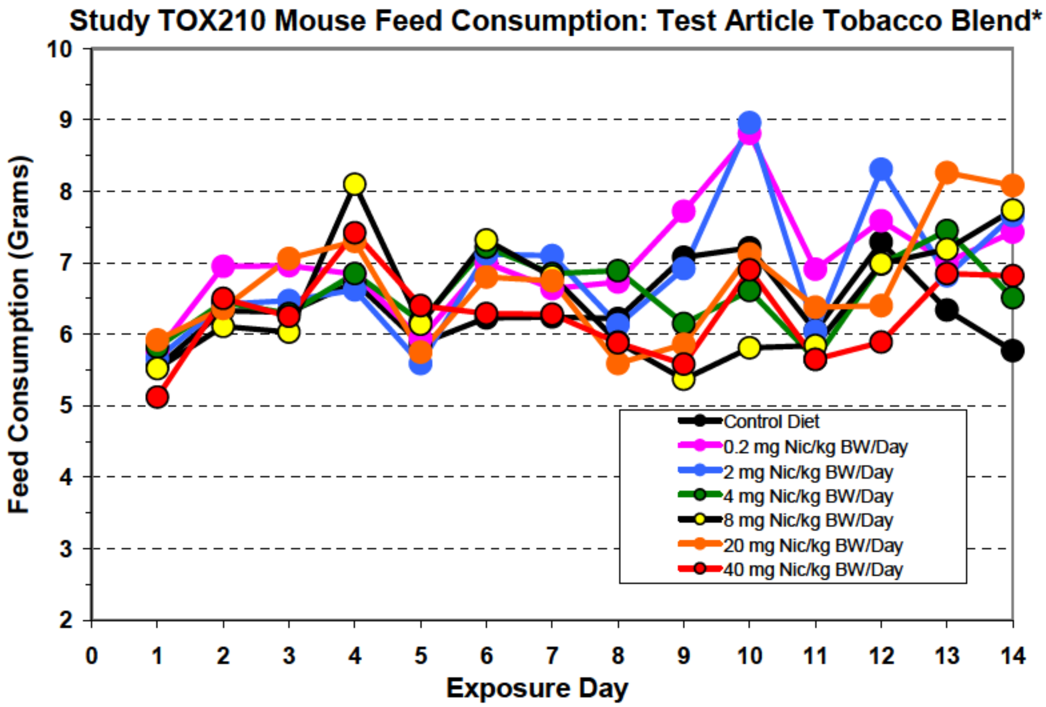
Terminal body weights determined on the last day of the study showed no statistically significant differences when compared to the control group for any of the groups fed feed containing the tobacco blend, the tobacco extract or nicotine hydrogen tartrate. The lack of statistical difference between the mice fed the high dose diets is associated with the high standard deviation produced by the single mouse in the control group that did not demonstrated the anticipated increases in body weight compared to the other members of this group.

Feed Consumption

Determination of the feed consumption of rodents fed powdered feed is notoriously difficult, especially for young mice and rats. These animals have a tendency to spill significant quantities of feed through playful exploratory activities and while feeding. Even though attempts were made to minimize spillage in this study, the feed consumption data can only be considered estimates. Daily feed consumption data are provided in Appendix X. Feed consumption data for mice fed feed containing the tobacco blend, tobacco extract or nicotine hydrogen tartrate are shown in Figures 7-9.

Feed consumption data for the mice provided feed formulated with the smokeless tobacco blend are provided in Figure 7.

Figure 7

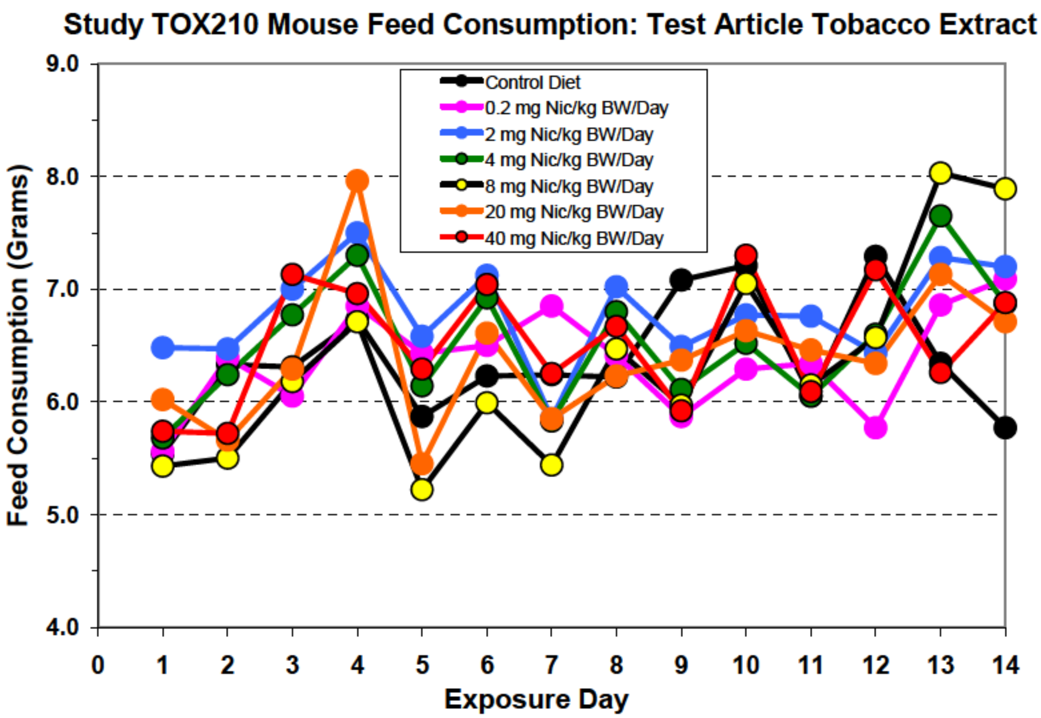


* Data represent the group mean feed consumption for mice fed different doses of nicotine in feed formulated with the tobacco blend.

Data for feed consumption for the mice in this study appear erratic irrespective of which feed they were provided, including the control group. Feed consumption in the groups provided feed formulated with the smokeless tobacco blend do not demonstrate any definitive trends compared to the control group. This is true even at the 40 mg nicotine/kg bw/day dose where there appeared to a slight decrease in cumulative percent body weight gain. This indicates that the inclusion of the tobacco blend at these doses did not affect the palatability of the feed to an extent that produced a detectable reduced feed intake for the mice.

Feed consumption data for the mice fed feed containing the tobacco extract are shown in Figure 8. As seen with the tobacco blend, the feed consumption data for the tobacco extract appears erratic with no obvious dose related trends. As seen with the tobacco blend, inclusion of the tobacco extract at the doses used in this study does not alter the palatability of the feed to an extent that produced a detectable reduction in the feed intake of the mice.

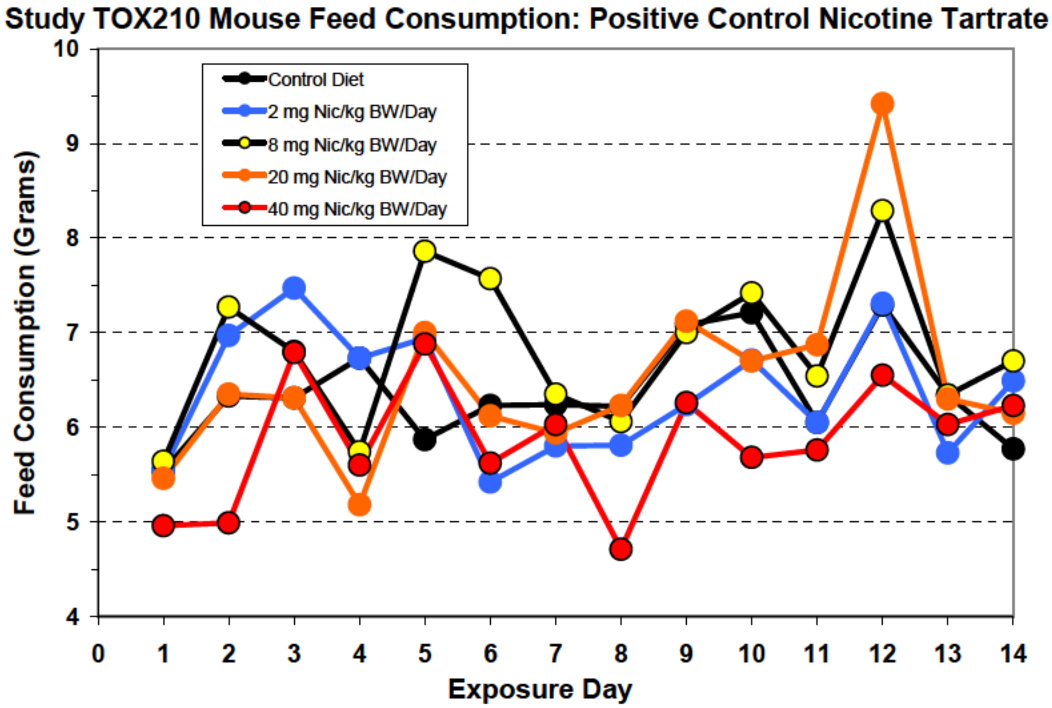
Figure 8



* Data represent the group mean feed consumption for mice fed different doses of nicotine in feed formulated with the tobacco extract.

Feed consumption data for the mice fed feed containing nicotine hydrogen tartrate are shown in Figure 9.

Figure 9



* Data represent the group mean feed consumption for mice fed different doses of nicotine in feed formulated with nicotine hydrogen tartrate.

As seen with the tobacco blend and the tobacco extract, the data for feed consumption for the mice dosed with nicotine hydrogen tartrate appear erratic. It is not possible to discern a definitive dose related trend in these data, even at the high nicotine dose. As seen with the tobacco blend and the tobacco extract, the addition of nicotine hydrogen tartrate to feed provided the mice did not alter its palatability to an extent that resulted in a detectable decrease in feed consumption.

DISCUSSION

Review of the feed formulation nicotine analysis data indicates that the formulation methodology developed for this study produced feed containing different and appropriate concentrations of the smokeless tobacco blend, aqueous extract of the tobacco blend and nicotine hydrogen tartrate suitable for use in this investigation study. Utilization of a trial feed formulation provided insight into improved technical changes to the mixing methodology. Investigation of the homogeneity of added materials within the NTP-2000 powdered rodent feed indicated the test articles and positive control were homogenously mixed within the feed to an extent that avoided the occurrence random areas of either low or higher concentrations different from the anticipated concentrations. This indicates the mice were provided dosed feed that produced consistent doses during the study. Analytical determinations of nicotine content in the feed produced for the various doses and test articles, including the positive control, indicated the formulated feeds provided appropriate doses of nicotine and tobacco components to the mice. To determine the stability of the test articles and positive control, measured as nicotine, to assess the needed frequency of preparation of the formulated feed, the stability of formulated feed stored at room temperature was ascertained. This study revealed the formulated feed was stable for at least ten-days. This confirmed the adequacy of the preparation of formulated feed weekly. An additional stability investigation indicated that the formulated feed was stable for at least one-month when stored at room temperature. This indicates that future studies would not require weekly feed formulations.

Overall, this preliminary investigation provided evidence of the adequacy of the dosed feed formulation methodology for this study in respect to homogeneity, confirmation of anticipated dose and formulated feed stability for this palatability study.

The male, CFW Swiss Webster mice used in this study were maintained under protocol specified conditions throughout the study and the data related to room temperature, humidity, room air exchanges and water quality demonstrated that there were no excursions that could impact the results of the study. In addition, serological testing of the sentinel mice indicated no antibodies to disease while pathological examination of the lungs indicated no evidence of the presence of contagious disease. These investigations provided data indicative that the environmental conditions and the health of the animals were appropriate for this study.

Evaluation of the body weight data from this study indicates that mice are highly resistance to body weight changes compared to the decreased body weight gains seen in rats at the higher doses when fed identical nicotine doses (TOX209). The control mice in this study demonstrated the expected body weight gains throughout this study, with the exception of a single mouse, indicating that there were no conditions that could unduly affect body weight during the study.

The effect on body weight produced by feeding diets formulated with the test articles and positive control is clearly seen when expressed as cumulative percent body weight gain. Mice provided feed formulated with the tobacco blend at doses of 0.2-20 mg nicotine/kg bw/day did not demonstrate dose related trends in body weight gain that were clearly different from that of the untreated control group. At a dose of 40 mg nicotine/kg bw/day, the tobacco blend produced a consistent trend toward reduced body weight gain that indicates a slight test article related effect. However, the final body weights at 40 mg nicotine/kg bw/day were not statistically different from control. This lack of statistical significance is, in most part, related to the large standard deviation seen in the control resulting from a single control mouse that did not demonstrate the usual gain in body weight.

Cumulative percent body weight gain in mice fed feed containing the tobacco extract at doses equivalent to those fed the tobacco blend did not demonstrate a definitive change in body weight gain at any dose. The reasons for the lack of concordance between the tobacco blend and the tobacco extract are not evident from the results of this study.

When mice were fed feed formulated with nicotine hydrogen tartrate, the trends seen in cumulative percent body weight gain at doses of 2 and 8 mg nicotine/kg bw/day appear not different from the control and there was no dose response. In contrast, at 40 mg nicotine/kg bw/day there is a definitive reduction in percent body weight gain. At 20 mg nicotine/kg bw/day, the trend is not as definitive and from day 10 of the study did not differ from that seen at 2 mg/kg/day. This may indicate that an initial aversion to the feed by the mice is overcome.

Evaluation of the data from this study indicates that mice fed the tobacco blend or tobacco extract at quantities that yield nicotine as high as 20 mg nicotine/kg bw/day show little or no effect in any of the variables determined in this study, especially cumulative percent body weight gain. At a nicotine dose of 40 mg/kg bw/day percent body weight gain tended to decrease with the tobacco blend and nicotine hydrogen tartrate but no definitive trend was seen with the tobacco extract. These data are in sharp contrast to those obtained with rats at identical nicotine doses (TOX209). This indicates that mice demonstrate less susceptibility to the reduced body weight gain seen with rats at identical doses.

The recommendation based upon the results of this study is that it should be repeated using higher doses of nicotine and that the low dose for a repeat of this study be 40 mg nicotine/kg bw/day. In addition, the number of control animals should be increased from five to ten to ensure a determination of statistical differences.

X. ACKNOWLEDGMENTS

The study director would like to acknowledge the following individuals for their efforts on this study: Ms. Jenny L. Smith, the original Study Director, Ms. Susan Pike, for her assistance in feed formulation, the Research Resources staff for their excellent conduct of the in-life portion of the study; Ms. Karen B. Kilby, Mr. Timothy A. Ellisor, Dr. Gary Byrd and others who conducted the chemical analysis for this study and Ms. Jessica Baker for her coordination of the animal resources staff involved on this study. In addition, the efforts of Dr. Chandra D. Williams, D.V.M., Attending Veterinarian are acknowledged.

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TOX209 Two Week Investigational Study of the Palatability of Smokeless Tobacco Blend and Extract Formulated in NTP-2000 Diets for Rats.

Appendix I

Study Protocol, Amendments to Protocol, Notes to Study File

RJReynolds

Research and Development
Preclinical Models of Disease
In Vivo Toxicology Division
Study Protocol

Protocol Identifier: TOX210

Investigational Study of the Palatability of Smokeless Tobacco Test Articles Formulated in NTP-2000 Diets for Mice

Scientist III,
Preclinical Models of Disease
In Vivo Toxicology:
Study Director:
Jenny L. Smith
Jenny L. Smith, B.S.
Date: 4-3-08

Senior Principal Scientist,
Preclinical Models of Disease
In Vivo Toxicology:
Chairman, Institutional Animal Care and Use Committee:
Paul H Ayres
Paul H. Ayres, Ph.D., DABT
Date: 3-31-08

Attending Veterinarian:
Preclinical Models of Disease
In-Vivo Toxicology
Chandra D. Williams, D.V.M.
Date: 3/31/08

Senior Director,
Stewardship:
Charles D. Garner, Ph.D., DABT
Date: 4/2/08

Senior Staff Toxicologist,
Stewardship:
Theophilus
Suzana Theophilus, Ph.D., DABT.
Date: 3/31/08

Senior Director;
Preclinical Models
Of Disease:
Natalie P Takenaka
Natalie Takenaka, Ph.D.
Date: 3 Apr 08

Vice-President,
Product Integrity:
Christopher J. Cook, Ph.D.
Date: 4/4/08

Anticipated Mouse Delivery Date: April 9, 2008
Anticipated Final Report Date: November 5, 2008

Table of Contents

Facilities and Administration

Sponsor

R. J. Reynolds Tobacco Company (RJRT)
Research and Development
Product Integrity
Bowman Gray Technical Center
Winston-Salem, NC, 27102

Testing Facility

R. J. Reynolds Tobacco Company
Research and Development
Preclinical Models of Disease
In-Vivo Toxicology Division
Building 630-2 Winston-Salem, NC, 27102

Contractors

Charles River Laboratories *Wilmington, MA*	Serology
Research Resources of North Carolina, Inc. *On-site*	Animal Husbandry and Quality Assurance

Study Administration

Study Director, *In Vivo* Toxicology	Jenny L. Smith, B.S.
Attending Veterinarian, Preclinical Models of Disease	Chandra D. Williams, D.V.M.
Program Manager, Research Resources of North Carolina, Inc.	Jessica Baker, B.S., LAT
Senior Principal Scientist, *In Vivo* Toxicology	Paul H. Ayres, Ph.D., DABT
Senior Director, Preclinical Models of Disease	Natalie Takenaka, Ph.D.
Vice-President, Product Integrity	Christopher J. Cook, Ph.D.

Investigational Study of the Mouse Palatability of Smokeless Tobacco Test Articles Formulated in NTP-2000 Diets

Executive Summary

A series of in vivo toxicology studies to investigate the potential toxicity of a blend of tobaccos and an aqueous extract of this tobacco blend along with a positive control (nicotine hydrogen tartrate) are planned to be sponsored by R. J. Reynolds Tobacco Co. and to be conducted in a Contract Research Organization. The route of exposure to the mice is oral through a formulation of the test articles into NTP-2000 feed to be provided to the mice. This raises a fundamental question concerning the acceptance of the formulated feed by the mice. Addition of the test articles to the feed may make it unpalatable to the mice. An unpalatable diet would severely compromise the results from the planned studies. The current study is designed to determine the palatability of formulated diets each of which contains one of the test articles or the positive control by comparison to the control diet. This will be accomplished by feeding the formulated diets and the control diet to Swiss Webster mice (the strain to be used in the toxicology studies) that are closely matched to the age of the mice to be used in the toxicology studies. Feed consumption and body weight will be determined during the 14-day study to assess what effect, if any, addition of the test articles to the feed has on its palatability and acceptance by the mice. Conducting the study in our laboratory will significantly decrease any potential delay in initiating the planned studies and the chance of obtaining data from these studies that are not useful. It will also produce data to allow the design of studies to overcome any potential problems associated with lack of palatability of the formulated diets.

Quality Assurance

As a preliminary investigational study, this study will not be subject to Quality Assurance (QA) review. All individuals assigned to the study will be properly trained in the performance of procedures identified as essential. Individual training records will be maintained according to the training program specified by the RJRT *In vivo* Toxicology Division.

Objective

The objective of this study will be to evaluate the palatability of diets formulated in NTP-2000 feed with a smokeless tobacco blend, an aqueous tobacco extract of the smokeless tobacco blend and nicotine tartrate as positive control when fed to Swiss Webster mice.

Experimental Design

A smokeless tobacco blend and an aqueous extract of the smokeless tobacco blend will be tested in a series of toxicology studies to be conducted in a Contract Research Organization (CRO) by RJRT. Also, a positive control, nicotine hydrogen tartrate will be used in some of the planned studies. The tobacco blend and aqueous tobacco extract test articles and the positive control will be incorporated into the mouse's feed (non-certified, NTP-2000 manufactured by Zeiglar Brothers, Inc., Gardners, PA). There is the possibility that incorporation of the test articles and positive control in the feed may alter its palatability to the mice. If the feed is less palatable than the control diet, the mice may consume less feed with a resulting decrease in body weight gain. This would also result in lower than anticipated doses during these studies. Therefore, it is necessary to ascertain the palatability of the dosed feed to mice. The time frame for the studies in the CRO is short to produce the required data at an appropriate time. A preliminary investigational study of the palatability of the diets will be conducted in RJRT facilities to expedite the CRO studies.

Palatability will be assessed by comparing the feed intake of mice fed the standard NTP-2000 diet (control group) to the feed intake of mice fed NTP-2000 diets formulated to contain different doses of the tobacco blend and different doses of the tobacco extract as well as the different doses of the positive control. Feed intake will be measured daily during the 14 day study. In addition, the body weights of the mice fed the control NTP-2000 diet will also be determined daily. Twice daily mortality and morbidity observations will be conducted on all study mice as will twice weekly standard clinical observations. No additional data will be collected. The duration of the feeding and data collection period is 14 days. The data from this study will be provided to the CRO for use in the planning and conduct of subsequent studies.

The experimental groups and the number of mice per group are provided in the following table:

Group Number	*Treatment Group (Doses based on Nicotine) (mg/kg body weight/day)*	*Number of Mice*	*Mouse ID Numbers*
	Control		
1	NTP-2000 feed	5	1-5
	Smokeless Tobacco Blend		
2	Dose 1 Tobacco in NTP-2000 feed (0.2)	5	6-10
3	Dose 2 Tobacco in NTP-2000 feed (2.0)	5	11-15
4	Dose 3 Tobacco in NTP-2000 feed (4.0)	5	16-20
5	Dose 4 Tobacco in NTP-2000 feed (8.0)	5	21-25
6	Dose 5 Tobacco in NTP-2000 feed (20.0)	5	26-30
7	Dose 6 Tobacco in NTP-2000 feed (40.0)	5	31-35
	Tobacco Extract		
8	Dose 1 Tobacco Extract in NTP-2000 feed (0.2)	5	36-40
9	Dose 2 Tobacco Extract in NTP-2000 feed (2.0)	5	41-45
10	Dose 3 Tobacco Extract in NTP-2000 feed (4.0)	5	46-50
11	Dose 4 Tobacco Extract in NTP-2000 feed (8.0)	5	51-55
12	Dose 5 Tobacco Extract in NTP-2000 feed (20.0)	5	56-60
13	Dose 6 Tobacco Extract in NTP-2000 feed (40.0)	5	61-65
	Positive Control		
14	Dose 1 Nicotine Tartrate in NTP-2000 feed (2.0)	5	66-70
15	Dose 2 Nicotine Tartrate in NTP-2000 feed (8.0)	5	71-75
16	Dose 3 Nicotine Tartrate in NTP-2000 feed (20.0)	5	76-80
17	Dose 4 Nicotine Tartrate in NTP-2000 feed (40.0)	5	81-85
	Sentinels		
	Sentinels (no treatment)	10	86-95

The doses to be used for the study are based upon doses used in a previous study of a tobacco product and the published literature. They are expected to bracket the anticipated doses to be used in a short-term repeated dosing study in the laboratory of the CRO. No undue toxicity is expected at these doses.

Experimental Use of Mice

This protocol was prepared with reference to ***SOP DAT030*** "Preparing Research Protocols".

Duplication of the Study

To determine if this proposed study duplicates any previous studies, a literature search was conducted (Appendix 2). The literature revealed one published study where snus tobacco was incorporated into the feed of mice (Stenstrom, et al., 2007). Although the data were not adequately reported, the authors noted that a short-term pilot study had indicated when snus tobacco was incorporated into the feed of the mice, body weight was decreased (data not provided). The relevance of this study to the present study cannot be ascertained because the feed was not the same, the tobacco was not the same, dosing was based on grams of tobacco instead of nicotine and no tobacco extract was used in the study nor was a positive control employed. Therefore, the current study is not a duplication of this study.

Rationale for the Use of Animals

The rationale provided by the National Research Council (NRC, 1988) for using animal studies to evaluate human health risk is that all mammalian species generally possess similar genetic, biochemical and physiologic characteristics; similarities extend to toxification and detoxification mechanisms, as well as to target sites for the adverse effects of toxicants. This study is designed to determine the palatability of the formulated diets. There is no known in vitro methodology to determine the palatability of rodent diets containing specific test articles to mice; therefore, mouse studies are necessary. In addition, there are no viable, relevant, and/or sufficiently validated alternate systems for comparing the potential palatability of rodent diets containing test articles to rodent diets without test article.

Animal Selection and Justification for Test System

Mice have been classically used in toxicology studies and mouse studies are required and accepted by U.S. regulatory agencies as well as international regulatory agencies. Specifically, mice are the animal model chosen for this study because they will be used in a toxicology program to be conducted at a CRO and sponsored by RJRT. This study is designed to provide preliminary information to the CRO for the design and planning of their studies.

Selection of the Swiss Webster Mouse

The Swiss Webster mouse, a hardy out bred strain, will be used in a toxicology assessment program in a CRO under development by RJRT. Since this study is a preliminary investigation that will support this toxicology assessment program, it is necessary to use the Swiss Webster mouse strain in this study.

Justification for Areas of Investigation

The test articles to be investigated in this preliminary investigational study will be used in a toxicology assessment program in a CRO sponsored by RJRT that will encompass both short-term and long-term studies. The test articles and positive control will be incorporated into the diets for the mice in these studies. This presents the possibility that they will alter the palatability of the diets to the extent that the mice will consume lower quantities of their feed. Lower feed consumption and the resulting lower body weight gain will complicate the interpretation of the data from these studies. This study is designed to determine the palatability of these formulated diets in a dose dependent manner compared to control feed and will provide important information for the design of the upcoming toxicology studies.

Animal Requirements

The number of mice to be used is the minimum associated with meaningful statistical analyses of the data. A minimum of 90 male, Swiss Webster, juvenile mice (5-7 weeks of age) will be received from Charles River Laboratories (Raleigh, NC) for conduct of this study. Assigned to the TOX209 Xybion protocol will be 85 male mice for the experimental groups, as well as 10 male mice (retired breeders) to be used for health screening and sentinels [***SOP TOX061***]; assessments of the sentinel population will occur at the conclusion of the feeding study. The design for the current study uses five male mice/study group and uses six dose groups for each of the tobacco test articles and a control group fed diet without the addition of test article. The positive control group uses four dose groups consisting of five male mice each. Extra mice (five male) will be utilized during the allocation and randomization process, i.e., to ensure that an adequate number of healthy animals are available for placement onto the study in the event that any of the mice demonstrate abnormal clinical signs, or die unexpectedly (e.g., are euthanized for humane reasons) during the quarantine/acclimation period. Any additional animals shipped by the vendor in excess of the number ordered will be used for studies approved by the Institutional Animal Care and Use Committee (IACUC) or euthanized using 70% carbon dioxide (CO_2) in air [***SOP TOX057***]. The final fate of each animal will be documented.

Quarantine/Acclimation and Serological Evaluation

Mice received into the facility (***SOP TOX015***) will be quarantined for a minimum of 7 days under conditions simulating those of the study (***SOP, TOX012***). All mice will be assigned a pre-allocation identification number, and that number will be indicated on the corresponding cage card. The pre-allocation identification number will include a coded reference to the age of the mouse.

At the termination of the 14-day feeding period, the 10 sentinel mice will be euthanized (see "Euthanasia," below) for health screening (***SOP TOX010***). Sera will be processed for routine measurement of the following antibodies to disease: Pneumonia virus of mice (PVM), Sendai virus (SEND), Minute Virus of Mice (MVM), Mouse Parvovirus (MPV)*1&2, REO-3, *Mycoplasma pulmonis* (MPUL), Lymphocytic choriomeningitis virus (LCMV), Mouse Adenovirus (MAV) 1&2, Hantaviruses (HANT), *Encephalitozoon cuniculi* (ECUN), Cilia Associated Respiratory Bacillus (CARB), K virus, GDVII (Murine Encephalomyelitis Virus), Mouse Hepatitis Virus (MHV), Ectromelia (Mousepox), Polyoma Virus, Epizootic Diarrhea of Infant Mice Virus (EDIM), Mouse Cytomegalovirus (MCMV), Mouse Thymic Virus (MTLV), and Murine Norovirus (MNV).

Mice euthanized for serological evaluation will then be necropsied to determine any evidence of disease. The carcasses of these mice will be stored frozen in airtight plastic bags until they are disposed of via a North Carolina-certified, medical waste-disposal firm (***SOP ADM002***) or other approved method.

The Attending Veterinarian will perform a health examination of all mice within four days after delivery. Commencement of mouse dosing is dependent upon a favorable review of the health examination, as well as a written statement from the Attending Veterinarian releasing the mice from quarantine.

Allocation of Animals to Study Groups

On the fourth day of the quarantine/acclimation period, mice will be assigned to dose groups according to body weight using the "A" module of the PATH/TOX software (version 4.2.2; Xybion Medical Systems; Cedar Knolls, NJ) (***SOPs TOX042, TOX068***). Body weights and detailed clinical signs will be recorded prior to conducting the allocation process. At the discretion of the Study Director, mice exhibiting positive clinical signs, demonstrating body weight loss (since the initial weighing), or representing low or high extremes of body weight may be excluded from the allocation process. Mice not selected during the allocation process will either be transferred to another IACUC-approved protocol, or euthanized using 70% CO_2 in air (***SOP TOX057***). The final fate of each mouse will be documented.

To ensure groups of similar mean body weight, all groups within the PATH/TOX protocol will be compared by analysis of variance (ANOVA) and least significant difference criteria, and demonstrated not to be significantly different at a 5 percent, two-sided risk level. Following allocation into groups, mice will be uniquely identified with their permanent identification number by tail tattoo [***SOP TOX041***]. Mice will be assigned to cages with permanent cage cards attached, recording the study number, Study Director's name, species of the animal, sex of the animal, group number, pre-allocation animal number, and the animal's permanent identification number (***SOP TOX068)***.

Animal Husbandry

Animals will be housed and cared for in accordance with the Institute of Laboratory Animal Research (ILAR), Commission of Life Sciences, National Research Council document entitled, *Guide for the Care and Use of Laboratory Animals* (1996).

The mice will be housed in a room of the vivarium with controlled lighting (12 hours of darkness, from 6:00 p.m. to 6:00 a m. +/- 30 minutes, Eastern Standard Time, except on days converting to and from daylight savings time), temperature (18-26ºC, or 64.4-78.8ºF), relative humidity (RH, 30-70%), and airflow (greater than 10 room air changes/hour). Seven-day, continuous chart-wheel recordings will be kept for room temperature and relative humidity (***SOP EQP064 or EQP019***). In addition, room airflow and light cycles will be monitored continuously and data recorded every 30 minutes to a computer file via an automated facility data collection system (***SOP DAT025***).

Mice will be individually housed in stainless-steel, wire-bottomed cages (3 ¾"W x 9"L x 5"H) suspended on stainless steel racks. Rack and cage maintenance will be conducted according to ***SOPs TOX016, TOX021, TOX022, TOX052, EQP002, EQP026, EQP027, EQP035, and EQP072.***

Mice will have *ad libitum* access to NTP-2000 feed, with the exception of the sentinel mice, which will be fed Lab Diet, Certified Rodent Diet #5002 feed (PMI Nutrition International), presented as pellets (***SOP TOX017***). Feed will be presented as a powdered diet formulated with the test articles, positive control or as a control diet with no test articles. Clean feeders will be provided daily, and feedlots monitored (***SOP TOX070***). Water will be provided to mice on an *ad libitum* basis through an automatic system (***SOP EQP048***). The water source originates from the municipal supply of the City of Winston-Salem, and is subsequently filtered through activated carbon and 5-micron particulate filters prior to mouse delivery. This water is analyzed semi-annually. There are no known contaminants expected to be present in the feed or water that would be anticipated to interfere with the outcome of the study.

Invasive Techniques

There are no invasive procedures anticipated during conduct of the present study (see "Survival Surgery", below).

Survival Surgery

No surgical interventions are planned during this study; hence, no survival surgery is scheduled.

Pain/Distress

Momentary pain and/or distress may be associated with the tail tattooing process; however, this procedure represents an acceptable identification method for rodents. Tail tattooing (***SOP TOX041***) will be performed without anesthesia or analgesics by trained technicians, in accordance with the tattoo equipment manufacturer's procedures. Tattooing will be performed after allocation to study groups.

Nicotine (a component of the test articles) may produce transitory toxicological effects in mice, including tremors, lethargy and increased sensory sensitivity; while unlikely, in some instances, the mice receiving the high doses may become prone and unresponsive, with an increased potential for death. In most instances, they will rapidly recover from these effects, which should diminish as the study progresses. During dosing and morbidity/mortality checks, mice are closely monitored by trained and experienced technical staff. While unlikely, if toxicological effects are excessive, the dosing regimen may be modified in consultation with the Attending Veterinarian and Study Director.

A literature search was conducted to identify potential alternatives to the test article exposure procedure, incorporating the principles of replacement, reduction and refinement. The keywords and databases searched (including periods covered) are provided in Appendix II. The literature search revealed mostly papers unrelated to the research focus. It was determined there were no *bona fide* alternatives identified that would replace, reduce, or refine the exposure procedure that would be consistent with the goals of this study.

Euthanasia

Sentinel mice will be anesthetized with 70% CO_2 in air and euthanized by exsanguination during serological evaluation (***SOPs TOX002, TOX004, TOX010***). Euthanasia by 70% CO_2 in air is used at terminal sacrifice and for mice in a moribund condition (***SOP TOX003***); this procedure is used to avoid any unnecessary pain or suffering.

Hazardous Materials and Safety

Hazardous Materials

The test articles which will contain nicotine will be stored in a refrigerator in Lab #78 until the study has been completed. The outside of the freezer will be labeled to indicate that it contains substances that pose a health-hazard. MSD sheets for all substances contained shall be attached to the outside of the freezer.

Because the test articles and positive control contain nicotine, there is some concern associated with breathing dust from the formulated diets containing the tobacco, tobacco extract or nicotine tartrate. Diet mixing will take place under a certified exhaust hood (***SOP EQP056***). The tobacco extract may present a hazard through skin exposure because nicotine can be absorbed through the skin. Therefore, gloves and safety glasses will be required along with appropriate attire to minimize the possibility of skin contact when working with the tobacco extract and positive control. Only the smallest quantities (of hazardous materials) needed for a particular procedure will be used. Excess material will be disposed of as described below.

Safety Procedures

Due to the use of materials with known and unknown toxic and carcinogenic potential, safety procedures will be employed for personal protection. These procedures adhere to the provisions of the RJRT R&D Chemical Hygiene Plan (*developed to comply with the OSHA Laboratory Standard, 29 CFR 1910.1450*). These include the use of protective clothing and eyewear, a certified exhaust of the test articles and the formulated diets containing the test articles (***SOP TOX150***).

During the diet mixing, two people will be present in case any direct exposures of personnel occur. In the event of any mishap (i.e., direct nicotine exposure), the individual will immediately wash the exposed areas with cold water for a period of no less than five minutes. While the injured person is washing the exposed area, the second person will call 1911 if it was determined that the injured person did in fact accidentally expose himself or herself.

Disposal of Contaminated Wastes

Disposal of chemical wastes, including feed not consumed by the mice, will be handled according to the RJRT R&D Chemical Hygiene Plan. Disposal of biohazard wastes will be handled according to ***SOP ADM002***.

Test Articles

Smokeless Tobacco Blend

The tobacco test article consists of natural tobaccos processed to a particle size suitable for mixing in the diet of the mice. It contains no additives and is adjusted to a typical water content. Information concerning the source, identity, processing and other characteristics of the tobacco test article will be on file. Because the tobacco is a complex mixture of natural components, its purity cannot be ascertained. The tobacco will be assayed for nicotine. The smokeless tobacco blend test article will be identified by Manufacture Date. A Material Safety Data Sheet for the tobacco test article will be made available. The test article will be stored frozen ($\leq 0^{o}$C). Before formulation of test article into the diet, an appropriate amount of the tobacco will be thawed at room temperature. An archival sample (~ 5 g) of tobacco used to formulate the mouse diets will be maintained frozen ($\leq 0^{o}$C).

Aqueous Tobacco Extract

The aqueous tobacco extract test article consists of a water extraction of the tobacco test article. It will contain no components not contained in the tobacco and the water used for extraction. Information concerning the identity, processing and other characteristics of the water extract will be on file. Because the tobacco extract is a complex mixture of natural components, its purity cannot be ascertained. The tobacco extract will be assayed for nicotine. The aqueous tobacco extract will be identified by Manufacture Date. A Material Safety Data Sheet for the tobacco extract test article will be provided. It will be stored frozen ($\leq 0^oC$). Quantities to be used for diet formulation will be thawed at room temperature before use. If after removal of the required aliquot for diet formulation, there is a significant amount of test article remaining, it should be subdivided into suitable aliquots for future use, and then re-frozen. An archival sample (~ 5 ml) of the extract used to prepare each diet formulation shall be maintained frozen ($\leq 0^oC$).

Positive Control

The test articles contain nicotine. Therefore, a positive control group will be fed diets containing nicotine hydrogen tartrate salt at a concentration equivalent to the nicotine concentration of selected doses of each test article. Nicotine hydrogen tartrate (98% purity) will be obtained from Sigma-Aldrich Co., St. Louis, MO. The nicotine free base is 35.1% of the bulk salt (2.85 g of salt contains 1 g of free nicotine). Mouse dosing will be based upon nicotine and not the bulk salt. A Certificate of Analysis and a Material Safety Data Sheet will be obtained from the supplier and maintained in the study file. The nicotine tartrate will be stored under conditions recommended by the supplier. An archival sample (~ 0.5 g) of the nicotine tartrate shall be maintained under the storage conditions recommended by the supplier.

Dosed Diet Formulation

The bulk NTP-2000 unformulated feed will be stored at refrigerator temperatures (approximately 4ºC) in Lab 95 before being aliquotted to the control group and before it is aliquotted to prepare the formulated feeds.

Diets will be formulated by the addition of the test article to a portion of the total diet to be formulated during a mixing process using a commercial mixer. This pre-mix will then be added to the bulk diet and mixed to obtain homogeneity. A preliminary test batch of diet formulated with each test article will be made to refine the techniques required and will not be provided to the mice. Upon satisfactory formulation, the technique will be used to prepare the diets to be used in the study. Diet formulation is planned to be conducted weekly during the study. Homogeneity will be determined by careful visual inspection of the diets. The formulated feeds will be stored at room temperature during their one week use. The control feed will be maintained identical to the formulated feed during each feeding period.

Test and Control Article Exposure

Dosing Regimen

The mice, with the exception of the sentinel group, will be provided NTP-2000 diets during the acclimation period. On day one of the study each experimental group will be provided the NTP-2000 diet with the appropriate quantity of test article or positive control mixed in the diet. Fresh feed and clean feeders will be provided daily throughout study. The formulated diets will be fed for a period of 14 days. All diets during the acclimation period and during the study period will be fed *ad libitum*.

Biological Effect Evaluation During In-Life Phase

Evaluation of Dead or Moribund Animals

Twice daily observations of all mice, once in the morning and once in the afternoon (at least 6 hours apart) will be performed to identify dead or moribund mice (***SOPs TOX062, DAT017***). Observations will be made five days per week (Monday through Friday, excluding holidays); during weekends and holidays, only one observation per day will be performed.

Mice whose condition makes it unlikely that they will survive until the next observation period, or appear to be in pain will be euthanized and necropsied at the discretion of the Attending Veterinarian or Study Director. Clinical observations will be recorded shortly before euthanasia.

Any pre-test study mouse, including sentinels, that is euthanized in a moribund condition during the quarantine/acclimation phase will have serum collected for serology and will be necropsied at the discretion of the Attending Veterinarian or Study Director (***SOPs TOX055, TOX056***).

Body Weights

Individual non-fasted body weights will be determined the day after delivery, again prior to study group allocation (i.e., prior to the initial dosing). Body weights will be recorded daily for the duration of the 14-day study (***SOPs EQP034, TOX038***). Weighing will take place at approximately the same time each day. Individual body weights will be used to calculate the mean body weight for each experimental group. The "A" module of the PATH/TOX system will be used for acquisition of body weight data. Unscheduled body weight determinations may be made at any time if deemed necessary by the Attending Veterinarian or Study Director. All mouse weights will be acquired using Mettler PM2000 balances (Mettler Instrument Corporation, Highstown, NJ) (***SOP EQP034).***

A non-fasted, terminal body weight will be obtained from mice euthanized at study completion. In addition, terminal weights will be taken for mice that are euthanized due to moribundity or for humane reasons. Data will be entered into the "A" module of the PATH/TOX computer software. No terminal body weight will be obtained for mice found dead.

Feed Consumption

The day before the start of the 14 day study period, each mouse's feed will be weighed into its tared feed cup. Each day of the study the uneaten feed will be weighed and discarded and the food consumption will be calculated. Data will be entered into the "A" module of the PATH/TOX computer software. Each mouse's feed consumption will be used to calculate the mean feed consumption for the group. In cases of excessive spillage of feed the weight will be recorded but not used to determine mean feed consumption for the group. In cases where the spillage is not excessive, the weight of the spilled feed can be estimated and added to the weight of feed in the feed cup and these data used in calculation of mean feed consumption. After determination of the feed consumed by a mouse, weighed fresh feed will be placed in a clean tared feed cup and provided to the mouse.

Clinical Observations

Except for weekends and holidays, daily observations for clinical signs will be taken. All positive findings will be recorded as unscheduled clinical observations using the "AINPUT" module of the PATH/TOX computer software (***SOP DAT004***). Negative findings (normal/no significant findings) will not be recorded.

In addition, detailed (scheduled) clinical observations will be performed the day after delivery, when collecting body weights for allocation to study groups and at twice weekly intervals, Monday and Friday, throughout the study (***SOPs DAT004, TOX047)***. Both positive and negative findings will be recorded. The "A" module of the PATH/TOX system will be used for acquisition of clinical signs data.

Biological Effect Evaluation at Termination of In-Life Phase

Terminal body weights will be the only data taken at this phase. Mice will not be necropsied.

Statistical Analyses

The following statistical tests will be used unless a statistician recommends other tests.

Body Weights

Statistical evaluations of group mean body weights and terminal body weights will be made using the tests built into the PATH/TOX software, including a one-way analysis of variance (ANOVA), followed by Bartlett's test for homogeneity of variance. If the data are homogeneous, then Dunnett's test will be performed; if the data are non-homogeneous, then Cochran and Cox's modified t-test will be used.

Feed Consumption

Statistical evaluations of group mean feed consumption will be made using the tests built into the PATH/TOX software, including a one-way analysis of variance (ANOVA), followed by Bartlett's test for homogeneity of variance. If the data are homogeneous, then Dunnett's test will be performed; if the data are non-homogeneous, then Cochran and Cox's modified t-test will be used.

Significance

Statistical tests will be carried out to 5%, two-sided criteria.

Records to be Maintained

Records that would be required to reconstruct the study, as well as to demonstrate adherence to the protocol will be maintained in the Toxicology archives. These will include, but will not be limited to the following:

- Study protocol and any amendments
- PATH/TOX protocols and any amendments
- Names, signatures and initials for study personnel
- Deviations from the study protocol and standard operating procedures (SOPs)
- Pertinent correspondences
- Mouse ordering, receipt and quarantine records
- Health screening data
- Records of allocation of mice to study groups
- Smokeless tobacco blend specifications CoA and MSDS
- Tobacco extract specifications CoA and MSDS
- Positive control (nicotine hydrogen tartrate) manufacturers specifications and MSDS
- Mouse identification (tattooing) records
- Test articles and positive control inventory and utilization records
- Feed and water analysis records
- Animal room temperature and relative humidity records
- Animal room light cycle and air flow records
- Animal housing and care records
- Equipment maintenance and calibration records
- Mortality, body weight, feed consumption and clinical observation records
- Statistical analysis results

Original laboratory notebooks will be stored in the archives at the sponsor's facilities.

Electronic files will be retained on diskette, compact disk and/or removable disk, and placed in the study file. Additionally, the version numbers of the software and operating systems will be documented in the study file, along with the type of hardware used to run the software.

The clinical observations, body weight and feed consumption will be entered into the PATHTOX software (*version 4.2.2; Xybion Medical Systems; Cedar Knolls, NJ*) running under the VMS operating system. This software is designed for the acquisition and management of toxicology and pathology data. System control is maintained by a computer resident protocol for data integrity, in compliance with FDA *Good Laboratory Practice* guidelines.

Reporting

Final Report

A written final report of the study will be prepared. The report will include, but will not be limited to the following:

- Name and address of the facility performing the study, and the dates on which the study was initiated and completed
- Objectives and procedures, as stated in the approved protocol
- Test articles and positive control will be identified by name and manufacture date.
- Materials and methods
- Description of the test system used, including the number of mice used, sex, body weight range, source of supply, species, strain and substrain, age, and the procedure used for identification
- Description of the dosage, dosage regimen, route of administration, and duration of test article and positive control and treatments
- Description of all circumstances that may have affected the quality and/or outcome of the study, or integrity of the data
- Name of the study director, the names of other scientists or professionals affiliated with the study, and the names of all supervisory personnel involved in the study
- Description of the transformations, calculations or operations performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analysis
- Signed and dated reports of each of the individual scientists or other professionals involved in the study.
- Location where specimens, raw data and the final report are to be stored

Statement By The Study Director

The study director assumes responsibility for ensuring that all work will be performed as described in the protocol. Every attempt will be made to perform the study as described. Any deviation or amendments to the approved protocol will be documented as such. Amendments involving significant modifications in the usage of animals will be referred to the IACUC, prior to implementation.

The Study Director assures that this study does not represent any unnecessary duplication of experimental studies using animal resources. The Study Director assures that this study will follow practices set forth in the *Guide for the Care and Use of Laboratory Animals* and IACUC policies.

Jenny L. Smith, B.S., Scientist III

R**eference**s

National Research Council (NRC). 1996. Guide for the Care and Use of Laboratory Animals. Institute of Laboratory Animal Resources, Commission on Life Sciences. Washington, DC, 1996.

Stenstrom, B., Zhao, C.-M., Rogers, A. B., Nilsson, H.-O., Sturegard, E., Lundgren, S., Fox, J. G., Wang, C., Wadstrom, T. M. and Chen, D. Sw3edish moist snuff accelerates gastric cancer development in Helicobacter pylori-infected wild-type and gastrin transgenic mice. Carcinogenesis, 28, 2041-2046, 2007.

U.S. Food and Drug Administration (FDA), Department of Health and Human Services. 2004. *Code of Federal Regulations*. 21 CFR Part 58 Good Laboratory Practice for Nonclinical Laboratory Studies. Office of the Federal Register, National Archives and Records Administration. Washington, DC.

Appendix I: Proposed Study Schedule

Test Article Receipt:	
Smokeless tobacco blend:	March 7-March 15, 2008
Tobacco Extract:	March 7-March 15, 2008
Nicotine hydrogen tartrate	March 7-March 20, 2008
Animal Quarantine/Acclimation Start:	April 9, 2008
Animal Randomization:	April 11, 2008
Animal Identification:	April 14-April 15, 2008
Initiation of Feeding Formulated Diets	April 16, 2008
Study Termination:	April 30, 2008
Report Dates:	
Data Report	May 8, 2008
Draft Report:	August 29, 2008
Final Report	November 5, 2008

Appendix II: Literature Search Strategies and Results

Duplication of Effort/Pain and Distress

Databases Searched Using Dialogue

File 155: MEDLINE(R) 1951-2005/Dec W4
File 156: ToxFile 1965-2004/Nov W2
File 159: Cancerlit 1975-2002/Oct
File 5: Biosis Previews(R) 1969-2005/Dec W4
File 35: Dissertation Abs Online 1861-2004/Dec
File 10: AGRICOLA 70-2004/Nov
File 71: ELSEVIER BIOBASE 1994-2005/Jan W1
File 73: EMBASE 1974-2005/Jan W1
File 162: Global Health 1983-2005/Dec
File 266: FEDRIP 2004/Sep

The following databases were searched up to February 27, 2008-March 5, 2008.

PubMed

Toxline

The following search terms were used and the total number of matching publications is provided:

Oral nicotine rat: 186 titles and abstracts retrieved

Oral nicotine mouse: 92 titles and abstracts retrieved

Diet nicotine mouse: 13 titles and abstracts retrieved

Diet tobacco mouse: 83 titles and abstracts retrieved

Diet tobacco rat: 79 titles and abstracts retrieved

Feed tobacco: 78 titles and abstracts retrieved

Oral tobacco rat: 66 titles and abstracts retrieved

Oral tobacco mouse: 51 titles and abstracts retrieved

Snuff diet: 4 titles and abstracts retrieved

Snuff diet rat: 76 titles and abstracts retrieved

Snuff diet mouse: 4 titles and abstracts retrieved

Palatability tobacco: 2 titles and abstracts retrieved

Palatability snuff: 0 titles and abstracts retrieved

Snus animal: 1 title and abstract retrieved

Snus mice: 2 titles and abstracts retrieved

Snus rats: 0 title and abstract retrieved

Snus: 78 titles and abstracts retrieved

Palatability nicotine: 6 titles and abstracts retrieved

Palatability nicotine refinement alternative: 0 titles and abstracts retrieved

Palatability study refinement alternative: 0 titles and abstracts retrieved

Feed palatability alternative: 6 titles and abstracts retrieved

Feeding study palatability refinement: 0 titles and abstracts retrieved

Feeding study palatability replacement alternative: 0 titles and abstracts retrieved

Nicotine palatability replacement alternative: 0 titles and abstracts retrieved

Nicotine palatability refinement alternative: 0 titles and abstracts retrieved

Palatability study nicotine: 2 titles and abstracts retrieved

Palatability study tobacco: 1 title and abstract retrieved

Palatability study snus: 0 titles and abstracts retrieved

Palatability study: 588 titles and abstracts retrieved

Review of the titles or abstracts retrieved during these literature searches revealed only one publication relevant to the study design in this protocol (Stenstrom et al., 2007). It was determined that this publication contained no data that would negate the need to conduct the current study.

R. J. Reynolds Tobacco Company
Research and Development
Preclinical Models of Disease
Protocol Amendment

Amendment # 1

Protocol Identifier: TOX210
Protocol Title: Investigational Study of the Palatability of Smokeless Tobacco Test Articles Formulated in NTP-2000 Diets for Mice
Study Director: Jenny L. Smith
Animal Husbandry: Change this section to read.

On the day of arrival and the following day (April 9-10, 2008), all groups of mice will have *ad libitum* access to Certified Rodent Diet #5002 feed (PMI Nutrition International), presented as pellets. Starting on April 11, 2008 and continuing through April 15, 2008, all groups with the exception of the sentinel mice will have *ad libitum* access to NTP-2000 feed. The sentinel mice will have *ad libitum* access to Certified Rodent Diet #5002 feed (PMI Nutrition International), presented as pellets.

Reason for change: The diet change will aid in the acclimation to the new laboratory environment.

Approval

Jenny L. Smith, Study Director

Jenny L. Smith 4-8-08

R. J. Reynolds Tobacco Company
Research and Development
Preclinical Models of Disease

Protocol Amendment

Amendment # 2

Protocol Identifier: TOX210
Protocol Title: Investigational Study of the Palatability of Smokeless Tobacco Test Articles Formulated in NTP-2000 Diets for Mice
Study Director: Jenny L. Smith
Allocation: Change this section to read.

On the third day of the quarantine/acclimation period, rats will be assigned to dose groups according to body weight using the "A" module of the PATH/TOX software (version 4.2.2; Xybion Medical Systems; Cedar Knolls, NJ) (*SOPs TOX042, TOX067*).

Reason for change: Date of Arrival for Mice changed; acclimation must be performed during the normal work week.

Approval

Jenny L. Smith, Study Director

Jenny L. Smith 4-8-08

R. J. Reynolds Tobacco Company
Research and Development
Preclinical Models of Disease

Protocol Amendment

Amendment # 3

Protocol Identifier: TOX210
Protocol Title: Investigational Study of the Palatability of Smokeless Tobacco Test Articles Formulated in NTP-2000 Diets for Mice
Study Director: Jenny L. Smith
Allocation: Change this section to read.

On the sixth day of the quarantine/acclimation period, rats will be assigned to dose groups according to body weight using the "A" module of the PATH/TOX software (version 4.2.2; Xybion Medical Systems; Cedar Knolls, NJ) (*SOPs TOX042, TOX067*). Body weights and detailed clinical signs will be recorded prior to conducting the allocation process.

Reason for change: Date of Arrival for Mice changed; allocation must be performed during the normal work week.

Approval

Jenny L. Smith, Study Director

Jenny L. Smith 4-10-08

R. J. Reynolds Tobacco Company
Research and Development
Preclinical Models of Disease

Protocol Amendment

Amendment # 4

Protocol Identifier: TOX210
Protocol Title: Investigational Study of the Palatability of Smokeless Tobacco Test Articles Formulated in NTP-2000 Diets for Mice
Study Director: Jenny L. Smith
Body Weights: Changed the following.

Individual non-fasted body weights will be determined two days after delivery, again prior to study group allocation (i.e., prior to the initial dosing).

Clinical Observations: Changed the following.

In addition, detailed (scheduled) clinical observations will be performed two days after delivery, when collecting body weights for allocation to study groups and at twice weekly intervals, Monday and Friday, throughout the study (***SOPs DAT004, TOX047***).

Reason for change: Due to the short duration of the study, the body weights and clinical observations will be performed on April 11, 2008 in order to acclimate the mice to their new environment.

Approval

Jenny L. Smith, Study Director

Jenny L. Smith 4-10-08

R. J. Reynolds Tobacco Company
Research and Development
Preclinical Models of Disease

Protocol Amendment

Amendment # 5

Protocol Identifier: TOX210

Protocol Title: Investigational Study of the Palatability of Smokeless Tobacco Test Articles Formulated in NTP-2000 Diets for Mice

Study Director: Jenny L. Smith

Protocol Amendment:

The protocol for Study TOX210 is amended as follows:

The Study Director is changed from Jenny L. Smith to Paul Ayres, PhD., DABT.

Reason for Amendment:

Jenny Smith left R. J. Reynolds on September 30, 2008. Therefore, the Study Director is being changed to Dr. Paul Ayres for completion of the study.

Protocol Amendment Approval:

Paul Ayres

Paul Ayres, PhD, DBAT

1-20-2009

Date

1.0 STUDY TITLE

Investigational Study of the Palatability of Smokeless Tobacco Test Articles Formulated in NTP-2000 Diets for Mice

Smokeless Tobacco Blend and Extract Groups

1.1 Purpose of Study

The objective of this study will be to evaluate the palatability of diets formulated in NTP-2000 feed with a smokeless tobacco blend, an aqueous tobacco extract of the smokeless tobacco blend and nicotine tartrate as positive control when fed to Swiss Webster Mice.

1.2 Sponsor

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

1.3 Test Facility

R.J. Reynolds Tobacco Company
Research and Development
Preclinical Models of Disease
In-Vivo Toxicology Division
Winston-Salem, NC 27102

2.0 STUDY PERSONNEL

2.1 Study Director________________________ Approval date: Tue. 01-Apr-08
JENNY SMITH

2.2 Reviewer _____________________________ Approval date: Tue. 01-Apr-08
DANIEL R. MECKLEY

2.3

Consultant	JOHNNIE R. HAYES
Attending Veterinarian	CHANDRA D. WILLIAMS, DVM
Senior Principal Scientist	PAUL AYRES, PH.D., DABT
Animal Resources Supervisor	JESSICA BAKER, BS, LAT
Animal Care Technician:	PAMELA SMOOT
Animal Care Technician:	KIM STANLEY, BS, LAT
Animal Care Technician:	WALDEN HEARN, JR.
Animal Care Technician:	Abraham Doby
Animal Care Technician:	ANDRE BRYANT
Animal Care Technician:	MONICA L. PAITSEL
Animal Care Technician:	PATRICIA BATCHELOR

Animal Care Technician: TABATHA GALLIMORE
Animal Care Technician: JAYSON HULL
Animal Care Technician: LIZ CHIASSON
Animal Care Technician: DEBORA TRAIL
QA Investigator Jeffrey Dwayne Hedrick

3.0 PROPOSED DATES

Dosing initiation date - - - - - - - - - - - - Wed. 16-Apr-08
Study completion date - - - - - - - - - - - - - Thu. 08-May-08

4.0 STUDY TYPE AND SPECIES SPECIFICATIONS

4.1 Study Type - - - - - - - - - - - - - - - - - - FEEDING STUDY
Study Category - - - - - - - - - - - - - - - - PALATABILITY

4.2 Species - - - - - - - - - - - - - - - - - - - MOUSE
Strain - SWISS WEBSTER
Method of identification - - - - - - - - - - - Tail Tattoo

4.3 Animal Supplier

CHARLES RIVER BREEDING LABS,INC.; RALEIGH, NC

5.0 NUMBER OF ANIMALS ON STUDY

Pretest: 68 # Males: 68 # Females:
Study: 65 # Males: 65 # Females:

5.1 Number of Animals Per Group

Group	1	2	3	4	5	6	7	8	9	10	11	12	13
Males	5	5	5	5	5	5	5	5	5	5	5	5	5

5.2 Starting Animal Number Per Group

Group	1	2	3	4	5	6	7	8	9	10	11	12	13
Males	1	6	11	16	21	26	31	36	41	46	51	56	61

6.0 Test Article Descriptions

6.1 Test Article: TOB BLEND

Test article identification - - TOB BLEND

6.2 Test Article: TOB EXTRACT

Test article identification - - TOB EXTRACT

7.0 Control Article Descriptions

7.1 CONTROL ARTICLE NTP-2000

10.0 Study Phases, Laboratory Determinations, and Schedules

Quarantine/Acclimation	09-Apr-08 (Receipt date)	M 1 F 1 MFS NDZ
Exposure phase	21-Apr-08 (Start of dosing)	M 1 F 1 MFS NDZ
	08-May-08 (Final sacrifice day)	

Key: M=males/cage,F=females/cage,MFT=males and females caged together, MFS=males and females caged separately,D&P= dams and pups caged together,NDZ= no day zero on phase, DZ=day zero on phase.

10.1 ANIMAL ROOM FUNCTIONS (Quarantine/Acclimation)

10.1.1 BODY WEIGHT FUNCTIONS

Scheduled Days: 3 6

			Parameter range limits				#
			Male		Female		Dec
Abv	Parameter	Units	Low	High	Low	High	Pts
BW	BODY WEIGHTS	GRAMS	20.000	50.000	20.000	50.000	2

10.1.2 FULL FEEDER WEIGHT FUNCTIONS

Scheduled Days: 6

			Parameter range limits				#
			Male		Female		Dec
Abv	Parameter	Units	Low	High	Low	High	Pts
FF	FULL FEEDER WEIGHTS	GRAMS	380.00	420.00	380.00	420.00	1

10.1.3 EMPTY FEEDER WEIGHT FUNCTIONS

Scheduled Days: 7

			Parameter range limits				#
			Male		Female		Dec
Abv	Parameter	Units	Low	High	Low	High	Pts
EF	EMPTY FEEDER/FEED CONSUMED	GRAMS	10.000	35.000	10.000	35.000	1

10.1.4 CLINICAL SIGNS 2 /day

Scheduled Days: 3 6

Abv	Parameter
CS	CLINICAL SIGNS

10.2 ANIMAL ROOM FUNCTIONS (Exposure phase)

10.2.1 BODY WEIGHT FUNCTIONS

Starting on day 1 every day through day 19

			Parameter range limits				#
			Male		Female		Dec
Abv	Parameter	Units	Low	High	Low	High	Pts
BW	BODY WEIGHTS	GRAMS	20.000	50.000	20.000	50.000	2

10.2.2 FULL FEEDER WEIGHT FUNCTIONS

Starting on day 1 every day through day 19

			Parameter range limits				#
			Male		Female		Dec
Abv	Parameter	Units	Low	High	Low	High	Pts
FF	FULL FEEDER WEIGHTS	GRAMS	300.00	375.00	300.00	375.00	2

10.2.3 EMPTY FEEDER WEIGHT FUNCTIONS

Starting on day 1 every day through day 20

			Parameter range limits				#
			Male		Female		Dec
Abv	Parameter	Units	Low	High	Low	High	Pts
EF	EMPTY FEEDER/FEED CONSUMED	GRAMS	5.0000	25.000	5.0000	25.000	2

10.2.4 CLINICAL SIGNS 2 /day

Scheduled Days: 6 10 13 17

Abv	Parameter
CS	CLINICAL SIGNS

10.3 DOSING (Exposure phase)

10.3.1 DOSED FEED

Starting on day 1 every day through day 14

			#
			Dec
Abv	Parameter Name	Parameter Type	Pts

FD   DOSED FEEDING   Solid Dose Units - mg/kg   1

10.4   Necropsy Procedures (F0 - Exposure phase)

Method of sacrifice - - - - - - - - - - - - -	Carbon dioxide inhalation
Anesthetic - - - - - - - - - - - - - - - - - -	CO 2
Randomization algorithm for sacrifices - - -	No
Skip unscheduled dead during selection - - -	Yes
Select animals from top of groups - - - - -	Yes
At final, sacrifice all remaining animals -	Yes
Final phase sacrifice on day - - - - - - - - -	15

11.0   Treatment Groups and Dosages

11.1   Doses: Exposure phase

Group No./ No.	Group	Sex	Dosage in mg/kg * Articles A	B	C
1	5	M	------	------	------
2	5	M	0.2	------	------
3	5	M	2.0	------	------
4	5	M	4.0	------	------
5	5	M	8.0	------	------
6	5	M	20.0	------	------
7	5	M	40.0	------	------
8	5	M	------	0.2	------
9	5	M	------	2.0	------
10	5	M	------	4.0	------
11	5	M	------	8.0	------
12	5	M	------	20.0	------
13	5	M	------	40.0	------

* Article codes: A=TOB BLEND
B=TOB EXTRACT
C=NTP-2000

11.0 Protocol amendments:

11.1 Date: 07-Apr-08 Approved by: JENNY SMITH

Time: 10:54

Reason: changed schedule to reflect protocol

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o CLINICAL SIGNS 2 /day

Scheduled Days: 3 6 10 13

11.2 Date: 09-Apr-08 Approved by: JENNY SMITH

Time: 16:21

Reason: changed body weight to accomodate current schedule

o ANIMAL ROOM FUNCTIONS (Quarantine/Acclimation)

o BODY WEIGHT FUNCTIONS

Scheduled Days: 3 6

11.3 Date: 09-Apr-08 Approved by: JENNY SMITH

Time: 16:23

Reason: changed clinical to accomodate current schedule

o ANIMAL ROOM FUNCTIONS (Quarantine/Acclimation)

o CLINICAL SIGNS 2 /day

Scheduled Days: 3 6

11.4 Date: 18-Apr-08 Approved by: JENNY SMITH

Time: 14:29

Reason: Delayed exposure phase

o PROPOSED DATES

Dosing initiation date - - - - - - - - - - - - Wed. 16-Apr-08
Study completion date - - - - - - - - - - - - - Thu. 08-May-08

11.5 Date: 18-Apr-08 Approved by: JENNY SMITH

Time: 14:33

Reason: Delayed exposure phase

Edited phase description
F0 - Exposure phase 21-Apr-08 (Start of dosing) M 1 F 1 MFS NDZ
08-May-08 (Final sacrifice day)

11.6 Date: 21-Apr-08 Approved by: JENNY SMITH

Time: 08:43

Reason: Updated full feeder weight range limits

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o FULL FEEDER WEIGHT FUNCTIONS

			Parameter range limits				#
			Male		Female		Dec
Abv	Parameter	Units	Low	High	Low	High	Pts
FF	FULL FEEDER WEIGHTS	GRAMS	300.00	375.00	300.00	375.00	1

11.7 Date: 21-Apr-08 Approved by: JENNY SMITH

Time: 08:44

Reason: Updated full feeder weight range limits

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o EMPTY FEEDER WEIGHT FUNCTIONS

			Parameter range limits				#
			Male		Female		Dec
Abv	Parameter	Units	Low	High	Low	High	Pts
EF	EMPTY FEEDER/FEED CONSUMED	GRAMS	300.00	375.00	10.000	35.000	1

11.8 Date: 21-Apr-08 Approved by: JENNY SMITH

Time: 08:45

Reason: Updated empty feeder weight range limits

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o EMPTY FEEDER WEIGHT FUNCTIONS

			Parameter range limits				#
			Male		Female		Dec
Abv	Parameter	Units	Low	High	Low	High	Pts
EF	EMPTY FEEDER/FEED CONSUMED	GRAMS	275.00	370.00	10.000	35.000	1

11.9 Date: 21-Apr-08 Approved by: JENNY SMITH

Time: 08:46

Reason: Updated clinical schedule due to delay of exposure phase

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o CLINICAL SIGNS 2 /day

Scheduled Days: 1 5 8 12

11.10 Date: 21-Apr-08 Approved by: JENNY SMITH

Time: 09:44

Reason: Updated clinical schedule

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o CLINICAL SIGNS 2 /day

Scheduled Days: 6 10 13 17

11.11 Date: 25-Apr-08 Approved by: JENNY SMITH

Time: 21:12

Reason: Updated full feeder weight targets

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o FULL FEEDER WEIGHT FUNCTIONS

			Parameter range limits				#
			Male		Female		Dec
Abv	Parameter	Units	Low	High	Low	High	Pts
FF	FULL FEEDER WEIGHTS	GRAMS	340.00	375.00	340.00	375.00	1

11.12 Date: 25-Apr-08 Approved by: JENNY SMITH

Time: 21:14

Reason: Updated empty feeder weight targets

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o ANIMAL ROOM FUNCTIONS (Exposure phase)

o EMPTY FEEDER WEIGHT FUNCTIONS

Abv	Parameter	Units	Parameter range limits Male Low	Male High	Female Low	Female High	# Dec Pts
EF	EMPTY FEEDER/FEED CONSUMED	GRAMS	310.00	375.00	310.00	375.00	1

11.13 Date: 26-Apr-08 Approved by: JENNY SMITH

Time: 16:29

Reason: Updated full feeder weight limitations

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o FULL FEEDER WEIGHT FUNCTIONS

Abv	Parameter	Units	Parameter range limits Male Low	Male High	Female Low	Female High	# Dec Pts
FF	FULL FEEDER WEIGHTS	GRAMS	300.00	375.00	300.00	375.00	1

11.14 Date: 26-Apr-08 Approved by: JENNY SMITH

Time: 16:30

Reason: Updated empty feeder weight limitations

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o EMPTY FEEDER WEIGHT FUNCTIONS

Abv	Parameter	Units	Parameter range limits Male Low	Male High	Female Low	Female High	# Dec Pts
EF	EMPTY FEEDER/FEED CONSUMED	GRAMS	275.00	375.00	275.00	375.00	2

11.15 Date: 26-Apr-08 Approved by: JENNY SMITH

Time: 16:31

Reason: updated full feeder weight limitations

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o ANIMAL ROOM FUNCTIONS (Exposure phase)

o FULL FEEDER WEIGHT FUNCTIONS

			Parameter range limits				#
			Male		Female		Dec
Abv	Parameter	Units	Low	High	Low	High	Pts
FF	FULL FEEDER WEIGHTS	GRAMS	300.00	375.00	300.00	375.00	2

11.16 Date: 29-Apr-08 Approved by: JENNY SMITH

Time: 10:28

Reason: Updated empty feeder weight limitations

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o EMPTY FEEDER WEIGHT FUNCTIONS

			Parameter range limits				#
			Male		Female		Dec
Abv	Parameter	Units	Low	High	Low	High	Pts
EF	EMPTY FEEDER/FEED CONSUMED	GRAMS	5.0000	25.000	5.0000	25.000	2

11.17 Date: 30-Apr-08 Approved by: DANIEL R. MECKLEY

Time: 08:11

Corrected schedule due to change in starting date.

11.18 Date: 30-Apr-08 Approved by: DANIEL R. MECKLEY

Time: 08:12

Corrected schedule due to change in starting date.

11.19 Date: 30-Apr-08 Approved by: DANIEL R. MECKLEY

Time: 08:12

Corrected schedule due to change in starting date.

11.20 Date: 30-Apr-08 Approved by: JENNY SMITH

Time: 13:23

Reason: Updated body weight function schedule

---

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o BODY WEIGHT FUNCTIONS

11.21 Date: 30-Apr-08 Approved by: JENNY SMITH

Time: 13:24

Reason: Updated body weight schedule

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o BODY WEIGHT FUNCTIONS

Starting on day 1 every day through day 19

11.22 Date: 30-Apr-08 Approved by: JENNY SMITH

Time: 13:25

Reason: Updated full feeder weight schedule

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o FULL FEEDER WEIGHT FUNCTIONS

Starting on day 1 every day through day 19

11.23 Date: 30-Apr-08 Approved by: JENNY SMITH

Time: 13:26

Reason: Updated empty feeder weight schedule

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o EMPTY FEEDER WEIGHT FUNCTIONS

Starting on day 1 every day through day 20

1.0 STUDY TITLE

Investigational Study of the Palatablility of Smokeless Tobacco Test Articles Formulated in NTP-2000 Diets for Mice

Positive Control and Sentinel Groups

1.1 Purpose of Study

The objective of this study will be to evaluate the palatablity of diets formulated in NTP-2000 feed with a smokeless tobacco blend, an aqueous tobacco extract of the smokeless tobacco blend and nicotine tartrate as positive control when fed to Swill Webster Mice.

1.2 Sponsor

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

1.3 Test Facility

R.J. Reynolds Tobacco Company
Research and Development
Preclinical Models of Disease
In-Vivo Toxicology Division
Winston-Salem, NC 27102

2.0 STUDY PERSONNEL

2.1 Study Director________________________ Approval date: Tue. 01-Apr-08
JENNY SMITH

2.2 Reviewer _____________________________ Approval date: Tue. 01-Apr-08
DANIEL R. MECKLEY

2.3

Consultant	JOHNNIE R. HAYES
Attending Veterinarian	CHANDRA D. WILLIAMS, DVM
Senior Principal Scientist	PAUL AYRES, PH.D., DABT
Animal Resources Supervisor	JESSICA BAKER, BS, LAT
Animal Care Technician:	PAMELA SMOOT
Animal Care Technician:	KIM STANLEY, BS, LAT
Animal Care Technician:	WALDEN HEARN, JR.
Animal Care Technician:	Abraham Doby
Animal Care Technician:	ANDRE BRYANT
Animal Care Technician:	PATRICIA BATCHELOR
Animal Care Technician:	TABATHA GALLIMORE

Animal Care Technician:	JAYSON HULL
Animal Care Technician:	LIZ CHIASSON
Animal Care Technician:	DEBORA TRAIL
Animal Care Technician:	MONICA L. PAITSEL
QA Investigator	Jeffrey Dwayne Hedrick

3.0 PROPOSED DATES

Dosing initiation date - - - - - - - - - - - - Wed. 16-Apr-08
Study completion date - - - - - - - - - - - - - Thu. 08-May-08

4.0 STUDY TYPE AND SPECIES SPECIFICATIONS

4.1 Study Type - - - - - - - - - - - - - - - - - - FEEDING STUDY
Study Category - - - - - - - - - - - - - - - - PALATABILITY

4.2 Species - - - - - - - - - - - - - - - - - - - MOUSE
Strain - SWISS WEBSTER
Method of identification - - - - - - - - - - - Tail Tattoo

4.3 Animal Supplier

CHARLES RIVER BREEDING LABS,INC.; RALEIGH, NC

5.0 NUMBER OF ANIMALS ON STUDY

Pretest: 32 # Males: 32 # Females:
Study: 30 # Males: 30 # Females:

5.1 Number of Animals Per Group

Group	1	2	3	4	5
Males	5	5	5	5	10

5.2 Starting Animal Number Per Group

Group	1	2	3	4	5
Males	66	71	76	81	86

6.0 Test Article Descriptions

6.1 Test Article: NIC TARTRATE

Test article identification - - NIC TARTRATE

7.0 Control Article Descriptions

7.1 CONTROL ARTICLE SENTINEL

10.0 Study Phases, Laboratory Determinations, and Schedules

Quarantine/Acclimation	09-Apr-08 (Receipt date)	M 1 F 1 MFS NDZ
Exposure phase	21-Apr-08 (Start of dosing)	M 1 F 1 MFS NDZ
	08-May-08 (Final sacrifice day)	

Key: M=males/cage,F=females/cage,MFT=males and females caged together, MFS=males and females caged separately,D&P= dams and pups caged together,NDZ= no day zero on phase, DZ=day zero on phase.

10.1 ANIMAL ROOM FUNCTIONS (Quarantine/Acclimation)

10.1.1 BODY WEIGHT FUNCTIONS

Scheduled Days: 3 6

			Parameter range limits				#
			Male		Female		Dec
Abv	Parameter	Units	Low	High	Low	High	Pts
BW	BODY WEIGHTS	GRAMS	20.000	50.000	20.000	50.000	2

10.1.2 FULL FEEDER WEIGHT FUNCTIONS

Scheduled Days: 6

			Parameter range limits				#
			Male		Female		Dec
Abv	Parameter	Units	Low	High	Low	High	Pts
FF	FULL FEEDER WEIGHTS	GRAMS	380.00	420.00	380.00	420.00	1

10.1.3 EMPTY FEEDER WEIGHT FUNCTIONS

Scheduled Days: 7

			Parameter range limits				#
			Male		Female		Dec
Abv	Parameter	Units	Low	High	Low	High	Pts
EF	EMPTY FEEDER/FEED CONSUMED	GRAMS	10.000	35.000	10.000	35.000	1

10.1.4 CLINICAL SIGNS 2 /day

Scheduled Days: 3 6

Abv	Parameter
CS	CLINICAL SIGNS

10.2 ANIMAL ROOM FUNCTIONS (Exposure phase)

10.2.1 BODY WEIGHT FUNCTIONS

Starting on day 1 every day through day 19

Abv	Parameter	Units	Male Low	Male High	Female Low	Female High	# Dec Pts
BW	BODY WEIGHTS	GRAMS	20.000	50.000	20.000	50.000	2

10.2.2 FULL FEEDER WEIGHT FUNCTIONS

Starting on day 1 every day through day 19

Abv	Parameter	Units	Male Low	Male High	Female Low	Female High	# Dec Pts
FF	FULL FEEDER WEIGHTS	GRAMS	300.00	375.00	300.00	375.00	2

10.2.3 EMPTY FEEDER WEIGHT FUNCTIONS

Starting on day 2 every day through day 20

Abv	Parameter	Units	Male Low	Male High	Female Low	Female High	# Dec Pts
EF	EMPTY FEEDER/FEED CONSUMED	GRAMS	5.0000	25.000	5.0000	25.000	2

(Parameter range limits)

10.2.4 CLINICAL SIGNS 2 /day

Scheduled Days: 6 10 13 17

Abv	Parameter
CS	CLINICAL SIGNS

10.3 DOSING (Exposure phase)

10.3.1 DOSED FEED

Starting on day 1 every day through day 14

Abv	Parameter Name	Parameter Type	# Dec Pts

FD   DOSED FEEDING   Solid Dose Units - mg/kg   1

10.4   Necropsy Procedures (F0 - Exposure phase)

Method of sacrifice - - - - - - - - - - - - -	Carbon dioxide inhalation
Anesthetic - - - - - - - - - - - - - - - - - -	CO 2
Randomization algorithm for sacrifices - - -	No
Skip unscheduled dead during selection - - -	Yes
Select animals from top of groups - - - - -	Yes
At final, sacrifice all remaining animals -	Yes
Final phase sacrifice on day - - - - - - - - -	15

11.0   Treatment Groups and Dosages

11.1   Doses: Exposure phase

Group No.	No. Group	Sex	Dosage in mg/kg * Articles A	B
1	5	M	2.0	------
2	5	M	8.0	------
3	5	M	20.0	------
4	5	M	40.0	------
5	10	M	------	------

* Article codes: A=NIC TARTRATE
  B=SENTINEL

11.0 Protocol amendments:

11.1 Date: 07-Apr-08 Approved by: JENNY SMITH

Time: 10:55

Reason: changed schedule to reflect protocol

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o CLINICAL SIGNS 2 /day

Scheduled Days: 3 6 10 13

11.2 Date: 09-Apr-08 Approved by: JENNY SMITH

Time: 16:30

Reason: changed body weight to accomodate current schedule

o ANIMAL ROOM FUNCTIONS (Quarantine/Acclimation)

o BODY WEIGHT FUNCTIONS

Scheduled Days: 3 6

11.3 Date: 09-Apr-08 Approved by: JENNY SMITH

Time: 16:31

Reason: changed clinical signs to accomodate current schedule

o ANIMAL ROOM FUNCTIONS (Quarantine/Acclimation)

o CLINICAL SIGNS 2 /day

Scheduled Days: 3 6

11.4 Date: 18-Apr-08 Approved by: JENNY SMITH

Time: 14:38

Reason: Delayed study start

o PROPOSED DATES

Dosing initiation date - - - - - - - - - - - - Wed. 16-Apr-08
Study completion date - - - - - - - - - - - - - Thu. 08-May-08

11.5 Date: 18-Apr-08 Approved by: JENNY SMITH

Time: 14:40

Reason: Delayed exposure phase

Edited phase description
F0 - Exposure phase   21-Apr-08 (Start of dosing)   M 1 F 1 MFS NDZ
                             08-May-08 (Final sacrifice day)

11.6   Date: 18-Apr-08   Approved by: JENNY SMITH

Time: 14:42

Reason: Delayed exposure phase

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o CLINICAL SIGNS   2 /day

Scheduled Days: 1   5   8   12

11.7   Date: 21-Apr-08   Approved by: JENNY SMITH

Time: 08:49

Reason: Updated full feeder weight range limits

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o FULL FEEDER WEIGHT FUNCTIONS

Abv	Parameter	Units	Parameter range limits Male Low	High	Female Low	High	# Dec Pts
FF	FULL FEEDER WEIGHTS	GRAMS	300.00	375.00	380.00	420.00	1

11.8   Date: 21-Apr-08   Approved by: JENNY SMITH

Time: 08:50

Reason: Updated empty feeder weight range limits

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o EMPTY FEEDER WEIGHT FUNCTIONS

Abv	Parameter	Units	Parameter range limits Male Low	High	Female Low	High	# Dec Pts
EF	EMPTY FEEDER/FEED CONSUMED	GRAMS	275.00	370.00	10.000	35.000	1

11.9   Date: 21-Apr-08   Approved by: JENNY SMITH

Time: 09:47

Reason: Updated clinical schedule

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o CLINICAL SIGNS 2 /day

Scheduled Days: 6 10 13 17

11.10 Date: 25-Apr-08 Approved by: JENNY SMITH

Time: 21:17

Reason: Updated full feeder weight targets

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o FULL FEEDER WEIGHT FUNCTIONS

			Parameter range limits				#
			Male		Female		Dec
Abv	Parameter	Units	Low	High	Low	High	Pts
FF	FULL FEEDER WEIGHTS	GRAMS	340.00	375.00	340.00	375.00	1

11.11 Date: 25-Apr-08 Approved by: JENNY SMITH

Time: 21:18

Reason: Updated empty feeder weight targets

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o EMPTY FEEDER WEIGHT FUNCTIONS

			Parameter range limits				#
			Male		Female		Dec
Abv	Parameter	Units	Low	High	Low	High	Pts
EF	EMPTY FEEDER/FEED CONSUMED	GRAMS	310.00	375.00	310.00	375.00	1

11.12 Date: 26-Apr-08 Approved by: JENNY SMITH

Time: 16:41

Reason: updated full feeder weight limitations

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o FULL FEEDER WEIGHT FUNCTIONS

			Parameter range limits				#
			Male		Female		Dec
Abv	Parameter	Units	Low	High	Low	High	Pts
FF	FULL FEEDER WEIGHTS	GRAMS	300.00	375.00	300.00	375.00	2

11.13 Date: 26-Apr-08 Approved by: JENNY SMITH

Time: 16:41

Reason: updated empty feeder weight limitations

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o EMPTY FEEDER WEIGHT FUNCTIONS

			Parameter range limits				#
			Male		Female		Dec
Abv	Parameter	Units	Low	High	Low	High	Pts
EF	EMPTY FEEDER/FEED CONSUMED	GRAMS	300.00	375.00	300.00	375.00	2

11.14 Date: 26-Apr-08 Approved by: JENNY SMITH

Time: 16:43

Reason: empty feeder weight limitations changed

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o EMPTY FEEDER WEIGHT FUNCTIONS

			Parameter range limits				#
			Male		Female		Dec
Abv	Parameter	Units	Low	High	Low	High	Pts
EF	EMPTY FEEDER/FEED CONSUMED	GRAMS	275.00	375.00	275.00	375.00	2

11.15 Date: 29-Apr-08 Approved by: JENNY SMITH

Time: 10:29

Reason: Updated empty feeder weight limitations

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o EMPTY FEEDER WEIGHT FUNCTIONS

			Parameter range limits				#
			Male		Female		Dec
Abv	Parameter	Units	Low	High	Low	High	Pts
EF	EMPTY FEEDER/FEED CONSUMED	GRAMS	5.0000	25.000	5.0000	25.000	2

11.16 Date: 30-Apr-08 Approved by: DANIEL R. MECKLEY

Time: 08:13

Corrected schedule due to change in starting date.

11.17 Date: 30-Apr-08 Approved by: DANIEL R. MECKLEY

Time: 08:13

Corrected schedule due to change in starting date.

11.18 Date: 30-Apr-08 Approved by: DANIEL R. MECKLEY

Time: 08:14

Corrected schedule due to change in starting date.

11.19 Date: 30-Apr-08 Approved by: JENNY SMITH

Time: 13:30

Reason: g

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o BODY WEIGHT FUNCTIONS

11.20 Date: 30-Apr-08 Approved by: JENNY SMITH

Time: 13:30

Body Weight Schedule Updated

11.21 Date: 30-Apr-08 Approved by: JENNY SMITH

Time: 13:32

Reason: Updated Full feeder weight schedule

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o FULL FEEDER WEIGHT FUNCTIONS

Starting on day 1 every day through day 20

11.22 Date: 30-Apr-08 Approved by: JENNY SMITH

Time: 13:38

Reason: Updated empty feeder weight schedule

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o EMPTY FEEDER WEIGHT FUNCTIONS

Starting on day 2 every day through day 20

11.23 Date: 30-Apr-08 Approved by: JENNY SMITH

Time: 19:16

Reason: Updated full feeder weight schedule

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o FULL FEEDER WEIGHT FUNCTIONS

Starting on day 1 every day through day 19

TOX210 Preliminary Report

Preliminary Data Report for TOX210

Investigational Study of the Palatability of Smokeless Tobacco Test Articles Formulated in NTP-2000 Diets for Mice

R. J. Reynolds Tobacco Company
Research and Development
Preclinical Models of Disease
In-Vivo Toxicology Division
Building 630-2
Winston-Salem, N.C. 27102

Report Prepared by:

Jenny L. Smith, B.S.
Study Director
Scientist III
Preclinical Models of Disease
In Vivo Toxicology

Jenny L. Smith, B.S.	*Date*

Study termination date: May 5, 2008
Preliminary data report date: May 22, 2008

Facilities and Administration

Sponsor

R. J. Reynolds Tobacco Company
Research and Development
Product Integrity
Bowman Gray Technical Center
Winston-Salem, N.C.

Testing Facility

R. J. Reynolds Tobacco Company
Research and Development
Preclinical Models of Disease
In-Vivo Toxicology Division
Building 630-2
Winston-Salem, N.C. 27102

Contractors

Charles River Laboratories Wilmington, MA	Serology
Research Resources of North Carolina, Inc. On-site	Animal Husbandry, Quality Assurance

Study Administration

Study Director, In Vivo Toxicology	Jenny L. Smith, B.S.
Attending Veterinarian, Preclinical Models of Disease	Chandra D. Williams, D.V.M.
Program Manager, Research Resources of N.C., Inc.	Jessica Baker, B.S., L.A.T
Director Product Integrity, Preclinical Models of Disease, In Vivo Toxicology	Paul H. Ayres, Ph.D., D.A.B.T.
Senior Director, Product Integrity Preclinical Models of Disease	Natalie Takenaka, Ph.D.
Vice-President, Product Integrity	Christopher J. Cook, Ph.D.

Executive Summary

The objective of this study was to evaluate the palatability of diets formulated in NTP-2000 rodent feed with a tobacco blend, an aqueous tobacco extract of the tobacco blend or nicotine hydrogen tartrate as positive control when fed to CD-1 Swiss Webster mice. A tobacco blend and an aqueous extract of the tobacco blend will be tested in an upcoming series of toxicology studies. Also, a positive control, nicotine hydrogen tartrate will be used in some of the planned studies.

Doses for the current study were based upon the nicotine content of the tobacco, tobacco extract and the nicotine tartrate. The tobacco and tobacco extract are complex mixtures of components occurring naturally in the tobacco plant. Nicotine is a significant component of tobacco and is known to be toxic at higher doses. The tobacco blend and aqueous tobacco extract test articles and the positive control were incorporated into the mouse's feed. There is the possibility that incorporation of the test articles and positive control in the feed may alter its palatability to mice. If the feed is less palatable than the control diet, the mice may consume less feed with a resulting decrease in body weight gain. This would also result in lower than anticipated doses during toxicology studies. Therefore, it was necessary to ascertain the palatability of the dosed feed to mice.

Palatability was assessed by comparing the feed intake and body weight of mice fed the standard NTP-2000 diet (control group) to the feed intake and body weight of mice fed NTP-2000 diets formulated to contain different doses of the tobacco blend and different doses of the tobacco extract, as well as different doses of the positive control. The duration of the feeding and data collection period was 14 days. Feed intake and body weight were measured daily during the study. Twice daily mortality and morbidity observations were conducted on all study mice as well as twice weekly standard clinical observations. No additional data were collected.

The control mice demonstrated normal body weights and body weight gains for male mice of the age used in the study. Mice fed feed formulated with the tobacco blend, did not demonstrate a strong dose response. The high dose (40 mg nicotine/kg body weight/day) was consistently below that of the control and other doses and could be used as a dose in additional studies. Unlike the tobacco blend, the tobacco extract produced little or no dose response in respect to body weight gains. Mice exposed to feed containing nicotine hydrogen tartrate at nicotine doses equivalent to those of the tobacco blend and extract demonstrated evidence of decreased body weight gain at 20 and 40 mg nicotine/kg body weight/day with the high dose producing a greater change than that seen with the tobacco blend. Feed consumption among the mice from all treatment groups and the control were too erratic to produce useful data.

No effects were seen during clinical observations. This indicates that at the doses used in this study exposure of mice to the tobacco blend, tobacco extract or nicotine hydrogen tartrate produced no observable nicotinic effects.

Study Objectives

The objective of this study was to evaluate the palatability of diets formulated in NTP-2000 rodent feed with a tobacco blend, an aqueous tobacco extract of the tobacco blend and nicotine hydrogen tartrate as positive control when fed to CD-1 Swiss Webster mice.

Materials and Methods

Study Design: A tobacco blend and an aqueous extract of the tobacco blend will be tested in an upcoming series of toxicology studies. Also, a positive control, nicotine hydrogen tartrate, will be used in some of the planned studies. Doses for this study were based upon the nicotine content of the tobacco, tobacco extract and the nicotine tartrate. The tobacco and tobacco extract are complex mixtures of components occurring naturally in the tobacco plant. Nicotine is a significant component of tobacco and is known to be toxic at higher doses and was used as the tobacco component on which the doses were based.

The tobacco blend and aqueous tobacco extract test articles and the positive control were incorporated into the feed for the mice (NTP-2000 rodent feed manufactured by Zeiglar Brothers, Inc., Gardners, PA). There is the possibility that incorporation of the test articles and positive control in the feed may alter its palatability to the mice. If the feed is less palatable than the control diet, the mice may consume less feed with a resulting decrease in body weight gain. This would also result in lower than anticipated doses during toxicology studies. Therefore, it was necessary to ascertain the palatability of the dosed feed to mice.

Palatability was assessed by comparing the feed intake and body weight gain of mice fed the standard NTP-2000 diet (control group) to the feed intake and body weight gain of mice fed NTP-2000 diets formulated to contain different doses of the tobacco blend and different doses of the tobacco extract as well as different doses of the positive control. The duration of the feeding and data collection period was 14 days. Twice daily mortality and morbidity observations were conducted on all study mice as well as twice weekly standard clinical observations. No additional data were collected.

The doses used for the study were chosen to match a concurrent rat study (TOX209) whose doses were based upon those used in a previous rat study of a tobacco material and the published literature. They are expected to assist in the development of doses to be used in a planned mouse short-term repeated dosing study. No undue toxicity was expected at these doses.

The experimental groups and the number of mice per group are provided in Table 1.

Table 1: **Treatment Groups and Doses**[1]

Group Number	Treatment Group (mg nicotine/kg body weight/day)	Number of Mice	Mouse ID Numbers
	Control		
1	NTP-2000 feed	5	1-5
	Tobacco Blend		
2	Dose 1 Tobacco in NTP-2000 feed (0.2)	5	6-10
3	Dose 2 Tobacco in NTP-2000 feed (2.0)	5	11-15
4	Dose 3 Tobacco in NTP-2000 feed (4.0)	5	16-20
5	Dose 4 Tobacco in NTP-2000 feed (8.0)	5	21-25
6	Dose 5 Tobacco in NTP-2000 feed (20.0)	5	26-30
7	Dose 6 Tobacco in NTP-2000 feed (40.0)	5	31-35
	Tobacco Extract		
8	Dose 1 Tobacco Extract in NTP-2000 feed (0.2)	5	36-40
9	Dose 2 Tobacco Extract in NTP-2000 feed (2.0)	5	41-45
10	Dose 3 Tobacco Extract in NTP-2000 feed (4.0)	5	46-50
11	Dose 4 Tobacco Extract in NTP-2000 feed (8.0)	5	51-55
12	Dose 5 Tobacco Extract in NTP-2000 feed (20.0)	5	56-60
13	Dose 6 Tobacco Extract in NTP-2000 feed (40.0)	5	61-65
	Positive Control		
14	Dose 1 Nicotine Tartrate in NTP-2000 feed (2.0)	5	66-70
15	Dose 2 Nicotine Tartrate in NTP-2000 feed (8.0)	5	71-75
16	Dose 3 Nicotine Tartrate in NTP-2000 feed (20.0)	5	76-80
17	Dose 4 Nicotine Tartrate in NTP-2000 feed (40.0)	5	81-85
	Sentinels		
	Sentinels (no treatment)	10	86-95

[1] Doses are based upon mg nicotine/kg body weight/day

Test Articles and Positive Control: Two test articles and a positive control were used for the study. Test Article 1 was identified as Tobacco Blend Lot#0T162AF and consisted of a blend of natural tobaccos ground to a powder, which contained no preservatives or other additives. Test Article 1 contained 2.63% nicotine. The Certificate of Analysis (CofA) for Test Article 1 is on file with the Sponsor. Because the tobacco is a complex mixture of natural components, its purity can not be ascertained. Upon arrival at the testing facility, the tobacco blend was stored at 4 ^{O}C for no more than three weeks before use for the last feed formulation. Test Article 1 was mixed to ensure uniformity before aliquots were removed for feed formulation.

Test Article 2 was identified as Tobacco Extract Lot#0T162AE and consisted of an aqueous extract of Test Article 1. Its water content was adjusted to result in 1 ml of Test Article 2 being equivalent to 1 g of Test Article 1. It contained no components not contained in the tobacco blend and the water used for extraction. The water used for extraction of the tobacco blend was analyzed for a series of components and the results are on file with the sponsor. Because the aqueous extract is a complex mixture of materials extracted from the tobacco, its purity can not be ascertained. Upon arrival at the testing facility, Test Article 2 was maintained frozen at approximately -25 ^{O}C for no more than three weeks before use for the last feed formulation. Before each use for feed formulation, the extract was thawed at room temperature, required aliquots removed and then refrozen. Test Article 2 contained 2.30% nicotine. The CofA for Test Article 2 is on file with the sponsor. Preliminary determination of the density of Test Article 2 revealed a density of 1.203 g/ml.

The positive control used in the study was nicotine hydrogen tartrate (Lot#077K1810). It was obtained from Sigma-Aldrich Co., St. Louis, MO. The CofA for the nicotine salt stated it was 98% pure. Preliminary analysis of the salt at RJRT indicated it was at least 98% pure, if not of higher purity than reported. The nicotine free base is 35.1% of the bulk salt (2.85 g salt contains 1 g of free nicotine). Feed formulation was based upon nicotine and not the bulk salt. The nicotine hydrogen tartrate was stored at room temperature, as recommended by the supplier. After formulation of the first test diet, the nicotine hydrogen tartrate was stored desiccated.

Animals: The protocol and the use of animals for this study were reviewed and approved by the RJRT Institutional Animal Use and Care Committee (IACUC) before arrival of the animals into the facility. Ninety male, juvenile CD-1 Swiss Webster mice (5-7 weeks of age) from Charles River Laboratories (Portage, Maine) were received into the facility on April 09, 2008. An additional 10 male, retired breeder mice were received for use as sentinel animals. These mice were maintained under identical conditions as the study animals, except they were fed Lab Diet, Certified Rodent Diet #5002 feed (PMI Nutrition International), provided as pellets throughout the study.

The mice were housed and cared for in accordance with the Institute of Laboratory Animal Research (ILAR), Commission of Life Sciences, National Research Council document entitled, *Guide for the Care and Use of Laboratory Animals* (1996) in an AAALAC accredited facility.

The mice were housed in a room in the vivarium with controlled lighting (12 hours of darkness, from 6:00 p.m. to 6:00 a.m. +/- 30 minutes. The room temperature was set to maintain 18-26ºC with a relative humidity of 30-70%. Room airflow was greater than 10 room air changes/hour. Seven-day, continuous chart-wheel recordings were kept for room temperature and relative humidity. In addition, room airflow and light cycles were monitored continuously and data recorded every 30 minutes to a computer file via an automated facility data collection system.

Mice were individually housed with no bedding in stainless steel, wire bottomed cages 9 in. (L) x 3.75 in. (W) x 5 in. (H) suspended on stainless steel racks. Mice had *ad libitum* access to NTP-2000 feed during the acclimation period. After acclimation and throughout the dosing period, NTP-2000 feed was provided as a powdered diet formulated with the test articles, positive control or as a control diet with no test article. Data for mice that spilled or contaminated their feed were censored for days when spillage was reported or when the data were unreasonable for the specific animal based upon group means and previous and subsequent feed intake for that specific animal. Water was provided to mice on an *ad libitum* basis through an automatic system. The water source originates from the municipal supply of the City of Winston-Salem, and is subsequently filtered through activated carbon and 5-micron particulate filters prior to delivery to the mice. This water is analyzed semi-annually. There are no known contaminants expected to be present in the feed or water that would be anticipated to interfere with the outcome of the study.

Mice were quarantined and acclimated to the facility for seven days prior to initiation of the study and fed NTP-2000 feed. On the sixth day of the quarantine/acclimation period, mice were assigned to dose groups according to body weight using the “A” module of the PATH/TOX software (version 4.2.2; Xybion Medical Systems; Cedar Knolls, NJ). Body weights and detailed clinical signs were recorded before allocation. To ensure groups of similar mean body weight, all groups within the PATH/TOX protocol were compared by analysis of variance (ANOVA) and least significant difference criteria, and demonstrated not to be significantly different at a 5 percent, two-sided risk level. Following allocation into groups, mice were uniquely identified with their permanent identification number by tail tattoo and assigned to cages with permanent cage cards attached, which recorded the study number, Study Director’s name, species, gender of the animal, group number, pre-allocation animal number, and the animal’s permanent identification number.

The Attending Veterinarian performed a health examination of all mice within four days after delivery. Commencement of mouse dosing was dependent upon a favorable review of the health examination, as well as a written statement from the Attending Veterinarian releasing the mice from quarantine.

After allocation to study groups during the acclimation period, it was noted that some animals demonstrated excessive spillage, manipulated the feed and demonstrated other behaviors that could effect the measurement of feed consumption. To further refine the feeding techniques, the start of the study was delayed five days. During this period there was some drift of the animals’ body weights resulting in group means being different on the first day of formulated feed administration.

Dosed Diet Formulation: The bulk NTP-2000 unformulated feed was stored at refrigerator temperatures (4ºC) in Lab 95 before being aliquotted to the control group and before it was aliquotted to prepare the formulated feeds.

Diets were formulated by the addition of the test article to a portion (premix representing approximately 25% of the total feed mix) of the total diet to be formulated. Mixing was done using commercial mixers. The pre-mix was then added to the bulk diet and mixed to obtain homogeneity. A preliminary test batch of feed formulated with each test article at the high and low dose was produced to refine the formulation techniques required and was not used in the study. Feed formulations were conducted twice during the study. Preparation of formulated feed for the first formulation (Series 1, used during the first week of the study) was based upon extrapolated body weight and feed consumption (30 g body weight, 5 g feed consumed/day) while the data from the first study week were used for these variables for the second formulation (Series 2, used during the second week of the study) (30 g body weight, 6.5 g feed consumed/day). Formulated feeds were stored at room temperature. The control feed was maintained identical to the formulated feed during each feeding period.

Analysis of formulated feed: The preliminary test batch of formulated feed at the high dose and low dose was submitted for analysis of nicotine to determine homogeneity and dose confirmation. Analysis was based upon a previously reported method that involved treating the formulated feed with base followed by solvent extraction and GC-FID quantitation. The initial analysis revealed that this method was appropriate only for the high dose used in the trial formulation. The nicotine concentration in the low dose was below the limit of quantitation for the GC-FID method. Therefore, a new analytical method was rapidly developed at RJRT that allowed quantitation of the nicotine content in the diets at all dose concentrations. This method used GC-MS for quantitation and is not only more sensitive but decreased the sample requirements and analysis time.

Specific subsequent feed formulations at the high dose were assessed for homogeneity and stability and all doses were analyzed for dose confirmation.

Evaluation of Dead or Moribund Animals: Twice daily observations of all mice, once in the morning and once in the afternoon (at least 6 hours apart) were performed to identify dead or moribund mice. Observations were made five days per week, during weekends only one observation per day was performed.

Mice whose condition made it unlikely that they would survive until the next observation period, or appeared to be in pain were to be euthanized and necropsied at the discretion of the Attending Veterinarian or Study Director. Clinical observations were to be recorded shortly before euthanasia.

Any pre-test study mouse, including sentinels, euthanized in a moribund condition during the quarantine/acclimation phase were to have serum collected for serology and necropsied at the discretion of the Attending Veterinarian or Study Director.

Body Weights: Individual non-fasted body weights were determined two days after delivery and again prior to study group allocation (i.e., prior to the initial dosing). Body weights were recorded daily for the duration of the 14-day study. The “A” module of the Xybion PATH/TOX system was used for acquisition of body weight data. Weighing took place at approximately the same time each day. Individual body weights were used to calculate the mean body weight for each experimental group and percent body weight gain. Unscheduled body weight determinations were made at any time, if deemed necessary by the Attending Veterinarian or Study Director. Mouse weights were acquired using Mettler PM2000 balances (Mettler Instrument Corporation, Highstown, NJ)**.** A non-fasted, terminal body weight was obtained from mice euthanized at study completion.

Feed Consumption: On each day of the study, feed was placed into the feed bowl and its weight determined and recorded. The next day, the bowl with uneaten feed was weighed and the feed consumption calculated. Data were entered into the “A” module of the PATH/TOX computer software. Each mouse’s feed consumption was used to calculate the mean feed consumption for the group. In cases of excessive spillage or other inconsistencies, feed weight was recorded but not used to determine mean feed consumption for the group. After determination of the feed consumed by a mouse, additional, fresh feed was placed in a bowl and provided to the mouse after recording the weight in the PATH/TOX software.

Clinical Observations: Except for weekends, daily observations for clinical signs were taken. All positive findings were recorded as unscheduled clinical observations using the "AINPUT" module of the PATH/TOX computer software. Negative findings (normal/no significant findings) were not recorded.

In addition, detailed (scheduled) clinical observations were performed the two days after delivery, when collecting body weights for allocation to study groups and at twice weekly intervals, Monday and Friday, throughout the study. Both positive and negative findings were recorded. The "A" module of the PATH/TOX system was used for acquisition of clinical signs data.

Results and Discussion

Feed Formulation Analysis: During the course of the study three feed formulations were conducted. The first formulation was a trial run to refine the formulation methodology and determine the homogeneity obtained at the high and low doses. The trial run used dosing information for rats to formulate the diet. Even though the rat formulation was used it directly relates to mouse diets because the feed was identical, the test articles were identical and the formulation methodology was identical for rats and mice. Feed from the trial run was not fed to the mice. This was followed by Series 1 formulation for the first week of the study, then Series 2 formulation for the second week of the study. Calculations of feed requirements (feed consumption and body weight) for Series 1 were based upon extrapolation of the growth of the mice. Calculations for Series 2 formulation were based upon data collected during the first week of the study. The formulated feed from certain of these preparations has undergone preliminary analyses for nicotine to determine homogeneity of the test articles and positive control in the diet and for nicotine concentration to confirm that the feed contained the anticipated concentration of nicotine.

Homogeneity data from the trial run are presented in Table 2.

Table 2**: Feed Formulation Trial Run: Homogeneity Data**[1]
(40 mg nicotine/kg body weight/day)

Target Concentration (mg nic/g feed)	Top	Sample Location Middle (mg nic/g feed)	Bottom	Average Concentration (mg nic/g feed)
		Tobacco Blend		
0.50	0.46 ± 0.02 (8%)[2]	0.47 ± 0.01 (6%)	0.45 ± 0.02 (10%)	0.46 ± 0.01 (8% ± 2%)
		Tobacco Extract		
0.50	0.40 ± 0.04 (20%)	0.43 ± 0.06 (14%)	0.39 ± 0.02 (22%)	0.41 ± 0.02 (18% ± 4%)
		Nicotine Tartrate		
0.50	0.42 ± 0.2 (16%)	0.41 ± 0.01 (18%)	0.40 ± 0.01 (20%)	0.41 ± 0.02 (18% ± 2%)

[1] Analytical method uncertainty for nicotine analysis = ± 5.2%, data represent the mean ± SD of triplicate assays. [2] % difference from target concentration

These data are from the high dose preparation for rats (40 mg nicotine/kg body weight/day) but can equally be used for mice since the methodology for diet formulation is identical for each species with the exception of nicotine concentration. Samples were obtained from the top of the formulated feed mixture as well as the middle and bottom of the mixture. Although the low dose preparation (0.2 mg nicotine/kg body weight/day) was also analyzed for nicotine content, the nicotine concentration was below the limit of quantitation for the GC-FID analysis method.

Feed formulated with the tobacco blend demonstrated good homogeneity with the samples being within ± 10% of each other. The mean concentration of nicotine in the feed indicated that it was within ± 10% of the anticipated concentration, indicating adequate dose confirmation. Visual inspection of the formulated feed indicated no change in the color of the feed and there were no visible evidence of tobacco particles.

Feed formulated with the aqueous tobacco extract demonstrated adequate homogeneity but was below the anticipated nicotine concentration. The problem appears to occur at the pre-mix stage. The extract is viscous and tends to stick to the blade of the mixer and to some extent the mixing bowl. This would result in a lower than expected concentration. Based upon these data, the mixing methodology was altered to decrease the potential for the extract to contact the blending device.

The nicotine hydrogen tartrate also demonstrated adequate homogeneity in feed but the nicotine concentration was lower than anticipated. Based upon these data, the mixing methodology was modified by placing a small portion of the diet in a mortar and pestle to which the nicotine salt was added. Lumps of the salt were gently broken and mixed with the feed. When there were no longer any visible lumps, the feed was then added to the pre-mix for mechanical mixing. Also, the nicotine salt was stored in a desiccator to minimize moisture absorption.

Homogeneity data for the Series 1 and Series 2 formulations are currently unavailable.

Dose Confirmation Data: Series 1 analytical data were collected during an early stage of development of the GC-MS analytical methodology and may be unreliable and are not presented in this preliminary report. It is anticipated that these samples will be reanalyzed. Dose confirmation data for Series 2 formulations are presented in Tables 3 – 5, respectively. These data are presented as a general comparison not as absolute quantitative data. The nicotine quantitation data are from an analytical method that is currently under development and has not been extensively validated. The data are useful to confirm that the diets contained increasing quantities of nicotine and indicate that there were no major errors in formulation. Additional data may be available in the near future and will be incorporated into the final report for the study. These data in combination with the dose responses seen in the study indicate that the proper formulated feeds were fed to the mice.

Table 3: **Series 2 Dose Confirmation Data Tobacco Blend**

Target Dose (mg of nicotine/kg of bw/day)	**Target Concentration** (mg of nicotine/g of feed)	**Feed Nicotine Concentration** (mg of nicotine/g of feed)
0.2	0.0009	0.0011
2.0	0.0092	0.009
4.0	0.0185	0.016
8.0	0.0369	0.030
20.0	0.0923	0.090
40.0	0.1846	0.184

Table 4: **Series 2 Dose Confirmation Data Tobacco Extract**

Target Dose (mg of nicotine/kg of bw/day)	**Target Concentration** (mg of nicotine/g of feed)	**Feed Nicotine Concentration** (mg of nicotine/g of feed)
0.2	0.0009	0.0008
2.0	0.0092	0.004
4.0	0.0185	0.020
8.0	0.0369	0.018
20.0	0.0923	0.062
40.0	0.1846	0.205

Table 5: **Series 2 Dose Confirmation Data Nicotine Tartrate**

Target Dose (mg of nicotine/kg of bw/day)	**Target Concentration** (mg of nicotine/g of feed)	**Feed Nicotine Concentration** (mg of nicotine/g of feed)
2.0	0.0092	0.008
8.0	0.0369	0.031
20.0	0.0923	-
40.0	0.1846	0.233

These data indicate that the concentrations of nicotine in the formulated feeds were adequate for the purposes of this study.

Stability: Preliminary stability data for nicotine from each test article and the positive control are available for the high dose formulation made during the prestudy trial run. A sample set was allowed to remain at room temperature for 30 days after initial formulation. The 30-day old samples are compared to the fresh samples in Table 6. These data provide no indication of instability of the nicotine in the 30-day old feed samples.

Table 6: **Prestudy Trial Formulations 30 Day Stability Data**[1]

Dose (mg nic/kg bw/day	Target Concentration (mg nic/g feed)	"0" day (mg nic/g feed)	30 Day (mg nic/g feed)	% Difference
		Tobacco Blend		
40	0.50	0.47 ± 0.01	0.44	-6.4
		Tobacco Extract		
40	0.50	0.43 ± 0.06	0.44	+2.3
		Nic. Tartrate		
40	0.50	0.41 ± 0.01	0.42	+2.4

[1] Formulated feed stored at room temperature in the mixing room.

Preliminary stability data are also available for nicotine from low and high dose of each test article and the positive control for the rat Series 2 feed formulations. Because the mouse formulated feed was identical to that of the rat except for the exact nicotine concentrations, these data should also apply for the mouse formulations. These data compare the nicotine content of freshly prepared feed with that of feed maintained in the animal room for one week. There is no indication of a lack of stability of nicotine in these samples. The variability seen in the low dose tobacco blend is believed to be due to the analytical variability and not to a loss of nicotine because no differences were seen with the extract and positive control.

Table 7: **Series 2 Formulations One Week Stability Data**[1]

Dose (mg nic/kg bw/day	Target Concentration (mg nic/g feed)	"0" day (mg nic/g feed)	One Week (mg nic/g feed)	% Difference
		Tobacco Blend		
0.2	0.002	0.004	0.002	-50
40	0.433	0.36	0.41	+14
		Tobacco Extract		
0.2	0.002	0.001	0.001	0
40	0.433	0.31	0.37	+19
		Nic. Tartrate		
2.0	0.021	0.019	0.019	0
40	0.433	0.036	0.036	0

[1] Formulated feed stored in the animal room for one week.

Body Weight Data: Percent body weight gain data for mice fed the tobacco blend at different doses of nicotine are provided in Figure 1, while absolute body weight gain is presented in Figure 2. The data in Figure 1 are presented in terms of percent body weight gain that normalizes the body weights, which differed slightly at the initiation of the study. Individual body weights, group mean body weights and their standard deviations as well as additional graphs are presented in the Appendix of this report.

Figure 1

Study TOX210 Mouse Body Weight Gain : Test Article Tobacco Blend

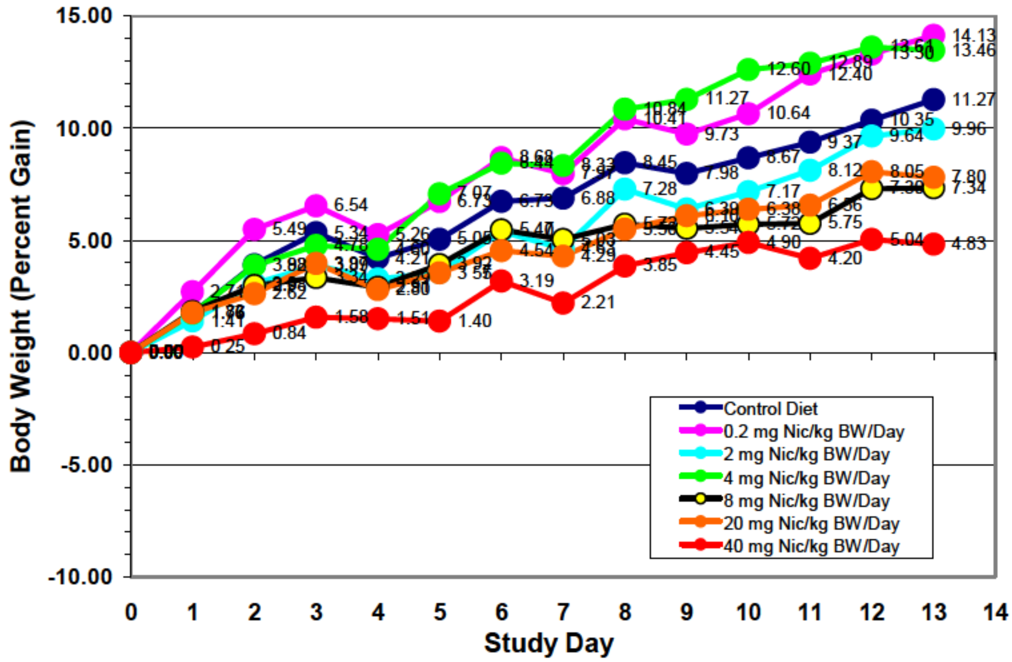
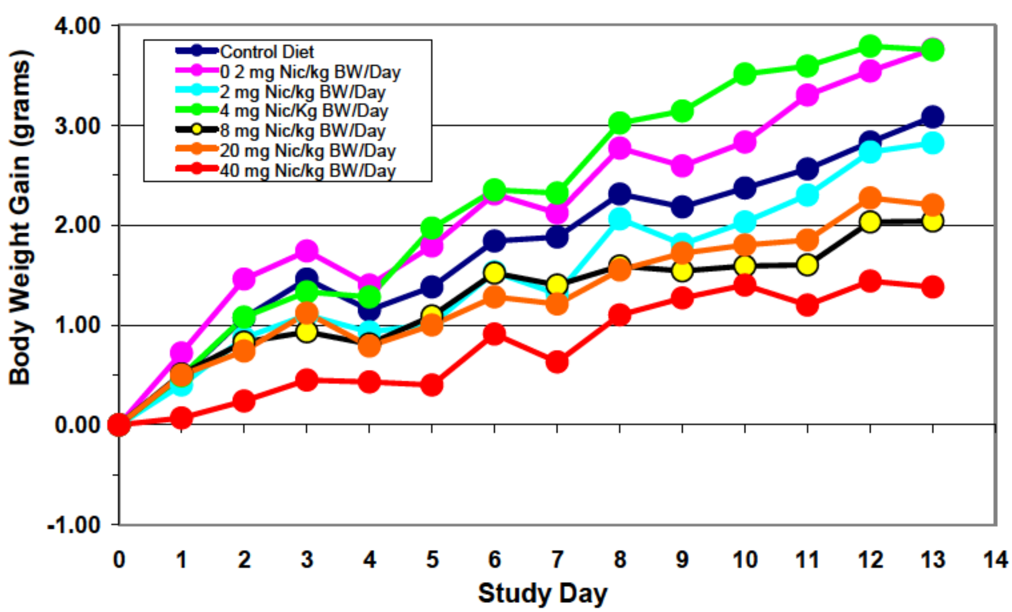


Figure 2

Study TOX210 Mouse Body Weights: Test Article Tobacco Blend
Absolute Gain in Body Weight (grams)



The control demonstrated normal body weight gains for male mice of this age. There is not a strong dose response demonstrated in the data. For two of the nicotine doses, 0.2 and 4 mg nicotine/kg body weight/day, the trend in percent body weight gain actually surpassed that of the control group. The 2 mg nicotine dose trend for body weight gain was slightly below that of the control group, while the 8 and 20 mg nicotine/kg body weight/day were almost identical and their trend in body weight gain was below that of the control. The high dose, 40 mg nicotine/kg body weight/day, was consistently below that of the control and the other doses. The increase in percent body weight gain at the high dose was about one half that of the control, indicating a definitive effect at this dose.

These data indicate that the mice could detect, either at an organoleptic level or a neurophysiological level, the presence of the tobacco blend at the high dose used in this study. These data do not provide evidence for a dose that would produce unwarranted reductions in percent body weight gain but do indicate that a dose of 40 mg nicotine/kg body weight/day could be a potential lower dose for additional studies.

At least two possibilities should be considered in respect to explaining these data. First, at an organoleptic level, the mice may consider diets containing the tobacco to lack palatability and consume them at a lower rate than the control diet. As the dose increased the palatability of the feed became lower resulting in less feed consumption with the resulting decrease in body weight gain. Second, at the neurophysiological level, it is possible that the nicotine in the tobacco blend produced changes in the peripheral or central nervous system that were undetected in this palatability study. These changes could have produced an appetite depression or other effect that may have altered feed intake and body weight gain.

Percent body weight gain data for mice fed the feed formulated with the tobacco extract are presented in Figure 3, while absolute body weight gain data are presented in Figure 4.

Figure 3

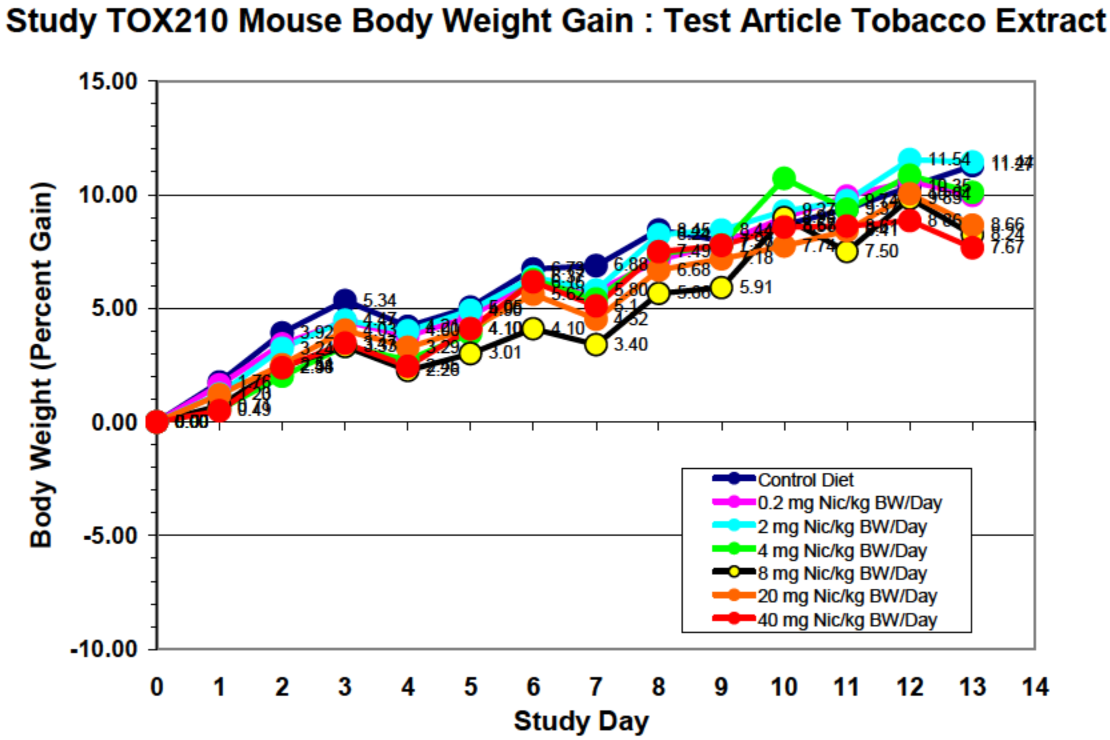
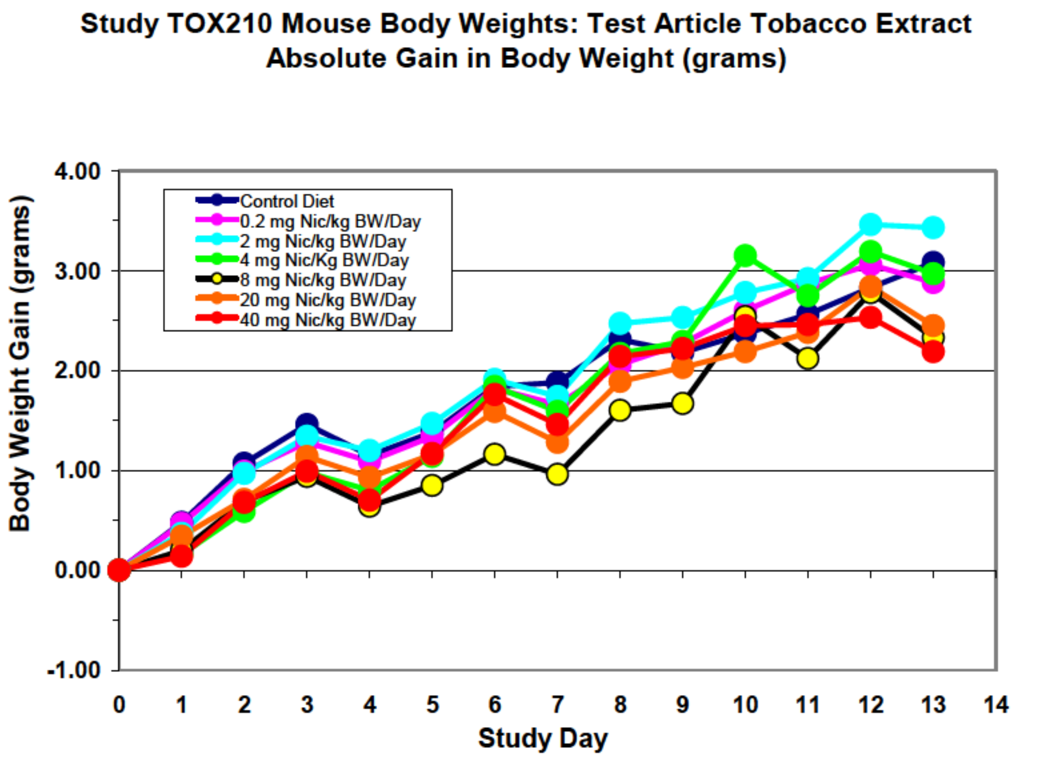


Figure 4



Although there was a trend toward the mice receiving the feed formulated with different doses of the tobacco extract to have slightly reduced body weight gain, there is little evidence of dose related differences. Unlike the tobacco blend there is no dose that shows a definitive trend toward reduced body weight percent gain. The reasons for the different response compared to the tobacco blend are not apparent from the results of this study.

Figure 5 provides the percent body weight gain data for mice fed diets containing the positive control, nicotine hydrogen tartrate, while Figure 6 provides the absolute body weight gains.

Figure 5

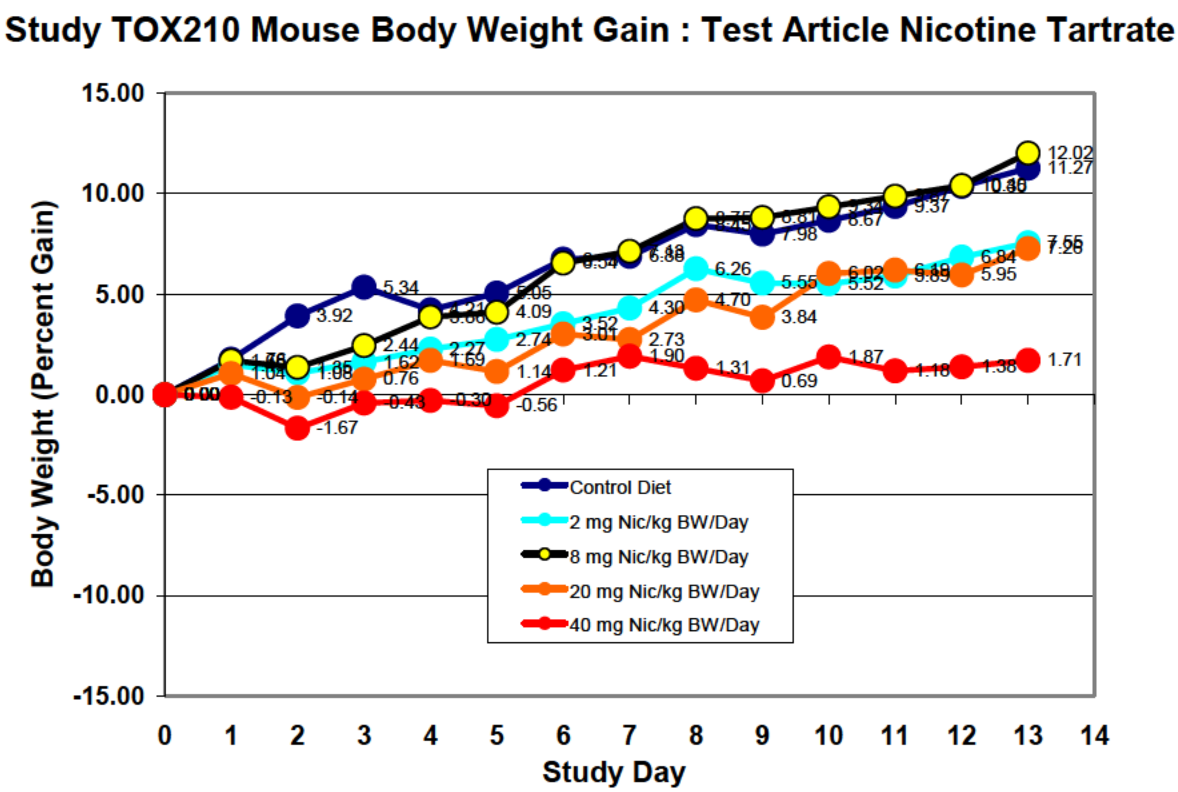
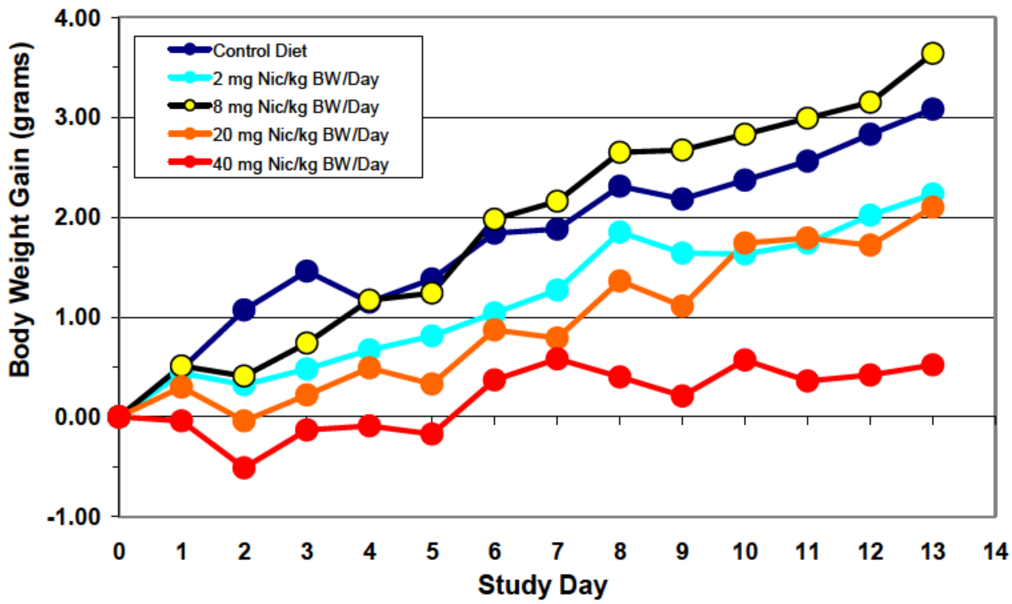


Figure 6

Study TOX210 Mouse Body Weights: Test Article Nicotine Tartrate Absolute Gain in Body Weight (grams)



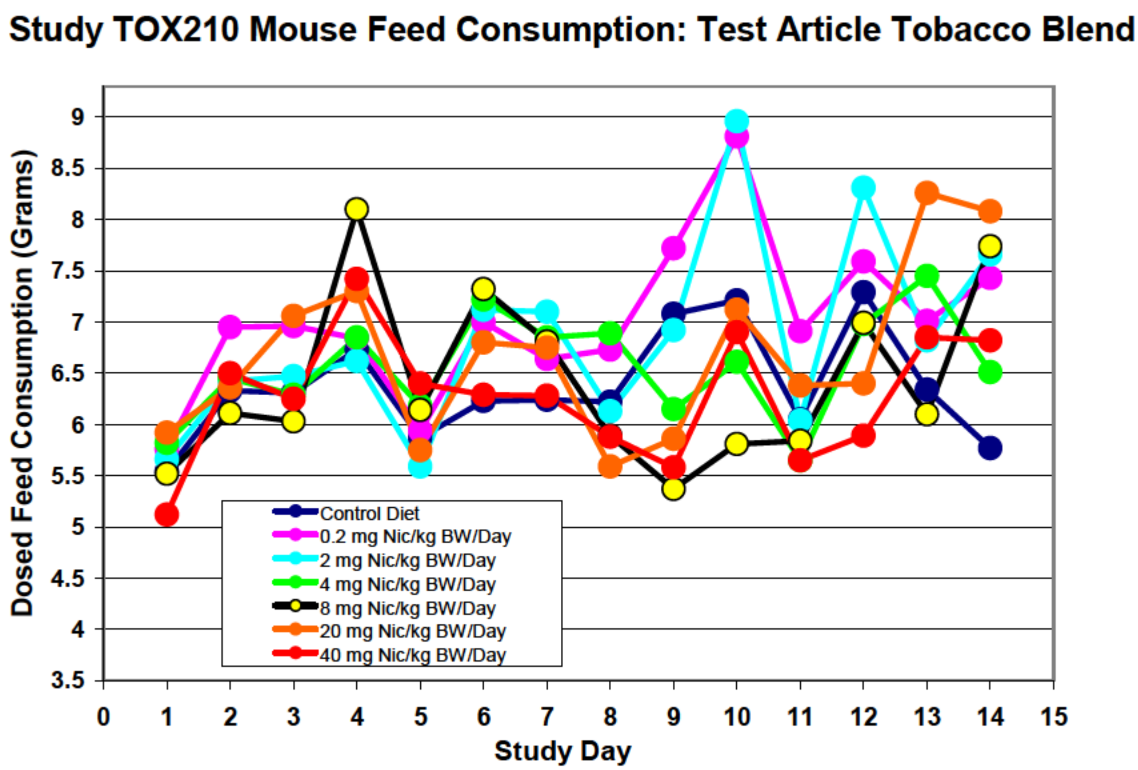
Mice fed feed containing nicotine tartrate demonstrated a definitive dose response in percent body weight gain at the 20 and 40 mg nicotine/kg body weight/day doses. At the high dose there was no increase in percent body weight gain until day six of the study and from day six to study termination there was little change in percent body weight gain, while the control demonstrated a normal increase in percent body weight gain. At 20 mg nicotine/kg body weight, there was a lag of 2-3 days before body weight gain began to increase. At this dose, body weight gain continued to increase throughout the remainder of the study at a rate similar to the control but remained quantitatively below that of the control. The 2 mg nicotine/kg body weight/day dose followed a trend similar to the 20 mg nicotine dose but remained quantitatively below that of the control. At 8 mg nicotine/kg body weight/day, there was a depression in body weight on days 2 and 3 compared to the control while on day 4 body weight was equivalent to the control. During the second week of the study, this dose group's mean body weight was similar, if not slightly higher, than that of the control group. Nicotine hydrogen tartrate produced a somewhat greater effect at 40 mg nicotine/kg body weight that did the tobacco blend. The overall trends in the data follow those seen with the tobacco blend as opposed to the tobacco extract. Whether or not this is due to a lack of palatability of the feed or to a physiological effect of nicotine can not be ascertained from this study.

Based upon these nicotine hydrogen tartrate data, doses substantially higher than 40 mg nicotine/kg body weigh/day for long durations may result in unwarranted decreases in body weight in mice fed diets formulated with this nicotine salt.

Because the nicotine tartrate dosed group contained no tobacco, these data may indicate that the decreased percent body weight gains seen in this study in the various treatment groups may be associated with their nicotine content more than with the presence of other tobacco components.

Feed Consumption: Feed consumption data for mice fed feed containing the tobacco blend are shown in Figure 7.

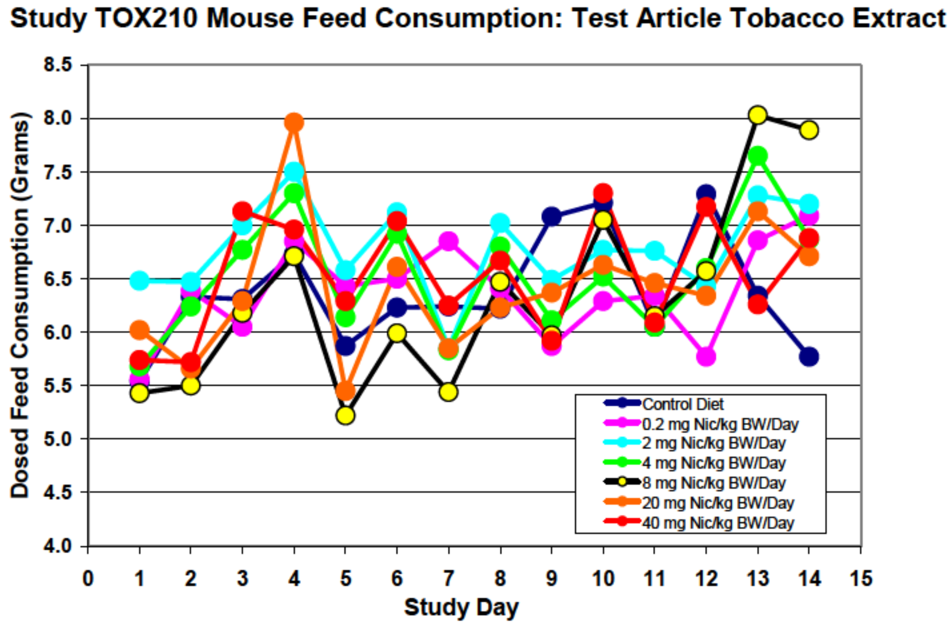
Figure 7



Data for feed consumption for the mice in this study appears highly erratic, even for the control group. No dose related trends can be ascertained.

Feed consumption data for mice fed feed containing the tobacco extract are shown in Figure 8.

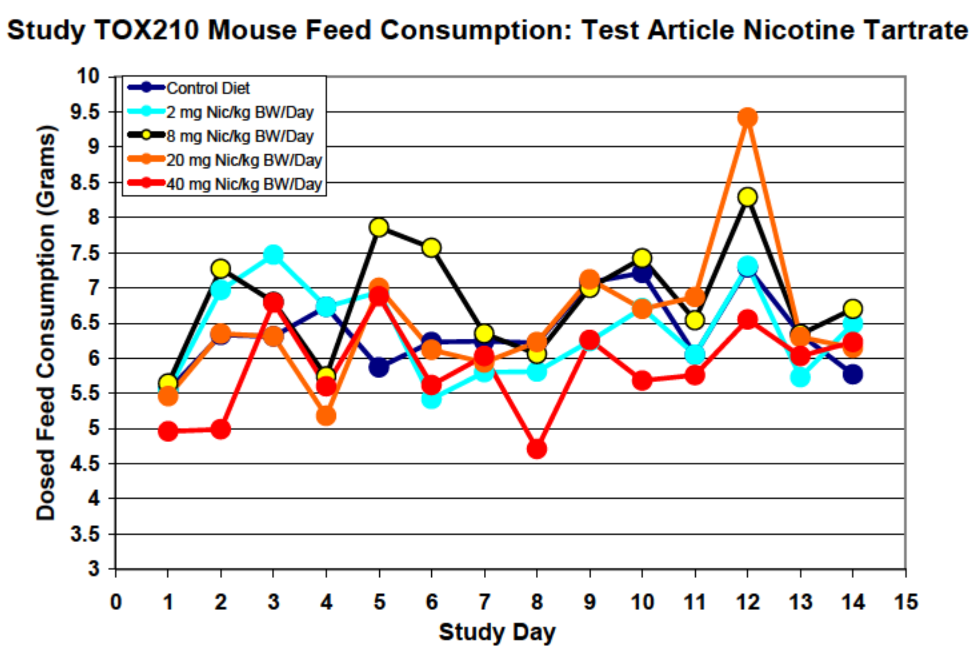
Figure 8



As seen with the tobacco blend, the feed consumption data for the tobacco extract appears erratic and no dose trends are obvious.

Feed consumption data for the mice fed feed containing nicotine hydrogen tartrate are shown in Figure 9.

Figure 9



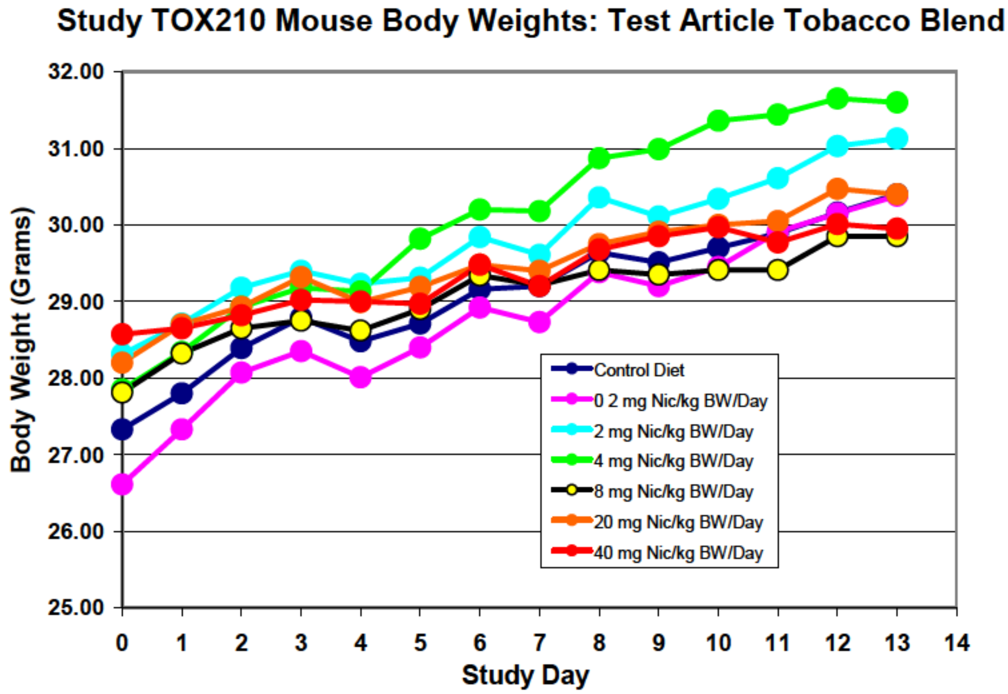
As seen with the tobacco blend and the tobacco extract, the data for feed consumption for the mice dosed with nicotine hydrogen tartrate are too erratic to discern dose related effects.

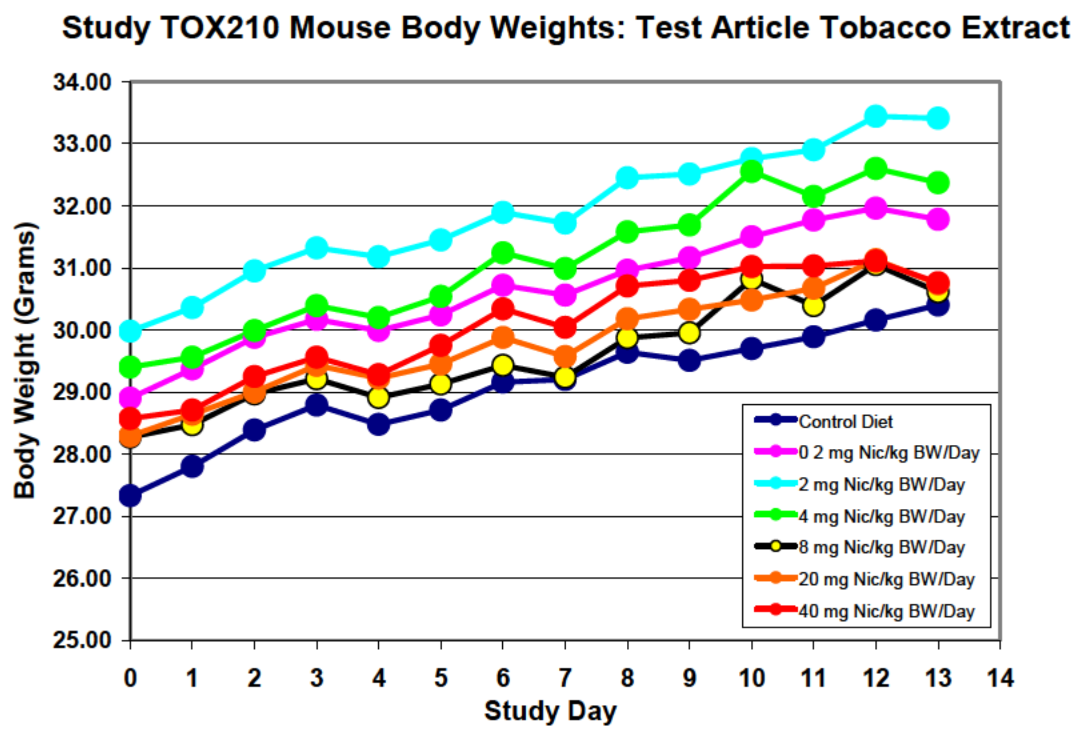
Clinical Observations: There were no clinical observations indicating altered behavior or any other evidence of nicotine toxicity during the study. This indicates that the doses used in the study were below those that may elicit nicotinic effects in the animals detectable by clinical observations.

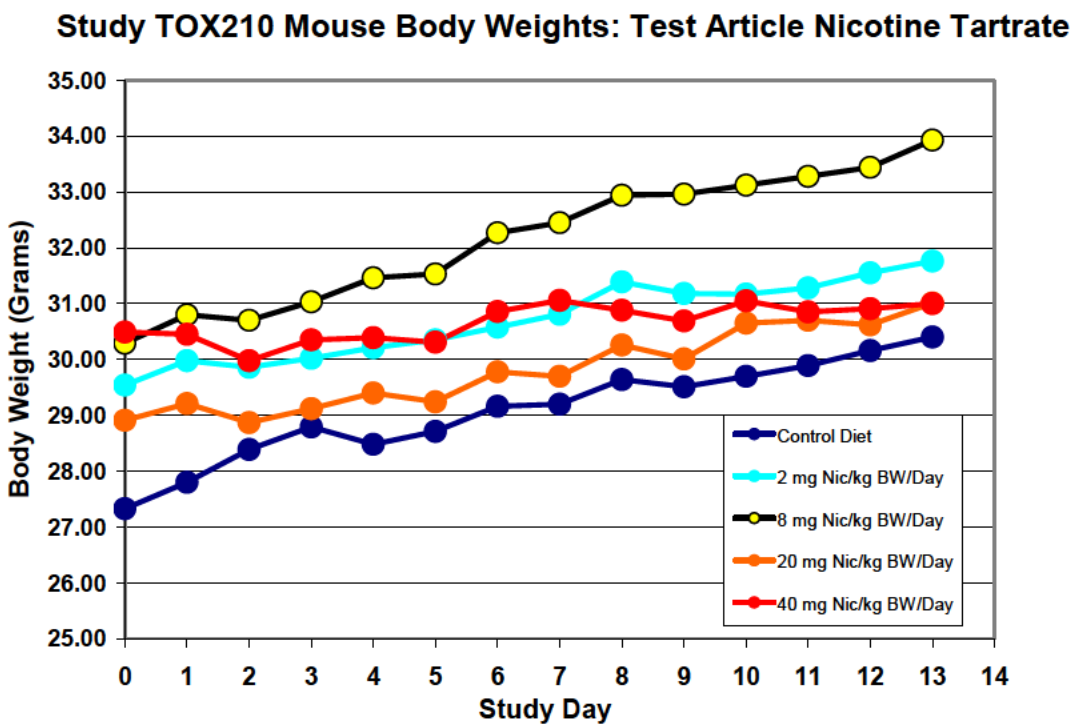
Serology: Serological screening provided no indication of disease in the sentinel mice.

Appendix

Additional Graphs of Data






Study TOX210 Mouse Body Weights: Test Article Nicotine Tartrate
Body Weight (Grams)
35.00
34.00
33.00
32.00
31.00
30.00
29.00
28.00
27.00
26.00
25.00
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Study Day
Control Diet
2 mg Nic/kg BW/Day
8 mg Nic/kg BW/Day
20 mg Nic/kg BW/Day
40 mg Nic/kg BW/Day

Comments Concerning Path/Tox Data Outputs

Data associated with the use of mice on study were acquired with the aid of the Path/Tox (Xybion Medical Systems, Cedar Knolls, NJ) software version 4.2.2 resident on a VAX operating system under the Path/Tox protocols referred to as TOX210A and TOX210B.

The Xybion data collection protocols TOX210A and TOX210B were used for body weights and feed consumption of mice used on this study. Body weight data and feed consumption data were input into the Xybion Path/Tox collection protocols under "A" module, "AINPUT'.

Because of the limitations in the Path/Tox system, two protocols were created to accommodate all 17 dosed groups. TOX210A contains Dose Groups 1-13. TOX210B contains the four Nicotine Tartrate Positive Control Groups .

Xybion Group Number	*Treatment Group (Doses based on Nicotine) (mg/kg body weight/day)*	*Number of Mice*	*Mouse ID Numbers*
	Xybion Protocol TOX210A Control		
1	NTP-2000 feed	5	1-5
	Xybion Protocol TOX210A Tobacco Blend		
2	Dose 1 Tobacco in NTP-2000 feed (0.2)	5	6-10
3	Dose 2 Tobacco in NTP-2000 feed (2.0)	5	11-15
4	Dose 3 Tobacco in NTP-2000 feed (4.0)	5	16-20
5	Dose 4 Tobacco in NTP-2000 feed (8.0)	5	21-25
6	Dose 5 Tobacco in NTP-2000 feed (20.0)	5	26-30
7	Dose 6 Tobacco in NTP-2000 feed (40.0)	5	31-35
	Xybion Protocol TOX210A Tobacco Extract		
8	Dose 1 Tobacco Extract in NTP-2000 feed (0.2)	5	36-40
9	Dose 2 Tobacco Extract in NTP-2000 feed (2.0)	5	41-45
10	Dose 3 Tobacco Extract in NTP-2000 feed (4.0)	5	46-50
11	Dose 4 Tobacco Extract in NTP-2000 feed (8.0)	5	51-55
12	Dose 5 Tobacco Extract in NTP-2000 feed (20.0)	5	56-60
13	Dose 6 Tobacco Extract in NTP-2000 feed (40.0)	5	61-65
	Xybion Protocol TOX210B Nicotine Tartrate		
1	Dose 1 Nicotine Tartrate in NTP-2000 feed (2.0)	5	66-70
2	Dose 2 Nicotine Tartrate in NTP-2000 feed (8.0)	5	71-75
3	Dose 3 Nicotine Tartrate in NTP-2000 feed (20.0)	5	76-80
4	Dose 4 Nicotine Tartrate in NTP-2000 feed (40.0)	5	81-85
	Sentinels		
	Sentinels (no treatment)	10	86-95

Because the start of the exposure phase was delayed five days for both TOX210A and TOX210B studies, Exposure Phase Day 6 of the Xybion data output is study Day 0 when the dosed feed was first administered to the mice (April 21, 2008). Day 1 (Exposure Phase Day 7 of the Xybion data output) represents data collected after a full 24-hr exposure either to the control feed, or the tobacco blend, or tobacco extract or nicotine tartrate formulated feed.

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
MOUSE/SWISS WEBSTER

Animal body weights in (g)
Study number: TOX210A

Dosing start date: 16-Apr-08

PRINTED: 08-May-08
Page: 1

FEEDING STUDY/PALATABILITY

Animal	Group		Day of Phase 6	7	8	9	10	11	12	13	14	15	16	17	18
			Male Animals												
1	1		30.41	30.65	31.36	32.00	31.68	31.71	31.86	31.98	32.52	32.44	32.69	33.05	33.33
2			28.83	29.47	30.23	30.52	30.12	30.55	31.30	31.17	32.05	31.85	32.27	32.41	33.20
3			29.52	30.19	30.61	31.17	31.06	31.24	32.15	32.42	32.70	32.89	33.47	33.11	33.51
4			25.75	26.46	26.97	27.49	27.33	27.69	28.07	28.32	28.46	28.25	28.06	28.52	28.41
5			22.12	22.24	22.79	22.76	22.20	22.36	22.44	22.12	22.45	22.11	22.00	22.34	22.35
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	27.33	27.80	28.39	28.79	28.48	28.71	29.16	29.20	29.64	29.51	29.70	29.89	30.16
		Sdevs	3.40	3.51	3.55	3.78	3.88	3.88	4.10	4.27	4.37	4.52	4.79	4.63	4.86
6	2		29.34	30.71	31.59	31.90	31.38	32.20	32.32	32.08	32.90	32.72	32.90	33.64	34.01
7			26.14	26.61	27.19	27.63	27.29	27.78	28.60	28.34	29.29	29.07	29.50	29.89	30.02
8			23.21	23.81	24.55	24.93	24.64	24.53	25.20	25.09	25.83	25.61	25.79	26.24	26.50
9			26.64	27.17	27.85	28.02	27.86	28.32	28.74	28.75	28.99	28.72	29.09	29.01	29.37
10			27.73	28.36	29.18	29.29	28.87	29.17	29.73	29.39	29.91	29.89	29.95	30.76	30.85
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	26.61	27.33	28.07	28.35	28.01	28.40	28.92	28.73	29.38	29.20	29.45	29.91	30.15
		Sdevs	2.26	2.52	2.59	2.54	2.45	2.76	2.56	2.50	2.52	2.55	2.53	2.69	2.71
11	3		33.30	33.94	34.32	34.97	34.99	35.14	35.56	35.24	36.11	35.87	36.41	36.69	37.59
12			26.55	26.65	27.16	27.02	27.35	27.30	28.22	28.27	29.15	28.91	28.91	29.38	29.53
13			28.86	29.03	29.78	30.36	29.44	29.52	30.47	30.00	30.45	30.14	30.69	30.65	31.02
14			28.56	29.13	29.71	29.61	29.26	29.13	29.36	29.19	29.57	29.42	29.55	29.84	30.02
15			24.26	24.80	24.91	25.05	25.13	25.48	25.58	25.36	26.54	26.22	26.14	26.49	27.01
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	28.31	28.71	29.18	29.40	29.23	29.31	29.84	29.61	30.36	30.11	30.34	30.61	31.03
		Sdevs	3.35	3.43	3.51	3.76	3.66	3.63	3.68	3.60	3.53	3.54	3.78	3.74	3.95
16	4		25.66	26.13	27.02	27.34	27.50	28.66	28.69	28.63	29.47	29.62	29.38	29.54	29.42
17			30.00	30.83	31.42	32.01	31.80	32.28	32.99	33.23	33.96	34.19	34.88	34.85	35.20
18			29.85	30.42	31.02	31.53	31.42	32.02	32.38	32.44	33.60	33.76	34.41	34.26	34.58
19			27.74	28.09	28.49	28.45	28.26	29.10	29.49	29.74	30.08	30.13	30.63	30.62	30.87
20			26.01	26.23	26.69	26.58	26.69	27.04	27.44	26.84	27.25	27.26	27.50	27.95	28.16
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	27.85	28.34	28.93	29.18	29.13	29.82	30.20	30.18	30.87	30.99	31.36	31.44	31.65
		Sdevs	2.05	2.23	2.20	2.46	2.33	2.26	2.39	2.65	2.86	2.93	3.20	3.00	3.12
21	5		27.97	28.31	28.69	28.70	28.63	28.88	29.10	29.22	29.73	29.31	29.21	29.12	29.60
22			30.10	30.61	31.09	30.93	30.79	31.12	31.81	31.78	31.47	31.68	31.77	31.80	32.11
23			28.03	28.20	28.15	28.68	28.56	29.15	29.41	29.19	29.24	29.44	29.52	29.44	30.09
24			26.08	26.76	27.08	27.04	26.99	27.37	27.72	27.37	27.79	28.09	28.04	28.08	28.88

Note: Data for Exposure phase

R.J.R. TOBACCO | Animal body weights in (g) | PRINTED: 08-May-08
TOXICOLOGY DIVISION | Study number: TOX210A | Page: 2
Building 630/2
MOUSE/SWISS WEBSTER | Dosing start date: 16-Apr-08 | FEEDING STUDY/PALATABILITY

Animal	Group		6	7	8	9	10	11	12	13	14	15	16	17	18
							Male Animals								
25	5		26.89	27.74	28.22	28.38	28.14	27.99	28.65	28.51	28.81	28.24	28.49	28.61	28.56
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	27.81	28.32	28.65	28.75	28.62	28.90	29.34	29.21	29.41	29.35	29.41	29.41	29.85
		Sdevs	1.51	1.42	1.49	1.40	1.38	1.43	1.52	1.62	1.36	1.44	1.44	1.43	1.40
26	6		32.03	32.81	32.84	33.49	33.41	33.65	34.03	33.82	34.06	34.34	34.54	34.77	35.20
27			27.12	27.44	27.50	27.91	27.71	28.23	28.77	28.73	29.20	29.30	29.43	29.68	30.12
28			24.87	25.17	25.34	25.60	25.28	25.40	25.46	25.35	25.80	25.92	26.05	26.10	26.32
29			28.76	29.22	29.94	30.19	29.64	30.06	30.08	30.24	30.78	30.87	31.07	31.01	31.33
30			28.20	28.86	29.04	29.41	28.91	28.63	29.06	28.87	28.90	29.13	28.89	28.69	29.36
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	28.20	28.70	28.93	29.32	28.99	29.19	29.48	29.40	29.75	29.91	30.00	30.05	30.47
		Sdevs	2.61	2.79	2.80	2.91	2.97	3.01	3.08	3.06	3.01	3.06	3.12	3.19	3.23
31	7		29.30	29.70	29.91	30.14	29.80	29.70	30.09	29.90	30.16	30.26	30.09	29.95	30.56
32			29.07	28.94	28.83	28.90	29.30	28.97	30.08	29.88	30.34	30.86	30.84	30.36	30.76
33			28.47	28.23	28.93	29.20	29.48	29.49	29.98	29.85	30.18	30.57	30.90	30.91	30.93
34			31.03	31.06	30.82	31.27	30.79	31.24	31.25	30.61	31.61	31.49	31.72	31.40	31.43
35			24.99	25.30	25.59	25.59	25.63	25.47	26.02	25.77	26.09	26.05	26.30	26.23	26.39
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	28.57	28.65	28.82	29.02	29.00	28.97	29.48	29.20	29.68	29.85	29.97	29.77	30.01
		Sdevs	2.22	2.14	1.98	2.13	1.97	2.13	2.01	1.94	2.09	2.17	2.13	2.05	2.05
36	8		24.01	24.47	24.90	25.06	24.72	24.92	25.69	25.42	25.75	25.62	26.16	26.15	26.32
37			30.93	31.29	32.22	32.34	32.83	33.45	33.51	33.06	33.80	33.81	34.27	34.35	34.51
38			29.34	29.68	30.22	30.40	29.97	30.00	30.50	30.54	30.56	31.01	31.33	31.40	31.94
39			31.70	32.33	32.66	33.23	32.63	32.93	33.62	33.52	34.05	34.31	34.56	35.14	34.97
40			28.51	29.06	29.42	29.84	29.79	29.90	30.29	30.26	30.64	31.07	31.17	31.80	32.06
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	28.90	29.37	29.88	30.17	29.99	30.24	30.72	30.56	30.96	31.16	31.50	31.77	31.96
		Sdevs	3.01	3.03	3.10	3.18	3.27	3.39	3.23	3.22	3.35	3.45	3.38	3.53	3.44
41	9		28.19	28.93	29.29	29.86	29.70	29.81	30.24	29.81	30.65	30.83	30.97	31.06	31.44
42			28.31	28.86	29.21	29.65	29.90	30.28	30.35	30.41	30.71	30.36	30.99	30.69	31.69
43			33.90	34.08	35.08	35.22	35.51	35.87	36.08	36.08	36.82	36.79	37.28	37.36	37.63
44			32.64	32.80	33.35	33.83	33.77	33.57	34.81	35.12	35.88	35.45	35.24	35.90	36.10
45			26.87	27.11	27.83	28.04	27.03	27.73	27.96	27.19	28.19	29.13	29.34	29.51	30.36
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	29.98	30.36	30.95	31.32	31.18	31.45	31.89	31.72	32.45	32.51	32.76	32.90	33.44
		Sdevs	3.09	2.94	3.10	3.05	3.41	3.24	3.41	3.76	3.72	3.38	3.34	3.49	3.21

Column heading spanning days 6–18: Day of Phase

Note: Data for Exposure phase

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			Day of Phase												
Animal	Group		6	7	8	9	10	11	12	13	14	15	16	17	18
			Male Animals												
46	10		26.17	26.67	27.19	27.32	27.04	27.78	28.69	27.91	29.28	29.03	29.16	29.40	29.70
47			30.29	30.27	30.70	30.97	30.61	30.73	31.59	30.97	31.17	31.32	34.58	30.85	31.06
48			30.78	31.40	31.59	32.08	32.16	32.42	33.17	33.46	34.10	34.34	34.52	35.22	35.48
49			28.27	28.23	28.45	28.95	28.82	29.18	29.66	29.57	29.76	29.75	29.79	30.24	30.64
50			31.50	31.22	32.01	32.61	32.36	32.59	33.10	33.05	33.57	34.02	34.69	35.03	36.10
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	29.40	29.56	29.99	30.39	30.20	30.54	31.24	30.99	31.58	31.69	32.55	32.15	32.60
		Sdevs	2.17	2.05	2.08	2.21	2.27	2.08	2.02	2.34	2.18	2.42	2.81	2.77	2.97
51	11		31.46	31.61	32.36	32.90	32.28	32.65	33.05	32.72	33.31	33.38	33.68	33.88	34.42
52			27.06	27.18	27.67	28.03	27.79	27.60	27.87	27.68	27.98	28.16	28.49	28.60	29.01
53			27.85	28.18	28.47	28.52	28.24	28.59	28.64	28.84	29.42	29.31	29.99	27.17	27.75
54			25.70	25.73	26.07	26.60	26.40	26.56	27.11	26.41	27.36	27.46	30.01	30.26	30.84
55			29.30	29.65	30.27	30.00	29.86	30.23	30.50	30.54	31.30	31.42	31.91	32.05	33.24
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	28.27	28.47	28.97	29.21	28.91	29.13	29.43	29.24	29.87	29.95	30.82	30.39	31.05
		Sdevs	2.21	2.26	2.43	2.39	2.25	2.39	2.38	2.47	2.45	2.44	2.01	2.67	2.80
56	12		32.54	33.01	33.40	33.55	33.58	33.53	33.69	33.17	33.10	33.06	33.58	33.59	33.89
57			26.90	27.65	28.04	28.56	28.28	28.88	28.94	28.81	28.98	29.08	29.07	28.81	29.31
58			30.30	30.35	30.87	30.96	30.78	31.34	31.89	31.97	32.57	32.84	33.01	33.95	34.16
59			23.42	23.69	24.14	24.46	24.38	24.37	24.76	23.86	24.99	25.34	25.25	25.30	26.04
60			28.30	28.48	28.55	29.62	29.09	29.15	30.13	30.06	31.28	31.31	31.49	31.71	32.27
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	28.29	28.64	29.00	29.43	29.22	29.45	29.88	29.57	30.18	30.33	30.48	30.67	31.13
		Sdevs	3.46	3.45	3.45	3.35	3.38	3.41	3.38	3.61	3.31	3.21	3.40	3.63	3.44
61	13		28.45	28.79	28.92	29.19	28.90	29.25	30.27	29.87	30.37	30.40	30.28	30.32	30.21
62			26.17	26.19	27.03	27.28	27.29	27.60	28.29	28.24	28.57	28.75	29.23	29.24	29.35
63			26.97	27.38	27.48	27.65	27.37	27.92	28.74	27.97	29.31	29.42	29.78	29.74	30.05
64			28.93	28.27	29.04	29.35	29.16	29.59	29.83	29.66	29.66	29.78	30.06	30.06	29.93
65			32.35	32.93	33.79	34.33	33.66	34.37	34.56	34.44	35.65	35.64	35.75	35.79	36.00
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	28.57	28.71	29.25	29.56	29.28	29.75	30.34	30.04	30.71	30.80	31.02	31.03	31.11
		Sdevs	2.38	2.56	2.68	2.82	2.60	2.72	2.49	2.60	2.84	2.77	2.67	2.69	2.75

Note: Data for Exposure phase

Animal	Group		Day of Phase 19
			Male Animals
1	1		33.76
2			33.40
3			33.84
4			28.54
5			22.47
		(n)	5
		Means	30.40
		Sdevs	4.96
6	2		34.04
7			30.14
8			26.79
9			29.66
10			31.25
		(n)	5
		Means	30.38
		Sdevs	2.63
11	3		37.81
12			29.66
13			31.12
14			30.29
15			26.76
		(n)	5
		Means	31.13
		Sdevs	4.08
16	4		29.40
17			35.21
18			34.26
19			31.26
20			27.88
		(n)	5
		Means	31.60
		Sdevs	3.12
21	5		29.78
22			32.09
23			30.22
24			28.86

Note: Data for Exposure phase

R.J.R. TOBACCO
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MOUSE/SWISS WEBSTER

Animal body weights in (g)
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FEEDING STUDY/PALATABILITY

Animal	Group		Day of Phase 19
			Male Animals
25	5		28.32
		(n)	5
		Means	29.85
		Sdevs	1.46
26	6		34.71
27			30.13
28			26.23
29			31.37
30			29.56
		(n)	5
		Means	30.40
		Sdevs	3.07
31	7		30.45
32			30.51
33			30.76
34			31.76
35			26.28
		(n)	5
		Means	29.95
		Sdevs	2.12
36	8		25.88
37			34.46
38			31.54
39			35.05
40			31.97
		(n)	5
		Means	31.78
		Sdevs	3.63
41	9		31.34
42			31.11
43			37.66
44			36.29
45			30.64
		(n)	5
		Means	33.41
		Sdevs	3.30

Note: Data for Exposure phase

Animal	Group		Day of Phase 19
			Male Animals
46	10		29.86
47			30.80
48			35.05
49			30.42
50			35.74
		(n)	5
		Means	32.37
		Sdevs	2.79
51	11		33.91
52			28.36
53			27.85
54			30.22
55			32.69
		(n)	5
		Means	30.61
		Sdevs	2.65
56	12		33.73
57			28.75
58			34.05
59			25.57
60			31.61
		(n)	5
		Means	30.74
		Sdevs	3.58
61	13		29.39
62			28.96
63			29.93
64			29.25
65			36.27
		(n)	5
		Means	30.76
		Sdevs	3.10

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
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MOUSE/SWISS WEBSTER

Animal body weights in (g)
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FEEDING STUDY/PALATABILITY

Animal	Group		Day of Phase 6	7	8	9	10	11	12	13	14	15	16	17	18
								Male Animals							
66	1		30.63	30.70	30.44	30.75	31.01	31.11	31.02	31.50	31.87	31.26	31.58	31.75	32.43
67			28.17	28.68	28.60	28.65	28.81	29.10	29.23	29.25	29.57	29.41	29.71	29.62	29.98
68			29.68	29.96	29.58	29.68	29.70	29.54	29.68	29.98	31.18	30.61	30.02	30.58	30.49
69			31.92	32.29	32.43	32.76	33.15	33.18	33.76	34.00	34.50	35.02	34.79	34.76	34.98
70			27.29	28.25	28.23	28.26	28.37	28.81	29.22	29.32	29.82	29.59	29.73	29.69	29.89
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	29.54	29.98	29.86	30.02	30.21	30.35	30.58	30.81	31.39	31.18	31.17	31.28	31.55
		Sdevs	1.86	1.62	1.68	1.81	1.93	1.81	1.92	2.00	1.98	2.28	2.17	2.13	2.17
71	2		32.34	32.69	32.16	32.73	33.31	33.55	34.14	34.46	34.79	35.02	34.91	35.23	36.36
72			29.98	30.87	30.72	31.19	31.40	31.44	32.70	32.75	33.42	33.66	33.89	34.57	34.08
73			30.11	30.65	30.42	30.26	30.70	30.76	31.50	31.48	31.89	31.50	31.73	31.90	31.96
74			29.54	29.82	29.60	29.99	30.26	30.12	30.68	30.89	31.55	31.78	31.87	31.53	31.75
75			29.48	29.96	30.58	30.98	31.63	31.77	32.34	32.68	33.06	32.85	33.21	33.15	33.03
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	30.29	30.80	30.70	31.03	31.46	31.53	32.27	32.45	32.94	32.96	33.12	33.28	33.44
		Sdevs	1.18	1.15	0.93	1.07	1.17	1.30	1.30	1.37	1.29	1.44	1.35	1.62	1.88
76	3		29.35	29.45	29.42	29.86	30.29	30.35	30.89	30.94	31.10	30.78	31.36	31.56	31.21
77			29.09	29.69	29.18	29.53	29.61	29.44	29.83	30.09	30.49	30.54	31.10	30.82	30.82
78			30.10	30.02	29.85	30.02	30.25	29.82	30.47	30.62	31.36	31.43	31.93	32.07	32.23
79			27.14	27.49	27.11	27.24	27.32	27.32	28.10	27.86	28.76	28.05	28.72	28.84	28.90
80			28.86	29.38	28.79	28.97	29.54	29.25	29.60	29.00	29.61	29.27	30.14	30.20	29.96
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	28.91	29.21	28.87	29.12	29.40	29.24	29.78	29.70	30.26	30.01	30.65	30.70	30.62
		Sdevs	1.09	0.99	1.06	1.13	1.21	1.15	1.07	1.27	1.08	1.35	1.26	1.26	1.26
81	4		30.04	30.22	29.81	30.02	29.96	29.89	30.47	30.43	30.68	30.56	31.63	30.93	31.68
82			29.18	28.93	28.16	28.36	28.66	28.65	29.33	29.68	29.23	29.01	29.26	28.81	28.87
83			30.15	29.51	29.45	30.53	30.08	30.24	30.26	30.10	30.33	30.36	30.69	30.54	31.26
84			30.97	31.44	30.79	31.02	31.34	31.13	31.90	32.13	31.79	31.39	31.43	31.40	30.76
85			32.09	32.14	31.69	31.84	31.93	31.66	32.33	32.97	32.39	32.15	32.26	32.55	31.97
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	30.49	30.45	29.98	30.35	30.39	30.31	30.86	31.06	30.88	30.69	31.05	30.85	30.91
		Sdevs	1.10	1.33	1.34	1.30	1.28	1.17	1.23	1.42	1.24	1.18	1.15	1.37	1.23

Note: Data for Exposure phase

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TOXICOLOGY DIVISION
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MOUSE/SWISS WEBSTER

Animal body weights in (g)
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FEEDING STUDY/PALATABILITY

Animal	Group		Day of Phase 19
			Male Animals
66	1		32.33
67			30.19
68			30.57
69			35.52
70			30.21
		(n)	5
		Means	31.76
		Sdevs	2.28
71	2		36.18
72			35.14
73			32.23
74			32.32
75			33.77
		(n)	5
		Means	33.93
		Sdevs	1.73
76	3		32.08
77			31.10
78			32.59
79			29.26
80			30.01
		(n)	5
		Means	31.01
		Sdevs	1.39
81	4		31.74
82			29.00
83			30.92
84			31.04
85			32.31
		(n)	5
		Means	31.00
		Sdevs	1.25

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
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MOUSE/SWISS WEBSTER

Individual Animal Feed Consumed/day (g)
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FEEDING STUDY/PALATABILITY

Animal	Group		Day of Phase 7	8	9	10	11	12	13	14	15	16	17	18	19
			Male Animals												
1	1		4.85	5.94	6.48	6.99	5.87	5.96	6.45	5.68	6.93	6.33	6.30	6.55	6.24
2			6.35	7.20	7.17	7.17	7.12	7.36	7.31	7.20	7.56	7.30	6.91	7.30	7.34
3			5.38	5.94	6.53	6.57	6.20	6.47	6.22	6.93	7.72	6.98	5.86	6.82	5.66
4			6.47	7.34	6.98	7.68	5.88	6.17	6.21	6.09	6.40		5.93	7.17	6.38
5			4.66	5.22	4.41	5.23	4.28	5.21	4.99	5.22	6.81	8.21	5.25	8.62	6.06
		(n)	5	5	5	5	5	5	5	5	5	4	5	5	5
		Means	5.54	6.33	6.31	6.73	5.87	6.23	6.24	6.22	7.08	7.21	6.05	7.29	6.34
		Sdevs	0.84	0.91	1.10	0.93	1.02	0.78	0.83	0.83	0.55	0.78	0.61	0.80	0.62
6	2		6.28	5.95	7.34	7.58	7.32	6.93	6.73	7.85	7.67	11.78	7.68	8.53	7.15
7			4.49	6.70	7.59	6.21	7.58	7.31	6.04	6.16	7.47	8.88	6.43	6.44	5.96
8			6.29		7.51	7.74	4.99	7.43	7.18	8.68	8.28		7.41		9.18
9			5.96	7.62	6.04	6.22	5.19	6.45	7.89	5.53	8.45	8.33	6.66	8.14	6.97
10				7.54	6.34	6.45	4.62	6.86	5.36	5.44	6.73	6.25	6.39	7.23	5.79
		(n)	4	4	5	5	5	5	5	5	5	4	5	4	5
		Means	5.76	6.95	6.96	6.84	5.94	7.00	6.64	6.73	7.72	8.81	6.91	7.59	7.01
		Sdevs	0.86	0.79	0.72	0.76	1.40	0.39	0.98	1.46	0.69	2.28	0.59	0.94	1.35
11	3		6.40	7.64	7.28	7.39	5.75	8.13	6.59	6.93	7.17	9.78	7.44	10.47	8.53
12				6.49	6.92	8.35	6.03	6.78	6.05	6.05	6.94	8.63	5.68	7.50	6.62
13			5.63	6.67	7.28	5.80	5.42	7.55	6.39	6.37	7.09	7.45	5.78	7.78	6.31
14			5.37	6.31	5.86	5.69	5.51	7.28	8.73	5.26	7.02	9.97	5.57	7.17	7.10
15			5.25	5.00	4.99	5.89	5.25	5.88	7.75	6.02	6.36		5.75	8.62	5.53
		(n)	4	5	5	5	5	5	5	5	5	4	5	5	5
		Means	5.66	6.42	6.47	6.62	5.59	7.12	7.10	6.13	6.92	8.96	6.04	8.31	6.82
		Sdevs	0.52	0.95	1.01	1.19	0.30	0.85	1.11	0.61	0.32	1.17	0.78	1.32	1.11
16	4		5.74	6.15	6.04	7.62	6.86	8.10	7.68	7.58	5.91	5.45	4.52	6.33	6.98
17			6.33	6.65	6.81	6.88	6.50	7.12	6.57	6.95	6.48	6.36	5.90	6.49	6.59
18			5.71	7.42	7.75	7.78	6.45	6.59	6.55	7.40	6.81	8.91	6.71	7.12	9.83
19			5.97	6.30	5.36	6.32	5.88	6.64	6.12	6.16	6.23	6.19	5.46	5.80	6.87
20			5.36	5.73	5.51	5.65	5.21	7.67	7.34	6.35	5.32	6.14		9.16	6.98
		(n)	5	5	5	5	5	5	5	5	5	5	4	5	5
		Means	5.82	6.45	6.29	6.85	6.18	7.22	6.85	6.89	6.15	6.61	5.65	6.98	7.45
		Sdevs	0.36	0.63	0.99	0.89	0.65	0.66	0.64	0.63	0.57	1.33	0.91	1.31	1.34
21	5		5.29	5.78	6.85	9.12	7.44	7.73	7.41	5.70	4.93	5.61	4.63	6.83	6.06
22			5.39	6.54	6.69	8.49	5.90	6.67	7.30	5.19	5.59	5.64	5.50	6.26	5.94
23			5.14	5.30	4.88	8.22	6.52	7.91	5.81	5.34	5.51	5.70	7.47	7.80	6.79
24			5.85	6.64	6.04	8.34	5.04	6.43	5.66	7.09	5.18	6.68	6.09	8.45	11.57

Note: Data for Exposure phase

R.J.R. TOBACCO | Individual Animal Feed Consumed/day (g) | PRINTED: 08-May-08
TOXICOLOGY DIVISION | Study number: TOX210A | Page: 2
Building 630/2
MOUSE/SWISS WEBSTER | Dosing start date: 16-Apr-08 | FEEDING STUDY/PALATABILITY

Day of Phase

Animal	Group		7	8	9	10	11	12	13	14	15	16	17	18	19
								Male Animals							
25	5		5.93	6.27	5.71	6.34	5.82	7.86	7.86	6.14	5.64	5.42	5.53	5.61	5.60
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	5.52	6.11	6.03	8.10	6.14	7.32	6.81	5.89	5.37	5.81	5.84	6.99	7.19
		Sdevs	0.35	0.56	0.80	1.04	0.90	0.71	1.00	0.76	0.30	0.50	1.05	1.15	2.49
26	6		7.14	6.96	8.18	9.28	5.82	6.19	6.08	5.38	5.71	6.17	5.77	6.49	6.96
27			5.37	5.83	6.65	6.71	6.62	6.52	7.60	6.17	5.66	6.20	6.98	6.23	7.81
28			5.17	5.50	6.73	6.63	4.64	6.89	5.55	5.55	4.91	9.77	7.39	5.90	
29			6.12	6.52	6.90	7.38	6.46	5.52	8.22	5.84	6.14	6.94	6.89	6.14	9.95
30			5.78	7.01	6.83	6.50	5.23	8.87	6.31	4.99	6.87	6.54	4.89	7.23	8.32
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	4
		Means	5.92	6.36	7.06	7.30	5.75	6.80	6.75	5.59	5.86	7.12	6.38	6.40	8.26
		Sdevs	0.78	0.68	0.63	1.16	0.83	1.26	1.11	0.45	0.72	1.51	1.03	0.51	1.26
31	7		5.56	6.15	5.63	6.16	4.62	5.51	5.35	5.15	5.06	5.08	4.84	5.37	5.00
32			5.12	5.87	5.59	6.94	5.81	6.31	5.87	6.63	6.02	6.82	5.65	5.91	6.43
33			5.11	7.24	6.08	6.97	6.85	7.07	5.96	5.79	5.59	6.08	5.40	5.64	7.07
34			4.44	6.63	6.03	9.70	8.87	6.36	8.81	6.44	5.91	7.15	5.55	6.71	7.95
35			5.36	6.60	7.91	7.33	5.87	6.22	5.40	5.38	5.33	9.37	6.80	5.83	7.81
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	5.12	6.50	6.25	7.42	6.40	6.29	6.28	5.88	5.58	6.90	5.65	5.89	6.85
		Sdevs	0.42	0.52	0.96	1.34	1.59	0.55	1.44	0.65	0.40	1.59	0.72	0.50	1.20
36	8		4.95	5.79	5.86	5.94	8.08	6.29	8.27	5.15	4.97	5.62	6.06	4.77	6.09
37			5.99	7.09	6.07	7.95	5.71	6.43	6.80	6.02	6.23	6.65	6.95	6.50	7.40
38			6.06	6.78	7.11	6.84	5.01	6.53	6.70	7.74	5.75		5.52	5.31	7.00
39			5.65	6.25	5.59	6.48	7.57	6.25	5.65	6.91	6.07	6.20	6.11	5.84	
40			5.15	6.05	5.62	7.04	5.80	6.99	6.85	6.21	6.32	6.68	7.07	6.42	6.94
		(n)	5	5	5	5	5	5	5	5	5	4	5	5	4
		Means	5.56	6.39	6.05	6.85	6.43	6.50	6.85	6.41	5.87	6.29	6.34	5.77	6.86
		Sdevs	0.50	0.53	0.62	0.74	1.32	0.30	0.93	0.97	0.55	0.50	0.65	0.74	0.55
41	9		5.73	5.82	8.75	5.80	5.19	6.24	5.72	7.45	6.15		7.85	6.78	7.77
42			8.42	6.82	6.22	8.85	6.85	8.36	6.01	7.43	5.57	6.81	5.02	5.75	6.75
43			6.82	7.42	6.96	8.61	6.65	7.04	6.21	6.72	6.41	6.74	6.05	6.53	7.09
44			6.00	6.29	6.32	7.31	5.90	7.00	6.93	6.82	6.59	6.24	6.06	6.65	7.50
45			5.42	6.00	6.73	6.92	8.31	6.96	4.39	6.67	7.73	7.28	8.81		
		(n)	5	5	5	5	5	5	5	5	5	4	5	4	4
		Means	6.48	6.47	7.00	7.50	6.58	7.12	5.85	7.02	6.49	6.77	6.76	6.43	7.28
		Sdevs	1.20	0.65	1.03	1.26	1.17	0.77	0.93	0.39	0.79	0.43	1.53	0.46	0.45

Note: Data for Exposure phase

R.J.R. TOBACCO Individual Animal Feed Consumed/day (g) PRINTED: 08-May-08
TOXICOLOGY DIVISION Study number: TOX210A Page: 3
Building 630/2
MOUSE/SWISS WEBSTER Dosing start date: 16-Apr-08 FEEDING STUDY/PALATABILITY

Animal	Group		Day of Phase 7	8	9	10	11	12	13	14	15	16	17	18	19
			Male Animals												
46	10		6.14	6.55	6.51	7.56		8.35	6.16	8.30	5.90	6.84	7.37	6.17	7.19
47			5.38	5.83	6.17	8.20	6.90	7.07	5.17	6.14	5.74	5.68	5.17	6.68	8.62
48			5.93	6.40	6.35	6.89	6.08	6.62	6.30	6.96	6.42	6.38	6.06	5.85	6.81
49			4.93	5.44	5.22	6.36	5.62	5.98	5.33	5.88	5.50	6.28	5.15	6.48	6.94
50			6.03	6.98	9.60	7.47	5.97	6.60	6.21	6.73	6.99	7.43	6.49	7.82	8.67
		(n)	5	5	5	5	4	5	5	5	5	5	5	5	5
		Means	5.68	6.24	6.77	7.30	6.14	6.92	5.83	6.80	6.11	6.52	6.05	6.60	7.65
		Sdevs	0.51	0.61	1.66	0.70	0.54	0.89	0.54	0.94	0.60	0.65	0.94	0.75	0.92
51	11		5.01	5.36	5.46	5.79	4.73	5.16	5.23	6.10	5.40	5.72	5.07	5.56	5.94
52			5.37	5.12	5.68	6.32	4.57	5.38	5.49	5.99	5.73	6.55	7.03	5.91	8.89
53			6.05	5.98	6.98	7.50	6.68	6.89		6.73	6.43	8.41	5.54	6.44	9.93
54			4.81	5.15	6.79	7.43	4.43	6.59	4.94	6.45	6.04	7.85	6.89	8.00	8.83
55			5.89	5.87	5.98	6.53	5.71	5.93	6.09	7.06	6.27	6.72	6.20	6.92	6.56
		(n)	5	5	5	5	5	5	4	5	5	5	5	5	5
		Means	5.43	5.50	6.18	6.71	5.22	5.99	5.44	6.47	5.97	7.05	6.15	6.57	8.03
		Sdevs	0.54	0.40	0.67	0.74	0.96	0.75	0.49	0.44	0.42	1.07	0.85	0.95	1.70
56	12		6.27	6.41	5.81	7.63	5.67	7.56	5.40	5.61	5.30	6.86	5.39	6.03	6.59
57			6.38	6.02	6.81	7.76	6.83	5.55	7.37	5.66	6.09	6.91	5.63	4.94	6.12
58			5.65	5.54	6.28	8.58	5.87	5.75	6.15	6.37	6.57	6.23	6.01	6.22	6.63
59			6.17	5.29	6.33	8.48	4.83	6.89	4.56	6.45	6.79	6.67	8.01	7.85	7.71
60			5.62	5.05	6.23	7.33	4.05	7.28	5.79	7.06	7.09	6.48	7.26	6.66	8.59
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	6.02	5.66	6.29	7.96	5.45	6.61	5.85	6.23	6.37	6.63	6.46	6.34	7.13
		Sdevs	0.36	0.55	0.36	0.55	1.06	0.91	1.03	0.61	0.70	0.28	1.13	1.06	1.00
61	13		6.03	5.88	7.39	6.60	5.96	7.67	5.94	6.54	5.57	5.44	5.32	5.79	5.34
62			5.86	5.78	7.37	7.36	6.67	7.00	6.32	6.43	5.97	7.00	7.10	7.97	7.31
63			5.00	4.73	6.68	6.70	6.85	7.28	6.69	6.90	6.39	8.77	5.82	6.69	6.14
64			5.30	5.71	7.08	7.29	5.95	6.45	6.51	6.17	5.70		5.81	9.50	
65			6.50	6.49		6.85	6.04	6.82	5.79	7.33	5.99	7.98	6.42	5.92	
		(n)	5	5	4	5	5	5	5	5	5	4	5	5	3
		Means	5.74	5.72	7.13	6.96	6.29	7.04	6.25	6.67	5.92	7.30	6.09	7.17	6.26
		Sdevs	0.60	0.63	0.33	0.35	0.43	0.46	0.38	0.45	0.32	1.43	0.68	1.56	0.99

Note: Data for Exposure phase

Animal	Group		Day of Phase 20
			Male Animals
1	1		6.17
2			5.93
3			6.22
4			4.93
5			5.62
		(n)	5
		Means	5.77
		Sdevs	0.53
6	2		5.20
7			6.60
8			9.41
9			6.17
10			9.79
		(n)	5
		Means	7.43
		Sdevs	2.05
11	3		8.98
12			7.85
13			5.87
14			7.83
15			7.76
		(n)	5
		Means	7.66
		Sdevs	1.12
16	4		5.99
17			6.19
18			6.65
19			6.31
20			7.41
		(n)	5
		Means	6.51
		Sdevs	0.56
21	5		7.70
22			6.13
23			7.54
24			8.05

Note: Data for Exposure phase

Animal	Group		Day of Phase 20
			Male Animals
25	5		9.26
		(n)	5
		Means	7.74
		Sdevs	1.12
26	6		5.99
27			8.19
29			10.17
30			7.96
		(n)	4
		Means	8.08
		Sdevs	1.71
31	7		6.13
32			6.33
33			6.64
34			7.19
35			7.79
		(n)	5
		Means	6.82
		Sdevs	0.68
36	8		6.45
37			8.57
38			6.79
39			7.49
40			6.15
		(n)	5
		Means	7.09
		Sdevs	0.97
41	9		6.56
42			5.99
43			5.99
44			7.54
45			9.94
		(n)	5
		Means	7.20
		Sdevs	1.66

Note: Data for Exposure phase

Animal	Group		Day of Phase 20
			Male Animals
46	10		8.58
47			6.15
48			6.18
49			6.90
50			6.52
		(n)	5
		Means	6.87
		Sdevs	1.01
51	11		6.05
52			5.73
53			10.25
54			11.02
55			6.39
		(n)	5
		Means	7.89
		Sdevs	2.53
56	12		6.65
57			6.59
58			7.39
59			5.28
60			7.64
		(n)	5
		Means	6.71
		Sdevs	0.92
61	13		5.58
62			6.07
63			7.36
64			8.49
		(n)	4
		Means	6.88
		Sdevs	1.31

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
MOUSE/SWISS WEBSTER

Individual Animal Feed Consumed/day (g)
Study number: TOX210B

Dosing start date: 16-Apr-08

PRINTED: 08-May-08
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FEEDING STUDY/PALATABILITY

			Day of Phase												
Animal	Group		7	8	9	10	11	12	13	14	15	16	17	18	19
			Male Animals												
66	1		5.49	6.06	6.73	4.84	6.38	5.77	5.27	5.42	5.61	5.63	6.57	7.34	5.49
67			5.44	7.01	8.89	7.02		6.50	5.44	5.41	7.19	7.72	6.01		5.52
68			5.55		7.71	9.71	6.90	6.46	5.93	6.59	5.97	7.48	6.18	7.13	5.73
69			6.25	7.49	8.05	6.28	7.54	6.18	6.92	6.03	6.82	5.54	5.28	7.04	5.53
70			5.02	7.31	5.97	5.79	6.95	2.17	5.45	5.60	5.60	7.19	6.19	7.74	6.36
		(n)	5	4	5	5	4	5	5	5	5	5	5	4	5
		Means	5.55	6.97	7.47	6.73	6.94	5.42	5.80	5.81	6.24	6.71	6.05	7.31	5.73
		Sdevs	0.44	0.64	1.14	1.85	0.47	1.84	0.67	0.50	0.73	1.05	0.47	0.31	0.37
71	2		5.94	6.30	6.36	5.85	7.30	6.85	6.11	6.29	7.53	7.35	6.17	8.91	6.05
72			5.81	9.08	7.45	6.02	10.24	9.90	6.09	7.25	8.06	9.15	8.26	9.93	6.68
73			5.26	6.58	5.48	4.95	6.90	6.84	6.36	4.99	5.75	7.06	6.57	8.26	5.44
74			5.88	7.03	6.43	5.07	6.60	7.50	6.25	5.91	6.82	6.34	5.79	6.66	7.08
75			5.29	7.38	8.30	6.82	8.27	6.74	6.92	5.88	6.85	7.19	5.90	7.69	6.43
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	5.64	7.27	6.80	5.74	7.86	7.57	6.35	6.06	7.00	7.42	6.54	8.29	6.34
		Sdevs	0.33	1.09	1.09	0.76	1.47	1.34	0.34	0.82	0.87	1.04	1.01	1.23	0.63
76	3		6.63	6.14	6.77	5.85	7.32	5.95	5.12		6.51	6.19	7.80	9.47	9.02
77			5.94	6.90	6.47	4.76	3.81	6.46	6.63	2.81	9.56	7.10	7.33		5.46
78			5.08	7.45	6.26		7.53	6.06	6.11	12.44	6.84	6.87	7.29	8.71	7.11
79			4.56	5.11	6.67	5.36	8.40	7.07	6.49	5.31	6.99	7.36	6.02	10.07	5.78
80			5.10	6.13	5.40	4.75	7.92	5.07	5.35	4.34	5.70	5.99	5.92		4.14
		(n)	5	5	5	4	5	5	5	4	5	5	5	3	5
		Means	5.46	6.35	6.31	5.18	7.00	6.12	5.94	6.23	7.12	6.70	6.87	9.42	6.30
		Sdevs	0.82	0.89	0.55	0.53	1.83	0.73	0.68	4.27	1.45	0.59	0.85	0.68	1.85
81	4		4.85	5.59	6.74	5.22	6.20	3.15	5.35	4.98	6.59	6.57	5.28	6.91	6.66
82			4.57	0.00		6.02	7.04	7.42	7.16	5.02	7.28	5.69	6.61	7.36	6.81
83			4.28	6.52	7.13	6.37	7.47	5.40	5.95	4.41	6.39	5.87	5.27	6.91	4.45
84			5.53	5.99	5.61	4.74	5.54	5.55	5.47	3.83	5.22	4.38	4.26	4.84	5.83
85			5.56	6.85	7.67	5.63	8.13	6.56	6.22	5.32	5.83	5.91	7.37	6.72	6.38
		(n)	5	5	4	5	5	5	5	5	5	5	5	5	5
		Means	4.96	4.99	6.79	5.60	6.88	5.62	6.03	4.71	6.26	5.68	5.76	6.55	6.03
		Sdevs	0.57	2.83	0.87	0.64	1.02	1.60	0.72	0.59	0.78	0.80	1.23	0.98	0.96

Note: Data for Exposure phase

Animal	Group		Day of Phase 20
			Male Animals
66	1		5.34
67			7.29
68			7.60
69			5.61
70			6.59
		(n)	5
		Means	6.49
		Sdevs	1.00
71	2		6.15
72			6.83
73			6.85
74			6.94
75			6.74
		(n)	5
		Means	6.70
		Sdevs	0.32
76	3		5.57
77			6.92
78			7.69
79			5.63
80			4.96
		(n)	5
		Means	6.15
		Sdevs	1.12
81	4		7.68
82			7.00
83			5.85
84			4.71
85			5.91
		(n)	5
		Means	6.23
		Sdevs	1.15

Note: Data for Exposure phase

Appendix II

Density Determination of Tobacco Extract

Density Worksheet

Sample Type:	Test Article 2
Study Number:	TOX209 + TOX210
Cigarette ID:	Tobacco Extract
Aliquot Number:	Manuf. Date 3-5-08

Densito Configuration/Calibration

Serial Number:	MIB31A63	Calibration Verified:	yes

Density Measurements

density 1:	1.2031	~~m~~g/ml
density 2:	1.2028	~~m~~g/ml
density 3:	1.2036	~~m~~g/ml
Average:	1.2032	~~m~~g/ml
Standard Deviation:	0.4	

Comments:

Density Measured By: S. Pike	Date: 4-3-08
Verified By: G Smith	Date: 4.3.08

Appendix III

Analytical Chemistry Data for Homogeneity and Dose Confirmation

Calculations of Formulation of Dosed Feed

TOX210 TEST ARTICLE NEEDED FOR UPCOM NG PALATAB LITY STUDY FOR**MICE** Series 1

	Tobacco Blend (mg nic/kg bw/day)	Assumed Average BW kg	Needed mg of NIC	Needed g of tob	Assumed g of feed consumed per mouse per day	Amount of tob (g) in g feed	Total g Feed	Total g Tobacco in Diet Prep	Percent Tobacco in Feed	mg of nic/g of feed
Group 2	0 2	0 03	0 00572	0 00022	5 000	0 000044	800	0 0348	0.0044	0 0011
Group 3	2 0	0 03	0 06	0 00228	5 000	0 000457	800	0 3653	0.0457	0 0120
Group 4	4 0	0 03	0 12	0 00457	5 000	0 000913	800	0.7307	0.0913	0 0240
Group 5	8 0	0 03	0 24	0 00913	5 000	0 001827	800	1.4614	0.1827	0 0480
Group 6	20 0	0 03	0 6	0 02283	5 000	0 004567	800	3 6535	0.4567	0.1200
Group 7	40 0	0 03	1 2	0 04567	5 000	0 009134	800	7 3069	0.9134	0 2400

26.3 mg of nic per g of tobacco blend

	Tobacco Extract (mg nic/kg bw/day)	Assumed Average BW kg	Needed mg of Nic	Needed g of tob	Assumed g of feed consumed per mouse per day	Amount of tob (g) in g feed	Total g Feed	Total g obacco Ext in Diet Pre	Percent Tobacco in Feed	mg of nic/g of feed
Group 8	0 2	0 03	0 006	0 00026	5 000	0 00005	800	0 0417	0.0052	0 0012
Group 9	2 0	0 03	0 06	0 00261	5 000	0 00052	800	0.4174	0.0522	0 0120
Group 10	4 0	0 03	0 12	0 00522	5 000	0 00104	800	0 8348	0.1043	0 0240
Group 11	8 0	0 03	0 24	0 01043	5 000	0 00209	800	1 6696	0.2087	0 0480
Group 12	20 0	0 03	0 6	0 02609	5 000	0 00522	800	4.1739	0.5217	0.1200
Group 13	40 0	0 03	1 2	0 05217	5 000	0 01043	800	8 3478	1.0435	0 2400

23.0 mg of nic per g of tobacco extract

	Nicotine Hydrogen Tartr (mg nic/kg bw/day)	Assumed Average BW kg	Needed mg of Nic	Needed mg of nic tartrate	Assumed g of feed consumed per mouse per day	mg amount of nic tar per g feed	Total g Feed	Total mg of nic tartrate in Diet Prep	Total g of nic tartrate in Diet Prep	mg of nic/g of feed
Group 14	2 0	0 03	0 06	0.171	5 000	0 0342	800	27.350	0.02735	0 0120
Group 15	8 0	0 03	0 24	0 684	5 000	0 1368	800	109.402	0.10940	0 0480
Group 16	20 0	0 03	0.6	1.709	5 000	0 3419	800	273 504	0.27350	0.1200
Group 17	40 0	0 03	1.2	3.419	5 000	0 6838	800	547 009	0.54701	0 2400

1 g of NIC in 2 85 g of NICOT NE HYDROGEN TARTRATE

0.351 Proportion of Nicotine in Nicotine Hydrogen Tartrate

TOX210 MICE

g of feed per day	Number of Mice	Number of Days	Total g Feed
10	5	8	400

TOX210 TEST ARTICLE NEEDED FOR UPCOMING PALATABILITY STUDY FOR **MICE** Series 2

Group Number	Tobacco Blend (mg nic/kg bw/day)	Assumed Average BW kg	Needed mg of NIC	Needed g of tob	Assumed g of feed consumed per mouse per day	Amount of tob (g) in g feed	Total g Feed	Total g Tobacco in Diet Prep	Percent Tobacco in Feed	mg of nic/g of feed
Group 2	0 2	0.03	0 00572	0 00022	6 500	0 000033	1000	0.0335	0.0033	0.0009
Group 3	2 0	0.03	0 06	0 00228	6 500	0 000351	1000	0.3513	0.0351	0.0092
Group 4	4 0	0.03	0 12	0 00457	6 500	0 000703	1000	0.7026	0.0703	0.0185
Group 5	8 0	0.03	0 24	0 00913	6 500	0 001405	1000	1.4052	0.1405	0.0369
Group 6	20 0	0.03	0 6	0 02283	6 500	0 003513	1000	3.5129	0.3513	0.0923
Group 7	40 0	0.03	1 2	0 04567	6 500	0 007026	1000	7.0259	0.7026	0.1846

26.3 mg of nic per g of tobacco blend

	Tobacco Extract (mg nic/kg bw/day)	Assumed Average BW kg	Needed mg of Nic	Needed g of tob	Assumed g of feed consumed per mouse per day	Amount of tob (g) in g feed	Total g Feed	Total g Tobacco Ext in Diet Prep	Percent Tobacco in Feed	mg of nic/g of feed
Group 8	0 2	0.03	0 006	0 00026	6 500	0 00004	1000	0.0401	0.0040	0.0009
Group 9	2 0	0.03	0 06	0 00261	6 500	0 00040	1000	0.4013	0.0401	0.0092
Group 10	4 0	0.03	0 12	0 00522	6 500	0 00080	1000	0.8027	0.0803	0.0185
Group 11	8 0	0.03	0 24	0 01043	6 500	0 00161	1000	1.6054	0.1605	0.0369
Group 12	20 0	0.03	0 6	0 02609	6 500	0 00401	1000	4.0134	0.4013	0.0923
Group 13	40 0	0.03	1 2	0 05217	6 500	0 00803	1000	8.0268	0.8027	0.1846

23.0 mg of nic per g of tobacco extract

	Nicotine Hydrogen Tartrate (mg nic/kg bw/day)	Assumed Average BW kg	Needed mg of Nic	Needed mg of nic tartrate	Assumed g of feed consumed per mouse per day	mg amount of nic tar per g feed	Total g Feed	Total mg of nic tartrate in Diet Prep	Total g of nic tartrate in Diet Prep	mg of nic/g of feed
Group 14	2 0	0.03	0.06	0.171	6 500	0 0263	1000	26.298	0.02630	0.0092
Group 15	8 0	0.03	0.24	0.684	6 500	0 1052	1000	105.194	0.10519	0.0369
Group 16	20 0	0.03	0.6	1.709	6 500	0 2630	1000	262.985	0.26298	0.0923
Group 17	40 0	0.03	1.2	3.419	6 500	0 5260	1000	525.970	0.52597	0.1846

1 g of NIC in 2.85 g of NICOTINE HYDROGEN TARTRATE

0.351 Proportion of Nicotine in Nicotine Hydrogen Tartrate

Analytical Chemistry Data

Notes concerning TOX210 Analytical Data

In the TOX210 report, data for dose levels of 0.2, 2.0, 4.0, 8.0 and 20 for the Series 1 feed formulation represent an average of raw data from GN#76276 and GN#77622 from PAD-MKBK 218 report.

Data from dose level 40 mg/kg bw/day comes only from GN#77622. Data for this dose from GN#76276 were provided by high volume GC/FID lab and were considered to be unreliable.

Series 2 feed formulation dose confirmation data for the TOX210 report were derived from average of raw data from GN#77624 and GN#76747 from PAD-MKBK 218 report.

TOX210

Investigational Study of the Palatability of Smokeless Tobacco Test Articles Formulated in NTP-2000 Diets for Mice

The following Tables list the GN Numbers assigned to Nicotine Dose Articles (Tobacco Blend, Tobacco Extract and Nicotine Tartrate). The GN Numbers were located on sample cups submitted to Analytical Chemistry for analysis for Study TOX210.

Signature

9-Feb-09
Date

TOX210		
Study Number	**GN Number**	**Description**
TOX210A	GN76747AA	Article NTP-2000/Tobacco Blend Dose 40.0 mg of nic/kg of bwt
TOX210A	GN76747AB	Article NTP-2000/Tobacco Blend Dose 20.0 mg of nic/kg of bwt (Top)
TOX210A	GN76747AC	Article NTP-2000/Tobacco Blend Dose 8.0 mg of nic/kg of bwt
TOX210A	GN76747AD	Article NTP-2000/Tobacco Blend Dose 4.0 mg of nic/kg of bwt
TOX210A	GN76747AE	Article NTP-2000/Tobacco Blend Dose 2.0 mg of nic/kg of bwt
TOX210A	GN76747AF	Article NTP-2000/Tobacco Blend Dose 0.2 mg of nic/kg of bwt (Bottom)
TOX210A	GN76747AG	Article NTP-2000/Tobacco Extract Dose 40.0 mg of nic/kg of bwt
TOX210A	GN76747AH	Article NTP-2000/Tobacco Extract Dose 20.0 mg of nic/kg of bwt (Top)
TOX210A	GN76747AI	Article NTP-2000/Tobacco Extract Dose 8.0 mg of nic/kg of bwt
TOX210A	GN76747AJ	Article NTP-2000/Tobacco Extract Dose 4.0 mg of nic/kg of bwt
TOX210A	GN76747AK	Article NTP-2000/Tobacco Extract Dose 2.0 mg of nic/kg of bwt
TOX210A	GN76747AL	Article NTP-2000/Tobacco Extract Dose 0.2 mg of nic/kg of bwt (Top)
TOX210B	GN76747AM	Article NTP-2000/Nicotine Tartrate Dose 40.0 mg of nic/kg of bwt
TOX210B	GN76747AN	Article NTP-2000/Nicotine Tartrate Dose 20.0 mg of nic/kg of bwt
TOX210B	GN76747AO	Article NTP-2000/Nicotine Tartrate Dose 8.0 mg of nic/kg of bwt
TOX210B	GN76747AP	Article NTP-2000/Nicotine Tartrate Dose 2.0 mg of nic/kg of bwt
TOX210A	GN77622AA	Article NTP-2000/Tobacco Blend Dose 0.2 mg of nic/kg of bwt
TOX210A	GN77622AB	Article NTP-2000/Tobacco Blend Dose 2.0 mg of nic/kg of bwt
TOX210A	GN77622AC	Article NTP-2000/Tobacco Blend Dose 4.0 mg of nic/kg of bwt
TOX210A	GN77622AD	Article NTP-2000/Tobacco Blend Dose 8.0 mg of nic/kg of bwt
TOX210A	GN77622AE	Article NTP-2000/Tobacco Blend Dose 20.0 mg of nic/kg of bwt
TOX210A	GN77622AF	Article NTP-2000/Tobacco Blend Dose 40.0 mg of nic/kg of bwt
TOX210A	GN77622AG	Article NTP-2000/Tobacco Extract Dose 0.2 mg of nic/kg of bwt
TOX210A	GN77622AH	Article NTP-2000/Tobacco Extract Dose 2.0 mg of nic/kg of bwt
TOX210A	GN77622AI	Article NTP-2000/Tobacco Extract Dose 4.0 mg of nic/kg of bwt
TOX210A	GN77622AJ	Article NTP-2000/Tobacco Extract Dose 8.0 mg of nic/kg of bwt
TOX210A	GN77622AK	Article NTP-2000/Tobacco Extract Dose 20.0 mg of nic/kg of bwt
TOX210A	GN77622AL	Article NTP-2000/Tobacco Extract Dose 40.0 mg of nic/kg of bwt
TOX210B	GN77622AM	Article NTP-2000/Nicotine Tartrate Dose 2.0 mg of nic/kg of bwt
TOX210B	GN77622AN	Article NTP-2000/Nicotine Tartrate Dose 8.0 mg of nic/kg of bwt
TOX210B	GN77622AO	Article NTP-2000/Nicotine Tartrate Dose 20.0 mg of nic/kg of bwt
TOX210B	GN77622AP	Article NTP-2000/Nicotine Tartrate Dose 40.0 mg of nic/kg of bwt
TOX210A	GN77624AA	Article NTP-2000/Tobacco Blend Dose 0.2 mg of nic/kg of bwt
TOX210A	GN77624AB	Article NTP-2000/Tobacco Blend Dose 2.0 mg of nic/kg of bwt
TOX210A	GN77624AC	Article NTP-2000/Tobacco Blend Dose 4.0 mg of nic/kg of bwt
TOX210A	GN77624AD	Article NTP-2000/Tobacco Blend Dose 8.0 mg of nic/kg of bwt
TOX210A	GN77624AE	Article NTP-2000/Tobacco Blend Dose 20.0 mg of nic/kg of bwt
TOX210A	GN77624AF	Article NTP-2000/Tobacco Blend Dose 40.0 mg of nic/kg of bwt
TOX210A	GN77624AG	Article NTP-2000/Tobacco Extract Dose 0.2 mg of nic/kg of bwt
TOX210A	GN77624AH	Article NTP-2000/Tobacco Extract Dose 2.0 mg of nic/kg of bwt
TOX210A	GN77624AI	Article NTP-2000/Tobacco Extract Dose 4.0 mg of nic/kg of bwt
TOX210A	GN77624AJ	Article NTP-2000/Tobacco Extract Dose 8.0 mg of nic/kg of bwt
TOX210A	GN77624AK	Article NTP-2000/Tobacco Extract Dose 20.0 mg of nic/kg of bwt
TOX210A	GN77624AL	Article NTP-2000/Tobacco Extract Dose 40.0 mg of nic/kg of bwt
TOX210B	GN77624AM	Article NTP-2000/Nicotine Tartrate Dose 2.0 mg of nic/kg of bwt
TOX210B	GN77624AN	Article NTP-2000/Nicotine Tartrate Dose 8.0 mg of nic/kg of bwt
TOX210B	GN77624AO	Article NTP-2000/Nicotine Tartrate Dose 20.0 mg of nic/kg of bwt
TOX210B	GN77624AP	Article NTP-2000/Nicotine Tartrate Dose 40.0 mg of nic/kg of bwt

TOX210		
Study Number	**GN Number**	**Discription**
TOX210A	GN76276AA	Article NTP-2000/Tobacco Blend Dose 0.2 mg of nic/kg of bwt (Bottom)
TOX210A	GN76276AB	Article NTP-2000/Tobacco Blend Dose 0.2 mg of nic/kg of bwt (Middle)
TOX210A	GN76276AC	Article NTP-2000/Tobacco Blend Dose 0.2 mg of nic/kg of bwt (Top)
TOX210A	GN76276AD	Article NTP-2000/Tobacco Extract Dose 0.2 mg of nic/kg of bwt (Top)
TOX210A	GN76276AE	Article NTP-2000/Tobacco Extract Dose 0.2 mg of nic/kg of bwt (Middle)
TOX210A	GN76276AF	Article NTP-2000/Tobacco Extract Dose 0.2 mg of nic/kg of bwt (Bottom)
TOX210B	GN76276AG	Article NTP-2000/Nicotine Tartrate Dose 2.0 mg of nic/kg of bwt (Top)
TOX210B	GN76276AH	Article NTP-2000/Nicotine Tartrate Dose 2.0 mg of nic/kg of bwt (Middle)
TOX210B	GN76276AI	Article NTP-2000/Nicotine Tartrate Dose 2.0 mg of nic/kg of bwt (Bottom)
TOX210A	GN76276AS	Article NTP-2000/Tobacco Blend Dose 2.0 mg of nic/kg of bwt
TOX210A	GN76276AT	Article NTP-2000/Tobacco Blend Dose 4.0 mg of nic/kg of bwt
TOX210A	GN76276AU	Article NTP-2000/Tobacco Blend Dose 8.0 mg of nic/kg of bwt
TOX210A	GN76276AV	Article NTP-2000/Tobacco Extract Dose 2.0 mg of nic/kg of bwt
TOX210A	GN76276AW	Article NTP-2000/Tobacco Extract Dose 4.0 mg of nic/kg of bwt
TOX210A	GN76276AX	Article NTP-2000/Tobacco Extract Dose 8.0 mg of nic/kg of bwt
TOX210A	GN76276AY	Article NTP-2000/Tobacco Extract Dose 20.0 mg of nic/kg of bwt
TOX210B	GN76276BA	Article NTP-2000/Nicotine Tartrate Dose 8.0 mg of nic/kg of bwt
TOX210B	GN76276BB	Article NTP-2000/Nicotine Tartrate Dose 20.0 mg of nic/kg of bwt
TOX210A	GN76276BC	Article NTP-2000/Tobacco Blend Dose 20.0 mg of nic/kg of bwt

TOX210 Analytical Chemistry Summary

Trial Run TOX209**

Sub. Date	GN#	Test Article	Dose (mg/kg/day)	Nicotine (FID)* (mg/g)	Nicotine (MS)# (mg/g)	Expected (mg/g)
4/3/2008	75963AA	Blend Top	40 mg/kg	0.46	0.49	
	75963AB	Blend Mid	40 mg/kg	0.47	0.46	
	75963AC	Blend Bot	40 mg/kg	0.45	0.41	
	Mean			0.460	0.45	0.5
	75963AD	Blend Top	0.2 mg/kg	BLQ^	0.003	
	75963AE	Blend Mid	0.2 mg/kg	BLQ	0.003	
	75963AF	Blend Bot	0.2 mg/kg	BLQ	0.003	
	Mean				0.003	0.0025
	75963AG	Tartrate Top	40 mg/kg	0.42	0.413	
	75963AH	Tartrate Mid	40 mg/kg	0.41	0.424	
	75963AI	Tartrate Bot	40 mg/kg	0.40	0.411	
	Mean			0.410	0.416	0.5
	75963AJ	Tartrate Top	2.0 mg/kg	BLQ	0.022	
	75963AK	Tartrate Mid	2.0 mg/kg	BLQ	0.021	
	75963AL	Tartrate Bot	2.0 mg/kg	BLQ	0.02	
	Mean				0.021	0.025
4/4/2008	76002AA	Extract Top	40 mg/kg	0.400		
	76002AB	Extract Mid	40 mg/kg	0.430		
	76002AC	Extract Bot	40 mg/kg	0.390		
	Mean			0.407		0.5
	76002AD	Control	0			0
	76002AE	Extract Top	0.2 mg/kg	BLQ		
	76002AF	Extract Mid	0.2 mg/kg	BLQ		
	76002AG	Extract Bot	0.2 mg/kg	BLQ		0.0025

* Data acquired by GC/FID method
# Data acquired by GC/MS method
^ Below Limit of Quantitation

**These data are presented in the TOX210 report and listed here in support of that report.
Raw data are a part of the Study File for TOX209.

Trial Run: TOX209 1-Month Stability Data Feed Prepared on 4-2 & 4-3

Sub. Date	GN#	Test Article	Dose (mg/kg/day)	Initial Data@ Nicotine (FID)* (mg/g)	Initial Data@ Nicotine (MS)# (mg/g)	Final Data Nicotine (MS)# (mg/g)	Expected (mg/g)
5/2/2008	76749AA	Blend	40.0	0.460	0.450	0.438	0.500
	76749AB	Blend	0.2	BLQ	0.003	0.002	0.003
	76749AC	Extract	40.0	0.407	\	0.436	0.500
	76749AD	Extract	0.2	BLQ	\	0.002	0.003
	76749AE	Tartrate	40.0	0.410	0.416	0.411	0.500
	76749AF	Tartrate	2.0	BLQ	0.021	0.020	0.025

@ Data from initial analysis of trial run formulation

TOX209 Series 2 one month stability data: 1-Month Stability Data Feed Prepared on 4-2 & 4-3

Sub. Date	GN#	Test Article	Dose (mg/kg/day)	Initial Data@ Nicotine (FID)* (mg/g)	Final Data Nicotine (FID)* (mg/g)	Expected (mg/g)
4/30/2008	76677AA	Blend	40.0	0.460	0.50	0.500
	76677AB	Extract	40.0	0.407	0.51	0.500
	76677AC	Tartrate	40.0	0.410	0.45	0.500
	76677AD	Blend	0.2	BLQ	BLQ	0.003
	76677AE	Extract	0.2	BLQ	BLQ	0.003
	76677AF	Tartrate	2.0	BLQ	BLQ	0.025

@ Data from initial analysis of feed formulation

TOX210 Analytical Chemistry Summary

TOX210 Series 1 Feed Formulation 40 mg/kg/day Dose Homogeneity

Sub. Date	GN#	Test Article	Dose (mg/kg/day)	Nicotine (FID)* (mg/g)	Nicotine (MS)# (mg/g)	Expected (mg/g)
4/18/2008	76364AA	Blend Top	40 mg/kg	BLQ		0.240
	76364AB	Blend Mid	40 mg/kg	BLQ		0.240
	76364AC	Blend Bot	40 mg/kg	BLQ		0.240
	Mean					
	76364AD	Extract Top	40 mg/kg	BLQ		0.240
	76364AE	Extract Mid	40 mg/kg	BLQ		0.240
	76364AF	Extract Bot	40 mg/kg	BLQ		0.240
	Mean					
	76364AG	Tartrate Top	40 mg/kg	BLQ		0.240
	76364AH	Tartrate Mid	40 mg/kg	BLQ		0.240
	76364AI	Tartrate Bot	40 mg/kg	BLQ		0.240
	Mean					

TOX210 Series 1 Feed Formulation 0.2-2.0 Dose Homogeneity

Sub. Date	GN#	Test Article	Dose (mg/kg/day)	Nicotine (FID)* (mg/g)	Nicotine (MS)# (mg/g)	Expected (mg/g)
4/18/2008	76276AC	Blend Top	0.2 mg/kg		0.001	0.001
	76276AB	Blend Mid	0.2 mg/kg		0.002	0.001
	76276AA	Blend Bot	0.2 mg/kg		0.001	0.001
	Mean				0.001	
	76276AD	Extract Top	0.2 mg/kg		0.001	0.001
	76276AE	Extract Mid	0.2 mg/kg		0.001	0.001
	76276AF	Extract Bot	0.2 mg/kg		0.001	0.001
	Mean				0.001	
	76276AG	Tartrate Top	2.0 mg/kg		0.012	0.012
	76276AH	Tartrate Mid	2.0 mg/kg		0.011	0.012
	76276AI	Tartrate Bot	2.0 mg/kg		0.010	0.012
	Mean				0.011	

TOX210 Analytical Chemistry Summary

TOX210 Series 1 Feed Formulation: First Submission

Sub. Date	GN#	Test Article	Dose (mg/kg/day)	Nicotine (FID)* (mg/g)	Nicotine (MS)# (mg/g)	Expected (mg/g)
4/16/2008	76276	Blend	0.2		0.001+	0.001
	76276AS	Blend	2.0		0.009	0.012
	76276AT	Blend	4.0		0.018	0.024
	76276AU	Blend	8.0		0.036	0.048
	76276BC	Blend	20.0		0.093	0.120
		Blend	40.0			0.240
	76276	Extract	0.2		0.001+	0.001
	76276AV	Extract	2.0		0.003	0.012
	76276AW	Extract	4.0		0.014	0.024
	76276AX	Extract	8.0		0.030	0.048
	76276AY	Extract	20.0		0.067	0.120
	77622AL	Extract	40.0		0.284	0.240
	76276	Tartrate	2.0		0.011+	0.012
	76276BA	Tartrate	8.0		0.033	0.048
	76276BB	Tartrate	20.0		0.089	0.120
	76276	Tartrate	40.0		0.206	0.240

`=+ Data from Homogeneity Table

TOX210 Series 1 Feed Formulation: Second Submission

Sub. Date	GN#	Test Article	Dose (mg/kg/day)	Nicotine (FID)* (mg/g)	Nicotine (MS)# (mg/g)	Expected (mg/g)
6/12/2008	77622AA	Blend	0.2		0.002	0.001
	77622AB	Blend	2.0		0.012	0.012
	77622AC	Blend	4.0		0.014	0.024
	77622AD	Blend	8.0		0.029	0.048
	77622AE	Blend	20.0		0.085	0.120
	77622AF	Blend	40.0		0.135	0.240
	77622AG	Extract	0.2		0.001	0.001
	77622AH	Extract	2.0		0.006	0.012
	77622AI	Extract	4.0		0.014	0.024
	77622AJ	Extract	8.0		0.046	0.048
	77622AK	Extract	20.0		0.054	0.120
	77622AL	Extract	40.0		0.284	0.240
	77622AM	Tartrate	2.0		0.011	0.012
	77622AN	Tartrate	8.0		0.030	0.048
	77622AO	Tartrate	20.0		0.001	0.120
	77622AP	Tartrate	40.0		0.206	0.240

TOX210 Analytical Chemistry Summary

TOX210 Series 2 Feed Formulation: Dose Confirmation First Submission

Sub. Date	GN#	Test Article	Dose (mg/kg/day)	Nicotine (FID)* (mg/g)	Nicotine (MS)# (mg/g)	Expected (mg/g)
5/2/2008	76747AF	Blend	0.2		0.001	0.001
	76747AE	Blend	2.0		0.009	0.009
	76747AD	Blend	4.0		0.016	0.019
	76747AC	Blend	8.0		0.029	0.037
	76747AB	Blend	20.0		0.089	0.092
	76747AA	Blend	40.0		0.182	0.185
	76747AL	Extract	0.2		0.001	0.001
	76747AK	Extract	2.0		0.003	0.009
	76747AJ	Extract	4.0		0.013	0.019
	76747AI	Extract	8.0		0.018	0.037
	76747AH	Extract	20.0		0.061	0.092
	76747AG	Extract	40.0		0.203	0.185
	76747AP	Tartrate	2.0		0.008	0.009
	76747AO	Tartrate	8.0		0.031	0.037
	76747AN	Tartrate	20.0		BLQ	0.092
	76747AM	Tartrate	40.0		0.231	0.185

TOX210 Series 2 Feed Formulation: Dose Confirmation Second Submission

Sub. Date	GN#	Test Article	Dose (mg/kg/day)	Nicotine (FID)* (mg/g)	Nicotine (MS)# (mg/g)	Expected (mg/g)
6/12/2008	77624AA	Blend	0.2		0.001	0.001
	77624AB	Blend	2.0		0.011	0.009
	77624AC	Blend	4.0		0.018	0.019
	77624AD	Blend	8.0		0.032	0.037
	77624AE	Blend	20.0		0.077	0.092
	77624AF	Blend	40.0		0.161	0.185
	77624AG	Extract	0.2		0.001	0.001
	77624AH	Extract	2.0		0.003	0.009
	77624AI	Extract	4.0		0.068	0.019
	77624AJ	Extract	8.0		0.018	0.037
	77624AK	Extract	20.0		0.041	0.092
	77624AL	Extract	40.0		0.090	0.185
	77624AM	Tartrate	2.0		0.007	0.009
	77624AN	Tartrate	8.0		0.027	0.037
	77624AO	Tartrate	20.0		0.066	0.092
	77624AP	Tartrate	40.0		0.130	0.185

Analytical Chemistry Data

Series 1 Low Dose Feed Formulation Homogeneity Data Analysis By GC/MS

Christine R.
Deleted Verse
GN #5

SAMPLE SUBMISSION RECORD

Study Number: TOX210

The following sample(s) are being submitted for analysis:
Submitted by: Jenny Smith   Submitted on: 4-16-08

Sample Identification (GN number)	Analysis	Sample Identification (GN number)	Analysis
GN76276 AA	46	GN76276 AL	
GN76276 AB	46	GN76276 AM	
GN76276 AC	46	GN76276 AN	
GN76276 AD	46	GN76276 AO	
GN76276 AE	46	GN76276 AP	
GN76276 AF	46	GN76276 ① AQ	
GN76276 AG	46	GN76276 ~~AB~~ AR	
GN76276 AH	46	GN76276 AS	
GN76216 AI	46	GN76276 AT	
GN76276 AJ	46	GN76276 AU	
GN76276 AK	46	GN76276 AV	

Comments:

Sample(s) received by Analytical Chemistry personnel and request for analysis acknowledged.

Sample(s) Received By: Gregory J Bowman   Received On: 4/16/08

TOX215.003.091406

① Entry Error GS 4-16-08

Submission Date Was
April 16, 08

Series 1 Doses formulated on
April 14, 08 - TB ++
April 15, 08 - Nic Tartrate ++
April 18, 08 - TE ++ +

TOX210 Series 1
Formulation data
including some
stability data (1 week)

Data Set A

SAMPLE SUBMISSION RECORD

Study Number: TOX210

The following sample(s) are being submitted for analysis:
Submitted by: Gerry Smith Submitted on: 4-16-08

Sample Identification (GN number)	Analysis	Sample Identification (GN number)	Analysis
GN76276 AW	46	GN 76276BI	46
GN76276 AX	46		
GN 76276 AY	46		
GN 76276 BA	46		
GN 76276 BB	46		
GN 76276 BC	46		
GN 76276 BD	46		
GN 76276 BE	46		
GN 76276 BF	46		
GN 76276BG	46		
GN 76276BH	46		

Comments:

Sample(s) received by Analytical Chemistry personnel and request for analysis acknowledged.

Sample(s) Received By: Gary J Born Received On: 4/16/08

TOX215.003.091406

Testno: GN76276 Prog no: 900 Protocol: GENERAL Needed: 04/17/08
Requester: SMITH, JENNY L Phone: 741-0125
Description: TOX210 FEED STUDY
PDR Number: TOX210
Lab Instructions:
Please Run Duplicates

Part: GN76276AA Points: 1 Butt Len: 0 Part Name: TOB BLEND 0.2mg TOP
Date: 20080415 Shift: Comments:

Part: GN76276AB Points: 1 Butt Len: 0 Part Name: TOB BLEND 0.2mg MID
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AC Points: 1 Butt Len: 0 Part Name: TOB BLEND 0.2mg BOT
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AD Points: 1 Butt Len: 0 Part Name: TOB EXTRAC 0.2 TOP
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AE Points: 1 Butt Len: 0 Part Name: TOB EXTRAC 0.2 MID
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AF Points: 1 Butt Len: 0 Part Name: TOB EXTRAC 0.2 BOT
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AG Points: 1 Butt Len: 0 Part Name: NIC TAR 2.0 TOP
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AH Points: 1 Butt Len: 0 Part Name: NIC TAR 2.0 MID
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AI Points: 1 Butt Len: 0 Part Name: NIC TAR 2.0 BOT
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AJ Points: 1 Butt Len: 0 Part Name: NIC TAR 40.0mg TOP
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AK Points: 1 Butt Len: 0 Part Name: NIC TAR 40.0mg MID
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AL Points: 1 Butt Len: 0 Part Name: NIC TAR 40.0mg BOT
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AM Points: 1 Butt Len: 0 Part Name: TOB BLEND 40.0mg TOP
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AN Points: 1 Butt Len: 0 Part Name: TOB BLEND 40.0mg MID
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AO Points: 1 Butt Len: 0 Part Name: TOB BLEND 40.0mg BOT
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AP Points: 1 Butt Len: 0 Part Name: TOB EXTRACT 40.0 TOP
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AQ Points: 1 Butt Len: 0 Part Name: TOB EXTRACT 40.0 MID
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AR Points: 1 Butt Len: 0 Part Name: TOB EXTRACT 40.0 BOT
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AS Points: 1 Butt Len: 0 Part Name: TOB BLEND 2.0mg
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AT Points: 1 Butt Len: 0 Part Name: TOB BLEND 4.0mg
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AU Points: 1 Butt Len: 0 Part Name: TOB BLEND 8.0mg
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AV Points: 1 Butt Len: 0 Part Name: TOB EXTRACT 2.0mg
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AW Points: 1 Butt Len: 0 Part Name: TOB EXTRACT 4.0mg
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AX Points: 1 Butt Len: 0 Part Name: TOB EXTRACT 8.0mg
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AY Points: 1 Butt Len: 0 Part Name: TOB EXTRACT 20.0mg
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276AZ Points: 1 Butt Len: 0 Part Name: NIC TAR 2.0mg
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276BA Points: 1 Butt Len: 0 Part Name: NIC TAR 8.0mg
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276BB Points: 1 Butt Len: 0 Part Name: NIC TAR 20.0mg
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276BC Points: 1 Butt Len: 0 Part Name: TOB BLEND 20.0mg ✓
Date: 20080416 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276BD Points: 1 Butt Len: 0 Part Name: tob ext 40mg 4-3 STA ✓
Date: 20080416 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276BE Points: 1 Butt Len: 0 Part Name: tob ext 0.2 4-3 STA ✓
Date: 20080416 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276BF Points: 1 Butt Len: 0 Part Name: nic tar 40.0 4-2 STA ✓

Date: 20080416 Shift: Comments:

(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276BG Points: 1 Butt Len: 0 Part Name: nic tar 2.0 4-2 STA
Date: 20080416 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276BH Points: 1 Butt Len: 0 Part Name: tob blen 40 4-2 STA
Date: 20080416 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76276BI Points: 1 Butt Len: 0 Part Name: tob blen 0.2 4-2 STA
Date: 20080416 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Series 1 Data

- Date for Submission corresponds
- GN #'s correspond
- Data has been averaged by JRH

TOX 209 RAT FEEDING ST TOX 209

Sample #	Test Article	Dose	Sample #	Rep 1 Nicotine (mg/gm)	Rep 2 Nicotine (mg/gm)	Average	
GN76269AA	Tobacco Blend	0.2	GN76269AA	0.00124	0.0015	0.00137	
GN76269AB	Tobacco Blend	0.2	GN76269AB	<0.0001	0.0011	0.00108	Top
GN76269AC	Tobacco Blend	0.2	GN76269AC	0.00119	0.0013	0.00122	
GN76269AD	Tobacco Blend	40.0	GN76269AD	analyzed by High Volume GC area			
GN76269AE	Tobacco Blend	40.0	GN76269AE				
GN76269AF	Tobacco Blend	40.0	GN76269AF				
GN76269AK	Tobacco Extract	0.2	GN76269AK	*	0.0008	0.00075	
GN76269AL	Tobacco Extract	0.2	GN76269AL	0.00061	0.0009	0.00077	
GN76269AM	Tobacco Extract	0.2	GN76269AM	0.00055	0.0005	0.00054	
GN76269AQ	Tobacco Extract	40.0	GN76269AQ	analyzed by High Volume GC area			
GN76269AR	Tobacco Extract	40.0	GN76269AR				
GN76269AS	Tobacco Extract	40.0	GN76269AS				
GN76269AN	Nicotine Tartrate	2.0	GN76269AN	0.18463	0.0184	0.10152	
GN76269AO	Nicotine Tartrate	2.0	GN76269AO	0.01382	0.0154	0.01459	
GN76269AP	Nicotine Tartrate	2.0	GN76269AP	0.01286	0.0143	0.01358	
GN76269AT	Nicotine Tartrate	40.0	GN76269AT	analyzed by High Volume GC area			
GN76269AU	Nicotine Tartrate	40.0	GN76269AU				
GN76269AV	Nicotine Tartrate	40.0	GN76269AV				

TOX 210 MOUSE FEEDING TOX 210 MOUSE FEEDING STUDY

Sample #	Test Article	Dose	Sample #					mean
GN76276AC	Tobacco Blend	0.2	GN76276AC	0.00115	0.0011	0.00115	* not average- rep 1 only- see note below	Bottom 0.0011
GN76276AB	Tobacco Blend	0.2	GN76276AB	0.00209	0.0011	0.00209	* not average- rep 1 only- see note below	middle 0.00159
GN76276AA	Tobacco Blend	0.2	GN76276AA	0.00111	*	0.00111	* not average- rep 1 only- see note below	Top
GN76276AM	Tobacco Blend	40.0	GN76276AM	analyzed by High Volume GC area				
GN76276AN	Tobacco Blend	40.0	GN76276AN					
GN76276AO	Tobacco Blend	40.0	GN76276AO					
GN76276AD	Tobacco Extract	0.2	GN76276AD	0.00069	0.0015	0.00069	* not average- rep 1 only- see note below	Top 0.00109
GN76276AE	Tobacco Extract	0.2	GN76276AE	0.00093	*	0.00093	* not average- rep 1 only- see note below	middle
GN76276AF	Tobacco Extract	0.2	GN76276AF	0.00126	*	0.00126	* not average- rep 1 only- see note below	Bottom
GN76276AP	Tobacco Extract	40.0	GN76276AP	analyzed by High Volume GC area				
GN76276AQ	Tobacco Extract	40.0	GN76276AQ					
GN76276AR	Tobacco Extract	40.0	GN76276AR					
GN76276AG	Nicotine Tartrate	2.0	GN76276AG	0.01137	0.0118	0.01160	Top	
GN76276AH	Nicotine Tartrate	2.0	GN76276AH	0.01002	0.0114	0.01070	Middle	
GN76276AI	Nicotine Tartrate	2.0	GN76276AI	0.01057	0.0094	0.00999	Bottom	
GN76276AJ	Nicotine Tartrate	40.0	GN76276AJ	analyzed by High Volume GC area				
GN76276AK	Nicotine Tartrate	40.0	GN76276AK					
GN76276AL	Nicotine Tartrate	40.0	GN76276AL					

JRH 02/04/09

TOX 209 RAT FEEDING ST TOX 209 RAT FEEDING STUDY

Sample #	Test Article	Dose	Sample #			
GN76269BC	NTP-2000 Diet	0	GN76269BC	<0.0001	<0.0001	<0.0001
GN76269AG	Tobacco Blend	2.0	GN76269AG	0.02244	0.0120	0.01722
GN76269AH	Tobacco Blend	4.0	GN76269AH	0.03699	0.0352	0.03608
GN76269AI	Tobacco Blend	8.0	GN76269AI	0.07517	0.0612	0.06817
GN76269AJ	Tobacco Blend	20.0	GN76269AJ	0.14557	0.1479	0.14672
GN76269AY	Tobacco Extract	2.0	GN76269AY	0.00848	0.0435	0.02599
GN76269AZ	Tobacco Extract	4.0	GN76269AZ	0.02748	0.0160	0.02174
GN76269BA	Tobacco Extract	8.0	GN76269BA	0.02382	0.1619	0.09284
GN76269BB	Tobacco Extract	20.0	GN76269BB	0.21407	0.1178	0.16593
GN76269BE	Nicotine Tartrate	8.0	GN76269BE	0.06808	0.0680	0.06802
GN76269AX	Nicotine Tartrate	20.0	GN76269AX	0.15292	1.6443	0.89860

TOX 210 MOUSE FEEDING TOX 210 MOUSE FEEDING STUDY

Sample #	Test Article	Dose	Sample #					Mean
	NTP-2000 Diet	0						
GN76276AS	Tobacco Blend	2.0	GN76276AS	0.00864	0.0097	0.00864	* not average- rep 1 only- see note below	0.0092
GN76276AT	Tobacco Blend	4.0	GN76276AT	0.01348	0.0222	0.01348	* not average- rep 1 only- see note below	0.0178
GN76276AU	Tobacco Blend	8.0	GN76276AU	0.03199	0.0397	0.03199	* not average- rep 1 only- see note below	0.0358
GN76276BC	Tobacco Blend	20.0	GN76276BC	0.10260	0.0824	0.10260	* not average- rep 1 only- see note below	0.0925
GN76276AV	Tobacco Extract	2.0	GN76276AV	0.00220	0.0029	0.00220	* not average- rep 1 only- see note below	0.0026
GN76276AW	Tobacco Extract	4.0	GN76276AW	0.01106	0.0161	0.01106	* not average- rep 1 only- see note below	0.0136
GN76276AX	Tobacco Extract	8.0	GN76276AX	0.03376	0.0267	0.03376	* not average- rep 1 only- see note below	0.0302
GN76276AY	Tobacco Extract	20.0	GN76276AY	0.08161	0.0527	0.08161	* not average- rep 1 only- see note below	0.0672
GN76276BA	Nicotine Tartrate	8.0	GN76276BA	0.03114	0.0349	0.03114	* not average- rep 1 only- see note below	0.0330
GN76276BB	Nicotine Tartrate	20.0	GN76276BB	0.08844	0.0885	0.08844	* not average- rep 1 only- see note below	0.0885

JKd 2/4/09

PLUS PLUS

TOX 209 RAT FEEDING ST TOX 209 RAT FEEDING STUDY

1 week stability data JKd 02/04/09

Sample #	Test Article	Dose	Sample #					
GN76276BD	Tobacco Extract	40.0	GN76276BD	0.46699	0.3951	0.46699	* not average- rep 1 only- see note below	0.431[illegible]
GN76276BE	Tobacco Extract	0.2	GN76276BE	0.00160	0.0021	0.00160	* not average- rep 1 only- see note below	0.001[illegible]
GN76276BF	Nicotine Tartrate	40.0	GN76276BF	0.37349	0.3828	0.37349	* not average- rep 1 only- see note below	0.37[illegible]
GN76276BG	Nicotine Tartrate	2.0	GN76276BG	0.02017	0.0179	0.02017	* not average- rep 1 only- see note below	0.0[illegible]0
GN76276BH	Tobacco Blend	40.0	GN76276BH	0.39827	0.3805	0.39827	* not average- rep 1 only- see note below	0.389[illegible]
GN76276BI	Tobacco Blend	0.2	GN76276BI	0.00594	0.0018	0.00594	* not average- rep 1 only- see note below	0.003[illegible]7

Notes

Series 1 Feed Formulation Dose Confirmation
Second Sample Submission

SAMPLE SUBMISSION RECORD

Study Number: TOX ~~209~~ 210 Series 1

The following sample(s) are being submitted for analysis:

Submitted by: GSmith

Submitted on: 6-12-08

Sample Identification (GN number)	Analysis	Sample Identification (GN number)	Analysis
GN 77622AA	46	GN 77622AL	46
GN 77622AB	46	GN 77622AM	46
GN 77622AC	46	GN 77622AN	46
GN 77622AD	46	GN 77622AO	46
GN 77622AE	46	GN 77622AP	46
GN 77622AF	46		
GN 77622AG	46		
GN 77622AH	46		
GN 77622AI	46		
GN 77622AJ	46		
GN 77622AK	46		

Comments:

Sample(s) received by Analytical Chemistry personnel and request for analysis acknowledged.

Sample(s) Received By: [signature]

Received On: 6-12-08

TOX215.004 041808

Testno: GN77622 Prog no: 900 Protocol: GENERAL Needed: //
Requester: SMITH, JENNY L Phone: 741-0125
Description: TOX210 SERIES 1
PDR Number: TOX210
Lab Instructions:
please run duplicates

Part: GN77622AA Points: 1 Butt Len: 0 Part Name: Gr 2 Dose 1 0.2 mg
Date: 20080612 Shift: Comments:

Part: GN77622AB Points: 1 Butt Len: 0 Part Name: Gr 3 Dose 2 2.0 mg
Date: 20080612 Shift: Comments:

Part: GN77622AC Points: 1 Butt Len: 0 Part Name: Gr 4 Dose 3 4.0 mg
Date: 20080612 Shift: Comments:

Part: GN77622AD Points: 1 Butt Len: 0 Part Name: Gr 5 Dose 4 8.0 mg
Date: 20080612 Shift: Comments:

Part: GN77622AE Points: 1 Butt Len: 0 Part Name: Gr 6 Dose 5 20.0 mg
Date: 20080612 Shift: Comments:

Part: GN77622AF Points: 1 Butt Len: 0 Part Name: Gr 7 Dose 6 40.0 mg
Date: 20080612 Shift: Comments:

Part: GN77622AG Points: 1 Butt Len: 0 Part Name: Gr 8 Dose 1 0.2 mg
Date: 20080612 Shift: Comments:

Part: GN77622AH Points: 1 Butt Len: 0 Part Name: Gr 9 Dose 2 2.0 mg
Date: 20080612 Shift: Comments:

Part: GN77622AI Points: 1 Butt Len: 0 Part Name: Gr 10 Dose 3 4.0 mg
Date: 20080612 Shift: Comments:

Part: GN77622AJ Points: 1 Butt Len: 0 Part Name: Gr 11 Dose 4 8.0 mg
Date: 20080612 Shift: Comments:

Part: GN77622AK Points: 1 Butt Len: 0 Part Name: Gr 12 Dose 5 20.0 mg
Date: 20080612 Shift: Comments:

Part: GN77622AL Points: 1 Butt Len: 0 Part Name: Gr 14 Dose 7 2.0 mg
Date: 20080612 Shift: Comments:

Part: GN77622AM Points: 1 Butt Len: 0 Part Name: GR 14 Dose 1 2.0mg
Date: 20080612 Shift: Comments:

Part: GN77622AN Points: 1 Butt Len: 0 Part Name: GR 15 Dose 2 8.0mg
Date: 20080612 Shift: Comments:

Part: GN77622AO Points: 1 Butt Len: 0 Part Name: GR 16 Dose 3 20.0mg
Date: 20080612 Shift: Comments:

Part: GN77622AP Points: 1 Butt Len: 0 Part Name: GR 17 Dose 4 40.0mg
Date: 20080612 Shift: Comments:

Testno: GN77622 Prog no: 900 Protocol: GENERAL Needed: //

Requester: SMITH, JENNY L Phone: 741-0125

Description: TOX210 SERIES 1

PDR Number: TOX210

Lab Instructions:

please run duplicates

Part: GN77622AA Points: 1 Butt Len: 0 Part Name: Gr 2 Dose 1 0.2 mg

Date: 20080612 Shift: Comments:

Part: GN77622AB Points: 1 Butt Len: 0 Part Name: Gr 3 Dose 2 2.0 mg

Date: 20080612 Shift: Comments:

Part: GN77622AC Points: 1 Butt Len: 0 Part Name: Gr 4 Dose 3 4.0 mg

Date: 20080612 Shift: Comments:

Part: GN77622AD Points: 1 Butt Len: 0 Part Name: Gr 5 Dose 4 8.0 mg

Date: 20080612 Shift: Comments:

Part: GN77622AE Points: 1 Butt Len: 0 Part Name: Gr 6 Dose 5 20.0 mg

Date: 20080612 Shift: Comments:

Part: GN77622AF Points: 1 Butt Len: 0 Part Name: Gr 7 Dose 6 40.0 mg

Date: 20080612 Shift: Comments:

Part: GN77622AG Points: 1 Butt Len: 0 Part Name: Gr 8 Dose 1 0.2 mg

Date: 20080612 Shift: Comments:

Part: GN77622AH Points: 1 Butt Len: 0 Part Name: Gr 9 Dose 2 2.0 mg

Date: 20080612 Shift: Comments:

Part: GN77622AI Points: 1 Butt Len: 0 Part Name: Gr 10 Dose 3 4.0 mg

Date: 20080612 Shift: Comments:

Part: GN77622AJ Points: 1 Butt Len: 0 Part Name: Gr 11 Dose 4 8.0 mg

Date: 20080612 Shift: Comments:

Part: GN77622AK Points: 1 Butt Len: 0 Part Name: Gr 12 Dose 5 20.0 mg

Date: 20080612 Shift: Comments:

Part: GN77622AL Points: 1 Butt Len: 0 Part Name: Gr 14 Dose 7 ~~2.0~~ mg

Date: 20080612 Shift: Comments:

40

Part: GN77622AM Points: 1 Butt Len: 0 Part Name: GR 14 Dose 1 2.0mg
Date: 20080612 Shift: Comments:

Part: GN77622AN Points: 1 Butt Len: 0 Part Name: GR 15 Dose 2 8.0mg
Date: 20080612 Shift: Comments:

Part: GN77622AO Points: 1 Butt Len: 0 Part Name: GR 16 Dose 3 20.0mg
Date: 20080612 Shift: Comments:

Part: GN77622AP Points: 1 Butt Len: 0 Part Name: GR 17 Dose 4 40.0mg
Date: 20080612 Shift: Comments:

PAD TEST MEMORANDUM

AUTHOR: Karen B. Kilby
Timothy A. Ellisor

DATE: August 5, 2008

DEPARTMENT: Product Quality

DIVISION: Product Assessment

CLIENTS: Jenny Smith
Suzanne Theophilus

PREVIOUS REPORTS: PAD-MKBK 2008, 217

PROJECT CHARTER: Smokeless Tobacco Stewardship Animal Feed Palatability Project

MANHOURS: 40

Determination of the amount of Nicotine Applied to Rat/Mouse Feed Samples

OBJECTIVE:

The purpose of this study was to determine the amount nicotine added to rat/mouse feed samples to support the Smokeless Tobacco Stewardship Feeding Studies Project. Eleven sets of samples (152 samples, 2 reps each) were submitted for analysis.

SUMMARY:

Rat and mouse feed samples with varying dosing levels of nicotine were submitted for analysis. The samples were logged into LIMS under the following identification numbers: GN76749 (AA-AF), GN76750 (AA-AF), GN76747 (AA-AP), GN77202 (AA-AR), GN77172 (AA-AI), GN77522 (AA-AO), GN77615 (AA-AQ), GN77622 (AA-AP), GN77620 (AA-AO), and GN77624 (AA-AP). The samples were analyzed, in duplicate, using the method outlined in PAD-MKBK 2008, 217.

STATUS:

The determination of the amount of nicotine applied to rat/mouse feed samples is complete.

KEYWORDS:
GC/MS, nicotine, smokeless tobacco, SNUS, Feed, Diet

Karen B. Kilby, Scientist III

Timothy A. Ellisor, Technician III

Reviewed by:

S. Mark DeBusk, Lead Manager Product Quality

INTRODUCTION:

Various levels of nicotine were applied to rat and mouse feed using various sources of nicotine (nicotine, nicotine extract, and nicotine tartrate). The samples were submitted for the determination of nicotine in feed to verify the level of nicotine applied to each sample. The samples were logged into LIMS under the following identification numbers: GN76749 (AA-AF), GN76750 (AA-AF), GN76747 (AA-AP), GN77202 (AA-AR), GN77172 (AA-AI), GN77522 (AA-AO), GN77615 (AA-AQ), GN77622 (AA-AP), GN77620 (AA-AO), and GN77624 (AA-AP).

EXPERIMENTAL:

Samples were prepared, in duplicate, according to the procedures outlined in PAD-MKBK 2008, 217, Appendix A. To summarize:

- Accurately weigh approximately 1 gram of each feed sample.
- Put sample in a tube containing 5 mL of NaOH solution.
- Mix to ensure complete saturation of sample.
- Wait 30 minutes.
- Add 5 mL of methyl tert butyl ether (MTBE) extraction solution to each sample.
- Shake on a wrist action shaker for 2 hours.
- Allow sample to separate into two layers.
- Transfer the top layer of sample to GC vial.
- Seal vial using crimp top cap.
- Analyze using GC/MS technology.

RESULTS AND DISCUSSION:

The results measured for each sample are shown in Tables 1-4. The results measured showed levels of nicotine in the expected range for this study.

Typ°

Table 1: Nicotine Results GN77624, GN77620, and GN77622

	GN7762 4	mg/g	Average	GN7762 0	mg/g	Average		GN7762 2	mg/g	Dose Average
control ?	AA1	<0.001		AA1	0.001		TB	AA1	0.002	0.2
	AA2	<0.001	<0.001	AA2	<0.001	0.001		AA2	0.001	0.002
	AB1	0.008		AB1	0.009		TB	AB1	0.012	2.0
	AB2	0.013	0.011	AB2	0.008	0.009		AB2	0.013	0.012
	AC1	0.021		AC1	0.015		TB	AC1	0.015	4.0
	AC2	0.015	0.018	AC2	0.017	0.016		AC2	0.013	0.014
	AD1	0.034		AD1	0.077		TB	AD1	0.029	8.0
	AD2	0.030	0.032	AD2	0.078	0.077		AD2	0.029	0.029
	AE1	0.082		AE1	0.153		TB	AE1	0.092	20.0
	AE2	0.071	0.077	AE2	0.157	0.155		AE2	0.078	0.085
	AF1	0.171		AF1	0.337		TB	AF1	0.125	40.0
	AF2	0.151	0.161	AF2	0.339	0.338		AF2	0.144	0.135
	AG1	0.001		AG1	0.001		TE	AG1	0.001	0.2
	AG2	0.000	0.001	AG2	0.001	0.001		AG2	0.001	0.001
	AH1	0.003		AH1	0.011		TE	AH1	0.006	2.0
	AH2	0.004	0.003	AH2	0.016	0.014		AH2	0.005	0.006
	AI1	0.064		AI1	0.018		TE	AI1	0.011	4.0
	AI2	0.072	0.068	AI2	0.024	0.021		AI2	0.017	0.014
	AJ1	0.019		AJ1	0.058		TE	AJ1	0.044	8.0
	AJ2	0.017	0.018	AJ2	0.048	0.053		AJ2	0.047	0.046
	AK1	0.048		AK1	0.108		TE	AK1	0.011	20.0
	AK2	0.034	0.041	AK2	0.176	0.142		AK2	0.096	0.054
	AL1	0.118		AL1	0.354		TE	AL1	0.288	40.0
	AL2	0.062	0.090	AL2	0.393	0.374		AL2	0.280	0.284
	AM1	0.007		AM1	0.017		NT	AM1	0.011	2.0
	AM2	0.007	0.007	AM2	0.017	0.017		AM2	0.010	0.011
	AN1	0.027		AN1	0.066		NT	AN1	0.030	8.0
	AN2	0.027	0.027	AN2	0.065	0.065		AN2	0.030	0.030
	AO1	0.068		AO1	0.156		NT	AO1	0.001	20.0
	AO2	0.064	0.066	AO2	0.151	0.154		AO2	0.001	0.001
	AP1	0.137		AP1	0.303		NT	AP1	0.209	40.0
	AP2	0.123	0.130	AP2	0.310	0.306		AP2	0.203	0.206
				AQ1	0.045			Submission Sheet label TOX210 series 1 Date June 12, 08 Have sample cups		
				AQ2	0.038	0.042				

Decoded from Sample cups sent to Chemistry group.

AL

Table 2:Nicotine Results GN77615, GN77522, And GN77172

GN77615	mg/g	Average	GN77522	mg/g	Average	GN77172	mg/g	Average
AA1	<0.001		AA1	0.176		AA1	0.166	
AA2	<0.001	<0.001	AA2	0.134	0.155	AA2	0.173	0.170
AB1	0.002		AB1	1.415		AB1	0.140	
AB2	0.003	0.002	AB2	1.481	1.448	AB2	0.156	0.148
AC1	0.015		AC1	0.304		AC1	0.121	
AC2	0.012	0.014	AC2	0.311	0.307	AC2	0.145	0.133
AD1	0.028		AD1	0.648		AD1	0.271	
AD2	0.042	0.035	AD2	0.577	0.612	AD2	0.334	0.302
AE1	0.050		AE1	0.875		AE1	0.628	
AE2	0.075	0.062	AE2	0.969	0.922	AE2	0.597	0.613
AF1	0.122		AF1	0.159		AF1	0.974	
AF2	0.153	0.138	AF2	0.156	0.158	AF2	0.821	0.898
AG1	0.307		AG1	1.581		AG1	1.503	
AG2	0.298	0.302	AG2	1.546	1.563	AG2	1.695	1.599
AH1	0.002		AH1*	0.647		AH1	1.732	
AH2	0.003	0.002	AH2*	0.683	0.665	AH2	1.654	1.693
AI1	0.068		AI1*	0.380		AI1	1.679	
AI2	0.032	0.050	AI2*	0.322	0.351	AI2	1.610	1.644
AJ1	0.054		AJ1	0.853				
AJ2	0.052	0.053	AJ2	0.879	0.866			
AK1	0.052		AK1	0.018				
AK2	0.067	0.060	AK2	0.016	0.017			
AL1	0.191		AL1	1.379				
AL2	0.164	0.178	AL2	1.340	1.359			
AM1	0.291		AM1	0.261				
AM2	0.239	0.265	AM2	0.259	0.260			
AN1	0.017		AN1	0.542				
AN2	0.018	0.018	AN2	0.539	0.540			
AO1	0.061		AO1	0.779				
AO2	0.064	0.063	AO2	0.789	0.784			
AP1	0.152							
AP2	0.155	0.153						
AQ1	0.287							
AQ2	0.306	0.296						

*Sample parts GN77522AH and AI appear to be mixed up.

Table 3: Nicotine Results GN77202, GN76746, and GN76747

GN77202	mg/g	Average	GN76746	mg/g	Average	GN76747	mg/g	Average
AA1	0.243		AA1	0.350		AA1	0.167	
AA2	0.148	0.196	AA2	0.363	0.357	AA2	0.197	0.182
AB1	0.148		AB1	0.176		AB1	0.095	
AB2	0.171	0.159	AB2	0.204	0.190	AB2	0.082	0.089
AC1	0.170		AC1	0.072		AC1	0.027	
AC2	0.198	0.184	AC2	0.070	0.071	AC2	0.031	0.029
AD1	0.512		AD1	0.048		AD1	0.014	
AD2	0.571	0.541	AD2	0.034	0.041	AD2	0.018	0.016
AE1	0.308		AE1	0.019		AE1	0.008	
AE2	0.335	0.322	AE2	0.010	0.014	AE2	0.010	0.009
AF1	0.907		AF1	0.002		AF1	0.001	
AF2	0.972	0.940	AF2	0.005	0.004	AF2	0.001	0.001
AG1	1.593		AG1	0.281		AG1	0.183	
AG2	1.589	1.591	AG2	0.337	0.309	AG2	0.224	0.203
AH1	1.491		AH1	0.154		AH1	0.056	
AH2	1.596	1.544	AH2	0.115	0.134	AH2	0.066	0.061
AI1	1.740		AI1	0.099		AI1	0.022	
AI2	1.591	1.665	AI2	0.144	0.122	AI2	0.014	0.018
AJ1	0.017		AJ1**	0.068		AJ1**	0.020	
AJ2	0.016	0.016	AJ2**	0.019	0.043	AJ2**	0.007	0.013
AK1	0.017		AK1**	0.011		AK1	0.004	
AK2	0.017	0.017	AK2**	0.069	0.040	AK2	0.003	0.003
AL1	0.017		AL1	0.001		AL1	0.001	
AL2	0.016	0.016	AL2	0.001	0.001	AL2	0.001	0.001
AM1	0.278		AM1	0.019		AM1	0.234	
AM2	0.280	0.279	AM2	0.019	0.019	AM2	0.228	0.231
AN1	0.589		AN1	0.074		AN1	<0.001	
AN2	0.599	0.594	AN2	0.075	0.075	AN2	<0.001	<0.001
AO1	0.851		AO1	0.178		AO1	0.031	
AO2	0.868	0.859	AO2	0.183	0.181	AO2	0.031	0.031
AP1	1.497		AP1	0.355		AP1	0.007	
AP2	1.536	1.517	AP2	0.356	0.356	AP2	0.009	0.008
AQ3	1.483							
AQ2	1.487	1.485						
AR1	1.520							
AR2	1.490	1.505						

**Unexpected difference in replicate results. The chromatograms were checked and results confirmed. Additional sample needed for further verification.

Table 4: Nicotine Results GN76749 and GN76750

GN76749	mg/g	Average		GN76750	mg/g	Average
AA1	0.466			AA1	0.416	
AA2	0.411	0.438		AA2	0.402	0.409
AB1	0.002			AB1	0.002	
AB2	0.002	0.002		AB2	0.001	0.002
AC1	0.448			AC1	0.408	
AC2	0.424	0.436		AC2	0.325	0.367
AD1	0.002			AD1	0.001	
AD2	0.002	0.002		AD2	0.001	0.001
AE1	0.415			AE1	0.360	
AE2	0.407	0.411		AE2	0.348	0.354
AF1	0.019			AF1	0.019	
AF2	0.021	0.020		AF2	0.019	0.019

CONCLUSION:
The determination of nicotine applied to rat/mouse feed is complete. The results reported in this study showed levels of nicotine in the expected range for this study.

Series 2 Feed Formulation Dose Confirmation Data
First Submission

Testno: GN76747 Prog no: 900 Protocol: GENERAL Needed: 05/05/08
Requester: SMITH, JENNY L Phone: 741-0125
scription: ~~TOX209~~ FEED Formulated 4-23/4-24
R Number: TX210MOUS TOX210 JS. 5-2-08
Lab Instructions:
Please run duplicates

Part: GN76747AA Points: 1 Butt Len: 0 Part Name: TOB BLE 40.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AB Points: 1 Butt Len: 0 Part Name: TOB BLE 20.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AC Points: 1 Butt Len: 0 Part Name: TOB BLE 8.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AD Points: 1 Butt Len: 0 Part Name: TOB BLE 4.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AE Points: 1 Butt Len: 0 Part Name: TOB BLE 2.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AF Points: 1 Butt Len: 0 Part Name: TOB BLE 0.20 MG
te: 20080502 Shift: Comments:

Part: GN76747AG Points: 1 Butt Len: 0 Part Name: TOB EXT 40.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AH Points: 1 Butt Len: 0 Part Name: TOB EXT 20.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AI Points: 1 Butt Len: 0 Part Name: TOB EXT 8.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AJ Points: 1 Butt Len: 0 Part Name: TOB EXT 4.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AK Points: 1 Butt Len: 0 Part Name: TOB EXT 2.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AL Points: 1 Butt Len: 0 Part Name: TOB EXT 0.2 MG
Date: 20080502 Shift: Comments:

Part: GN76747AM Points: 1 Butt Len: 0 Part Name: NIC TAR 40.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AN Points: 1 Butt Len: 0 Part Name: NIC TAR 20.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AO Points: 1 Butt Len: 0 Part Name: NIC TAR 8.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AP Points: 1 Butt Len: 0 Part Name: NIC TAR 2.0 MG
Date: 20080502 Shift: Comments:

Testno: GN76747 Prog no: 900 Protocol: GENERAL Needed: 05/05/08
Requester: SMITH, JENNY L Phone: 741-0125
scription: TOX~~209~~ FEED Formulated 4-23/4-24
R Number: TX210MOUS TOX210
Lab Instructions:
Please run duplicates

Part: GN76747AA Points: 1 Butt Len: 0 Part Name: TOB BLE 40.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AB Points: 1 Butt Len: 0 Part Name: TOB BLE 20.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AC Points: 1 Butt Len: 0 Part Name: TOB BLE 8.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AD Points: 1 Butt Len: 0 Part Name: TOB BLE 4.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AE Points: 1 Butt Len: 0 Part Name: TOB BLE 2.0 MG
Date: 20080502 Shift: Comments:

rt: GN76747AF Points: 1 Butt Len: 0 Part Name: TOB BLE 0.20 MG
te: 20080502 Shift: Comments:

Part: GN76747AG Points: 1 Butt Len: 0 Part Name: TOB EXT 40.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AH Points: 1 Butt Len: 0 Part Name: TOB EXT 20.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AI Points: 1 Butt Len: 0 Part Name: TOB EXT 8.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AJ Points: 1 Butt Len: 0 Part Name: TOB EXT 4.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AK Points: 1 Butt Len: 0 Part Name: TOB EXT 2.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AL Points: 1 Butt Len: 0 Part Name: TOB EXT 0.2 MG
Date: 20080502 Shift: Comments:

Part: GN76747AM Points: 1 Butt Len: 0 Part Name: NIC TAR 40.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AN Points: 1 Butt Len: 0 Part Name: NIC TAR 20.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AO Points: 1 Butt Len: 0 Part Name: NIC TAR 8.0 MG
Date: 20080502 Shift: Comments:

Part: GN76747AP Points: 1 Butt Len: 0 Part Name: NIC TAR 2.0 MG
Date: 20080502 Shift: Comments:

Series 2 Preliminary Data

gn76747	TEST ARTICLE	mg/mL	Weight (g)	vol solution	mg/g		
AA1	TOB BLE 40.0 mg	0.034701	1.0314	5	0.168		
AA2		0.040161	1.0105	5	0.199	0.1835	
AB1	TOB BLE 20.0 mg	0.019463	1.0118	5	0.096		
AB2		0.016781	1.0094	5	0.083	0.0897	
AC1	TOB BLE 8.0 mg	0.005636	1.0345	5	0.027		
AC2		0.006562	1.0358	5	0.032	0.0295	
AD1	TOB BLE 4.0 mg	0.002831	1.0335	5	0.014		
AD2		0.003639	1.0069	5	0.018	0.0159	
AE1	TOB BLE 2.0 mg	0.001669	1.0241	5	0.008		
AE2		0.002075	1.0029	5	0.010	0.0092	
AF1	TOB BLE 0.2 mg	0.000113	1.0142	5	0.001		
AF2		0.000343	1.0121	5	0.002	0.0011	
AG1	TOB EXT 40.0 mg	0.037549	1.0166	5	0.185		
AG2		0.045985	1.0155	5	0.226	0.2055	
AH1	TOB EXT 20.0 mg	0.011609	1.0192	5	0.057		
AH2		0.013694	1.0308	5	0.066	0.0617	
AI1	TOB EXT 8.0 mg	0.004507	1.0297	5	0.022		
AI2		0.002772	1.0097	5	0.014	0.0178	
Aj1	TOB EXT 4.0 mg	0.004079	1.0225	5	0.020		
Aj2		0.001711	1.0221	5	0.008	0.0142	
Ak1	TOB EXT 2.0 mg	0.000715	1.007	5	0.004		
Ak2		0.000687	1.0064	5	0.003	0.0035	
Al1	TOB EXT 0.2 mg	0.000179	1.0014	5	0.001		
Al2		0.000159	1.0014	5	0.001	0.0008	
AM1	NIC TAR 40.0 mg	0.048473	1.024	5	0.237		
AM2		0.04792	1.0409	5	0.230	0.2334	
AN1	NIC TAR 20.0 mg	<0.0001	1.008	5	<0.0001		Resampled
AN2		<0.0001	1.0123	5	<0.0001	<0.0001	
AO1	NIC TAR 8.0 mg	0.006344	1.0135	5	0.031		
AO2		0.006414	1.0278	5	0.031	0.0313	
AP1	NIC TAR 2.0 mg	0.001541	1.0219	5	0.008		
AP2		0.00181	1.0168	5	0.009	0.0082	

TOX210

*results marked in red indicate an unexpected difference between the replicates.
The chromatograms were rechecked and confirmed the results reported.
Additional sample would need to be submitted for further verification.

Table 3: Nicotine Results GN77202, GN76746, and GN76747

GN77202	mg/g	Average		GN76746	mg/g	Average	→	GN76747	mg/g	Dobe Average
AA1	0.243			AA1	0.350		TB	AA1	0.167	40 mg
AA2	0.148	0.196		AA2	0.363	0.357		AA2	0.197	0.182
AB1	0.148			AB1	0.176		TB	AB1	0.095	20 mg
AB2	0.171	0.159		AB2	0.204	0.190		AB2	0.082	0.089
AC1	0.170			AC1	0.072		TB	AC1	0.027	8.0 mg
AC2	0.198	0.184		AC2	0.070	0.071		AC2	0.031	0.029
AD1	0.512			AD1	0.048		TB	AD1	0.014	4.0 mg
AD2	0.571	0.541		AD2	0.034	0.041		AD2	0.018	0.016
AE1	0.308			AE1	0.019		TB	AE1	0.008	2.0 mg
AE2	0.335	0.322		AE2	0.010	0.014		AE2	0.010	0.009
AF1	0.907			AF1	0.002		TB	AF1	0.001	0.2 mg
AF2	0.972	0.940		AF2	0.005	0.004		AF2	0.001	0.001
AG1	1.593			AG1	0.281		TE	AG1	0.183	40 mg
AG2	1.589	1.591		AG2	0.337	0.309		AG2	0.224	0.203
AH1	1.491			AH1	0.154		TE	AH1	0.056	20 mg
AH2	1.596	1.544		AH2	0.115	0.134		AH2	0.066	0.061
AI1	1.740			AI1	0.099		TE	AI1	0.022	8 mg
AI2	1.591	1.665		AI2	0.144	0.122		AI2	0.014	0.018
AJ1	0.017			AJ1**	0.068		TE	AJ1**	0.020	4 mg
AJ2	0.016	0.016		AJ2**	0.019	0.043		AJ2**	0.007	0.013
AK1	0.017			AK1**	0.011		TE	AK1	0.004	2.0 mg
AK2	0.017	0.017		AK2**	0.069	0.040		AK2	0.003	0.003
AL1	0.017			AL1	0.001		TE	AL1	0.001	0.2 mg
AL2	0.016	0.016		AL2	0.001	0.001		AL2	0.001	0.001
AM1	0.278			AM1	0.019		NT	AM1	0.234	40 mg
AM2	0.280	0.279		AM2	0.019	0.019		AM2	0.228	0.231
AN1	0.589			AN1	0.074		NT	AN1	<0.001	20 mg
AN2	0.599	0.594		AN2	0.075	0.075		AN2	<0.001	<0.001
AO1	0.851			AO1	0.178		NT	AO1	0.031	8 mg
AO2	0.868	0.859		AO2	0.183	0.181		AO2	0.031	0.031
AP1	1.497			AP1	0.355		NT	AP1	0.007	2.0 mg
AP2	1.536	1.517		AP2	0.356	0.356		AP2	0.009	0.008
AQ3	1.483									
AQ2	1.487	1.485								
AR1	1.520									
AR2	1.490	1.505								

- TOX210 Series 2 Feed Formulation Data
- Submission May 2
- Confirmed by data cups

**Unexpected difference in replicate results. The chromatograms were checked and results confirmed. Additional sample needed for further verification.

Series 2 Feed Formulation Dose Confirmation
Second Submission

SAMPLE SUBMISSION RECORD

Study Number: ~~TOX209~~ 210 Series 2

The following sample(s) are being submitted for analysis:

Submitted by: GSmith Submitted on: 6-12-08

Sample Identification (GN number)	Analysis	Sample Identification (GN number)	Analysis
GN77624AA	46	GN77624AL	46
GN77624AB	46	GN77624AM	46
GN77624AC	46	GN77624AN	46
GN77624AD	46	GN77624AO	46
GN77624AE	46	GN77624AP	46
GN77624AF	46		
GN77624AG	46		
GN77624AH	46		
GN77624AI	46		
GN77624AJ	46		
GN77624AK	46		

Comments:

Sample(s) received by Analytical Chemistry personnel and request for analysis acknowledged.

Sample(s) Received By: [signature] Received On: 6-12-08

TOX215.004 041808

Testno: GN77624 Prog no: 900 Protocol: GENERAL Needed: //
Requester: SMITH, JENNY L Phone: 741-0125
Description: TOX213 SERIES 2
PDR Number: TOX210 2
Lab Instructions:
Please run duplicates

Part: GN77624AA Points: 1 Butt Len: 0 Part Name: GR 2 DOSE 1 0.2
Date: 20080612 Shift: Comments:

Part: GN77624AB Points: 1 Butt Len: 0 Part Name: GR 3 DOSE 2 2.0
Date: 20080612 Shift: Comments:

Part: GN77624AC Points: 1 Butt Len: 0 Part Name: GR 4 DOSE 3 4.0
Date: 20080612 Shift: Comments:

Part: GN77624AD Points: 1 Butt Len: 0 Part Name: GR 5 DOSE 4 8.0
Date: 20080612 Shift: Comments:

Part: GN77624AE Points: 1 Butt Len: 0 Part Name: GR 6 DOSE 5 20.0
Date: 20080612 Shift: Comments:

Part: GN77624AF Points: 1 Butt Len: 0 Part Name: GR 7 DOSE 6 40.0
Date: 20080612 Shift: Comments:

Part: GN77624AG Points: 1 Butt Len: 0 Part Name: GR 8 DOSE 1 0.2
Date: 20080612 Shift: Comments:

Part: GN77624AH Points: 1 Butt Len: 0 Part Name: GR 9 DOSE 2 2.0
Date: 20080612 Shift: Comments:

Part: GN77624AI Points: 1 Butt Len: 0 Part Name: GR 10 DOSE 3 4.0
Date: 20080612 Shift: Comments:

Part: GN77624AJ Points: 1 Butt Len: 0 Part Name: GR 11 DOSE 4 8.0
Date: 20080612 Shift: Comments:

Part: GN77624AK Points: 1 Butt Len: 0 Part Name: GR 12 DOSE 5 20.0
Date: 20080612 Shift: Comments:

Part: GN77624AL Points: 1 Butt Len: 0 Part Name: GR 13 DOSE 6 40.0
Date: 20080612 Shift: Comments:

Part: GN77624AM	Points: 1	Butt Len: 0	Part Name: GR 14 DOSE 1 2.0
Date: 20080612	Shift:	Comments:	
Part: GN77624AN	Points: 1	Butt Len: 0	Part Name: GR 15 DOSE 2 8.0
Date: 20080612	Shift:	Comments:	
Part: GN77624AO	Points: 1	Butt Len: 0	Part Name: GR 16 DOSE 3 20.0
Date: 20080612	Shift:	Comments:	
Part: GN77624AP	Points: 1	Butt Len: 0	Part Name: GR 17 DOSE 4 40.0
Date: 20080612	Shift:	Comments:	

Testno: GN77624 Prog no: 900 Protocol: GENERAL Needed: //
Requester: SMITH, JENNY L Phone: 741-0125
Description: TOX213 SERIES 2
PDR Number: TOX210 2
Lab Instructions:
Please run duplicates

Part: GN77624AA Points: 1 Butt Len: 0 Part Name: GR 2 DOSE 1 0.2
Date: 20080612 Shift: Comments:

Part: GN77624AB Points: 1 Butt Len: 0 Part Name: GR 3 DOSE 2 2.0
Date: 20080612 Shift: Comments:

Part: GN77624AC Points: 1 Butt Len: 0 Part Name: GR 4 DOSE 3 4.0
Date: 20080612 Shift: Comments:

Part: GN77624AD Points: 1 Butt Len: 0 Part Name: GR 5 DOSE 4 8.0
Date: 20080612 Shift: Comments:

Part: GN77624AE Points: 1 Butt Len: 0 Part Name: GR 6 DOSE 5 20.0
Date: 20080612 Shift: Comments:

Part: GN77624AF Points: 1 Butt Len: 0 Part Name: GR 7 DOSE 6 40.0
Date: 20080612 Shift: Comments:

Part: GN77624AG Points: 1 Butt Len: 0 Part Name: GR 8 DOSE 1 0.2
Date: 20080612 Shift: Comments:

Part: GN77624AH Points: 1 Butt Len: 0 Part Name: GR 9 DOSE 2 2.0
Date: 20080612 Shift: Comments:

Part: GN77624AI Points: 1 Butt Len: 0 Part Name: GR 10 DOSE 3 4.0
Date: 20080612 Shift: Comments:

Part: GN77624AJ Points: 1 Butt Len: 0 Part Name: GR 11 DOSE 4 8.0
Date: 20080612 Shift: Comments:

Part: GN77624AK Points: 1 Butt Len: 0 Part Name: GR 12 DOSE 5 20.0
Date: 20080612 Shift: Comments:

Part: GN77624AL Points: 1 Butt Len: 0 Part Name: GR 13 DOSE 6 40.0
Date: 20080612 Shift: Comments:

Part: GN77624AM Points: 1 Butt Len: 0 Part Name: GR 14 DOSE 1 2.0
Date: 20080612 Shift: Comments:

Part: GN77624AN Points: 1 Butt Len: 0 Part Name: GR 15 DOSE 2 8.0
Date: 20080612 Shift: Comments:

Part: GN77624AO Points: 1 Butt Len: 0 Part Name: GR 16 DOSE 3 20.0
Date: 20080612 Shift: Comments:

Part: GN77624AP Points: 1 Butt Len: 0 Part Name: GR 17 DOSE 4 40.0
Date: 20080612 Shift: Comments:

Testno: GN77624 Prog no: 900 Protocol: GENERAL Needed: //
Requester: SMITH, JENNY L Phone: 741-0125
Description: TOX210 SERIES 2
PDR Number: TOX210 2
Lab Instructions:
Please run duplicates

Part: GN77624AA Points: 1 Butt Len: 0 Part Name: GR 2 DOSE 1 0.2
Date: 20080612 Shift: Comments:

Part: GN77624AB Points: 1 Butt Len: 0 Part Name: GR 3 DOSE 2 2.0
Date: 20080612 Shift: Comments:

Part: GN77624AC Points: 1 Butt Len: 0 Part Name: GR 4 DOSE 3 4.0
Date: 20080612 Shift: Comments:

Part: GN77624AD Points: 1 Butt Len: 0 Part Name: GR 5 DOSE 4 8.0
Date: 20080612 Shift: Comments:

Part: GN77624AE Points: 1 Butt Len: 0 Part Name: GR 6 DOSE 5 20.0
Date: 20080612 Shift: Comments:

Part: GN77624AF Points: 1 Butt Len: 0 Part Name: GR 7 DOSE 6 40.0
Date: 20080612 Shift: Comments:

Part: GN77624AG Points: 1 Butt Len: 0 Part Name: GR 8 DOSE 1 0.2
Date: 20080612 Shift: Comments:

Part: GN77624AH Points: 1 Butt Len: 0 Part Name: GR 9 DOSE 2 2.0
Date: 20080612 Shift: Comments:

Part: GN77624AI Points: 1 Butt Len: 0 Part Name: GR 10 DOSE 3 4.0
Date: 20080612 Shift: Comments:

Part: GN77624AJ Points: 1 Butt Len: 0 Part Name: GR 11 DOSE 4 8.0
Date: 20080612 Shift: Comments:

Part: GN77624AK Points: 1 Butt Len: 0 Part Name: GR 12 DOSE 5 20.0
Date: 20080612 Shift: Comments:

Part: GN77624AL Points: 1 Butt Len: 0 Part Name: GR 13 DOSE 6 40.0
Date: 20080612 Shift: Comments:

Testno: GN77624 Prog no: 900 Protocol: GENERAL Needed: //
Requester: SMITH, JENNY L Phone: 741-0125
Description: TOX210 SERIES 2
PDR Number: TOX210 2
Lab Instructions:
Please run duplicates

Part: GN77624AA Points: 1 Butt Len: 0 Part Name: GR 2 DOSE 1 0.2
Date: 20080612 Shift: Comments:

Part: GN77624AB Points: 1 Butt Len: 0 Part Name: GR 3 DOSE 2 2.0
Date: 20080612 Shift: Comments:

Part: GN77624AC Points: 1 Butt Len: 0 Part Name: GR 4 DOSE 3 4.0
Date: 20080612 Shift: Comments:

Part: GN77624AD Points: 1 Butt Len: 0 Part Name: GR 5 DOSE 4 8.0
Date: 20080612 Shift: Comments:

Part: GN77624AE Points: 1 Butt Len: 0 Part Name: GR 6 DOSE 5 20.0
Date: 20080612 Shift: Comments:

Part: GN77624AF Points: 1 Butt Len: 0 Part Name: GR 7 DOSE 6 40.0
Date: 20080612 Shift: Comments:

Part: GN77624AG Points: 1 Butt Len: 0 Part Name: GR 8 DOSE 1 0.2
Date: 20080612 Shift: Comments:

Part: GN77624AH Points: 1 Butt Len: 0 Part Name: GR 9 DOSE 2 2.0
Date: 20080612 Shift: Comments:

Part: GN77624AI Points: 1 Butt Len: 0 Part Name: GR 10 DOSE 3 4.0
Date: 20080612 Shift: Comments:

Part: GN77624AJ Points: 1 Butt Len: 0 Part Name: GR 11 DOSE 4 8.0
Date: 20080612 Shift: Comments:

Part: GN77624AK Points: 1 Butt Len: 0 Part Name: GR 12 DOSE 5 20.0
Date: 20080612 Shift: Comments:

Part: GN77624AL Points: 1 Butt Len: 0 Part Name: GR 13 DOSE 6 40.0
Date: 20080612 Shift: Comments:

Part: GN77624AM Points: 1 Butt Len: 0 Part Name: GR 14 DOSE 1 2.0
Date: 20080612 Shift: Comments:

Part: GN77624AN Points: 1 Butt Len: 0 Part Name: GR 15 DOSE 2 8.0
Date: 20080612 Shift: Comments:

Part: GN77624AO Points: 1 Butt Len: 0 Part Name: GR 16 DOSE 3 20.0
Date: 20080612 Shift: Comments:

Part: GN77624AP Points: 1 Butt Len: 0 Part Name: GR 17 DOSE 4 40.0
Date: 20080612 Shift: Comments:

Typo

Table 1: Nicotine Results GN77624, GN77620, and GN77622

	GN7762 4	mg/g	Dose (mg) Average		GN7762 0	mg/g	Dose Average		GN7766 2	mg/g	Dose Average	
~~Control?~~ TB	AA1	<0.001	0.2	Control	AA1	0.001	0	TB	AA1	0.002	0.2	
	AA2	<0.001	<0.001		AA2	<0.001	0.001		AA2	0.001	0.002	
TB	AB1	0.008	2.0	TB	AB1	0.009	0.2	TB	AB1	0.012	2.0	
	AB2	0.013	0.011		AB2	0.008	0.009		AB2	0.013	0.012	
TB	AC1	0.021	4.0	TB	AC1	0.015	2.0	TB	AC1	0.015	4.0	
	AC2	0.015	0.018		AC2	0.017	0.016		AC2	0.013	0.014	
TB	AD1	0.034	8.0	TB	AD1	0.077	8.0	TB	AD1	0.029	8.0	
	AD2	0.030	0.032		AD2	0.078	0.077		AD2	0.029	0.029	
TB	AE1	0.082	20.0	TB	AE1	0.153	20.0	TB	AE1	0.092	20.0	
	AE2	0.071	0.077		AE2	0.157	0.155		AE2	0.078	0.085	
TB	AF1	0.171	40.0	TB	AF1	0.337	40.0	TB	AF1	0.125	40.0	
	AF2	0.151	0.161		AF2	0.339	0.338	~~TB~~	AF2	0.144	0.135	
TE	AG1	0.001	0.2	TE	AG1	0.001	0.2	TE	AG1	0.001	0.2	
	AG2	0.000	0.001		AG2	0.001	0.001		AG2	0.001	0.001	
TE	AH1	0.003	2.0	TE	AH1	0.011	2.0	TE	AH1	0.006	2.0	
	AH2	0.004	0.003		AH2	0.016	0.014		AH2	0.005	0.006	
TE	AI1	0.064	4.0	TE	AI1	0.018	4.0	TE	AI1	0.011	4.0	
	AI2	0.072	0.068		AI2	0.024	0.021		AI2	0.017	0.014	
TE	AJ1	0.019	8.0	TE	AJ1	0.058	8.0	TE	AJ1	0.044	8.0	
	AJ2	0.017	0.018		AJ2	0.048	0.053		AJ2	0.047	0.046	
TE	AK1	0.048	20.0	TE	AK1	0.108	20.0	TE	AK1	0.011	20.0	
	AK2	0.034	0.041		AK2	0.176	0.142		AK2	0.096	0.054	
TE	AL1	0.118	40.0	TE	AL1	0.354	40.0	TE	AL1	0.288	40.0	
	AL2	0.062	0.090		AL2	0.393	0.374		AL2	0.280	0.284	
NT	AM1	0.007	2.0	NT	AM1	0.017	2.0	NT	AM1	0.011	2.0	
NT	AM2	0.007	0.007		AM2	0.017	0.017		AM2	0.010	0.011	
	AN1	0.027	8.0	NT	AN1	0.066	8.0	NT	AN1	0.030	8.0	
	AN2	0.027	0.027		AN2	0.065	0.065		AN2	0.030	0.030	
NT	AO1	0.068	20.0	NT	AO1	0.156	20.0	NT	AO1	0.001	20.0	*
	AO2	0.064	0.066		AO2	0.151	0.154		AO2	0.001	0.001	
NT	AP1	0.137	40.0	NT	AP1	0.303	40.0	NT	AP1	0.209	40.0	
	AP2	0.123	0.130		AP2	0.310	0.306		AP2	0.203	0.206	
	•TOX210 Series 2 Feed Formulation Data			TB	AQ1	0.045	4.0		•TOX 210 Series 1 Feed Formulation Data			
					AQ2	0.038	0.042					

- Submission date 06/12/08
- Decoded from sample cups sent to chemistry

AL

- Data from PAD report

- TOX209 Series 1
- Feed Formulation Dose Confirmation
- Decoded from sample cups
- Data from PAD report
- Reanalysis

- Submission Date 06/12/08
- Decoded from sample cups sent to Chemistry
- Reanalysis

* Sample problem - data not used

Analytical Chemistry Reports

PAD TEST MEMORANDUM

AUTHOR: Karen B. Kilby
Timothy A. Ellisor

DATE: August 5, 2008

DEPARTMENT: Product Quality

DIVISION: Product Assessment

CLIENTS: Jenny Smith
Suzanne Theophilus

PREVIOUS REPORTS: PAD-MKBK 2008, 217

PROJECT CHARTER: Smokeless Tobacco Stewardship Animal Feed Palatability Project

MANHOURS: 40

Determination of the amount of Nicotine Applied to Rat/Mouse Feed Samples

OBJECTIVE:
The purpose of this study was to determine the amount nicotine added to rat/mouse feed samples to support the Smokeless Tobacco Stewardship Feeding Studies Project. Eleven sets of samples (152 samples, 2 reps each) were submitted for analysis.

SUMMARY:
Rat and mouse feed samples with varying dosing levels of nicotine were submitted for analysis. The samples were logged into LIMS under the following identification numbers: GN76749 (AA-AF), GN76750 (AA-AF), GN76747 (AA-AP), GN77202 (AA-AR), GN77172 (AA-AI), GN77522 (AA-AO), GN77615 (AA-AQ), GN77622 (AA-AP), GN77620 (AA-AO), and GN77624 (AA-AP). The samples were analyzed, in duplicate, using the method outlined in PAD-MKBK 2008, 217.

STATUS:
The determination of the amount of nicotine applied to rat/mouse feed samples is complete.

KEYWORDS:
GC/MS, nicotine, smokeless tobacco, SNUS, Feed, Diet

Karen B. Kilby, Scientist III

Timothy A. Ellisor, Technician III

Reviewed by:

S. Mark DeBusk, Lead Manager Product Quality

INTRODUCTION:

Various levels of nicotine were applied to rat and mouse feed using various sources of nicotine (nicotine, nicotine extract, and nicotine tartrate). The samples were submitted for the determination of nicotine in feed to verify the level of nicotine applied to each sample. The samples were logged into LIMS under the following identification numbers: GN76749 (AA-AF), GN76750 (AA-AF), GN76747 (AA-AP), GN77202 (AA-AR), GN77172 (AA-AI), GN77522 (AA-AO), GN77615 (AA-AQ), GN77622 (AA-AP), GN77620 (AA-AO), and GN77624 (AA-AP).

EXPERIMENTAL:

Samples were prepared, in duplicate, according to the procedures outlined in PAD-MKBK 2008, 217, Appendix A. To summarize:

- Accurately weigh approximately 1 gram of each feed sample.
- Put sample in a tube containing 5 mL of NaOH solution.
- Mix to ensure complete saturation of sample.
- Wait 30 minutes.
- Add 5 mL of methyl tert butyl ether (MTBE) extraction solution to each sample.
- Shake on a wrist action shaker for 2 hours.
- Allow sample to separate into two layers.
- Transfer the top layer of sample to GC vial.
- Seal vial using crimp top cap.
- Analyze using GC/MS technology.

RESULTS AND DISCUSSION:

The results measured for each sample are shown in Tables 1-4. The results measured showed levels of nicotine in the expected range for this study.

Table 1: Nicotine Results GN77624, GN77620, and GN77622

	GN7762 4	mg/g	Dose (mg) Average	GN7762 0	mg/g	Average	GN7766 2	mg/g	Average
Control?	AA1	<0.001	0.2	AA1	0.001		AA1	0.002	
TB	AA2	<0.001	<0.001	AA2	<0.001	0.001	AA2	0.001	0.002
TB	AB1	0.008	2.0	AB1	0.009		AB1	0.012	
	AB2	0.013	0.011	AB2	0.008	0.009	AB2	0.013	0.012
TB	AC1	0.021	4.0	AC1	0.015		AC1	0.015	
	AC2	0.015	0.018	AC2	0.017	0.016	AC2	0.013	0.014
TB	AD1	0.034	8.0	AD1	0.077		AD1	0.029	
	AD2	0.030	0.032	AD2	0.078	0.077	AD2	0.029	0.029
TB	AE1	0.082	20.0	AE1	0.153		AE1	0.092	
	AE2	0.071	0.077	AE2	0.157	0.155	AE2	0.078	0.085
TB	AF1	0.171	40	AF1	0.337		AF1	0.125	
	AF2	0.151	0.161	AF2	0.339	0.338	AF2	0.144	0.135
TE	AG1	0.001	0.2	AG1	0.001		AG1	0.001	
	AG2	0.000	0.001	AG2	0.001	0.001	AG2	0.001	0.001
TE	AH1	0.003	2.0	AH1	0.011		AH1	0.006	
	AH2	0.004	0.003	AH2	0.016	0.014	AH2	0.005	0.006
TE	AI1	0.064	4.0	AI1	0.018		AI1	0.011	
	AI2	0.072	0.068	AI2	0.024	0.021	AI2	0.017	0.014
TE	AJ1	0.019	8.0	AJ1	0.058		AJ1	0.044	
	AJ2	0.017	0.018	AJ2	0.048	0.053	AJ2	0.047	0.046
TE	AK1	0.048	20.0	AK1	0.108		AK1	0.011	
	AK2	0.034	0.041	AK2	0.176	0.142	AK2	0.096	0.054
TE	AL1	0.118	40.0	AL1	0.354		AL1	0.288	
	AL2	0.062	0.090	AL2	0.393	0.374	AL2	0.280	0.284
NT	AM1	0.007	2.0	AM1	0.017		AM1	0.011	
	AM2	0.007	0.007	AM2	0.017	0.017	AM2	0.010	0.011
NT	AN1	0.027	8.0	AN1	0.066		AN1	0.030	
	AN2	0.027	0.027	AN2	0.065	0.065	AN2	0.030	0.030
NT	AO1	0.068	20.0	AO1	0.156		AO1	0.001	
	AO2	0.064	0.066	AO2	0.151	0.154	AO2	0.001	0.001
NT	AP1	0.137	40.0	AP1	0.303		AP1	0.209	
	AP2	0.123	0.130	AP2	0.310	0.306	AP2	0.203	0.206
				AQ1	0.045				
				AQ2	0.038	0.042			

- TOX210 Series 2
- Decoded from sample cup sent to chemistry
- Submission Date 06/12/08

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Table 2:Nicotine Results GN77615, GN77522, And GN77172

GN77615	mg/g	Average	GN77522	mg/g	Average	GN77172	mg/g	Average
AA1	<0.001		AA1	0.176		AA1	0.166	
AA2	<0.001	<0.001	AA2	0.134	0.155	AA2	0.173	0.170
AB1	0.002		AB1	1.415		AB1	0.140	
AB2	0.003	0.002	AB2	1.481	1.448	AB2	0.156	0.148
AC1	0.015		AC1	0.304		AC1	0.121	
AC2	0.012	0.014	AC2	0.311	0.307	AC2	0.145	0.133
AD1	0.028		AD1	0.648		AD1	0.271	
AD2	0.042	0.035	AD2	0.577	0.612	AD2	0.334	0.302
AE1	0.050		AE1	0.875		AE1	0.628	
AE2	0.075	0.062	AE2	0.969	0.922	AE2	0.597	0.613
AF1	0.122		AF1	0.159		AF1	0.974	
AF2	0.153	0.138	AF2	0.156	0.158	AF2	0.821	0.898
AG1	0.307		AG1	1.581		AG1	1.503	
AG2	0.298	0.302	AG2	1.546	1.563	AG2	1.695	1.599
AH1	0.002		AH1*	0.647		AH1	1.732	
AH2	0.003	0.002	AH2*	0.683	0.665	AH2	1.654	1.693
AI1	0.068		AI1*	0.380		AI1	1.679	
AI2	0.032	0.050	AI2*	0.322	0.351	AI2	1.610	1.644
AJ1	0.054		AJ1	0.853				
AJ2	0.052	0.053	AJ2	0.879	0.866			
AK1	0.052		AK1	0.018				
AK2	0.067	0.060	AK2	0.016	0.017			
AL1	0.191		AL1	1.379				
AL2	0.164	0.178	AL2	1.340	1.359			
AM1	0.291		AM1	0.261				
AM2	0.239	0.265	AM2	0.259	0.260			
AN1	0.017		AN1	0.542				
AN2	0.018	0.018	AN2	0.539	0.540			
AO1	0.061		AO1	0.779				
AO2	0.064	0.063	AO2	0.789	0.784			
AP1	0.152							
AP2	0.155	0.153						
AQ1	0.287							
AQ2	0.306	0.296						

*Sample parts GN77522AH and AI appear to be mixed up.

Table 3: Nicotine Results GN77202, GN76746, and GN76747

GN77202	mg/g	Average
AA1	0.243	
AA2	0.148	0.196
AB1	0.148	
AB2	0.171	0.159
AC1	0.170	
AC2	0.198	0.184
AD1	0.512	
AD2	0.571	0.541
AE1	0.308	
AE2	0.335	0.322
AF1	0.907	
AF2	0.972	0.940
AG1	1.593	
AG2	1.589	1.591
AH1	1.491	
AH2	1.596	1.544
AI1	1.740	
AI2	1.591	1.665
AJ1	0.017	
AJ2	0.016	0.016
AK1	0.017	
AK2	0.017	0.017
AL1	0.017	
AL2	0.016	0.016
AM1	0.278	
AM2	0.280	0.279
AN1	0.589	
AN2	0.599	0.594
AO1	0.851	
AO2	0.868	0.859
AP1	1.497	
AP2	1.536	1.517
AQ3	1.483	
AQ2	1.487	1.485
AR1	1.520	
AR2	1.490	1.505

GN76746	mg/g	Average
AA1	0.350	
AA2	0.363	0.357
AB1	0.176	
AB2	0.204	0.190
AC1	0.072	
AC2	0.070	0.071
AD1	0.048	
AD2	0.034	0.041
AE1	0.019	
AE2	0.010	0.014
AF1	0.002	
AF2	0.005	0.004
AG1	0.281	
AG2	0.337	0.309
AH1	0.154	
AH2	0.115	0.134
AI1	0.099	
AI2	0.144	0.122
AJ1**	0.068	
AJ2**	0.019	0.043
AK1**	0.011	
AK2**	0.069	0.040
AL1	0.001	
AL2	0.001	0.001
AM1	0.019	
AM2	0.019	0.019
AN1	0.074	
AN2	0.075	0.075
AO1	0.178	
AO2	0.183	0.181
AP1	0.355	
AP2	0.356	0.356

GN76747	mg/g	Average
AA1	0.167	
AA2	0.197	0.182
AB1	0.095	
AB2	0.082	0.089
AC1	0.027	
AC2	0.031	0.029
AD1	0.014	
AD2	0.018	0.016
AE1	0.008	
AE2	0.010	0.009
AF1	0.001	
AF2	0.001	0.001
AG1	0.183	
AG2	0.224	0.203
AH1	0.056	
AH2	0.066	0.061
AI1	0.022	
AI2	0.014	0.018
AJ1**	0.020	
AJ2**	0.007	0.013
AK1	0.004	
AK2	0.003	0.003
AL1	0.001	
AL2	0.001	0.001
AM1	0.234	
AM2	0.228	0.231
AN1	<0.001	
AN2	<0.001	<0.001
AO1	0.031	
AO2	0.031	0.031
AP1	0.007	
AP2	0.009	0.008

**Unexpected difference in replicate results. The chromatograms were checked and results confirmed. Additional sample needed for further verification.

Table 4: Nicotine Results GN76749 and GN76750

GN76749	mg/g	Average		GN76750	mg/g	Average
AA1	0.466			AA1	0.416	
AA2	0.411	0.438		AA2	0.402	0.409
AB1	0.002			AB1	0.002	
AB2	0.002	0.002		AB2	0.001	0.002
AC1	0.448			AC1	0.408	
AC2	0.424	0.436		AC2	0.325	0.367
AD1	0.002			AD1	0.001	
AD2	0.002	0.002		AD2	0.001	0.001
AE1	0.415			AE1	0.360	
AE2	0.407	0.411		AE2	0.348	0.354
AF1	0.019			AF1	0.019	
AF2	0.021	0.020		AF2	0.019	0.019

CONCLUSION:
The determination of nicotine applied to rat/mouse feed is complete. The results reported in this study showed levels of nicotine in the expected range for this study.

Decode 1

PAD TEST MEMORANDUM

AUTHOR: Karen B. Kilby
Timothy A. Ellisor

DEPARTMENT: Product Quality

DATE: August 5, 2008

DIVISION: Product Assessment

CLIENTS: Jenny Smith
Suzanne Theophilus

PREVIOUS REPORTS: PAD-MKBK 2008, 217

PROJECT CHARTER: Smokeless Tobacco Stewardship Animal Feed Palatability Project

MANHOURS: 40

Determination of the amount of Nicotine Applied to Rat/Mouse Feed Samples

OBJECTIVE:

The purpose of this study was to determine the amount nicotine added to rat/mouse feed samples to support the Smokeless Tobacco Stewardship Feeding Studies Project. Eleven sets of samples (152 samples, 2 reps each) were submitted for analysis.

GN76746

~~listed on report as GN76746, which correlates with submission~~ sheet.

SUMMARY:

Rat and mouse feed samples with varying dosing levels of nicotine were submitted for analysis. The samples were logged into LIMS under the following identification numbers: GN76749 (AA-AF), GN76750 (AA-AF), GN76747 (AA-AP), GN77202 (AA-AR), GN77172 (AA-AI), GN77522 (AA-AO), GN77615 (AA-AQ), GN77622 (AA-AP), GN77620 (AA-AO), and GN77624 (AA-AP). The samples were analyzed, in duplicate, using the method outlined in PAD-MKBK 2008, 217.

STATUS:

The determination of the amount of nicotine applied to rat/mouse feed samples is complete.

KEYWORDS:

GC/MS, nicotine, smokeless tobacco, SNUS, Feed, Diet

- TB = Tobacco Blend
- TE = Tobacco Extract
- NT = Nicotine Hydrogen Tartrate

Karen B. Kilby

Karen B. Kilby, Scientist III

Timothy A. Ellisor

Timothy A. Ellisor, Technician III

Reviewed by:

S. Mark D. Busk

S. Mark DeBusk, Lead Manager Product Quality

INTRODUCTION:

Various levels of nicotine were applied to rat and mouse feed using various sources of nicotine (nicotine, nicotine extract, and nicotine tartrate). The samples were submitted for the determination of nicotine in feed to verify the level of nicotine applied to each sample. The samples were logged into LIMS under the following identification numbers: GN76749 (AA-AF), GN76750 (AA-AF), GN76747 (AA-AP), GN77202 (AA-AR), GN77172 (AA-AI), GN77522 (AA-AO), GN77615 (AA-AQ), GN77622 (AA-AP), GN77620 (AA-AO), and GN77624 (AA-AP).

EXPERIMENTAL:

Samples were prepared, in duplicate, according to the procedures outlined in PAD-MKBK 2008, 217, Appendix A. To summarize:

- Accurately weigh approximately 1 gram of each feed sample.
- Put sample in a tube containing 5 mL of NaOH solution.
- Mix to ensure complete saturation of sample.
- Wait 30 minutes.
- Add 5 mL of methyl tert butyl ether (MTBE) extraction solution to each sample.
- Shake on a wrist action shaker for 2 hours.
- Allow sample to separate into two layers.
- Transfer the top layer of sample to GC vial.
- Seal vial using crimp top cap.
- Analyze using GC/MS technology.

RESULTS AND DISCUSSION:

The results measured for each sample are shown in Tables 1-4. The results measured showed levels of nicotine in the expected range for this study.

Typo

Table 1: Nicotine Results GN77624, GN77620, and GN77622

	GN7762 4	mg/g	Dose (mg) Average		GN7762 0	mg/g	Dose Average		GN7766 2	mg/g	Dose Average	
~~Control?~~ TB	AA1	<0.001	0.2	control	AA1	0.001	0	TB	AA1	0.002	0.2	
	AA2	<0.001	<0.001		AA2	<0.001	0.001		AA2	0.001	0.002	
TB	AB1	0.008	2.0	TB	AB1	0.009	0.2	TB	AB1	0.012	2.0	
	AB2	0.013	0.011		AB2	0.008	0.009		AB2	0.013	0.012	
TB	AC1	0.021	4.0	TB	AC1	0.015	2.0	TB	AC1	0.015	4.0	
	AC2	0.015	0.018		AC2	0.017	0.016		AC2	0.013	0.014	
TB	AD1	0.034	8.0	TB	AD1	0.077	8.0	TB	AD1	0.029	8.0	
	AD2	0.030	0.032		AD2	0.078	0.077		AD2	0.029	0.029	
TB	AE1	0.082	20.0	TB	AE1	0.153	20.0	TB	AE1	0.092	20.0	
	AE2	0.071	0.077		AE2	0.157	0.155		AE2	0.078	0.085	
TB	AF1	0.171	40.0	TB	AF1	0.337	40.0	TB	AF1	0.125	40.0	
	AF2	0.151	0.161		AF2	0.339	0.338		AF2	0.144	0.135	
TE	AG1	0.001	0.2	TE	AG1	0.001	0.2	TE	AG1	0.001	0.2	
	AG2	0.000	0.001		AG2	0.001	0.001		AG2	0.001	0.001	
TE	AH1	0.003	2.0	TE	AH1	0.011	2.0	TE	AH1	0.006	2.0	
	AH2	0.004	0.003		AH2	0.016	0.014		AH2	0.005	0.006	
TE	AI1	0.064	4.0	TE	AI1	0.018	4.0	TE	AI1	0.011	4.0	
	AI2	0.072	0.068		AI2	0.024	0.021		AI2	0.017	0.014	
TE	AJ1	0.019	8.0	TE	AJ1	0.058	8.0	TE	AJ1	0.044	8.0	
	AJ2	0.017	0.018		AJ2	0.048	0.053		AJ2	0.047	0.046	
TE	AK1	0.048	20.0	TE	AK1	0.108	20.0	TE	AK1	0.011	20.0	
	AK2	0.034	0.041		AK2	0.176	0.142		AK2	0.096	0.054	
TE	AL1	0.118	40.0	TE	AL1	0.354	40.0	TE	AL1	0.288	40.0	
	AL2	0.062	0.090		AL2	0.393	0.374		AL2	0.280	0.284	
NT	AM1	0.007	2.0	NT	AM1	0.017	2.0	NT	AM1	0.011	2.0	
NT	AM2	0.007	0.007		AM2	0.017	0.017		AM2	0.010	0.011	
L	AN1	0.027	8.0	NT	AN1	0.066	8.0	NT	AN1	0.030	8.0	
	AN2	0.027	0.027		AN2	0.065	0.065		AN2	0.030	0.030	
NT	AO1	0.068	20.0	NT	AO1	0.156	20.0	NT	AO1	0.001	20.0	*
	AO2	0.064	0.066		AO2	0.151	0.154		AO2	0.001	0.001	
NT	AP1	0.137	40.0	NT	AP1	0.303	40.0	NT	AP1	0.209	40.0	
	AP2	0.123	0.130		AP2	0.310	0.306		AP2	0.203	0.206	
	•TOX210 Series 2 Feed Formulation Data			TB	AQ1	0.045	4.0		•TOX 210 Series 1 Feed Formulation Data			
					AQ2	0.038	0.042					

- Submission date 06/12/08
- Decoded from sample cups sent to chemistry

AL

- Data from PAD report

- TOX209 Series 1
- Feed Formulation Dose Confirmation
- Decoded from sample cups
- Data from PAD report
- Reanalysis

- Submission Date 06/12/08
- Decoded from sample cups sent to Chemistry
- Reanalysis

* Sample problem - data not used

Table 2:Nicotine Results GN77615, GN77522, And GN77172

	GN77615	mg/g	Dose Average		GN77522	mg/g	Dose Average	GN77172	mg/g	Average
Control TB	AA1	<0.001	0.2	TB	AA1	0.176	40	AA1	0.166	
	AA2	<0.001	<0.001		AA2	0.134	0.155	AA2	0.173	0.170
TB	AB1	0.002	0.2	TB	AB1	1.415	400	AB1	0.140	
	AB2	0.003	0.002		AB2	1.481	1.448	AB2	0.156	0.148
TB	AC1	0.015	2.0	TB	AC1	0.304	80	AC1	0.121	
	AC2	0.012	0.014		AC2	0.311	0.307	AC2	0.145	0.133
TB	AD1	0.028	4.0	TB	AD1	0.648	160	AD1	0.271	
	AD2	0.042	0.035		AD2	0.577	0.612	AD2	0.334	0.302
TB	AE1	0.050	8.0	TB	AE1	0.875	240	AE1	0.628	
	AE2	0.075	0.062		AE2	0.969	0.922	AE2	0.597	0.613
TB	AF1	0.122	20.0	TB	AF1	0.159	40	AF1	0.974	
	AF2	0.153	0.138		AF2	0.156	0.158	AF2	0.821	0.898
TB	AG1	0.307	40.0	TB	AG1	1.581	400	AG1	1.503	
	AG2	0.298	0.302		AG2	1.546	1.563	AG2	1.695	1.599
TE	AH1	0.002	0.2	TE	AH1*	0.647	160	AH1	1.732	
	AH2	0.003	0.002		AH2*	0.683	0.665	AH2	1.654	1.693
TE	AI1	0.068	2.0	TE	AI1*	0.380	80	AI1	1.679	
	AI2	0.032	0.050		AI2*	0.322	0.351	AI2	1.610	1.644
TE	AJ1	0.054	4.0	TE	AJ1	0.853	240			
	AJ2	0.052	0.053		AJ2	0.879	0.866			
TE	AK1	0.052	8.0	NT	AK1	0.018	40			
	AK2	0.067	0.060		AK2	0.016	0.017			
TE	AL1	0.191	20.0		AL1	1.379	400			
	AL2	0.164	0.178		AL2	1.340	1.359			
TE	AM1	0.291	40.0		AM1	0.261	80			
	AM2	0.239	0.265		AM2	0.259	0.260			
NT	AN1	0.017	2.0		AN1	0.542	160			
	AN2	0.018	0.018		AN2	0.539	0.540			
NT	AO1	0.061	8.0		AO1	0.779	240			
	AO2	0.064	0.063		AO2	0.789	0.784			
NT	AP1	0.152	20.0							
	AP2	0.155	0.153							
NT	AQ1	0.287	40.0							
	AQ2	0.306	0.296							

*Sample parts GN77522AH and AI appear to be mixed up.

TOX209 series 1
- Feed Formulation Data
- Dose Confirmation
- Decoded from sample cups by Duane

IR

- Data from TOX 213
- Feed Formulation
- Dose Confirmation
- Decoded from KK email
- Only one diet formulated

~~listed as GN7674~~2 or not listed

Table 3: Nicotine Results GN77202, GN76746, and GN76747

GN77202	mg/g	Average		GN76746	mg/g	Dose Average		GN76747	mg/g	Dose Average
AA1	0.243		TB	AA1	0.350	40	TB	AA1	0.167	40 mg
AA2	0.148	0.196		AA2	0.363	0.357		AA2	0.197	0.182
AB1	0.148		TB	AB1	0.176	20	TB	AB1	0.095	20 mg
AB2	0.171	0.159		AB2	0.204	0.190		AB2	0.082	0.089
AC1	0.170		TB	AC1	0.072	8.0	TB	AC1	0.027	8 mg
AC2	0.198	0.184		AC2	0.070	0.071		AC2	0.031	0.029
AD1	0.512		TB	AD1	0.048	4.0	TB	AD1	0.014	4.0 mg
AD2	0.571	0.541		AD2	0.034	0.041		AD2	0.018	0.016
AE1	0.308		TB	AE1	0.019	2.0	TB	AE1	0.008	2.0 mg
AE2	0.335	0.322		AE2	0.010	0.014		AE2	0.010	0.009
AF1	0.907		TB	AF1	0.002	0.2	TB	AF1	0.001	0.2 mg
AF2	0.972	0.940		AF2	0.005	0.004		AF2	0.001	0.001
AG1	1.593		TE	AG1	0.281	40	TE	AG1	0.183	40 mg
AG2	1.589	1.591	~~TE~~	AG2	0.337	0.309		AG2	0.224	0.203
AH1	1.491		TE	AH1	0.154	20	TE	AH1	0.056	20 mg
AH2	1.596	1.544		AH2	0.115	0.134		AH2	0.066	0.061
AI1	1.740		TE	AI1	0.099	8.0	TE	AI1	0.022	8 mg
AI2	1.591	1.665		AI2	0.144	0.122		AI2	0.014	0.018
AJ1	0.017		TE	AJ1**	0.068	4.0	TE	AJ1**	0.020	4 mg
AJ2	0.016	0.016		AJ2**	0.019	0.043		AJ2**	0.007	0.013
AK1	0.017		TE	AK1**	0.011	2.0	TE	AK1	0.004	2.0 mg
AK2	0.017	0.017		AK2**	0.069	0.040		AK2	0.003	0.003
AL1	0.017		TE	AL1	0.001	0.2	NT TB	AL1	0.001	0.2 mg
AL2	0.016	0.016		AL2	0.001	0.001		AL2	0.001	0.001
AM1	0.278		NT	AM1	0.019	2.0	NT	AM1	0.234	40 mg
AM2	0.280	0.279		AM2	0.019	0.019		AM2	0.228	0.231
AN1	0.589		NT	AN1	0.074	8.0	NT	AN1	<0.001	20 mg
AN2	0.599	0.594		AN2	0.075	0.075		AN2	<0.001	<0.001
AO1	0.851		NT	AO1	0.178	20	NT	AO1	0.031	8 mg
AO2	0.868	0.859		AO2	0.183	0.181		AO2	0.031	0.031
AP1	1.497		NT	AP1	0.355	40	NT	AP1	0.007	2.0 mg
AP2	1.536	1.517		AP2	0.356	0.356		AP2	0.009	0.008
AQ3	1.483									
AQ2	1.487	1.485								
AR1	1.520									
AR2	1.490	1.505								

TOX209 Series 2 Feed Formulation Data
- Submission 5/02/08
- Feed formulated 4/24-25

- TOX210 Series 2 Feed Formulation Data
- Submission May 2
- Confirmed by sample cups

**Unexpected difference in replicate results. The chromatograms were checked and results confirmed. Additional sample needed for further verification.

JR

Table 4: Nicotine Results GN76749 and GN76750

	GN76749	mg/g	Dose (mg) Average		GN76750	mg/g	Dose (mg) Average
TB	AA1	0.466	40	TB	AA1	0.416	40
	AA2	0.411	0.438		AA2	0.402	0.409
TB	AB1	0.002	0.2	TB	AB1	0.002	0.2
	AB2	0.002	0.002		AB2	0.001	0.002
TE	AC1	0.448	40	TE	AC1	0.408	40
	AC2	0.424	0.436		AC2	0.325	0.367
TE	AD1	0.002	0.2	TE	AD1	0.001	0.2
	AD2	0.002	0.002		AD2	0.001	0.001
NT	AE1	0.415	40	NT	AE1	0.360	40
	AE2	0.407	0.411		AE2	0.348	0.354
NT	AF1	0.019	2.0	NT	AF1	0.019	2.0
	AF2	0.021	0.020		AF2	0.019	0.019

• TOX209 Stability Data (1 month)

• TOX209 ~~One-week~~ Stability Data (10-day stability)
• LIMS submission sheet dated 05/02/08

CONCLUSION:
The determination of nicotine applied to rat/mouse feed is complete. The results reported in this study showed levels of nicotine in the expected range for this study.

• According to dates on LIMS submission sheet, this would be a 10 day stability.

• LIMS Coversheet Confirmation

• According to dates on LIMS coversheet this would be a 1 month study

• LIMS Coversheet Dated 05/02/08

• Data from Trial Run Feed Formulations

IR

IR

PAD TEST MEMORANDUM

AUTHOR: Karen B. Kilby
Timothy A. Ellisor

DATE: August 4, 2008

DEPARTMENT: Product Quality

DIVISION: Product Assessment

CLIENTS: Jenny Smith
Suzanne Theophilus

PREVIOUS REPORTS: none

PROJECT CHARTER: Smokeless Tobacco Stewardship Animal Feed Palatability Project

MANHOURS: 40

Validation of a New Method for the Determination of the amount of Nicotine Applied to Rat/Mouse Feed

OBJECTIVE:

The purpose of this study was to develop a method to determine the amount of nicotine applied to rat/mouse feed samples to support the Smokeless Tobacco Stewardship Animal Feed Palatability Project.

SUMMARY:

A method was developed to determine the amount of nicotine applied to rat/mouse feed to support the Smokeless Tobacco Stewardship Animal Feed Palatability Project. The proposed method is detailed in Appendix A. The method was validated based on several factors including: linearity, accuracy, instrument precision, and method reproducibility.

The linearity of the end determination was determined by analyzing standard solutions with various concentrations of nicotine. Statistical analysis showed excellent linearity with an r^2 greater than 0.999. The accuracy of the method was determined by two standard addition experiments. These experiments showed percent recoveries of 92 to 106%. Statistical analysis of the results measured in the standard addition experiment showed excellent linearity of the method (including sample preparation, extraction, and end determination), with an r^2 greater than 0.995 for both experiments. The instrument precision was determined by calculating the variation from 6 replicate injections of the same sample vial. The result showed the instrument precision to be 1.1%RSD. The method reproducibility was determined by calculating the variation of 6 replicate preparations of the same sample. The method reproducibility was calculated to be 1.6%RSD.

Acceptable results were measured for all aspects of the validation study; therefore, the proposed method shall be used for the determination of nicotine in rat/mouse feed to support the Smokeless Tobacco Stewardship Animal Feed Palatability Project.

STATUS:
The validation of a method for the determination of the amount of nicotine applied to rat/mouse feed is complete.

KEYWORDS:
GC/MS, nicotine, smokeless tobacco, SNUS, tobacco extract, tobacco blend, nicotine tartrate

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Reviewed by:

S. Mark DeBusk
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Cc:
Bert Gordon
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INTRODUCTION:
The Product Integrity group requested the analysis of nicotine applied to rat/mouse feed samples to support the Smokeless Tobacco Stewardship Animal Feed Palatability Project. Nicotine was applied to rat/mouse feed samples using various forms of nicotine (nicotine tartrate, tobacco extract, or tobacco blend). The study described herein was designed to validate a new method for the determination of nicotine applied to rat/mouse feed samples.

EXPERIMENTAL:
The linearity of the end determination was evaluated by analyzing standard solutions with various concentrations (0.000099 mg/mL-0.198000 mg/mL) of nicotine. The instrument precision was determined by calculating the variation from 6 replicate injections of

t Launch Internet Explorer Browser.lnk he same sample vial. The method reproducibility was determined by calculating the variation of 6 replicates of the same sample. Samples for these experiments were prepared according to the procedures described in the proposed method (Appendix A).

The accuracy of the method and method linearity were determined by standard addition experiments (1). For this experiment, a standard solution was prepared by adding a known amount of nicotine to tert-butyl methyl ether (MTBE, concentration 0.289 mg/mL). Two sets of samples were prepared using the blank rat/mouse feed samples. One set of standard addition samples were prepared by adding known amounts of the nicotine solution to the samples at the beginning of the sample extraction procedure; while a second set of samples were prepared by adding known amounts of the nicotine solution to the samples at the end of the extraction procedure. Three levels (six reps each) were prepared for each set of standard addition samples. The first level contained 50 µL of the standard solution (0.0145 mg nicotine), the second level contained 500 µL of the standard solution (0.1445 mg nicotine), and the third level contained 1000 µL of the standard solution (0.2890 mg nicotine). The samples were extracted and analyzed according to the procedures described in the proposed method (Appendix A).

RESULTS AND DISCUSSION:
This method was validated based on several factors including: linearity, accuracy, instrument precision, and method reproducibility,. These factors are discussed individually below.

Linearity
The linearity of the end determination was evaluated by analyzing standard solutions with various concentrations (0.000099 mg/mL-0.198000 mg/mL) of nicotine. The quantitation limit of the method was determined to be equal to the lowest standard. Statistical analysis of the results from the standard solutions was performed using least squares regression of the concentration versus the peak area ratio of nicotine to quinoline-d_7. The regression coefficient, r^2 is a measure of random error associated with the calibration and a measure of the linearity of the responses. The results measured were directly proportional to the analyte concentration. Excellent linearity was observed for nicotine, with an r^2 greater than 0.999, which is within the typical range observed for other methods.

Accuracy
The accuracy of the method was determined by two standard addition experiments (1). For this experiment, a standard solution was prepared by adding a known amount of nicotine to MTBE

(concentration 0.289 mg/mL). Two sets of samples were prepared using the blank rat/mouse feed samples. Six replicates were prepared for each level. The amount of nicotine measured in the blank feed sample was below the quantitation limit for this method (0.000099 mg/mL or 0.00099 mg). One set of standard addition samples were prepared by adding known amounts of the nicotine solution to the samples at the beginning of the sample extraction procedure; while a second set of samples were prepared by adding known amounts of the nicotine solution to the samples at the end of the extraction procedure. Recovery was calculated as follows:

$$\%\text{Recovery} = \frac{\text{Feed Plus Standard (mg)} - \text{Blank Feed (mg)}}{\text{Amount Standard Added (mg)}} \times 100\%$$

Table 1 shows the calculated percent Recovery for each level to be 92 to 106% for both sets of samples.

Table 1: Accuracy- Standard Addition Experiment (n=6)

Level	Amount Added (mg)	Average Amount Standard Measured (mg)	Average %Recovery
Standard Added Before Extraction			
Level 0	0	<0.00099	
Level 1	0.0145	0.01532	106.0
Level 2	0.1445	0.14561	100.8
Level 3	0.2890	0.26675	92.3
		% Recovery	**99.7**
Standard Added After Extraction			
Level 0	0	<0.00099	
Level 1	0.0145	0.01501	103.9
Level 2	0.1445	0.13927	96.4
Level 3	0.2890	0.27408	94.8
		% Recovery	**98.4**

Further evaluation of the data collected during the standard addition experiment shows the linearity of the method (including sample preparation, extraction and the end determination). The results measured are directly proportional to the concentration of nicotine added to each sample. Statistical analysis of the results from the standard addition experiments were performed using least squares regression of concentration versus the peak area ratio of nicotine to quinoline-d_7 (Figures 1 and 2). A regression coefficient of 0.9974 and 1.0000, (r^2) indicates excellent linearity of the method. A near zero intercept (0.0060 and 0.0019) is an indication that a constant systematic error between the amount of standard added and the amount of standard measured is not present and that a co-elution is highly unlikely.

Figure 1: Method Linearity Standard Addition Test 1

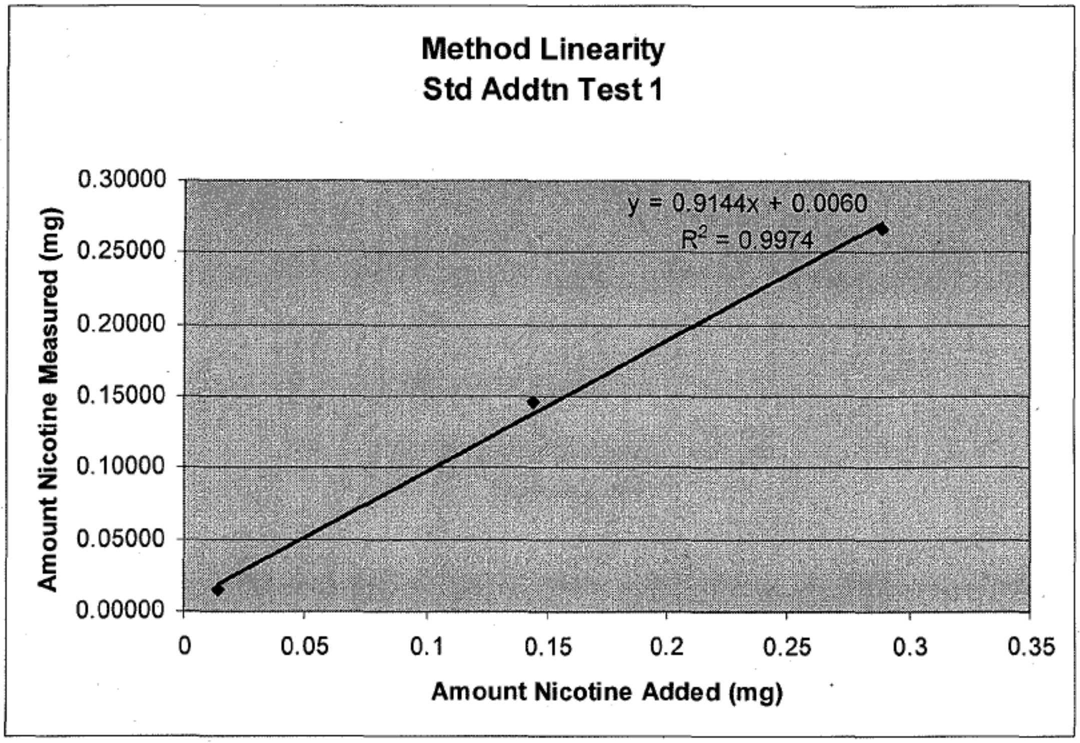
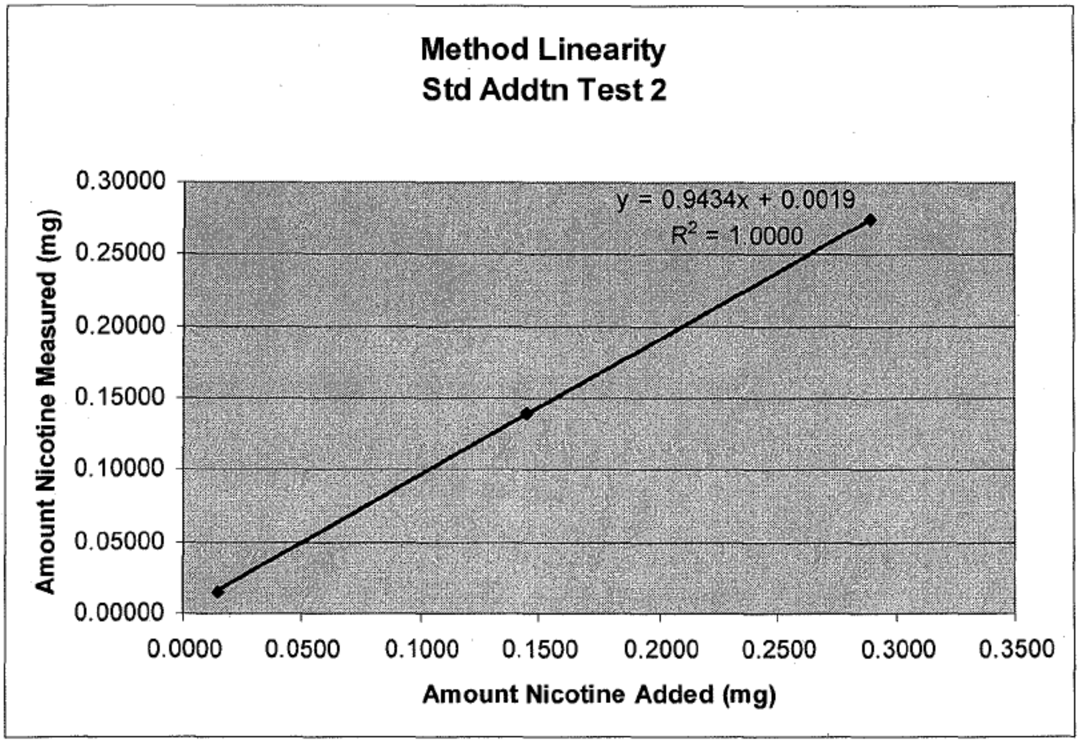


Figure 2: Method Linearity -Standard Addition Test 2



Instrument Precision

The instrument precision was determined by calculating the variation from 6 replicate injections of the same sample vial. The results showed the instrument precision to be 1.1%RSD.

Table 2: Instrument Precision

Sample	Nicotine (mg/g)
1	0.043
2	0.044
3	0.043
4	0.043
5	0.043
6	0.043
Average	0.043
Std Dev	0.0005
%RSD	1.1

Method Reproducibility
The method reproducibility was determined by calculating the variation of 6 replicate preparations of the same sample. The method reproducibility was calculated to be 1.6%RSD.

Table 3: Method Reproducibility

Sample	
	Nicotine (mg/g)
1	0.059
2	0.058
3	0.059
4	0.057
5	0.058
6	0.057
Avg.	0.058
Std dev.	0.0009
% RSD	**1.6**

CONCLUSION:
The validation of a new method for the determination of nicotine applied to rat/mouse feed samples is complete. The proposed method showed good linearity (r^2>0.999), accuracy (92-106%), instrument precision (1.1%RSD), and method reproducibility (1.6%RSD). The proposed method shall be used for the determination of nicotine in rat/mouse feed to support the Smokeless Tobacco Stewardship Animal Feed Palatability Project.

REFERENCES:

1. Meier, P. C. and Zünd, R. E., "Statistical Methods in Analytical Chemistry", John Wiley and Sons, New York, 1993, pp. 109-110.

Appendix A
Proposed Method

1. **Scope**
This method specifies procedures for the determination of the amount of nicotine in rat/mouse feed samples by gas chromatography/mass spectrometry (GC/MS)

2. **Principle**
Rat/mouse feed samples with known amounts of applied nicotine are treated with aqueous sodium hydroxide and the nicotine is extracted into tert-butyl methyl ether (MTBE). The amount of nicotine is then quantitated by gas chromatography/mass spectrometry. Results are reported in mg nicotine/g feed units.

3. **Equipment/Apparatus**

3.1 Equipment

3.1.1 Agilent Technologies 6890/5973 gas chromatograph/mass spectrometer with an Agilent Technologies 7683 automatic sampler, or equivalent.

3.1.2 Mettler AE 163 analytical balance, or equivalent.

3.1.3 Burrell wrist action shaker, model 75, or equivalent.

3.1.4 Thermolyne Maxi-Mix II, or equivalent.

3.1.5 Rainin Micropipettes- various dispense capabilities, or equivalent

3.2 Apparatus

3.2.1 Class A volumetric pipets –1 mL, 5 mL, 10 mL, 25 mL.

3.2.2 Class A volumetric flasks – 50 mL, 100 mL, 1 L.

3.2.3 Bottletop Dispensors- 5.0 mL capability, or equivalent.

3.2.4 Glass tubes 25 X 200 with screw caps, Kimax catalog # 45066-25200, or equivalent.

3.2.5 GC vials with crimp-top caps.

3.2.6 Fisherbrand 9" Pasteur Pipets flint glass (catalog no. 13-678-6B), or equivalent.

3.2.7 Liner, straight with glass wool in the middle (Agilent Technologies part no.19251-60540), or equivalent.

3.2.8 Column – J & W Scientific Co., DB-WAX, 30 m x 0.25 mm id, 0.5 micron film thickness (catalog no. 122-7033), or equivalent.

4. **Reagents/Safety**

4.1 Reagents

4.1.1 L-nicotine, minimum 99% purity, Acros, catalog # AC 18142-0250.

4.1.5 Quinoline-d_7 (Internal Standard) CDN Isotopes, catalog no. D-1450.

4.1.6 Tert-butyl methyl ether, MTBE-Aldrich catalog no. 29-321-0.

4.1.7 NaOH – Pellets, Fisher # S318-500.

4.2 Safety

The chemicals used in this method are possible carcinogens, mutagens, toxins, etc. The analysts shall refer to section 6.1 of this document and the Material Safety Data Sheets for each chemical for appropriate handling instructions.

5. Set Up GC:

5.1 Suitable chromatographic conditions for an Agilent Technologies 6890/5973 gas chromatograph/mass spectrometer with an Agilent Technologies 7683 automatic sampler and a J & W Scientific Co., DB-WAX, 30 m x 0.25 mm id, 0.5 micron film thickness, include:

Table 1: Oven Program

	°C/min	°C	Hold time (min)	Run Time (min)
Initial		60	1.00	
Ramp 1	15	230	0	12.33

Table 2: GC/MS Parameters

Gas Chromatograph	Agilent Technologies 6890
Mass Spectrometer	Agilent Technologies 5973
Data System	Agilent Technologies ChemStation
MS Source temperature	230 °C
Ionization Mode	EI
Injector	Agilent Technologies 7683 split/splitless
Injection Volume	1 µL
Syringe Size	10 µL
Washes	
Sample	1 pre-injection
Solvent A	1 pre-injection, 2 post-injection
Solvent B	1 pre-injection, 2 post-injection
Pumps	4 pre-injection

Inlet	
Injection Mode	Split
Gas	Helium
Heater	220 °C
Split Ratio	25:1
Split Flow	50 mL/min
Column	
Constant flow	2.0 mL/min
Detector	MSD
MSD Transfer line	150 °C
SIM Parameters	**m/z ***
Quinoline-d_7 (IS)	136
Nicotine	84

*The components and their selected ions listed above were identified as the optimal ions of interest for the quantification of nicotine in rat/mouse feed. See Figures 1-3 for example chromatograms of the calibration standards and product extracts. All ions are monitored concurrently for the entire run.

6. Preparation of Extraction Solution, Standards, and Checks:

6.1 Preparation of Solutions:

Safety Alert!

Nicotine is extremely toxic and readily absorbed through the skin, as well as a possible teratogen. Always wear nitrile gloves when handling and use appropriate glassware for pipetting.

6.1.1 Extraction Solution: Add approximately 0.0500 g of Quinoline-d_7 (Internal standard) in 4 liters of MTBE and mix well.

6.1.2 2N NaOH solution: Weigh 80 g of NaOH pellets into a 1 L volumetric flask. Dilute to volume with distilled water. Add a stir bar and stir to dissolve pellets. Mix well and transfer a portion of the solution to a container equipped with a bottle top dispenser to dispense 5 mL.

6.2 Prepare Standard Solutions:

6.2.1 Prepare Primary Standard Stock Solution
Weigh 0.4000 g (to the nearest 0.1 mg) nicotine into a 100 mL volumetric flask and dilute to volume with extraction solution.

Example calculation:

$$[0.4000 \text{ g nicotine} \times 1000 \frac{\text{mg}}{\text{g}} \times 0.99(\text{purity})] / 100 \text{ mL} = 3.96 \frac{\text{mg}}{\text{mL}} \text{ nicotine}$$

6.2.2 Prepare Diluted Standard Stock Solution
Pipette 5 mL of the Primary Standard Stock Solution into a 100 mL volumetric flask and dilute to volume with extraction solution. This solution is also used as the highest standard.

Example calculation:

$$(3.96 \frac{\text{mg}}{\text{mL}} \text{ nicotine} \times 5 \text{ mL}) / 100 \text{ mL} = 0.198 \frac{\text{mg}}{\text{mL}} \text{ nicotine}$$

6.2.3 Prepare Standard Solutions
Pipette (using a micro-pipette or Class A volumetric pipette) the following amounts of Standard Stock Solution to the appropriate 50 mL volumetric flask. Dilute to volume with extraction solution and mix well. Determine the concentration of nicotine for each standard as shown in the example below.

Table 3: Standard Preparation

Level	Amount of Standard Stock Solution	Nicotine Concentration mg/mL
L1 (Std 1)	25 µL	0.000099
L2 (Std 2)	50 µL	0.000198
L3 (Std 3)	100 µL	0.000396
L4 (Std 4)	500 µL	0.001980
L5 (Std 5)	1 mL	0.003960
L6 (Std 6)	5 mL	0.019800
L7 (Std 7)	10 mL	0.039600
L8 (Std 8)	25 mL	0.099000
L9 (Diluted stock)	Diluted Stock Solution	0.198000

Notes: All solutions shall be stored in a freezer, when not in use. New standards shall be prepared when extraction solution is made.

The calibration range concentrations may be expanded or changed to encompass varying levels, if necessary.

7. Process Standards:

7.1 Calibration is normally performed at the beginning of each week prior to sample analysis or when new extraction solution is prepared.

7.2 Using a Pasteur pipette, transfer an aliquot of each standard solution to GC vials and cap.

7.3 Prime the GC System.

7.4 Inject the standards in duplicate.

7.5 ChemStation performs a "quadratic regression" fit. Obtain a printout of the calibration report.

7.6 If the calibration curve is acceptable ($r^2 \geq 0.999$), continue with sample analysis. If it is not acceptable, take the necessary corrective action before continuing.

8. Prepare Test Portion(s):

8.1 Label glass tubes (25 X 200mm) to correspond to the samples to be analyzed.

8.2 Add 5 mL of 2N NaOH to each glass tube.

8.3 Accurately weigh approximately 1.0000 g (to the nearest 0.1 mg) of sample into the corresponding glass tube.

8.4 Shake each tube on the Maxi-Mix II (mini vortexer) to ensure saturation of the sample with the NaOH solution.

8.5 Allow the sample solutions to sit for 30 minutes.

8.6 Add 5.0 mL of extraction solution to each tube and cap tightly.

8.7 Shake each tube on the Maxi-Mix II shaker to ensure complete mixing.

8.8 Shake tubes for 2 hours on a wrist action shaker at full speed. (Make sure the extraction solution is completely mixing with the sample.)

8.9 After shaking, allow layers to separate (approximately 15 minutes).

8.10 Transfer a portion of the top layer into corresponding GC vials using a new disposable Pasteur pipette for each sample.

8.11 Use a crimper to cap the GC vials to ensure a good seal is formed.

9. Analyze Extracts:

9.1 Transfer the GC vials to the appropriate GC/MS system.

9.3 Results are expressed in mg nicotine per gram feed units and may be calculated manually according to the following equations:

9.3.1 $C\ (\text{mg/mL}) = ax^2 + bx + c$

where: C= Concentration of nicotine
a = quadratic term

b = linear term

c = constant term

x = component peak area/internal standard peak area

9.3.2 $$\frac{\text{mg nicotine}}{\text{g feed}} = \frac{C\ (\text{mg/mL}) \times 5\ \text{mL}}{\text{Feed Sample wt. (g)}}$$

10. Sample Disposal

Extracted samples are disposed of in accordance with the CHP. Sample Disposal shall be performed as follows:

10.1 Test tube caps are removed and placed in a container to be washed and re-used. The MTBE waste is poured into an appropriate chemical waste container labeled MTBE waste. When the container is full, it is transferred to the proper location. Test tubes are rinsed and transferred to the washroom to be washed and re-used.

10.2 Used GC vials are placed into plastic buckets obtained from the stockroom. When the containers are full, the buckets are transferred to the appropriate disposal location.

Figure 1: Selected Ion 136 Internal Standard Peak (Quinolined$_7$):

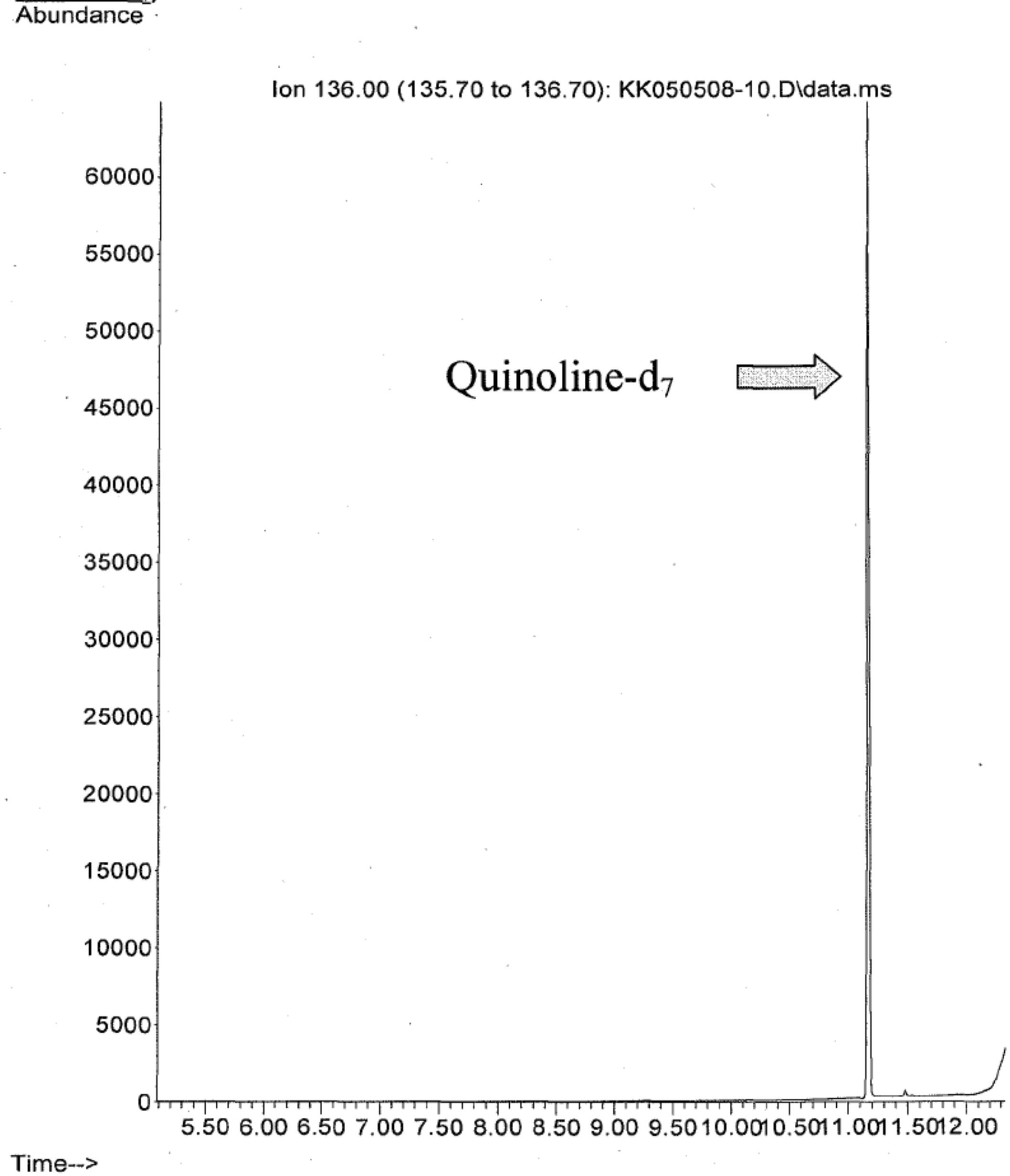


Figure 2: TIC Chromatogram of Calibration Standard (Std 5):

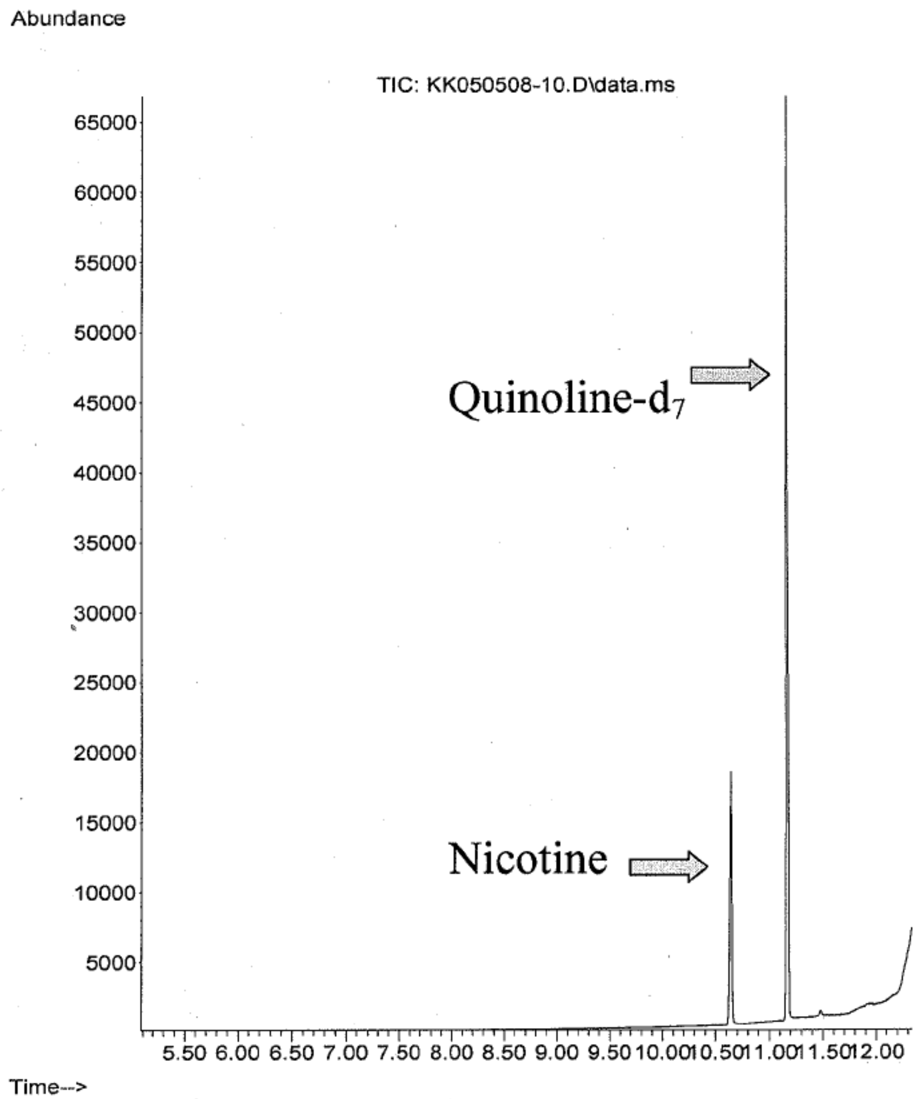
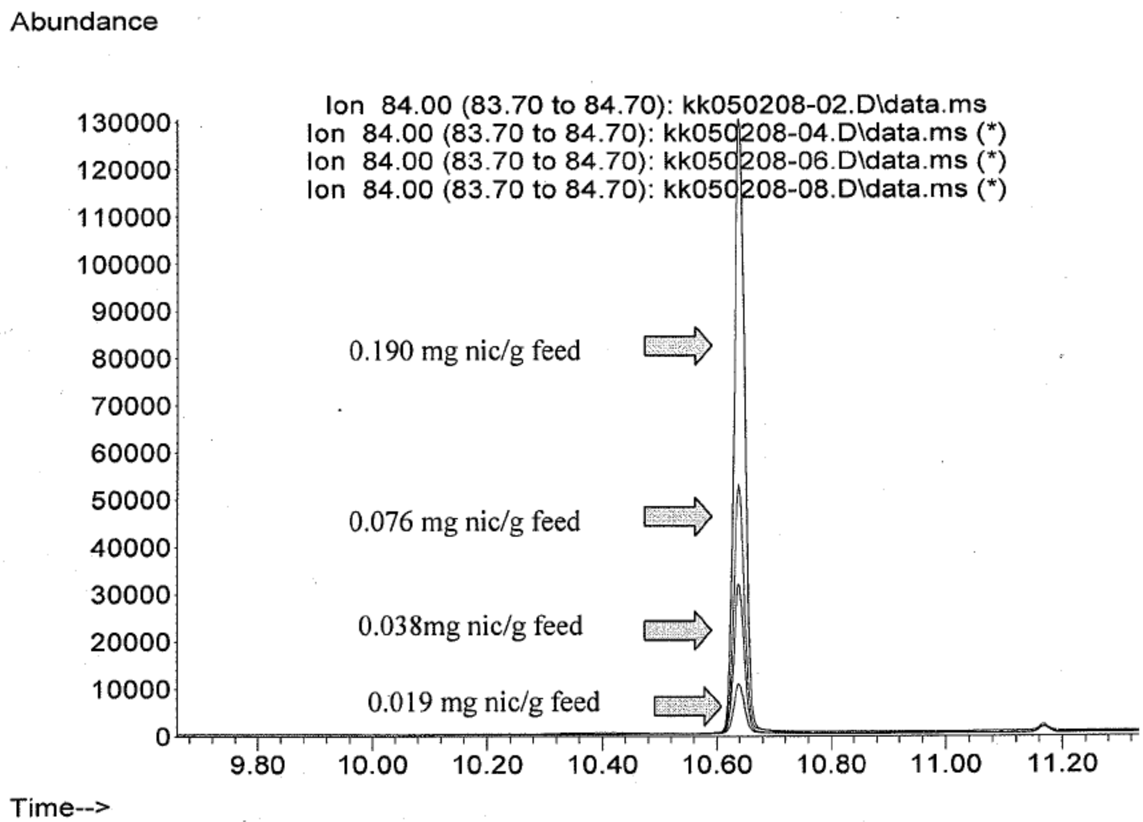


Figure 3: Overlaid Chromatograms (Selected Ion 84) of four Test Extracts Ranging from 0.019 to 0.19 mg nicotine per gram rat feed:



Appendix IV

Serology and Histopathology of the Lung

RJReynolds

Subject: Serology Results for TOX210

Date: May 9, 2008

To: Jenny Smith

From: Chandra Williams, DVM

Attached are the serology results of serum samples taken from animals on the TOX210 study. The samples were collected from 9 male (animal numbers 86-89 and 91-95) mice. Blood was not collected on animal number 90. Mice were euthanized for whole blood sample collection. Serum was removed from the whole blood samples and submitted on 9 animals (86-89 and 91-95). The samples were collected on May 6, 2008.

The serum was submitted to Charles River Laboratory (CRL) and was analyzed for the presence of antibodies to the rat pathogens (CRL Mouse Assessment Plus profile) listed below.

Sendai Virus	Polyoma Virus
Pneumonia Virus of Mice (PVM)	Mouse Adenovirus (MAV) 1 & 2
Mouse Hepatitis Virus (MHV)	Epizootic Diarrhea of Infant Mice Virus (EDIM)
Minute Virus of Mice (MVM)	Mouse Cytomegalovirus (MCMV)
GDVII (Murine Encephalomyelitis Virus)	Hantaviruses
REO-3	*Encephalitozoon cuniculi*
Mycoplasma pulmonis	Cilia Associated Respiratory Bacillus
Lymphocytic Choriomeningitis Virus	Mouse Parvovirus (MPV)* 1 & 2
Ectromelia (Mousepox)	Mouse Thymic Virus (MTLV)
K virus	Murine Norovirus (MNV)
Sendai Virus	Polyoma Virus

*MPV testing also includes testing for the non-structural protein of MPV, denoted as *ELISA NS1* on the CRL serology results report.

All results were negative.

Please contact me if you have questions or comments.

Cc: Jessica Baker
IACUC office

Printed: Friday, May 9, 2008 at 9:49

Charles River Research Animal Diagnostic Services
251 Ballardvale Street, Wilmington, MA 01887 USA
Tel: 800-338-9680 Fax: 978-658-7698

Sponsor: RJ Reynolds Tobacco Co **Accession #: 2008-025886**

Diagnostic Summary Report

PO Box 1236
Winston-Salem, NC 27102 USA

Attn: Dr. Chandra Williams

Tel: 336-741-0121

Received: 07 May 2008
Approved: 09 May 2008, 09:49
Bill Method: PO# P.O.4534185982
Test Specimen: Mouse

Sample Set	Service (# Tested)	Profile	Assay	Tested	+	+/-	?
#1	Serology (9)	All Results Negative					

+ = Positive, +/- = Equivocal, ? = Indeterminate

Service Approvals

Service	Approved By*	Date
Serology	Keith Provencher	09 May 2008, 09:49

**This report has been electronically signed by laboratory personnel. The name of the individual who approved these results appears in the header of this service report. All services are performed in accordance with and subject to General Terms and Conditions of Sale found in the Charles River Laboratories-Research Models and Services catalogue and on the back of invoices.*

Printed: Friday, May 9, 2008 at 9:49

Charles River Research Animal Diagnostic Services
251 Ballardvale Street, Wilmington, MA 01887 USA
Tel: 800-338-9680 Fax: 978-658-7698

Sponsor: RJ Reynolds Tobacco Co | **Accession #: 2008-025886**

Product: Not Indicated | **Test Specimen:** Mouse | **Received:** 07 May 2008

Serology Results Report

Department Review: Approved by Keith Provencher, 09 May 2008, 09:49*

Sample #: / Code :	1 TOX2 10M86	2 TOX2 10M87	3 TOX2 10M88	4 TOX2 10M89	6 TOX2 10M91	7 TOX2 10M92	8 TOX2 10M93	9 TOX2 10M94	10 TOX2 10M95
MFIA SEND	-	-	-	-	-	-	-	-	-
MFIA PVM	-	-	-	-	-	-	-	-	-
MFIA MHV	-	-	-	-	-	-	-	-	-
MFIA MVM	-	-	-	-	-	-	-	-	-
MFIA MPV-1	-	-	-	-	-	-	-	-	-
MFIA MPV-2	-	-	-	-	-	-	-	-	-
MFIA NS-1	-	-	-	-	-	-	-	-	-
MFIA MNV	-	-	-	-	-	-	-	-	-
MFIA GDVII	-	-	-	-	-	-	-	-	-
MFIA REO	-	-	-	-	-	-	-	-	-
MFIA EDIM	-	-	-	-	-	-	-	-	-
MFIA LCMV	-	-	-	-	-	-	-	-	-
MFIA ECTRO	-	-	-	-	-	-	-	-	-
MFIA MAV 1 & 2	-	-	-	-	-	-	-	-	-
MFIA MCMV	-	-	-	-	-	-	-	-	-
MFIA K	-	-	-	-	-	-	-	-	-
MFIA POLY	-	-	-	-	-	-	-	-	-
MFIA HANT	-	-	-	-	-	-	-	-	-
MFIA MPUL	-	-	-	-	-	-	-	-	-
MFIA ECUN	-	-	-	-	-	-	-	-	-
MFIA CARB	-	-	-	-	-	-	-	-	-
IFA MTLV	-	-	-	-	-	-	-	-	-
MFIA Anti-Ig	P	P	P	P	P	P	P	P	P

Remarks:
MFIA/ELISA/IFA Results: - = Negative; +/- = Equivocal; + = Moderate to strong positive; TC = Non-specific reaction with tissue control.
All Assays: IN = positive result interpreted as non-specific because not confirmed by other serologic assays, PDG = pending, QNS = Quantity not sufficient.

The anti-immunoglobulin (Anti-Ig) MFIA verifies that a serum specimen contains a sufficient concentration of immunoglobulin to be suitable for serologic testing. A result of P (for Pass) corresponds to a median fluorescence index (MFI) at or above the Anti-Ig assay cutoff (typically >= 7000 or higher). An Anti-Ig assay result of F (for Fail), assigned if the MFI is below the cutoff, might occur because the sample was received too dilute or was collected from an immunocompromised host. If a sample fails the Anti-Ig MFIA, then negative and borderline results in MFIA for microbial antibodies are considered I (for inconclusive).

**This report has been electronically signed by laboratory personnel. The name of the individual who approved these results appears in the header of this service report.*

May 18, 2008

Dr. Chandra Williams
R.J. Reynolds Tobacco Company
Toxicology Division
P. O. Box 1236
Winston Salem, NC 27102

REFERENCE: TOX-210 (Seventh Wave Study No. SW08-0143)

SUBJECT: Scheduled Health Screen, Histopathology, End Of Study

Dear Doctor Williams:

Formalin-preserved samples of infused lungs from ten male Swiss Webster mice were processed at Seventh Wave, beginning on May 8, 2008. Microscopic examination was performed on each of the five lung lobes from every mouse. As shown in the attached STARPATH Overall Incidence Table and Single Tabulated Animal Report, histopathologic changes in the lungs included congestion, hemorrhage, peribronchiolar/perivascular lymphocytic infiltrations, nonpigmented macrophages, chronic inflammation, and malignant lymphoma. The chronic inflammation was noted in only one lung lobe of two mice (Nos. 87 and 92). The occurrences of this change are regarded as random and nonspecific; they do not indicate the presence of contagious disease. The malignant lymphoma, observed in one mouse, is considered to be sporadic and noninfectious in origin.

The congestion and hemorrhage probably reflect the mode of anesthesia/euthanasia via carbon dioxide/exsanguination. The nonpigmented macrophages and lymphocytic infiltrations are anticipated background changes typically seen in mice of this age and strain.

CONCLUSION

Histopathologic examination revealed no evidence of intercurrent infectious disease in any of the mice examined.

John W. Sagartz, D.V.M.
Diplomate, A.C.V.P.

JWS: cjh

Seventh Wave Document No.: 312

cc: Jenny Smith
Paul Ayres
Jessica Baker
Sheri Bowman

SW08-0143 (TOX 210 HS)

QUALITY CONTOL STATEMENT

This study meets the Sponsor's requirements for quality control.

This study was performed without deviation from SOPs.

All SOPs used in this study were properly authorized.

The final report has been reviewed. The results accurately reflect the raw data of the study.

Any discrepancies are of an inconsequential nature or have been properly explained and documented.

The following phases of this study were inspected by Seventh Wave Laboratories Quality Control Unit. The dates of the inspections performed are as indicated below.

May 7, 2008	Part 1 of 9 - Project Sheet Review
May 13, 2008	Part 2 of 9 - Master Individual/Multiple Animal Worksheet
May 8, 2008	Part 3 of 9 - Histology Setup
May 13, 2008	Part 4 of 9 - Histology Completion
May 13, 2008	Part 5 of 9 - Slide/Block Match (100%)
May 13, 2008	Part 6 of 9 - Slide/Label Check (100%)
May 13, 2008	Part 7 of 9 - Wet Tissue Check (100%)
May 18, 2008	Part 8 of 9 - Rough Draft Report
May 18, 2008	Part 9 of 9 - Final Report

Vickie R Hocker 5/18/08

Vickie R. Hocker (Date)

The Starpath Project Documentation File
Project Title: Scheduled Health Screen, Histopathology, End Of Study
Institution: R. J. Reynolds Tobacco Company
Project Number: TOX-210 (SW08-0143) Species: Swiss Webster Mice Deaths will be reported in days.
This report was printed: 05-18-2008 This file was edited 05-18-2008 Reports will be paginated.

The Dosage Group Names

1 Health Screen	11	21
2	12	22
3	13	23
4	14	24
5	15	25
6	16	26
7	17	27
8	18	28
9	19	29
10	20	30

The Currently Defined Sacrifice Definitions

1 HS F Health Screen	11	21
2	12	22
3	13	23
4	14	24
5	15	25
6	16	26
7	17	27
8	18	28
9	19	29
10	20	30

The Project Organ File (No.=Organ Number S=Sex where M=male, F=female & B=both sexes)

No. S Name	No. S Name	No. S Name
186 B LUNG, LEFT LOBE, H&E		
187 B LUNG, INTERMEDIATE LOBE, H&E		
188 B LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E		
189 B LUNG, RIGHT CARDIAC LOBE, H&E		
190 B LUNG, RIGHT APICAL LOBE, H&E		

Single Tabulated Animal Report
Individual Macroscopic and Microscopic Observations
R. J. Reynolds Tobacco Company
Scheduled Health Screen, Histopathology, End Of Study

ANIMAL NUMBER: 86 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 05-18-2008.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

LUNG, LEFT LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD -INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD
LUNG, INTERMEDIATE LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT CARDIAC LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
LUNG, RIGHT APICAL LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL -HEMORRHAGE, MULTIFOCAL, MINIMAL

ANIMAL NUMBER: 87 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 05-18-2008.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

LUNG, LEFT LOBE, H&E	-CONGESTION, DIFFUSE, MILD -INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, INTERMEDIATE LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MINIMAL -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
LUNG, RIGHT CARDIAC LOBE, H&E	-INFLAMMATION, CHRONIC, FOCAL, MINIMAL -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL

SPECIES: Swiss Webster Mice
PROJECT NUMBER: TOX-210 (SW08-0143)

Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
R. J. Reynolds Tobacco Company
Scheduled Health Screen, Histopathology, End Of Study

ANIMAL NUMBER: 87 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 05-18-2008.

MICROSCOPIC OBSERVATIONS (continued):

LUNG, RIGHT APICAL LOBE, H&E	-CONGESTION, DIFFUSE, MINIMAL -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL -HEMORRHAGE, FOCAL, MINIMAL

ANIMAL NUMBER: 88 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 05-18-2008.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

LUNG, LEFT LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
LUNG, INTERMEDIATE LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
LUNG, RIGHT CARDIAC LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT APICAL LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MINIMAL -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL

ANIMAL NUMBER: 89 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 05-18-2008.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

LUNG, LEFT LOBE, H&E	-HEMORRHAGE, MULTIFOCAL, MINIMAL -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL

SPECIES: Swiss Webster Mice
PROJECT NUMBER: TOX-210 (SW08-0143)

Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
R. J. Reynolds Tobacco Company
Scheduled Health Screen, Histopathology, End Of Study

ANIMAL NUMBER: 89 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 05-18-2008.

MICROSCOPIC OBSERVATIONS (continued):

LUNG, INTERMEDIATE LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD -INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MINIMAL
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT CARDIAC LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL -HEMORRHAGE, MULTIFOCAL, MINIMAL
LUNG, RIGHT APICAL LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MINIMAL -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL -HEMORRHAGE, MULTIFOCAL, MINIMAL

ANIMAL NUMBER: 90 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 05-18-2008.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

LUNG, LEFT LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, INTERMEDIATE LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
LUNG, RIGHT CARDIAC LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT APICAL LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL

Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
R. J. Reynolds Tobacco Company
Scheduled Health Screen, Histopathology, End Of Study

ANIMAL NUMBER: 91 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 05-18-2008.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

LUNG, LEFT LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD
	-HEMORRHAGE, MULTIFOCAL, MILD
	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
LUNG, INTERMEDIATE LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
	-HEMORRHAGE, MULTIFOCAL, MINIMAL
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD
	-HEMORRHAGE, MULTIFOCAL, MILD
	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
LUNG, RIGHT CARDIAC LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
	-HEMORRHAGE, MULTIFOCAL, MINIMAL
LUNG, RIGHT APICAL LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
	-HEMORRHAGE, MULTIFOCAL, MINIMAL
	-CONGESTION, DIFFUSE, MINIMAL

ANIMAL NUMBER: 92 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 05-18-2008.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

LUNG, LEFT LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD
	-INFLAMMATION, CHRONIC, FOCAL, MINIMAL
	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, INTERMEDIATE LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD
	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD
	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
	-HEMORRHAGE, MULTIFOCAL, MINIMAL

Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
R. J. Reynolds Tobacco Company
Scheduled Health Screen, Histopathology, End Of Study

ANIMAL NUMBER: 92 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 05-18-2008.

MICROSCOPIC OBSERVATIONS (continued):

LUNG, RIGHT CARDIAC LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT APICAL LOBE, H&E	-CONGESTION, DIFFUSE, MINIMAL -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL

ANIMAL NUMBER: 93 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 05-18-2008.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

LUNG, LEFT LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, INTERMEDIATE LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL -INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	-CONGESTION, DIFFUSE, MILD -INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT CARDIAC LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT APICAL LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL

Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
R. J. Reynolds Tobacco Company
Scheduled Health Screen, Histopathology, End Of Study

ANIMAL NUMBER: 94 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 05-18-2008.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

LUNG, LEFT LOBE, H&E	-CONGESTION, DIFFUSE, MILD -HEMORRHAGE, MULTIFOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
LUNG, INTERMEDIATE LOBE, H&E	-CONGESTION, DIFFUSE, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MINIMAL -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT CARDIAC LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT APICAL LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL

ANIMAL NUMBER: 95 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 05-18-2008.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

LUNG, LEFT LOBE, H&E	-MALIGNANT LYMPHOMA, LYMPHOBLASTIC, MULTIFOCAL
LUNG, INTERMEDIATE LOBE, H&E	-MALIGNANT LYMPHOMA, LYMPHOBLASTIC, MULTIFOCAL
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	-MALIGNANT LYMPHOMA, LYMPHOBLASTIC, MULTIFOCAL
LUNG, RIGHT CARDIAC LOBE, H&E	-MALIGNANT LYMPHOMA, LYMPHOBLASTIC, MULTIFOCAL
LUNG, RIGHT APICAL LOBE, H&E	-MALIGNANT LYMPHOMA, LYMPHOBLASTIC, MULTIFOCAL

Overall Incidence for Males
R. J. Reynolds Tobacco Company
Scheduled Health Screen, Histopathology, End Of Study

PROJECT NUMBER: TOX-210 (SW08-0143)   SPECIES: Swiss Webster Mice
Printed on 05-18-2008.

Tissue/ Diagnosis/ Modifier(s)	Health Screen
LUNG, LEFT LOBE, H&E	(10)
CONGESTION	2
DIFFUSE, MILD	2
HEMORRHAGE	3
MULTIFOCAL, MINIMAL	1
MULTIFOCAL, MILD	2
INFILTRATION, LYMPHOCYTIC	6
PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD	1
PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD	5
INFLAMMATION, CHRONIC	1
FOCAL, MINIMAL	1
MACROPHAGES, NONPIGMENTED	9
MULTIFOCAL, MINIMAL	5
MULTIFOCAL, MILD	4
MALIGNANT LYMPHOMA	1
LYMPHOBLASTIC, MULTIFOCAL	1
LUNG, INTERMEDIATE LOBE, H&E	(10)
CONGESTION	1
DIFFUSE, MILD	1
HEMORRHAGE	1
MULTIFOCAL, MINIMAL	1
INFILTRATION, LYMPHOCYTIC	6
PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MINIMAL	2
PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD	2
PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD	2
MACROPHAGES, NONPIGMENTED	9
MULTIFOCAL, MINIMAL	5
MULTIFOCAL, MILD	4
MALIGNANT LYMPHOMA	1
LYMPHOBLASTIC, MULTIFOCAL	1
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	(10)
CONGESTION	1
DIFFUSE, MILD	1
HEMORRHAGE	2
MULTIFOCAL, MINIMAL	1
MULTIFOCAL, MILD	1
INFILTRATION, LYMPHOCYTIC	7
PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MINIMAL	1
PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD	6
MACROPHAGES, NONPIGMENTED	9
MULTIFOCAL, MINIMAL	4
MULTIFOCAL, MILD	5
MALIGNANT LYMPHOMA	1
LYMPHOBLASTIC, MULTIFOCAL	1

() = Number Of Animals Examined For This Tissue

All modifiers are printed.

Overall Incidence for Males (continued)
R. J. Reynolds Tobacco Company
Scheduled Health Screen, Histopathology, End Of Study

PROJECT NUMBER: TOX-210 (SW08-0143) SPECIES: Swiss Webster Mice
Printed on 05-18-2008.

Tissue/ Diagnosis/ Modifier(s)	Health Screen
LUNG, RIGHT CARDIAC LOBE, H&E	(10)
HEMORRHAGE	2
MULTIFOCAL, MINIMAL	2
INFILTRATION, LYMPHOCYTIC	6
PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD	3
PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD	3
INFLAMMATION, CHRONIC	1
FOCAL, MINIMAL	1
MACROPHAGES, NONPIGMENTED	9
MULTIFOCAL, MINIMAL	7
MULTIFOCAL, MILD	2
MALIGNANT LYMPHOMA	1
LYMPHOBLASTIC, MULTIFOCAL	1
LUNG, RIGHT APICAL LOBE, H&E	(10)
CONGESTION	3
DIFFUSE, MINIMAL	3
HEMORRHAGE	4
FOCAL, MINIMAL	1
MULTIFOCAL, MINIMAL	3
INFILTRATION, LYMPHOCYTIC	3
PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MINIMAL	2
PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD	1
MACROPHAGES, NONPIGMENTED	9
MULTIFOCAL, MINIMAL	9
MALIGNANT LYMPHOMA	1
LYMPHOBLASTIC, MULTIFOCAL	1

() = Number Of Animals Examined For This Tissue

Appendix V

Survival

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
MOUSE/SWISS WEBSTER

Dead Animal Status List for All Animals
Study number: TOX210A

Dosing start date: 16-Apr-08

PRINTED: 21-Oct-08
Page: 1

FEEDING STUDY/PALATABILITY

Animal Number	Grp	Sex	Study Phase	Date and Time Data was Entered		Oper. No.	Date of Death	Phase Day	Death Typ	Status	Term. Body Wt. (g)	Ow	Grs
1	1	M	Exposure phase	05-May-08	08:59	210	05-May-08	20	s	Final phase sacrifice	33.9	-	-
2	1	M	Exposure phase	05-May-08	09:00	210	05-May-08	20	s	Final phase sacrifice	33.3	-	-
3	1	M	Exposure phase	05-May-08	09:00	210	05-May-08	20	s	Final phase sacrifice	33.8	-	-
4	1	M	Exposure phase	05-May-08	09:01	210	05-May-08	20	s	Final phase sacrifice	28.5	-	-
5	1	M	Exposure phase	05-May-08	09:01	210	05-May-08	20	s	Final phase sacrifice	22.1	-	-
6	2	M	Exposure phase	05-May-08	09:01	210	05-May-08	20	s	Final phase sacrifice	33.7	-	-
7	2	M	Exposure phase	05-May-08	09:02	210	05-May-08	20	s	Final phase sacrifice	30.3	-	-
8	2	M	Exposure phase	05-May-08	09:02	210	05-May-08	20	s	Final phase sacrifice	26.8	-	-
9	2	M	Exposure phase	05-May-08	09:03	210	05-May-08	20	s	Final phase sacrifice	29.7	-	-
10	2	M	Exposure phase	05-May-08	09:03	210	05-May-08	20	s	Final phase sacrifice	30.8	-	-
11	3	M	Exposure phase	05-May-08	09:04	210	05-May-08	20	s	Final phase sacrifice	37.7	-	-
12	3	M	Exposure phase	05-May-08	09:04	210	05-May-08	20	s	Final phase sacrifice	29.8	-	-
13	3	M	Exposure phase	05-May-08	09:05	210	05-May-08	20	s	Final phase sacrifice	30.9	-	-
14	3	M	Exposure phase	05-May-08	09:05	210	05-May-08	20	s	Final phase sacrifice	30.5	-	-
15	3	M	Exposure phase	05-May-08	09:05	210	05-May-08	20	s	Final phase sacrifice	26.7	-	-
16	4	M	Exposure phase	05-May-08	09:06	210	05-May-08	20	s	Final phase sacrifice	30.2	-	-
17	4	M	Exposure phase	05-May-08	09:06	210	05-May-08	20	s	Final phase sacrifice	36.1	-	-
18	4	M	Exposure phase	05-May-08	09:06	210	05-May-08	20	s	Final phase sacrifice	34.8	-	-
19	4	M	Exposure phase	05-May-08	09:07	210	05-May-08	20	s	Final phase sacrifice	31.6	-	-
20	4	M	Exposure phase	05-May-08	09:07	210	05-May-08	20	s	Final phase sacrifice	28.5	-	-
21	5	M	Exposure phase	05-May-08	09:08	210	05-May-08	20	s	Final phase sacrifice	30.2	-	-
22	5	M	Exposure phase	05-May-08	09:08	210	05-May-08	20	s	Final phase sacrifice	32.0	-	-
23	5	M	Exposure phase	05-May-08	09:09	210	05-May-08	20	s	Final phase sacrifice	30.3	-	-
24	5	M	Exposure phase	05-May-08	09:09	210	05-May-08	20	s	Final phase sacrifice	29.4	-	-
25	5	M	Exposure phase	05-May-08	09:09	210	05-May-08	20	s	Final phase sacrifice	28.6	-	-
26	6	M	Exposure phase	05-May-08	09:11	210	05-May-08	20	s	Final phase sacrifice	35.0	-	-
27	6	M	Exposure phase	05-May-08	09:11	210	05-May-08	20	s	Final phase sacrifice	30.3	-	-
28	6	M	Exposure phase	05-May-08	09:12	210	05-May-08	20	s	Final phase sacrifice	26.6	-	-
29	6	M	Exposure phase	05-May-08	09:12	210	05-May-08	20	s	Final phase sacrifice	31.5	-	-
30	6	M	Exposure phase	05-May-08	09:13	210	05-May-08	20	s	Final phase sacrifice	30.0	-	-
31	7	M	Exposure phase	05-May-08	09:14	210	05-May-08	20	s	Final phase sacrifice	31.1	-	-
32	7	M	Exposure phase	05-May-08	09:14	210	05-May-08	20	s	Final phase sacrifice	31.1	-	-
33	7	M	Exposure phase	05-May-08	09:14	210	05-May-08	20	s	Final phase sacrifice	31.3	-	-
34	7	M	Exposure phase	05-May-08	09:15	210	05-May-08	20	s	Final phase sacrifice	32.1	-	-
35	7	M	Exposure phase	05-May-08	09:16	210	05-May-08	20	s	Final phase sacrifice	26.9	-	-
36	8	M	Exposure phase	05-May-08	09:16	210	05-May-08	20	s	Final phase sacrifice	27.0	-	-
37	8	M	Exposure phase	05-May-08	09:17	210	05-May-08	20	s	Final phase sacrifice	35.0	-	-
38	8	M	Exposure phase	05-May-08	09:17	210	05-May-08	20	s	Final phase sacrifice	32.4	-	-
39	8	M	Exposure phase	05-May-08	09:18	210	05-May-08	20	s	Final phase sacrifice	36.1	-	-
40	8	M	Exposure phase	05-May-08	09:18	210	05-May-08	20	s	Final phase sacrifice	32.3	-	-
41	9	M	Exposure phase	05-May-08	09:19	210	05-May-08	20	s	Final phase sacrifice	32.0	-	-
42	9	M	Exposure phase	05-May-08	09:19	210	05-May-08	20	s	Final phase sacrifice	31.8	-	-
43	9	M	Exposure phase	05-May-08	09:20	210	05-May-08	20	s	Final phase sacrifice	38.4	-	-
44	9	M	Exposure phase	05-May-08	09:20	210	05-May-08	20	s	Final phase sacrifice	37.1	-	-
45	9	M	Exposure phase	05-May-08	09:21	210	05-May-08	20	s	Final phase sacrifice	30.7	-	-

Note: * = pretest animal no.   P = partial data. C = complete data. - = no data.

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
MOUSE/SWISS WEBSTER

Dead Animal Status List for All Animals
Study number: TOX210A

Dosing start date: 16-Apr-08

PRINTED: 21-Oct-08
Page: 2

FEEDING STUDY/PALATABILITY

Animal Number	Grp	Sex	Study Phase	Date and Data was Entered	Time	Oper. No.	Date of Death	Phase Day	Death Typ	Status	Term. Body Wt. (g)	Ow	Grs
46	10	M	Exposure phase	05-May-08	09:21	210	05-May-08	20	s	Final phase sacrifice	30.6	-	-
47	10	M	Exposure phase	05-May-08	09:22	210	05-May-08	20	s	Final phase sacrifice	31.7	-	-
48	10	M	Exposure phase	05-May-08	09:22	210	05-May-08	20	s	Final phase sacrifice	36.3	-	-
49	10	M	Exposure phase	05-May-08	09:23	210	05-May-08	20	s	Final phase sacrifice	31.5	-	-
50	10	M	Exposure phase	05-May-08	09:23	210	05-May-08	20	s	Final phase sacrifice	36.0	-	-
51	11	M	Exposure phase	05-May-08	09:24	210	05-May-08	20	s	Final phase sacrifice	34.8	-	-
52	11	M	Exposure phase	05-May-08	09:25	210	05-May-08	20	s	Final phase sacrifice	29.3	-	-
53	11	M	Exposure phase	05-May-08	09:25	210	05-May-08	20	s	Final phase sacrifice	27.9	-	-
54	11	M	Exposure phase	05-May-08	09:25	210	05-May-08	20	s	Final phase sacrifice	31.0	-	-
55	11	M	Exposure phase	05-May-08	09:26	210	05-May-08	20	s	Final phase sacrifice	33.2	-	-
56	12	M	Exposure phase	05-May-08	09:26	210	05-May-08	20	s	Final phase sacrifice	34.3	-	-
57	12	M	Exposure phase	05-May-08	09:27	210	05-May-08	20	s	Final phase sacrifice	29.9	-	-
58	12	M	Exposure phase	05-May-08	09:28	210	05-May-08	20	s	Final phase sacrifice	35.0	-	-
59	12	M	Exposure phase	05-May-08	09:29	210	05-May-08	20	s	Final phase sacrifice	25.9	-	-
60	12	M	Exposure phase	05-May-08	09:29	210	05-May-08	20	s	Final phase sacrifice	32.5	-	-
61	13	M	Exposure phase	05-May-08	09:30	210	05-May-08	20	s	Final phase sacrifice	30.1	-	-
62	13	M	Exposure phase	05-May-08	09:30	210	05-May-08	20	s	Final phase sacrifice	29.8	-	-
63	13	M	Exposure phase	05-May-08	09:30	210	05-May-08	20	s	Final phase sacrifice	30.8	-	-
64	13	M	Exposure phase	05-May-08	09:31	210	05-May-08	20	s	Final phase sacrifice	29.5	-	-
65	13	M	Exposure phase	05-May-08	09:31	210	05-May-08	20	s	Final phase sacrifice	37.1	-	-

Note: * = pretest animal no.   P = partial data. C = complete data. - = no data.

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
MOUSE/SWISS WEBSTER

Dead Animal Status List for All Animals
Study number: TOX210B

Dosing start date: 16-Apr-08

PRINTED: 21-Oct-08
Page: 1

FEEDING STUDY/PALATABILITY

Animal Number	Grp	Sex	Study Phase	Date and Data was Entered	Time	Oper. No.	Date of Death	Phase Day	Death Typ	Status	Term. Body Wt. (g)	Ow	Grs
66	1	M	Exposure phase	05-May-08	08:45	210	05-May-08	20	s	Final phase sacrifice	32.4	-	-
67	1	M	Exposure phase	05-May-08	08:46	210	05-May-08	20	s	Final phase sacrifice	30.1	-	-
68	1	M	Exposure phase	05-May-08	08:46	210	05-May-08	20	s	Final phase sacrifice	30.8	-	-
69	1	M	Exposure phase	05-May-08	08:48	210	05-May-08	20	s	Final phase sacrifice	35.4	-	-
70	1	M	Exposure phase	05-May-08	08:48	210	05-May-08	20	s	Final phase sacrifice	30.3	-	-
71	2	M	Exposure phase	05-May-08	08:49	210	05-May-08	20	s	Final phase sacrifice	36.2	-	-
72	2	M	Exposure phase	05-May-08	08:49	210	05-May-08	20	s	Final phase sacrifice	35.1	-	-
73	2	M	Exposure phase	05-May-08	08:49	210	05-May-08	20	s	Final phase sacrifice	32.4	-	-
74	2	M	Exposure phase	05-May-08	08:50	210	05-May-08	20	s	Final phase sacrifice	32.3	-	-
75	2	M	Exposure phase	05-May-08	08:50	210	05-May-08	20	s	Final phase sacrifice	33.6	-	-
76	3	M	Exposure phase	05-May-08	08:51	210	05-May-08	20	s	Final phase sacrifice	32.0	-	-
77	3	M	Exposure phase	05-May-08	08:51	210	05-May-08	20	s	Final phase sacrifice	31.4	-	-
78	3	M	Exposure phase	05-May-08	08:52	210	05-May-08	20	s	Final phase sacrifice	32.8	-	-
79	3	M	Exposure phase	05-May-08	08:52	210	05-May-08	20	s	Final phase sacrifice	29.5	-	-
80	3	M	Exposure phase	05-May-08	08:52	210	05-May-08	20	s	Final phase sacrifice	30.0	-	-
81	4	M	Exposure phase	05-May-08	08:53	210	05-May-08	20	s	Final phase sacrifice	31.7	-	-
82	4	M	Exposure phase	05-May-08	08:53	210	05-May-08	20	s	Final phase sacrifice	29.3	-	-
83	4	M	Exposure phase	05-May-08	08:54	210	05-May-08	20	s	Final phase sacrifice	31.2	-	-
84	4	M	Exposure phase	05-May-08	08:55	210	05-May-08	20	s	Final phase sacrifice	31.3	-	-
85	4	M	Exposure phase	05-May-08	08:55	210	05-May-08	20	s	Final phase sacrifice	32.2	-	-
87	5	M	Exposure phase	06-May-08	07:35	210	06-May-08	21	s	Final phase sacrifice	40.1	-	-
88	5	M	Exposure phase	06-May-08	07:36	210	06-May-08	21	s	Final phase sacrifice	52.6	-	-
89	5	M	Exposure phase	06-May-08	07:37	210	06-May-08	21	s	Final phase sacrifice	43.4	-	-
90	5	M	Exposure phase	06-May-08	07:37	210	06-May-08	21	s	Final phase sacrifice	44.9	-	-
91	5	M	Exposure phase	06-May-08	07:38	210	06-May-08	21	s	Final phase sacrifice	46.3	-	-
92	5	M	Exposure phase	06-May-08	07:39	210	06-May-08	21	s	Final phase sacrifice	42.1	-	-
93	5	M	Exposure phase	06-May-08	07:40	210	06-May-08	21	s	Final phase sacrifice	44.3	-	-
94	5	M	Exposure phase	06-May-08	07:40	210	06-May-08	21	s	Final phase sacrifice	49.8	-	-
95	5	M	Exposure phase	06-May-08	07:41	210	06-May-08	21	s	Final phase sacrifice	39.8	-	-

Note: * = pretest animal no.   P = partial data. C = complete data. - = no data.

Appendix VI

Clinical Observations

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
MOUSE/SWISS WEBSTER

Summary of Clinical Signs
Study number: TOX210A
Exposure phase
Dosing start date: 16-Apr-08

PRINTED: 21-Oct-08
Page: 1
FEEDING STUDY/PALATABILITY

Interval: 3 - 17 Days

Group	Males 1 (5)		2 (5)		3 (5)		4 (5)		5 (5)		6 (5)		7 (5)	
Observation	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Normal														
Normal/no visible abnormalities	5	12.0	5	12.0	5	12.0	5	12.0	5	12.0	5	12.0	5	12.0

Key: () = Number of animals alive at start of interval
a = Number animals affected
b = Mean number of animal days with clinical sign

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
MOUSE/SWISS WEBSTER

Summary of Clinical Signs
Study number: TOX210A
Exposure phase
Dosing start date: 16-Apr-08

PRINTED: 21-Oct-08
Page: 2
FEEDING STUDY/PALATABILITY

Interval: 3 - 17 Days	Males											
Group	8		9		10		11		12		13	
Observation	(5)		(5)		(5)		(5)		(5)		(5)	
	a	b	a	b	a	b	a	b	a	b	a	b
Normal												
Normal/no visible abnormalities	5	12.0	5	12.0	5	12.0	5	12.0	5	12.0	5	12.0

Key: () = Number of animals alive at start of interval
a = Number animals affected
b = Mean number of animal days with clinical sign

Interval: 1 - 17 Days — Males

Group	1		2		3		4		5	
Observation	(5)		(5)		(5)		(5)		(10)	
	a	b	a	b	a	b	a	b	a	b
Normal										
Normal/no visible abnormalities	5	12.0	5	12.0	5	12.0	5	12.0	1	1.0
Body surface										
Unkempt hair	0	0.0	0	0.0	0	0.0	0	0.0	1	2.0
Abrasion	0	0.0	0	0.0	0	0.0	0	0.0	1	2.0

Key: () = Number of animals alive at start of interval
a = Number animals affected
b = Mean number of animal days with clinical sign

Appendix VII

Body Weights

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
MOUSE/SWISS WEBSTER

Animal body weights in (g)
Study number: TOX210A

Dosing start date: 16-Apr-08

PRINTED: 21-Nov-08
Page: 1

FEEDING STUDY/PALATABILITY

			Day of Phase												
Animal	Group		6!	1"	2	6	7	8	9	10	11	12	13	14	15
			Male Animals												
1	1		30.85	30.22	30.78	30.41	30.65	31.36	32.00	31.68	31.71	31.86	31.98	32.52	32.44
2			27.40	27.69	28.07	28.83	29.47	30.23	30.52	30.12	30.55	31.30	31.17	32.05	31.85
3			28.63	29.22	29.63	29.52	30.19	30.61	31.17	31.06	31.24	32.15	32.42	32.70	32.89
4			25.40	25.30	25.32	25.75	26.46	26.97	27.49	27.33	27.69	28.07	28.32	28.46	28.25
5			22.38	21.78	21.75	22.12	22.24	22.79	22.76	22.20	22.36	22.44	22.12	22.45	22.11
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	26.93	26.84	27.11	27.33	27.80	28.39	28.79	28.48	28.71	29.16	29.20	29.64	29.51
		Sdevs	3.22	3.38	3.63	3.40	3.51	3.55	3.78	3.88	3.88	4.10	4.27	4.37	4.52
6	2		28.88	28.66	28.60	29.34	30.71	31.59	31.90	31.38	32.20	32.32	32.08	32.90	32.72
7			28.25	24.89	25.09	26.14	26.61	27.19	27.63	27.29	27.78	28.60	28.34	29.29	29.07
8			23.65	23.38	23.49	23.21	23.81	24.55	24.93	24.64	24.53	25.20	25.09	25.83	25.61
9			26.35	26.52	26.62	26.64	27.17	27.85	28.02	27.86	28.32	28.74	28.75	28.99	28.72
10			26.99	27.25	27.55	27.73	28.36	29.18	29.29	28.87	29.17	29.73	29.39	29.91	29.89
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	26.82	26.14	26.27	26.61	27.33	28.07	28.35	28.01	28.40	28.92	28.73	29.38	29.20
		Sdevs	2.04	2.06	2.02	2.26	2.52	2.59	2.54	2.45	2.76	2.56	2.50	2.52	2.55
11	3		30.72	32.21	32.12	33.30	33.94	34.32	34.97	34.99	35.14	35.56	35.24	36.11	35.87
12			25.06	25.46	25.37	26.55	26.65	27.16	27.02	27.35	27.30	28.22	28.27	29.15	28.91
13			28.42	28.23	27.94	28.86	29.03	29.78	30.36	29.44	29.52	30.47	30.00	30.45	30.14
14			27.26	27.39	27.17	28.56	29.13	29.71	29.61	29.26	29.13	29.36	29.19	29.57	29.42
15			23.95	23.30	23.41	24.26	24.80	24.91	25.05	25.13	25.48	25.58	25.36	26.54	26.22
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	27.08	27.32	27.20	28.31	28.71	29.18	29.40	29.23	29.31	29.84	29.61	30.36	30.11
		Sdevs	2.69	3.33	3.26	3.35	3.43	3.51	3.76	3.66	3.63	3.68	3.60	3.53	3.54
16	4		17.32	23.47	24.73	25.66	26.13	27.02	27.34	27.50	28.66	28.69	28.63	29.47	29.62
17			29.60	29.71	29.31	30.00	30.83	31.42	32.01	31.80	32.28	32.99	33.23	33.96	34.19
18			28.18	28.07	28.31	29.85	30.42	31.02	31.53	31.42	32.02	32.38	32.44	33.60	33.76
19			26.93	26.67	26.41	27.74	28.09	28.49	28.45	28.26	29.10	29.49	29.74	30.08	30.13
20			25.66	25.50	25.04	26.01	26.23	26.69	26.58	26.69	27.04	27.44	26.84	27.25	27.26
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	25.54	26.68	26.76	27.85	28.34	28.93	29.18	29.13	29.82	30.20	30.18	30.87	30.99
		Sdevs	4.82	2.39	2.01	2.05	2.23	2.20	2.46	2.33	2.26	2.39	2.65	2.86	2.93
21	5		27.95	27.03	27.24	27.97	28.31	28.69	28.70	28.63	28.88	29.10	29.22	29.73	29.31
22			28.73	29.57	29.53	30.10	30.61	31.09	30.93	30.79	31.12	31.81	31.78	31.47	31.68
23			27.22	27.14	27.21	28.03	28.20	28.15	28.68	28.56	29.15	29.41	29.19	29.24	29.44
24			24.84	24.71	25.34	26.08	26.76	27.08	27.04	26.99	27.37	27.72	27.37	27.79	28.09

Note: ! = Quarantine/Acclimation; " = Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
MOUSE/SWISS WEBSTER

Animal body weights in (g)
Study number: TOX210A

Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

Animal	Group		6!	1"	2	6	7	8	9	10	11	12	13	14	15
							Day of Phase								
							Male Animals								
25	5		26.43	26.81	26.29	26.89	27.74	28.22	28.38	28.14	27.99	28.65	28.51	28.81	28.24
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	27.03	27.05	27.12	27.81	28.32	28.65	28.75	28.62	28.90	29.34	29.21	29.41	29.35
		Sdevs	1.49	1.72	1.56	1.51	1.42	1.49	1.40	1.38	1.43	1.52	1.62	1.36	1.44
26	6		29.63	30.21	30.41	32.03	32.81	32.84	33.49	33.41	33.65	34.03	33.82	34.06	34.34
27			26.50	25.87	26.20	27.12	27.44	27.50	27.91	27.71	28.23	28.77	28.73	29.20	29.30
28			24.09	24.34	24.30	24.87	25.17	25.34	25.60	25.28	25.40	25.46	25.35	25.80	25.92
29			28.39	27.92	27.61	28.76	29.22	29.94	30.19	29.64	30.06	30.08	30.24	30.78	30.87
30			26.89	27.05	27.41	28.20	28.86	29.04	29.41	28.91	28.63	29.06	28.87	28.90	29.13
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	27.10	27.08	27.19	28.20	28.70	28.93	29.32	28.99	29.19	29.48	29.40	29.75	29.91
		Sdevs	2.09	2.21	2.23	2.61	2.79	2.80	2.91	2.97	3.01	3.08	3.06	3.01	3.06
31	7		27.27	27.89	28.38	29.30	29.70	29.91	30.14	29.80	29.70	30.09	29.90	30.16	30.26
32			27.41	27.24	27.59	29.07	28.94	28.83	28.90	29.30	28.97	30.08	29.88	30.34	30.86
33			25.52	26.18	26.68	28.47	28.23	28.93	29.20	29.48	29.49	29.98	29.85	30.18	30.57
34			29.50	29.67	29.88	31.03	31.06	30.82	31.27	30.79	31.24	31.25	30.61	31.61	31.49
35			23.66	23.82	23.56	24.99	25.30	25.59	25.59	25.63	25.47	26.02	25.77	26.09	26.05
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	26.67	26.96	27.22	28.57	28.65	28.82	29.02	29.00	28.97	29.48	29.20	29.68	29.85
		Sdevs	2.20	2.17	2.36	2.22	2.14	1.98	2.13	1.97	2.13	2.01	1.94	2.09	2.17
36	8		25.78	23.34	23.04	24.01	24.47	24.90	25.06	24.72	24.92	25.69	25.42	25.75	25.62
37			18.03	26.06	27.83	30.93	31.29	32.22	32.34	32.83	33.45	33.51	33.06	33.80	33.81
38			26.62	27.32	27.89	29.34	29.68	30.22	30.40	29.97	30.00	30.50	30.54	30.56	31.01
39			28.65	29.35	29.85	31.70	32.33	32.66	33.23	32.63	32.93	33.62	33.52	34.05	34.31
40			27.40	27.81	27.91	28.51	29.06	29.42	29.84	29.79	29.90	30.29	30.26	30.64	31.07
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	25.30	26.78	27.30	28.90	29.37	29.88	30.17	29.99	30.24	30.72	30.56	30.96	31.16
		Sdevs	4.20	2.25	2.53	3.01	3.03	3.10	3.18	3.27	3.39	3.23	3.22	3.35	3.45
41	9		27.43	27.35	27.31	28.19	28.93	29.29	29.86	29.70	29.81	30.24	29.81	30.65	30.83
42			26.54	27.11	27.24	28.31	28.86	29.21	29.65	29.90	30.28	30.35	30.41	30.71	30.36
43			24.18	31.36	31.72	33.90	34.08	35.08	35.22	35.51	35.87	36.08	36.08	36.82	36.79
44			30.26	30.59	30.50	32.64	32.80	33.35	33.83	33.77	33.57	34.81	35.12	35.88	35.45
45			24.85	25.11	25.09	26.87	27.11	27.83	28.04	27.03	27.73	27.96	27.19	28.19	29.13
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	26.65	28.30	28.37	29.98	30.36	30.95	31.32	31.18	31.45	31.89	31.72	32.45	32.51
		Sdevs	2.40	2.60	2.69	3.09	2.94	3.10	3.05	3.41	3.24	3.41	3.76	3.72	3.38

Note: ! = Quarantine/Acclimation; " = Exposure phase

Animal body weights in (g)
Study number: TOX210A

Dosing start date: 16-Apr-08

			Day of Phase												
Animal	Group		6!	1"	2	6	7	8	9	10	11	12	13	14	15
							Male Animals								
46	10		23.51	25.70	25.36	26.17	26.67	27.19	27.32	27.04	27.78	28.69	27.91	29.28	29.03
47			29.36	29.18	29.70	30.29	30.27	30.70	30.97	30.61	30.73	31.59	30.97	31.17	31.32
48			28.61	29.04	29.38	30.78	31.40	31.59	32.08	32.16	32.42	33.17	33.46	34.10	34.34
49			26.76	26.91	26.93	28.27	28.23	28.45	28.95	28.82	29.18	29.66	29.57	29.76	29.75
50			25.76	27.71	28.70	31.50	31.22	32.01	32.61	32.36	32.59	33.10	33.05	33.57	34.02
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	26.80	27.71	28.01	29.40	29.56	29.99	30.39	30.20	30.54	31.24	30.99	31.58	31.69
		Sdevs	2.33	1.47	1.83	2.17	2.05	2.08	2.21	2.27	2.08	2.02	2.34	2.18	2.42
51	11		29.05	29.68	29.71	31.46	31.61	32.36	32.90	32.28	32.65	33.05	32.72	33.31	33.38
52			26.07	27.54	27.26	27.06	27.18	27.67	28.03	27.79	27.60	27.87	27.68	27.98	28.16
53			27.14	27.48	27.23	27.85	28.18	28.47	28.52	28.24	28.59	28.64	28.84	29.42	29.31
54			23.69	23.60	24.05	25.70	25.73	26.07	26.60	26.40	26.56	27.11	26.41	27.36	27.46
55			27.46	27.80	27.63	29.30	29.65	30.27	30.00	29.86	30.23	30.50	30.54	31.30	31.42
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	26.68	27.22	27.18	28.27	28.47	28.97	29.21	28.91	29.13	29.43	29.24	29.87	29.95
		Sdevs	1.98	2.22	2.03	2.21	2.26	2.43	2.39	2.25	2.39	2.38	2.47	2.45	2.44
56	12		29.17	30.08	30.44	32.54	33.01	33.40	33.55	33.58	33.53	33.69	33.17	33.10	33.06
57			26.51	25.95	26.63	26.90	27.65	28.04	28.56	28.28	28.88	28.94	28.81	28.98	29.08
58			28.38	28.73	29.02	30.30	30.35	30.87	30.96	30.78	31.34	31.89	31.97	32.57	32.84
59			22.74	22.41	22.66	23.42	23.69	24.14	24.46	24.38	24.37	24.76	23.86	24.99	25.34
60			26.90	26.89	27.06	28.30	28.48	28.55	29.62	29.09	29.15	30.13	30.06	31.28	31.31
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	26.74	26.81	27.16	28.29	28.64	29.00	29.43	29.22	29.45	29.88	29.57	30.18	30.33
		Sdevs	2.48	2.94	2.95	3.46	3.45	3.45	3.35	3.38	3.41	3.38	3.61	3.31	3.21
61	13		28.19	27.63	27.25	28.45	28.79	28.92	29.19	28.90	29.25	30.27	29.87	30.37	30.40
62			24.53	25.08	24.61	26.17	26.19	27.03	27.28	27.29	27.60	28.29	28.24	28.57	28.75
63			26.27	25.93	25.57	26.97	27.38	27.48	27.65	27.37	27.92	28.74	27.97	29.31	29.42
64			26.92	26.81	27.28	28.93	28.27	29.04	29.35	29.16	29.59	29.83	29.66	29.66	29.78
65			30.44	30.98	31.25	32.35	32.93	33.79	34.33	33.66	34.37	34.56	34.44	35.65	35.64
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	27.27	27.29	27.19	28.57	28.71	29.25	29.56	29.28	29.75	30.34	30.04	30.71	30.80
		Sdevs	2.21	2.27	2.54	2.38	2.56	2.68	2.82	2.60	2.72	2.49	2.60	2.84	2.77

Note: ! = Quarantine/Acclimation; " = Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
MOUSE/SWISS WEBSTER

Animal body weights in (g)
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FEEDING STUDY/PALATABILITY

Animal	Group		Day of Phase 16	17	18	19
			Male Animals			
1	1		32.69	33.05	33.33	33.76
2			32.27	32.41	33.20	33.40
3			33.47	33.11	33.51	33.84
4			28.06	28.52	28.41	28.54
5			22.00	22.34	22.35	22.47
		(n)	5	5	5	5
		Means	29.70	29.89	30.16	30.40
		Sdevs	4.79	4.63	4.86	4.96
6	2		32.90	33.64	34.01	34.04
7			29.50	29.89	30.02	30.14
8			25.79	26.24	26.50	26.79
9			29.09	29.01	29.37	29.66
10			29.95	30.76	30.85	31.25
		(n)	5	5	5	5
		Means	29.45	29.91	30.15	30.38
		Sdevs	2.53	2.69	2.71	2.63
11	3		36.41	36.69	37.59	37.81
12			28.91	29.38	29.53	29.66
13			30.69	30.65	31.02	31.12
14			29.55	29.84	30.02	30.29
15			26.14	26.49	27.01	26.76
		(n)	5	5	5	5
		Means	30.34	30.61	31.03	31.13
		Sdevs	3.78	3.74	3.95	4.08
16	4		29.38	29.54	29.42	29.40
17			34.88	34.85	35.20	35.21
18			34.41	34.26	34.58	34.26
19			30.63	30.62	30.87	31.26
20			27.50	27.95	28.16	27.88
		(n)	5	5	5	5
		Means	31.36	31.44	31.65	31.60
		Sdevs	3.20	3.00	3.12	3.12
21	5		29.21	29.12	29.60	29.78
22			31.77	31.80	32.11	32.09
23			29.52	29.44	30.09	30.22
24			28.04	28.08	28.88	28.86

Note: Data for Exposure phase

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Building 630/2
MOUSE/SWISS WEBSTER

Animal body weights in (g)
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FEEDING STUDY/PALATABILITY

Animal	Group		Day of Phase 16	17	18	19
			Male Animals			
25	5		28.49	28.61	28.56	28.32
		(n)	5	5	5	5
		Means	29.41	29.41	29.85	29.85
		Sdevs	1.44	1.43	1.40	1.46
26	6		34.54	34.77	35.20	34.71
27			29.43	29.68	30.12	30.13
28			26.05	26.10	26.32	26.23
29			31.07	31.01	31.33	31.37
30			28.89	28.69	29.36	29.56
		(n)	5	5	5	5
		Means	30.00	30.05	30.47	30.40
		Sdevs	3.12	3.19	3.23	3.07
31	7		30.09	29.95	30.56	30.45
32			30.84	30.36	30.76	30.51
33			30.90	30.91	30.93	30.76
34			31.72	31.40	31.43	31.76
35			26.30	26.23	26.39	26.28
		(n)	5	5	5	5
		Means	29.97	29.77	30.01	29.95
		Sdevs	2.13	2.05	2.05	2.12
36	8		26.16	26.15	26.32	25.88
37			34.27	34.35	34.51	34.46
38			31.33	31.40	31.94	31.54
39			34.56	35.14	34.97	35.05
40			31.17	31.80	32.06	31.97
		(n)	5	5	5	5
		Means	31.50	31.77	31.96	31.78
		Sdevs	3.38	3.53	3.44	3.63
41	9		30.97	31.06	31.44	31.34
42			30.99	30.69	31.69	31.11
43			37.28	37.36	37.63	37.66
44			35.24	35.90	36.10	36.29
45			29.34	29.51	30.36	30.64
		(n)	5	5	5	5
		Means	32.76	32.90	33.44	33.41
		Sdevs	3.34	3.49	3.21	3.30

Note: Data for Exposure phase

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MOUSE/SWISS WEBSTER

Animal body weights in (g)
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FEEDING STUDY/PALATABILITY

Animal	Group		Day of Phase 16	17	18	19
			Male Animals			
46	10		29.16	29.40	29.70	29.86
47			34.58	30.85	31.06	30.80
48			34.52	35.22	35.48	35.05
49			29.79	30.24	30.64	30.42
50			34.69	35.03	36.10	35.74
		(n)	5	5	5	5
		Means	32.55	32.15	32.60	32.37
		Sdevs	2.81	2.77	2.97	2.79
51	11		33.68	33.88	34.42	33.91
52			28.49	28.60	29.01	28.36
53			29.99	27.17	27.75	27.85
54			30.01	30.26	30.84	30.22
55			31.91	32.05	33.24	32.69
		(n)	5	5	5	5
		Means	30.82	30.39	31.05	30.61
		Sdevs	2.01	2.67	2.80	2.65
56	12		33.58	33.59	33.89	33.73
57			29.07	28.81	29.31	28.75
58			33.01	33.95	34.16	34.05
59			25.25	25.30	26.04	25.57
60			31.49	31.71	32.27	31.61
		(n)	5	5	5	5
		Means	30.48	30.67	31.13	30.74
		Sdevs	3.40	3.63	3.44	3.58
61	13		30.28	30.32	30.21	29.39
62			29.23	29.24	29.35	28.96
63			29.78	29.74	30.05	29.93
64			30.06	30.06	29.93	29.25
65			35.75	35.79	36.00	36.27
		(n)	5	5	5	5
		Means	31.02	31.03	31.11	30.76
		Sdevs	2.67	2.69	2.75	3.10

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
MOUSE/SWISS WEBSTER

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FEEDING STUDY/PALATABILITY

Animal	Group		Day of Phase 1	2	6	7	8	9	10	11	12	13	14	15	16
			Male Animals												
66	1		27.91	29.25	30.63	30.70	30.44	30.75	31.01	31.11	31.02	31.50	31.87	31.26	31.58
67			27.08	27.65	28.17	28.68	28.60	28.65	28.81	29.10	29.23	29.25	29.57	29.41	29.71
68			28.14	29.14	29.68	29.96	29.58	29.68	29.70	29.54	29.68	29.98	31.18	30.61	30.02
69			30.13	30.65	31.92	32.29	32.43	32.76	33.15	33.18	33.76	34.00	34.50	35.02	34.79
70			26.61	27.03	27.29	28.25	28.23	28.26	28.37	28.81	29.22	29.32	29.82	29.59	29.73
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	27.97	28.74	29.54	29.98	29.86	30.02	30.21	30.35	30.58	30.81	31.39	31.18	31.17
		Sdevs	1.35	1.43	1.86	1.62	1.68	1.81	1.93	1.81	1.92	2.00	1.98	2.28	2.17
71	2		30.00	30.70	32.34	32.69	32.16	32.73	33.31	33.55	34.14	34.46	34.79	35.02	34.91
72			28.17	28.47	29.98	30.87	30.72	31.19	31.40	31.44	32.70	32.75	33.42	33.66	33.89
73			28.22	29.10	30.11	30.65	30.42	30.26	30.70	30.76	31.50	31.48	31.89	31.50	31.73
74			27.73	28.58	29.54	29.82	29.60	29.99	30.26	30.12	30.68	30.89	31.55	31.78	31.87
75			28.01	28.75	29.48	29.96	30.58	30.98	31.63	31.77	32.34	32.68	33.06	32.85	33.21
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	28.43	29.12	30.29	30.80	30.70	31.03	31.46	31.53	32.27	32.45	32.94	32.96	33.12
		Sdevs	0.90	0.91	1.18	1.15	0.93	1.07	1.17	1.30	1.30	1.37	1.29	1.44	1.35
76	3		27.43	28.30	29.35	29.45	29.42	29.86	30.29	30.35	30.89	30.94	31.10	30.78	31.36
77			27.25	28.00	29.09	29.69	29.18	29.53	29.61	29.44	29.83	30.09	30.49	30.54	31.10
78			28.80	29.49	30.10	30.02	29.85	30.02	30.25	29.82	30.47	30.62	31.36	31.43	31.93
79			26.02	25.93	27.14	27.49	27.11	27.24	27.32	27.32	28.10	27.86	28.76	28.05	28.72
80			27.79	28.31	28.86	29.38	28.79	28.97	29.54	29.25	29.60	29.00	29.61	29.27	30.14
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	27.46	28.01	28.91	29.21	28.87	29.12	29.40	29.24	29.78	29.70	30.26	30.01	30.65
		Sdevs	1.00	1.29	1.09	0.99	1.06	1.13	1.21	1.15	1.07	1.27	1.08	1.35	1.26
81	4		28.63	28.46	30.04	30.22	29.81	30.02	29.96	29.89	30.47	30.43	30.68	30.56	31.63
82			27.47	27.96	29.18	28.93	28.16	28.36	28.66	28.65	29.33	29.68	29.23	29.01	29.26
83			27.74	28.25	30.15	29.51	29.45	30.53	30.08	30.24	30.26	30.10	30.33	30.36	30.69
84			29.38	30.02	30.97	31.44	30.79	31.02	31.34	31.13	31.90	32.13	31.79	31.39	31.43
85			29.90	30.33	32.09	32.14	31.69	31.84	31.93	31.66	32.33	32.97	32.39	32.15	32.26
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	28.62	29.00	30.49	30.45	29.98	30.35	30.39	30.31	30.86	31.06	30.88	30.69	31.05
		Sdevs	1.04	1.09	1.10	1.33	1.34	1.30	1.28	1.17	1.23	1.42	1.24	1.18	1.15

Note: Data for Exposure phase

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MOUSE/SWISS WEBSTER

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FEEDING STUDY/PALATABILITY

Animal	Group		Day of Phase 17	18	19
			Male Animals		
66	1		31.75	32.43	32.33
67			29.62	29.98	30.19
68			30.58	30.49	30.57
69			34.76	34.98	35.52
70			29.69	29.89	30.21
		(n)	5	5	5
		Means	31.28	31.55	31.76
		Sdevs	2.13	2.17	2.28
71	2		35.23	36.36	36.18
72			34.57	34.08	35.14
73			31.90	31.96	32.23
74			31.53	31.75	32.32
75			33.15	33.03	33.77
		(n)	5	5	5
		Means	33.28	33.44	33.93
		Sdevs	1.62	1.88	1.73
76	3		31.56	31.21	32.08
77			30.82	30.82	31.10
78			32.07	32.23	32.59
79			28.84	28.90	29.26
80			30.20	29.96	30.01
		(n)	5	5	5
		Means	30.70	30.62	31.01
		Sdevs	1.26	1.26	1.39
81	4		30.93	31.68	31.74
82			28.81	28.87	29.00
83			30.54	31.26	30.92
84			31.40	30.76	31.04
85			32.55	31.97	32.31
		(n)	5	5	5
		Means	30.85	30.91	31.00
		Sdevs	1.37	1.23	1.25

Note: Data for Exposure phase

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FEEDING STUDY/PALATABILITY

Day of Phase

Male Animals

Group(s)		6	7	8	9	10	11	12	13	14	15	16	17	18
1	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	27.33	27.80	28.39	28.79	28.48	28.71	29.16	29.20	29.64	29.51	29.70	29.89	30.16
	Sdevs	3.40	3.51	3.55	3.78	3.88	3.88	4.10	4.27	4.37	4.52	4.79	4.63	4.86
2	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	26.61	27.33	28.07	28.35	28.01	28.40	28.92	28.73	29.38	29.20	29.45	29.91	30.15
	Sdevs	2.26	2.52	2.59	2.54	2.45	2.76	2.56	2.50	2.52	2.55	2.53	2.69	2.71
3	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	28.31	28.71	29.18	29.40	29.23	29.31	29.84	29.61	30.36	30.11	30.34	30.61	31.03
	Sdevs	3.35	3.43	3.51	3.76	3.66	3.63	3.68	3.60	3.53	3.54	3.78	3.74	3.95
4	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	27.85	28.34	28.93	29.18	29.13	29.82	30.20	30.18	30.87	30.99	31.36	31.44	31.65
	Sdevs	2.05	2.23	2.20	2.46	2.33	2.26	2.39	2.65	2.86	2.93	3.20	3.00	3.12
5	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	27.81	28.32	28.65	28.75	28.62	28.90	29.34	29.21	29.41	29.35	29.41	29.41	29.85
	Sdevs	1.51	1.42	1.49	1.40	1.38	1.43	1.52	1.62	1.36	1.44	1.44	1.43	1.40
6	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	28.20	28.70	28.93	29.32	28.99	29.19	29.48	29.40	29.75	29.91	30.00	30.05	30.47
	Sdevs	2.61	2.79	2.80	2.91	2.97	3.01	3.08	3.06	3.01	3.06	3.12	3.19	3.23
7	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	28.57	28.65	28.82	29.02	29.00	28.97	29.48	29.20	29.68	29.85	29.97	29.77	30.01
	Sdevs	2.22	2.14	1.98	2.13	1.97	2.13	2.01	1.94	2.09	2.17	2.13	2.05	2.05
8	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	28.90	29.37	29.88	30.17	29.99	30.24	30.72	30.56	30.96	31.16	31.50	31.77	31.96
	Sdevs	3.01	3.03	3.10	3.18	3.27	3.39	3.23	3.22	3.35	3.45	3.38	3.53	3.44
9	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	29.98	30.36	30.95	31.32	31.18	31.45	31.89	31.72	32.45	32.51	32.76	32.90	33.44
	Sdevs	3.09	2.94	3.10	3.05	3.41	3.24	3.41	3.76	3.72	3.38	3.34	3.49	3.21
10	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	29.40	29.56	29.99	30.39	30.20	30.54	31.24	30.99	31.58	31.69	32.55	32.15	32.60
	Sdevs	2.17	2.05	2.08	2.21	2.27	2.08	2.02	2.34	2.18	2.42	2.81	2.77	2.97
11	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	28.27	28.47	28.97	29.21	28.91	29.13	29.43	29.24	29.87	29.95	30.82	30.39	31.05
	Sdevs	2.21	2.26	2.43	2.39	2.25	2.39	2.38	2.47	2.45	2.44	2.01	2.67	2.80

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
MOUSE/SWISS WEBSTER

Mean Animal Body Weights in (g)
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FEEDING STUDY/PALATABILITY

Group(s)		Day of Phase 6	7	8	9	10	11	12	13	14	15	16	17	18
12	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	28.29	28.64	29.00	29.43	29.22	29.45	29.88	29.57	30.18	30.33	30.48	30.67	31.13
	Sdevs	3.46	3.45	3.45	3.35	3.38	3.41	3.38	3.61	3.31	3.21	3.40	3.63	3.44
13	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	28.57	28.71	29.25	29.56	29.28	29.75	30.34	30.04	30.71	30.80	31.02	31.03	31.11
	Sdevs	2.38	2.56	2.68	2.82	2.60	2.72	2.49	2.60	2.84	2.77	2.67	2.69	2.75

Note: Data for Exposure phase
*(+) = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance
%($) = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

Group(s)		Day of Phase 19
		Male Animals
1	(N)	5
	Means	30.40
	Sdevs	4.96
2	(N)	5
	Means	30.38
	Sdevs	2.63
3	(N)	5
	Means	31.13
	Sdevs	4.08
4	(N)	5
	Means	31.60
	Sdevs	3.12
5	(N)	5
	Means	29.85
	Sdevs	1.46
6	(N)	5
	Means	30.40
	Sdevs	3.07
7	(N)	5
	Means	29.95
	Sdevs	2.12
8	(N)	5
	Means	31.78
	Sdevs	3.63
9	(N)	5
	Means	33.41
	Sdevs	3.30
10	(N)	5
	Means	32.37
	Sdevs	2.79
11	(N)	5
	Means	30.61
	Sdevs	2.65

Group(s)		Day of Phase 19
12	(N)	5
	Means	30.74
	Sdevs	3.58
13	(N)	5
	Means	30.76
	Sdevs	3.10

Note: Data for Exposure phase
*(+) = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance
%($) = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
MOUSE/SWISS WEBSTER

Mean Animal Body Weights in (g)
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FEEDING STUDY/PALATABILITY

Group(s)		6	7	8	9	10	11	12	13	14	15	16	17	18
						Day of Phase								
						Male Animals								
1	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	29.54	29.98	29.86	30.02	30.21	30.35	30.58	30.81	31.39	31.18	31.17	31.28	31.55
	Sdevs	1.86	1.62	1.68	1.81	1.93	1.81	1.92	2.00	1.98	2.28	2.17	2.13	2.17
2	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	30.29	30.80	30.70	31.03	31.46	31.53	32.27	32.45	32.94	32.96	33.12	33.28	33.44
	Sdevs	1.18	1.15	0.93	1.07	1.17	1.30	1.30	1.37	1.29	1.44	1.35	1.62	1.88
3	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	28.91	29.21	28.87	29.12	29.40	29.24	29.78	29.70	30.26	30.01	30.65	30.70	30.62
	Sdevs	1.09	0.99	1.06	1.13	1.21	1.15	1.07	1.27	1.08	1.35	1.26	1.26	1.26
4	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	30.49	30.45	29.98	30.35	30.39	30.31	30.86	31.06	30.88	30.69	31.05	30.85	30.91
	Sdevs	1.10	1.33	1.34	1.30	1.28	1.17	1.23	1.42	1.24	1.18	1.15	1.37	1.23
5	(N)													
	Means													
	Sdevs													

Note: Data for Exposure phase
*(+) = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance
%($) = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

R.J.R. TOBACCO
TOXICOLOGY DIVISION
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MOUSE/SWISS WEBSTER

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FEEDING STUDY/PALATABILITY

Group(s)		Day of Phase 19
		Male Animals
1	(N)	5
	Means	31.76
	Sdevs	2.28
2	(N)	5
	Means	33.93
	Sdevs	1.73
3	(N)	5
	Means	31.01
	Sdevs	1.39
4	(N)	5
	Means	31.00
	Sdevs	1.25
5	(N)	
	Means	
	Sdevs	

Note: Data for Exposure phase
*(+) = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance
%($) = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
MOUSE/SWISS WEBSTER

Mean Animal Body Weights in (g)
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FEEDING STUDY/PALATABILITY

Group(s)		Day of Phase 19
		Male Animals
1	(N)	5
	Means	30.40
	Sdevs	4.96
2	(N)	5
	Means	30.38
	Sdevs	2.63
3	(N)	5
	Means	31.13
	Sdevs	4.08
4	(N)	5
	Means	31.60
	Sdevs	3.12
5	(N)	5
	Means	29.85
	Sdevs	1.46
6	(N)	5
	Means	30.40
	Sdevs	3.07
7	(N)	5
	Means	29.95
	Sdevs	2.12
8	(N)	5
	Means	31.78
	Sdevs	3.63
9	(N)	5
	Means	33.41
	Sdevs	3.30
10	(N)	5
	Means	32.37
	Sdevs	2.79
11	(N)	5
	Means	30.61
	Sdevs	2.65

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FEEDING STUDY/PALATABILITY

Group(s)		Day of Phase 19
12	(N)	5
	Means	30.74
	Sdevs	3.58
13	(N)	5
	Means	30.76
	Sdevs	3.10

Note: Data for Exposure phase
*(+) = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance
%($) = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

Group(s)		Day of Phase 19
		Male Animals
1	(N)	5
	Means	31.76
	Sdevs	2.28
2	(N)	5
	Means	33.93
	Sdevs	1.73
3	(N)	5
	Means	31.01
	Sdevs	1.39
4	(N)	5
	Means	31.00
	Sdevs	1.25
5	(N)	
	Means	
	Sdevs	

Note: Data for Exposure phase
*(+) = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance
%($) = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX210A
Exposure phase (Day 7) (Reference Day 6)
Dosing start date: 16-Apr-08

M a l e   A n i m a l s
Data homogeneous by Bartlett's test   Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11	12	13
Number/group	5	5	5	5	5	5	5	5	5	5	5	5	5
Mean	0.48	0.72	0.40	0.49	0.51	0.50	0.07	0.47	0.37	0.16	0.20	0.34	0.14
Standard deviation	0.27	0.37	0.25	0.23	0.27	0.21	0.28	0.12	0.26	0.39	0.14	0.27	0.49
Group diff.@ P=.05		0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52
Group diff.@ P=.01		0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63

Analysis of variance: F ratio = 2.07 Df = 12/ 52 F probability = 0.036
Note: A * indicates group mean is significantly different from control at level of significance shown.

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Building 630/2
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Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX210A
Exposure phase (Day 8) (Reference Day 6)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

M a l e   A n i m a l s
Data homogeneous by Bartlett's test   Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11	12	13
Number/group	5	5	5	5	5	5	5	5	5	5	5	5	5
Mean	1.07	1.46	0.87	1.08	0.83	0.74	0.24	0.99	0.97	0.59	0.69	0.71	0.68
Standard deviation	0.28	0.47	0.23	0.34	0.45	0.32	0.43	0.17	0.18	0.33	0.24	0.33	0.50
Group diff.@ P=.05		0.63	0.63	0.63	0.63	0.63	0.63*	0.63	0.63	0.63	0.63	0.63	0.63
Group diff.@ P=.01		0.76	0.76	0.76	0.76	0.76	0.76*	0.76	0.76	0.76	0.76	0.76	0.76

Analysis of variance: F ratio = 3.57 Df = 12/ 52 F probability = 0.001
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX210A
Exposure phase (Day 9) (Reference Day 6)
Dosing start date: 16-Apr-08

M a l e   A n i m a l s

Data homogeneous by Bartlett's test   Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11	12	13
Number/group	5	5	5	5	5	5	5	5	5	5	5	5	5
Mean	1.46	1.74	1.10	1.33	0.93	1.12	0.45	1.28	1.34	0.98	0.94	1.14	0.99
Standard deviation	0.46	0.47	0.50	0.65	0.33	0.35	0.41	0.21	0.20	0.29	0.31	0.37	0.61
Group diff.@ P=.05		0.76	0.76	0.76	0.76	0.76	0.76*	0.76	0.76	0.76	0.76	0.76	0.76
Group diff.@ P=.01		0.92	0.92	0.92	0.92	0.92	0.92*	0.92	0.92	0.92	0.92	0.92	0.92

Analysis of variance: F ratio =   2.79   Df = 12/ 52   F probability = 0.005
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX210A
Exposure phase (Day 10) (Reference Day 6)
Dosing start date: 16-Apr-08

M a l e   A n i m a l s
Data homogeneous by Bartlett's test   Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11	12	13
Number/group	5	5	5	5	5	5	5	5	5	5	5	5	5
Mean	1.15	1.40	0.93	1.28	0.81	0.79	0.43	1.09	1.20	0.80	0.64	0.93	0.70
Standard deviation	0.62	0.38	0.44	0.63	0.28	0.37	0.47	0.52	0.61	0.40	0.17	0.33	0.48
Group diff.@ P=.05		0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83
Group diff.@ P=.01		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Analysis of variance: F ratio =   1.85   Df = 12/ 52   F probability = 0.064
Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX210A
Exposure phase (Day 11) (Reference Day 6)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

M a l e A n i m a l s
Data homogeneous by Bartlett's test   Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11	12	13
Number/group	5	5	5	5	5	5	5	5	5	5	5	5	5
Mean	1.38	1.79	1.01	1.97	1.09	1.00	0.40	1.34	1.47	1.14	0.85	1.16	1.17
Standard deviation	0.68	0.62	0.53	0.78	0.14	0.51	0.41	0.72	0.54	0.50	0.24	0.46	0.56
Group diff.@ P=.05		0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
Group diff.@ P=.01		1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19

Analysis of variance: F ratio = 2.71 Df = 12/ 52 F probability = 0.007
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX210A
Exposure phase (Day 12) (Reference Day 6)
Dosing start date: 16-Apr-08

M a l e   A n i m a l s

Data homogeneous by Bartlett's test   Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11	12	13
Number/group	5	5	5	5	5	5	5	5	5	5	5	5	5
Mean	1.84	2.31	1.53	2.35	1.52	1.28	0.91	1.82	1.91	1.84	1.16	1.59	1.76
Standard deviation	0.96	0.42	0.53	0.73	0.26	0.57	0.47	0.51	0.46	0.57	0.36	0.36	0.52
Group diff.@ P=.05		0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
Group diff.@ P=.01		1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20

Analysis of variance: F ratio =   2.86   Df = 12/ 52   F probability = 0.004

Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX210A
Exposure phase (Day 13) (Reference Day 6)
Dosing start date: 16-Apr-08

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M a l e   A n i m a l s
Data homogeneous by Bartlett's test   Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11	12	13
Number/group	5	5	5	5	5	5	5	5	5	5	5	5	5
Mean	1.88	2.12	1.31	2.32	1.40	1.21	0.63	1.66	1.74	1.59	0.96	1.28	1.46
Standard deviation	1.16	0.41	0.52	0.95	0.23	0.59	0.66	0.36	0.85	0.73	0.29	0.69	0.62
Group diff.@ P=.05		1.22	1.22	1.22	1.22	1.22	1.22*	1.22	1.22	1.22	1.22	1.22	1.22
Group diff.@ P=.01		1.47	1.47	1.47	1.47	1.47	1.47	1.47	1.47	1.47	1.47	1.47	1.47

Analysis of variance: F ratio =   2.33   Df = 12/ 52   F probability = 0.018
Note: A * indicates group mean is significantly different from control at level of significance shown.

M a l e   A n i m a l s
Data homogeneous by Bartlett's test   Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11	12	13
Number/group	5	5	5	5	5	5	5	5	5	5	5	5	5
Mean	2.31	2.77	2.06	3.02	1.59	1.55	1.10	2.06	2.47	2.17	1.60	1.89	2.14
Standard deviation	1.19	0.57	0.75	1.19	0.29	0.68	0.43	0.62	0.73	1.04	0.42	0.90	0.93
Group diff.@ P=.05		1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45
Group diff.@ P=.01		1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75

Analysis of variance: F ratio =   2.15   Df = 12/ 52   F probability = 0.029
Note: A * indicates group mean is significantly different from control at level of significance shown.

M a l e A n i m a l s

Data homogeneous by Bartlett's test   Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11	12	13
Number/group	5	5	5	5	5	5	5	5	5	5	5	5	5
Mean	2.18	2.59	1.81	3.14	1.54	1.72	1.27	2.27	2.53	2.29	1.67	2.03	2.22
Standard deviation	1.33	0.55	0.72	1.27	0.28	0.67	0.66	0.58	0.36	1.03	0.40	0.94	0.91
Group diff.@ P=.05		1.48	1.48	1.48	1.48	1.48	1.48	1.48	1.48	1.48	1.48	1.48	1.48
Group diff.@ P=.01		1.78	1.78	1.78	1.78	1.78	1.78	1.78	1.78	1.78	1.78	1.78	1.78

Analysis of variance: F ratio = 1.90 Df = 12/ 52 F probability = 0.055

Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX210A
Exposure phase (Day 16) (Reference Day 6)
Dosing start date: 16-Apr-08

M a l e   A n i m a l s

Data homogeneous by Bartlett's test   Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11	12	13
Number/group	5	5	5	5	5	5	5	5	5	5	5	5	5
Mean	2.37	2.83	2.03	3.51	1.59	1.80	1.40	2.60	2.78	3.15	2.54	2.19	2.45
Standard deviation	1.57	0.59	0.78	1.37	0.26	0.81	0.72	0.55	0.35	1.04	1.08	0.83	0.94
Group diff.@ P=.05		1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65
Group diff.@ P=.01		2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00

Analysis of variance: F ratio =   2.20   Df = 12/ 52   F probability = 0.025

Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX210A
Exposure phase (Day 17) (Reference Day 6)
Dosing start date: 16-Apr-08

M a l e   A n i m a l s
Data homogeneous by Bartlett's test   Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11	12	13
Number/group	5	5	5	5	5	5	5	5	5	5	5	5	5
Mean	2.56	3.30	2.30	3.59	1.60	1.85	1.20	2.87	2.92	2.75	2.12	2.38	2.46
Standard deviation	1.38	0.74	0.83	1.18	0.33	0.96	0.80	0.71	0.44	1.51	1.91	1.11	0.94
Group diff.@ P=.05		1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95
Group diff.@ P=.01		2.35	2.35	2.35	2.35	2.35	2.35	2.35	2.35	2.35	2.35	2.35	2.35

Analysis of variance: F ratio =   1.92   Df = 12/ 52   F probability = 0.053
Note: A * indicates group mean is significantly different from control at level of significance shown.

M a l e   A n i m a l s
Data nonhomogeneous by Bartlett's test   Modified T test of significance

Group	Control	2	3	4	5	6	7	8	9	10	11	12	13
Number/group	5	5	5	5	5	5	5	5	5	5	5	5	5
Mean	2.83	3.54	2.73	3.79	2.03	2.27	1.44	3.06	3.46	3.19	2.78	2.84	2.53
Standard deviation	1.62	0.76	1.05	1.22	0.47	0.91	0.74	0.58	0.18	1.65	2.00	1.09	1.11
Group diff.@ P=.05		2.22	2.40	2.53	2.10	2.31	2.22	2.14	2.03	2.88	3.20	2.43	2.44
Group diff.@ P=.01		3.71	4.00	4.21	3.50	3.85	3.70	3.56	3.38	4.79	5.33	4.05	4.07

Analysis of variance: F ratio =   1.60   Df = 12/ 52   F probability = 0.121
Note: A * indicates group mean is significantly different from control at level of significance shown.

M a l e   A n i m a l s

Data homogeneous by Bartlett's test   Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11	12	13
Number/group	5	5	5	5	5	5	5	5	5	5	5	5	5
Mean	3.08	3.76	2.82	3.75	2.04	2.20	1.38	2.88	3.43	2.97	2.33	2.45	2.19
Standard deviation	1.69	0.63	1.07	1.24	0.50	0.79	0.57	0.78	0.43	1.62	1.76	1.06	1.50
Group diff.@ P=.05		2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08
Group diff.@ P=.01		2.51	2.51	2.51	2.51	2.51	2.51	2.51	2.51	2.51	2.51	2.51	2.51

Analysis of variance: F ratio =   1.88   Df = 12/ 52   F probability = 0.059
Note: A * indicates group mean is significantly different from control at level of significance shown.

M a l e A n i m a l s

Data homogeneous by Bartlett's test Test of significance is Dunnett's test

Group	Control	2	3	4	5
Number/group	5	5	5	5	
Mean	0.44	0.51	0.30	-0.04	
Standard deviation	0.33	0.24	0.29	0.42	
Group diff.@ P=.05		0.54	0.54	0.54	
Group diff.@ P=.01		0.70	0.70	0.70	

Analysis of variance: F ratio = 2.75 Df = 3/ 16 F probability = 0.076

Note: A * indicates group mean is significantly different from control at level of significance shown.

M a l e   A n i m a l s
Data homogeneous by Bartlett's test   Test of significance is Dunnett's test

Group	Control	2	3	4	5
Number/group	5	5	5	5	
Mean	0.32	0.41	-0.04	-0.51	
Standard deviation	0.47	0.52	0.14	0.35	
Group diff.@ P=.05		0.65	0.65	0.65*	
Group diff.@ P=.01		0.85	0.85	0.85	

Analysis of variance: F ratio = 5.49 Df = 3/ 16 F probability = 0.009
Note: A * indicates group mean is significantly different from control at level of significance shown.

M a l e   A n i m a l s
Data homogeneous by Bartlett's test   Test of significance is Dunnett's test

Group	Control	2	3	4	5
Number/group	5	5	5	5	
Mean	0.48	0.74	0.22	-0.13	
Standard deviation	0.43	0.58	0.25	0.45	
Group diff.@ P=.05		0.72	0.72	0.72	
Group diff.@ P=.01		0.95	0.95	0.95	

Analysis of variance: F ratio = 3.56 Df = 3/ 16 F probability = 0.038
Note: A * indicates group mean is significantly different from control at level of significance shown.

M a l e A n i m a l s
Data homogeneous by Bartlett's test Test of significance is Dunnett's test

Group	Control	2	3	4	5
Number/group	5	5	5	5	
Mean	0.67	1.17	0.49	-0.09	
Standard deviation	0.50	0.63	0.34	0.32	
Group diff.@ P=.05		0.76	0.76	0.76*	
Group diff.@ P=.01		0.99	0.99	0.99	

Analysis of variance: F ratio = 6.30 Df = 3/ 16 F probability = 0.005
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX210B
Exposure phase (Day 11) (Reference Day 6)
Dosing start date: 16-Apr-08

M a l e   A n i m a l s
Data homogeneous by Bartlett's test   Test of significance is Dunnett's test

Group	Control	2	3	4	5
Number/group	5	5	5	5	
Mean	0.81	1.24	0.33	-0.17	
Standard deviation	0.66	0.70	0.46	0.31	
Group diff.@ P=.05		0.91	0.91	0.91*	
Group diff.@ P=.01		1.19	1.19	1.19	

Analysis of variance: F ratio =   6.06   Df = 3/ 16   F probability = 0.006
Note: A * indicates group mean is significantly different from control at level of significance shown.

M a l e   A n i m a l s
Data homogeneous by Bartlett's test   Test of significance is Dunnett's test

Group	Control	2	3	4	5
Number/group	5	5	5	5	
Mean	1.04	1.98	0.87	0.37	
Standard deviation	0.86	0.78	0.43	0.34	
Group diff.@ P=.05		1.05	1.05	1.05	
Group diff.@ P=.01		1.37	1.37	1.37	

Analysis of variance: F ratio = 5.55 Df = 3/ 16 F probability = 0.008
Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX210B
Exposure phase (Day 13) (Reference Day 6)
Dosing start date: 16-Apr-08

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M a l e   A n i m a l s
Data homogeneous by Bartlett's test   Test of significance is Dunnett's test

Group	Control	2	3	4	5
Number/group	5	5	5	5	
Mean	1.27	2.16	0.79	0.58	
Standard deviation	0.77	0.83	0.54	0.47	
Group diff.@ P=.05		1.10	1.10	1.10	
Group diff.@ P=.01		1.43	1.43	1.43	

Analysis of variance: F ratio =   5.53   Df =   3/ 16   F probability = 0.008
Note: A * indicates group mean is significantly different from control at level of significance shown.

M a l e A n i m a l s

Data homogeneous by Bartlett's test Test of significance is Dunnett's test

Group	Control	2	3	4	5
Number/group	5	5	5	5	
Mean	1.85	2.65	1.36	0.40	
Standard deviation	0.65	0.82	0.39	0.32	
Group diff.@ P=.05		0.95	0.95	0.95*	
Group diff.@ P=.01		1.25	1.25	1.25*	

Analysis of variance: F ratio = 13.16 Df = 3/ 16 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

M a l e   A n i m a l s
Data homogeneous by Bartlett's test   Test of significance is Dunnett's test

Group	Control	2	3	4	5
Number/group	5	5	5	5	
Mean	1.64	2.67	1.11	0.21	
Standard deviation	1.03	0.91	0.45	0.28	
Group diff.@ P=.05		1.21	1.21	1.21*	
Group diff.@ P=.01		1.58	1.58	1.58	

Analysis of variance: F ratio = 9.77 Df = 3/ 16 F probability = 0.001
Note: A * indicates group mean is significantly different from control at level of significance shown.

M a l e   A n i m a l s
Data homogeneous by Bartlett's test   Test of significance is Dunnett's test

Group	Control	2	3	4	5
Number/group	5	5	5	5	
Mean	1.63	2.83	1.74	0.57	
Standard deviation	1.04	0.97	0.31	0.60	
Group diff.@ P=.05		1.29	1.29	1.29	
Group diff.@ P=.01		1.69	1.69	1.69	

Analysis of variance: F ratio =   6.90   Df =   3/ 16   F probability = 0.003
Note: A * indicates group mean is significantly different from control at level of significance shown.

M a l e   A n i m a l s

Data homogeneous by Bartlett's test   Test of significance is Dunnett's test

Group	Control	2	3	4	5
Number/group	5	5	5	5	
Mean	1.74	2.99	1.79	0.36	
Standard deviation	0.84	1.17	0.33	0.46	
Group diff.@ P=.05		1.27	1.27	1.27*	
Group diff.@ P=.01		1.66	1.66	1.66	

Analysis of variance: F ratio =   9.66   Df =   3/ 16   F probability = 0.001

Note: A * indicates group mean is significantly different from control at level of significance shown.

M a l e   A n i m a l s

Data homogeneous by Bartlett's test   Test of significance is Dunnett's test

Group	Control	2	3	4	5
Number/group	5	5	5	5	
Mean	2.02	3.15	1.72	0.42	
Standard deviation	0.86	1.05	0.38	0.89	
Group diff.@ P=.05		1.37	1.37	1.37*	
Group diff.@ P=.01		1.79	1.79	1.79	

Analysis of variance: F ratio =   9.02   Df =   3/ 16   F probability = 0.001
Note: A * indicates group mean is significantly different from control at level of significance shown.

M a l e   A n i m a l s

Data homogeneous by Bartlett's test   Test of significance is Dunnett's test

Group	Control	2	3	4	5
Number/group	5	5	5	5	
Mean	2.23	3.64	2.10	0.52	
Standard deviation	1.06	1.21	0.60	0.75	
Group diff.@ P=.05		1.53	1.53	1.53*	
Group diff.@ P=.01		2.01	2.01	2.01	

Analysis of variance: F ratio =   9.32   Df =   3/ 16   F probability = 0.001

Note: A * indicates group mean is significantly different from control at level of significance shown.

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Animal weight gains per day in (g)
Study number: TOX210A

Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

			Day of Phase												
Animal	Group		6!	1"	2	6	7	8	9	10	11	12	13	14	15
			Male Animals												
1	1		0.940	-0.315	0.560	-0.093	0.240	0.710	0.640	-0.320	0.030	0.150	0.120	0.540	-0.080
2			0.367	0.145	0.380	0.190	0.640	0.760	0.290	-0.400	0.430	0.750	-0.130	0.880	-0.200
3			0.770	0.295	0.410	-0.027	0.670	0.420	0.560	-0.110	0.180	0.910	0.270	0.280	0.190
4			0.463	-0.050	0.020	0.108	0.710	0.510	0.520	-0.160	0.360	0.380	0.250	0.140	-0.210
5			0.240	-0.300	-0.030	0.093	0.120	0.550	-0.030	-0.560	0.160	0.080	-0.320	0.330	-0.340
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	0.556	-0.045	0.268	0.054	0.476	0.590	0.396	-0.310	0.232	0.454	0.038	0.434	-0.128
		Sdevs	0.290	0.269	0.259	0.113	0.275	0.142	0.271	0.182	0.161	0.365	0.256	0.288	0.200
6	2		0.643	-0.110	-0.060	0.185	1.370	0.880	0.310	-0.520	0.820	0.120	-0.240	0.820	-0.180
7			2.557	-1.680	0.200	0.262	0.470	0.580	0.440	-0.340	0.490	0.820	-0.260	0.950	-0.220
8			2.027	-0.135	0.110	-0.070	0.600	0.740	0.380	-0.290	-0.110	0.670	-0.110	0.740	-0.220
9			0.713	0.085	0.100	0.005	0.530	0.680	0.170	-0.160	0.460	0.420	0.010	0.240	-0.270
10			0.660	0.130	0.300	0.045	0.630	0.820	0.110	-0.420	0.300	0.560	-0.340	0.520	-0.020
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	1.320	-0.342	0.130	0.085	0.720	0.740	0.282	-0.346	0.392	0.518	-0.188	0.654	-0.182
		Sdevs	0.907	0.757	0.133	0.135	0.369	0.117	0.139	0.136	0.338	0.266	0.138	0.279	0.096
11	3		0.623	0.745	-0.090	0.295	0.640	0.380	0.650	0.020	0.150	0.420	-0.320	0.870	-0.240
12			0.620	0.200	-0.090	0.295	0.100	0.510	-0.140	0.330	-0.050	0.920	0.050	0.880	-0.240
13			0.517	-0.095	-0.290	0.230	0.170	0.750	0.580	-0.920	0.080	0.950	-0.470	0.450	-0.310
14			0.457	0.065	-0.220	0.347	0.570	0.580	-0.100	-0.350	-0.130	0.230	-0.170	0.380	-0.150
15			0.627	-0.325	0.110	0.213	0.540	0.110	0.140	0.080	0.350	0.100	-0.220	1.180	-0.320
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	0.569	0.118	-0.116	0.276	0.404	0.466	0.226	-0.168	0.080	0.524	-0.226	0.752	-0.252
		Sdevs	0.078	0.401	0.153	0.054	0.249	0.240	0.372	0.486	0.186	0.392	0.192	0.333	0.068
16	4		-1.023	3.075	1.260	0.233	0.470	0.890	0.320	0.160	1.160	0.030	-0.060	0.840	0.150
17			0.633	0.055	-0.400	0.173	0.830	0.590	0.590	-0.210	0.480	0.710	0.240	0.730	0.230
18			0.683	-0.055	0.240	0.385	0.570	0.600	0.510	-0.110	0.600	0.360	0.060	1.160	0.160
19			0.430	-0.130	-0.260	0.333	0.350	0.400	-0.040	-0.190	0.840	0.390	0.250	0.340	0.050
20			0.577	-0.080	-0.460	0.242	0.220	0.460	-0.110	0.110	0.350	0.400	-0.600	0.410	0.010
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	0.260	0.573	0.076	0.273	0.488	0.588	0.254	-0.048	0.686	0.378	-0.022	0.696	0.120
		Sdevs	0.723	1.400	0.717	0.085	0.232	0.189	0.317	0.172	0.321	0.241	0.348	0.334	0.089
21	5		0.803	-0.460	0.210	0.182	0.340	0.380	0.010	-0.070	0.250	0.220	0.120	0.510	-0.420
22			0.780	0.420	-0.040	0.142	0.510	0.480	-0.160	-0.140	0.330	0.690	-0.030	-0.310	0.210
23			-0.620	-0.040	0.070	0.205	0.170	-0.050	0.530	-0.120	0.590	0.260	-0.220	0.050	0.200
24			0.340	-0.065	0.630	0.185	0.680	0.320	-0.040	-0.050	0.380	0.350	-0.350	0.420	0.300

Note: ! = Quarantine/Acclimation; " = Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
MOUSE/SWISS WEBSTER

Animal weight gains per day in (g)
Study number: TOX210A

Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

			Day of Phase												
Animal	Group		6!	1"	2	6	7	8	9	10	11	12	13	14	15
			Male Animals												
25	5		0.283	0.190	-0.520	0.150	0.850	0.480	0.160	-0.240	-0.150	0.660	-0.140	0.300	-0.570
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	0.317	0.009	0.070	0.173	0.510	0.322	0.100	-0.124	0.280	0.436	-0.124	0.194	-0.056
		Sdevs	0.577	0.328	0.416	0.026	0.269	0.219	0.266	0.074	0.271	0.223	0.180	0.330	0.406
26	6		0.797	0.290	0.200	0.405	0.780	0.030	0.650	-0.080	0.240	0.380	-0.210	0.240	0.280
27			0.620	-0.315	0.330	0.230	0.320	0.060	0.410	-0.200	0.520	0.540	-0.040	0.470	0.100
28			0.303	0.125	-0.040	0.143	0.300	0.170	0.260	-0.320	0.120	0.060	-0.110	0.450	0.120
29			0.530	-0.235	-0.310	0.287	0.460	0.720	0.250	-0.550	0.420	0.020	0.160	0.540	0.090
30			0.580	0.080	0.360	0.198	0.660	0.180	0.370	-0.500	-0.280	0.430	-0.190	0.030	0.230
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	0.566	-0.011	0.108	0.253	0.504	0.232	0.388	-0.330	0.204	0.286	-0.078	0.346	0.164
		Sdevs	0.178	0.255	0.282	0.100	0.211	0.281	0.162	0.198	0.312	0.232	0.149	0.209	0.086
31	7		0.560	0.310	0.490	0.230	0.400	0.210	0.230	-0.340	-0.100	0.390	-0.190	0.260	0.100
32			0.203	-0.085	0.350	0.370	-0.130	-0.110	0.070	0.400	-0.330	1.110	-0.200	0.460	0.520
33			0.513	0.330	0.500	0.447	-0.240	0.700	0.270	0.280	0.010	0.490	-0.130	0.330	0.390
34			0.773	0.085	0.210	0.288	0.030	-0.240	0.450	-0.480	0.450	0.010	-0.640	1.000	-0.120
35			0.327	0.080	-0.260	0.358	0.310	0.290	0.000	0.040	-0.160	0.550	-0.250	0.320	-0.040
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	0.475	0.144	0.258	0.339	0.074	0.170	0.204	-0.020	-0.026	0.510	-0.282	0.474	0.170
		Sdevs	0.220	0.175	0.313	0.083	0.276	0.369	0.177	0.382	0.293	0.396	0.205	0.303	0.276
36	8		0.437	-1.220	-0.300	0.242	0.460	0.430	0.160	-0.340	0.200	0.770	-0.270	0.330	-0.130
37			-1.190	4.015	1.770	0.775	0.360	0.930	0.120	0.490	0.620	0.060	-0.450	0.740	0.010
38			0.313	0.350	0.570	0.363	0.340	0.540	0.180	-0.430	0.030	0.500	0.040	0.020	0.450
39			0.587	0.350	0.500	0.463	0.630	0.330	0.570	-0.600	0.300	0.690	-0.100	0.530	0.260
40			0.500	0.205	0.100	0.150	0.550	0.360	0.420	-0.050	0.110	0.390	-0.030	0.380	0.430
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	0.129	0.740	0.528	0.399	0.468	0.518	0.290	-0.186	0.252	0.482	-0.162	0.400	0.204
		Sdevs	0.744	1.947	0.777	0.242	0.124	0.244	0.196	0.427	0.229	0.280	0.198	0.266	0.257
41	9		0.417	-0.040	-0.040	0.220	0.740	0.360	0.570	-0.160	0.110	0.430	-0.430	0.840	0.180
42			0.760	0.285	0.130	0.267	0.550	0.350	0.440	0.250	0.380	0.070	0.060	0.300	-0.350
43			-1.397	3.590	0.360	0.545	0.180	1.000	0.140	0.290	0.360	0.210	0.000	0.740	-0.030
44			0.777	0.165	-0.090	0.535	0.160	0.550	0.480	-0.060	-0.200	1.240	0.310	0.760	-0.430
45			0.677	0.130	-0.020	0.445	0.240	0.720	0.210	-1.010	0.700	0.230	-0.770	1.000	0.940
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	0.247	0.826	0.068	0.402	0.374	0.596	0.368	-0.138	0.270	0.436	-0.166	0.728	0.062
		Sdevs	0.930	1.549	0.183	0.151	0.258	0.272	0.184	0.525	0.336	0.467	0.430	0.260	0.549

Note: ! = Quarantine/Acclimation; " = Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
MOUSE/SWISS WEBSTER

Animal weight gains per day in (g)
Study number: TOX210A
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

Animal	Group		6!	1"	2	6	7	8	9	10	11	12	13	14	15
							Day of Phase								
							Male Animals								
46	10		0.453	1.095	-0.340	0.202	0.500	0.520	0.130	-0.280	0.740	0.910	-0.780	1.370	-0.250
47			0.470	-0.090	0.520	0.148	-0.020	0.430	0.270	-0.360	0.120	0.860	-0.620	0.200	0.150
48			0.587	0.215	0.340	0.350	0.620	0.190	0.490	0.080	0.260	0.750	0.290	0.640	0.240
49			0.537	0.075	0.020	0.335	-0.040	0.220	0.500	-0.130	0.360	0.480	-0.090	0.190	-0.010
50			1.220	0.975	0.990	0.700	-0.280	0.790	0.600	-0.250	0.230	0.510	-0.050	0.520	0.450
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	0.653	0.454	0.306	0.347	0.156	0.430	0.398	-0.188	0.342	0.702	-0.250	0.584	0.116
		Sdevs	0.321	0.543	0.503	0.215	0.385	0.245	0.192	0.171	0.238	0.198	0.440	0.482	0.264
51	11		0.627	0.315	0.030	0.438	0.150	0.750	0.540	-0.620	0.370	0.400	-0.330	0.590	0.070
52			0.863	0.735	-0.280	-0.050	0.120	0.490	0.360	-0.240	-0.190	0.270	-0.190	0.300	0.180
53			0.360	0.170	-0.250	0.155	0.330	0.290	0.050	-0.280	0.350	0.050	0.200	0.580	-0.110
54			0.093	-0.045	0.450	0.413	0.030	0.340	0.530	-0.200	0.160	0.550	-0.700	0.950	0.100
55			0.217	0.170	-0.170	0.418	0.350	0.620	-0.270	-0.140	0.370	0.270	0.040	0.760	0.120
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	0.432	0.269	-0.044	0.275	0.196	0.498	0.242	-0.296	0.212	0.308	-0.196	0.636	0.072
		Sdevs	0.312	0.290	0.301	0.216	0.139	0.191	0.348	0.188	0.241	0.185	0.348	0.241	0.109
56	12		0.623	0.455	0.360	0.525	0.470	0.390	0.150	0.030	-0.050	0.160	-0.520	-0.070	-0.040
57			0.670	-0.280	0.680	0.068	0.750	0.390	0.520	-0.280	0.600	0.060	-0.130	0.170	0.100
58			0.677	0.175	0.290	0.320	0.050	0.520	0.090	-0.180	0.560	0.550	0.080	0.600	0.270
59			0.117	-0.165	0.250	0.190	0.270	0.450	0.320	-0.080	-0.010	0.390	-0.900	1.130	0.350
60			0.350	-0.005	0.170	0.310	0.180	0.070	1.070	-0.530	0.060	0.980	-0.070	1.220	0.030
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	0.487	0.036	0.350	0.283	0.344	0.364	0.430	-0.208	0.232	0.428	-0.308	0.610	0.142
		Sdevs	0.247	0.290	0.197	0.170	0.274	0.173	0.395	0.214	0.320	0.363	0.398	0.570	0.164
61	13		0.647	-0.280	-0.380	0.300	0.340	0.130	0.270	-0.290	0.350	1.020	-0.400	0.500	0.030
62			0.293	0.275	-0.470	0.390	0.020	0.840	0.250	0.010	0.310	0.690	-0.050	0.330	0.180
63			0.277	-0.170	-0.360	0.350	0.410	0.100	0.170	-0.280	0.550	0.820	-0.770	1.340	0.110
64			1.080	-0.055	0.470	0.412	-0.660	0.770	0.310	-0.190	0.430	0.240	-0.170	0.000	0.120
65			0.850	0.270	0.270	0.275	0.580	0.860	0.540	-0.670	0.710	0.190	-0.120	1.210	-0.010
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	0.629	0.008	-0.094	0.345	0.138	0.540	0.308	-0.284	0.470	0.592	-0.302	0.676	0.086
		Sdevs	0.350	0.254	0.431	0.058	0.490	0.390	0.139	0.247	0.162	0.364	0.293	0.577	0.076

Note: ! = Quarantine/Acclimation; " = Exposure phase

Animal	Group		Day of Phase 16	17	18	19
			Male Animals			
1	1		0.250	0.360	0.280	0.430
2			0.420	0.140	0.790	0.200
3			0.580	-0.360	0.400	0.330
4			-0.190	0.460	-0.110	0.130
5			-0.110	0.340	0.010	0.120
		(n)	5	5	5	5
		Means	0.190	0.188	0.274	0.242
		Sdevs	0.333	0.328	0.353	0.134
6	2		0.180	0.740	0.370	0.030
7			0.430	0.390	0.130	0.120
8			0.180	0.450	0.260	0.290
9			0.370	-0.080	0.360	0.290
10			0.060	0.810	0.090	0.400
		(n)	5	5	5	5
		Means	0.244	0.462	0.242	0.226
		Sdevs	0.152	0.353	0.129	0.148
11	3		0.540	0.280	0.900	0.220
12			0.000	0.470	0.150	0.130
13			0.550	-0.040	0.370	0.100
14			0.130	0.290	0.180	0.270
15			-0.080	0.350	0.520	-0.250
		(n)	5	5	5	5
		Means	0.228	0.270	0.424	0.094
		Sdevs	0.299	0.189	0.305	0.204
16	4		-0.240	0.160	-0.120	-0.020
17			0.690	-0.030	0.350	0.010
18			0.650	-0.150	0.320	-0.320
19			0.500	-0.010	0.250	0.390
20			0.240	0.450	0.210	-0.280
		(n)	5	5	5	5
		Means	0.368	0.084	0.202	-0.044
		Sdevs	0.383	0.233	0.188	0.284
21	5		-0.100	-0.090	0.480	0.180
22			0.090	0.030	0.310	-0.020
23			0.080	-0.080	0.650	0.130
24			-0.050	0.040	0.800	-0.020

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
MOUSE/SWISS WEBSTER

Animal weight gains per day in (g)
Study number: TOX210A

Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

Animal	Group		Day of Phase 16	17	18	19
			Male Animals			
25	5		0.250	0.120	-0.050	-0.240
		(n)	5	5	5	5
		Means	0.054	0.004	0.438	0.006
		Sdevs	0.137	0.088	0.329	0.164
26	6		0.200	0.230	0.430	-0.490
27			0.130	0.250	0.440	0.010
28			0.130	0.050	0.220	-0.090
29			0.200	-0.060	0.320	0.040
30			-0.240	-0.200	0.670	0.200
		(n)	5	5	5	5
		Means	0.084	0.054	0.416	-0.066
		Sdevs	0.184	0.192	0.168	0.259
31	7		-0.170	-0.140	0.610	-0.110
32			-0.020	-0.480	0.400	-0.250
33			0.330	0.010	0.020	-0.170
34			0.230	-0.320	0.030	0.330
35			0.250	-0.070	0.160	-0.110
		(n)	5	5	5	5
		Means	0.124	-0.200	0.244	-0.062
		Sdevs	0.210	0.198	0.256	0.227
36	8		0.540	-0.010	0.170	-0.440
37			0.460	0.080	0.160	-0.050
38			0.320	0.070	0.540	-0.400
39			0.250	0.580	-0.170	0.080
40			0.100	0.630	0.260	-0.090
		(n)	5	5	5	5
		Means	0.334	0.270	0.192	-0.180
		Sdevs	0.173	0.308	0.254	0.228
41	9		0.140	0.090	0.380	-0.100
42			0.630	-0.300	1.000	-0.580
43			0.490	0.080	0.270	0.030
44			-0.210	0.660	0.200	0.190
45			0.210	0.170	0.850	0.280
		(n)	5	5	5	5
		Means	0.252	0.140	0.540	-0.036
		Sdevs	0.327	0.343	0.361	0.337

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
MOUSE/SWISS WEBSTER

Animal weight gains per day in (g)
Study number: TOX210A

Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

Animal	Group		Day of Phase 16	17	18	19
			Male Animals			
46	10		0.130	0.240	0.300	0.160
47			3.260	-3.730	0.210	-0.260
48			0.180	0.700	0.260	-0.430
49			0.040	0.450	0.400	-0.220
50			0.670	0.340	1.070	-0.360
		(n)	5	5	5	5
		Means	0.856	-0.400	0.448	-0.222
		Sdevs	1.366	1.869	0.355	0.229
51	11		0.300	0.200	0.540	-0.510
52			0.330	0.110	0.410	-0.650
53			0.680	-2.820	0.580	0.100
54			2.550	0.250	0.580	-0.620
55			0.490	0.140	1.190	-0.550
		(n)	5	5	5	5
		Means	0.870	-0.424	0.660	-0.446
		Sdevs	0.951	1.340	0.304	0.310
56	12		0.520	0.010	0.300	-0.160
57			-0.010	-0.260	0.500	-0.560
58			0.170	0.940	0.210	-0.110
59			-0.090	0.050	0.740	-0.470
60			0.180	0.220	0.560	-0.660
		(n)	5	5	5	5
		Means	0.154	0.192	0.462	-0.392
		Sdevs	0.235	0.452	0.211	0.245
61	13		-0.120	0.040	-0.110	-0.820
62			0.480	0.010	0.110	-0.390
63			0.360	-0.040	0.310	-0.120
64			0.280	0.000	-0.130	-0.680
65			0.110	0.040	0.210	0.270
		(n)	5	5	5	5
		Means	0.222	0.010	0.078	-0.348
		Sdevs	0.234	0.033	0.194	0.438

Note: Data for Exposure phase

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Building 630/2
MOUSE/SWISS WEBSTER

Animal weight gains per day in (g)
Study number: TOX210B

Dosing start date: 16-Apr-08

PRINTED: 21-Nov-08
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FEEDING STUDY/PALATABILITY

			Day of Phase												
Animal	Group		6!	1"	2	6	7	8	9	10	11	12	13	14	15
			Male Animals												
66	1		0.567	0.380	1.340	0.345	0.070	-0.260	0.310	0.260	0.100	-0.090	0.480	0.370	-0.610
67			0.460	-0.015	0.570	0.130	0.510	-0.080	0.050	0.160	0.290	0.130	0.020	0.320	-0.160
68			0.417	0.100	1.000	0.135	0.280	-0.380	0.100	0.020	-0.160	0.140	0.300	1.200	-0.570
69			0.807	0.435	0.520	0.318	0.370	0.140	0.330	0.390	0.030	0.580	0.240	0.500	0.520
70			0.477	-0.135	0.420	0.065	0.960	-0.020	0.030	0.110	0.440	0.410	0.100	0.500	-0.230
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	0.546	0.153	0.770	0.199	0.438	-0.120	0.164	0.188	0.140	0.234	0.228	0.578	-0.210
		Sdevs	0.156	0.248	0.388	0.125	0.333	0.204	0.145	0.142	0.232	0.262	0.179	0.357	0.454
71	2		0.613	0.395	0.700	0.410	0.350	-0.530	0.570	0.580	0.240	0.590	0.320	0.330	0.230
72			0.420	0.265	0.300	0.378	0.890	-0.150	0.470	0.210	0.040	1.260	0.050	0.670	0.240
73			0.827	0.020	0.880	0.253	0.540	-0.230	-0.160	0.440	0.060	0.740	-0.020	0.410	-0.390
74			0.670	0.175	0.850	0.240	0.280	-0.220	0.390	0.270	-0.140	0.560	0.210	0.660	0.230
75			0.300	0.655	0.740	0.182	0.480	0.620	0.400	0.650	0.140	0.570	0.340	0.380	-0.210
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	0.566	0.302	0.694	0.293	0.508	-0.102	0.334	0.430	0.068	0.744	0.180	0.490	0.020
		Sdevs	0.208	0.240	0.233	0.097	0.237	0.429	0.285	0.190	0.140	0.298	0.160	0.162	0.299
76	3		0.433	0.045	0.870	0.263	0.100	-0.030	0.440	0.430	0.060	0.540	0.050	0.160	-0.320
77			0.880	-0.085	0.750	0.273	0.600	-0.510	0.350	0.080	-0.170	0.390	0.260	0.400	0.050
78			0.573	-0.110	0.690	0.153	-0.080	-0.170	0.170	0.230	-0.430	0.650	0.150	0.740	0.070
79			0.523	0.250	-0.090	0.302	0.350	-0.380	0.130	0.080	0.000	0.780	-0.240	0.900	-0.710
80			0.523	-0.110	0.520	0.138	0.520	-0.590	0.180	0.570	-0.290	0.350	-0.600	0.610	-0.340
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	0.586	-0.002	0.548	0.226	0.298	-0.336	0.254	0.278	-0.166	0.542	-0.076	0.562	-0.250
		Sdevs	0.172	0.155	0.378	0.075	0.285	0.233	0.134	0.217	0.202	0.179	0.347	0.290	0.323
81	4		0.600	0.490	-0.170	0.395	0.180	-0.410	0.210	-0.060	-0.070	0.580	-0.040	0.250	-0.120
82			0.420	-0.130	0.490	0.305	-0.250	-0.770	0.200	0.300	-0.010	0.680	0.350	-0.450	-0.220
83			0.353	-0.075	0.510	0.475	-0.640	-0.060	1.080	-0.450	0.160	0.020	-0.160	0.230	0.030
84			0.680	0.335	0.640	0.237	0.470	-0.650	0.230	0.320	-0.210	0.770	0.230	-0.340	-0.400
85			0.407	0.420	0.430	0.440	0.050	-0.450	0.150	0.090	-0.270	0.670	0.640	-0.580	-0.240
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	0.492	0.208	0.380	0.370	-0.038	-0.468	0.374	0.040	-0.080	0.544	0.204	-0.178	-0.190
		Sdevs	0.140	0.289	0.317	0.098	0.424	0.271	0.396	0.316	0.170	0.301	0.318	0.391	0.159

Note: ! = Quarantine/Acclimation; " = Exposure phase

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Building 630/2
MOUSE/SWISS WEBSTER

Animal weight gains per day in (g)
Study number: TOX210B
Dosing start date: 16-Apr-08

PRINTED: 21-Nov-08
Page: 2
FEEDING STUDY/PALATABILITY

Animal	Group		Day of Phase 16	17	18	19
			Male Animals			
66	1		0.320	0.170	0.680	-0.100
67			0.300	-0.090	0.360	0.210
68			-0.590	0.560	-0.090	0.080
69			-0.230	-0.030	0.220	0.540
70			0.140	-0.040	0.200	0.320
		(n)	5	5	5	5
		Means	-0.012	0.114	0.274	0.210
		Sdevs	0.391	0.268	0.280	0.242
71	2		-0.110	0.320	1.130	-0.180
72			0.230	0.680	-0.490	1.060
73			0.230	0.170	0.060	0.270
74			0.090	-0.340	0.220	0.570
75			0.360	-0.060	-0.120	0.740
		(n)	5	5	5	5
		Means	0.160	0.154	0.160	0.492
		Sdevs	0.179	0.385	0.603	0.472
76	3		0.580	0.200	-0.350	0.870
77			0.560	-0.280	0.000	0.280
78			0.500	0.140	0.160	0.360
79			0.670	0.120	0.060	0.360
80			0.870	0.060	-0.240	0.050
		(n)	5	5	5	5
		Means	0.636	0.048	-0.074	0.384
		Sdevs	0.144	0.190	0.213	0.300
81	4		1.070	-0.700	0.750	0.060
82			0.250	-0.450	0.060	0.130
83			0.330	-0.150	0.720	-0.340
84			0.040	-0.030	-0.640	0.280
85			0.110	0.290	-0.580	0.340
		(n)	5	5	5	5
		Means	0.360	-0.208	0.062	0.094
		Sdevs	0.413	0.382	0.673	0.267

Note: Data for Exposure phase

Appendix VIII

Data Used for Preparation of Body Weight Figures

Tobacco Blend

Body Weight (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	0.2 (2A)	SD	2.0 (3A)	SD	4.0 (4A)	SD	8.0 (5A)	SD	20.0 (6A)	SD	40.0 (7A)	SD
0	27.33	3.40	26.61	2.26	28.31	3.35	27.85	2.05	27.81	1.51	28.20	2.61	28.57	2.22
1	27.80	3.51	27.33	2.52	28.71	3.43	28.34	2.23	28.32	1.42	28.70	2.79	28.65	2.14
2	28.39	3.55	28.07	2.59	29.18	3.51	28.93	2.20	28.65	1.49	28.93	2.80	28.82	1.98
3	28.79	3.78	28.35	2.54	29.40	3.76	29.18	2.46	28.75	1.40	29.32	2.91	29.02	2.13
4	28.48	3.88	28.01	2.45	29.23	3.66	29.13	2.33	28.62	1.38	28.99	2.97	29.00	1.97
5	28.71	3.88	28.40	2.76	29.31	3.63	29.82	2.26	28.90	1.43	29.19	3.01	28.97	2.13
6	29.16	4.10	28.92	2.56	29.84	3.68	30.20	2.39	29.34	1.52	29.48	3.08	29.48	2.01
7	29.20	4.27	28.73	2.50	29.61	3.60	30.18	2.65	29.21	1.62	29.40	3.06	29.20	1.94
8	29.64	4.37	29.38	2.52	30.36	3.53	30.87	2.86	29.41	1.36	29.75	3.01	29.68	2.09
9	29.51	4.52	29.20	2.55	30.11	3.54	30.99	2.93	29.35	1.44	29.91	3.06	29.85	2.17
10	29.70	4.79	29.45	2.53	30.34	3.78	31.36	3.20	29.41	1.44	30.00	3.12	29.97	2.13
11	29.89	4.63	29.91	2.69	30.61	3.74	31.44	3.00	29.41	1.43	30.05	3.19	29.77	2.05
12	30.16	4.86	30.15	2.71	31.03	3.95	31.65	3.12	29.85	1.40	30.47	3.23	30.01	2.05
13	30.40	4.96	30.38	2.63	31.13	4.08	31.60	3.12	29.85	1.46	30.40	3.07	29.95	2.12

Tobacco Extract

Body Weight (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	0.2 (8A)	SD	2.0 (9A)	SD	4.0 (10A)	SD	8.0 (11A)	SD	20.0 (12A)	SD	40.0 (13A)	SD
0	27.33	3.40	28.90	3.01	29.98	3.09	29.40	2.17	28.27	2.21	28.29	3.46	28.57	2.38
1	27.80	3.51	29.37	3.03	30.36	2.94	29.56	2.05	28.47	2.26	28.64	3.45	28.71	2.56
2	28.39	3.55	29.88	3.10	30.95	3.10	29.99	2.08	28.97	2.43	29.00	3.45	29.25	2.68
3	28.79	3.78	30.17	3.18	31.32	3.05	30.39	2.21	29.21	2.39	29.43	3.35	29.56	2.82
4	28.48	3.88	29.99	3.27	31.18	3.41	30.20	2.27	28.91	2.25	29.22	3.38	29.28	2.60
5	28.71	3.88	30.24	3.39	31.45	3.24	30.54	2.08	29.13	2.39	29.45	3.41	29.75	2.72
6	29.16	4.10	30.72	3.23	31.89	3.41	31.24	2.02	29.43	2.38	29.88	3.38	30.34	2.49
7	29.20	4.27	30.56	3.22	31.72	3.76	30.99	2.34	29.24	2.47	29.57	3.61	30.04	2.60
8	29.64	4.37	30.96	3.35	32.45	3.72	31.58	2.18	29.87	2.45	30.18	3.31	30.71	2.84
9	29.51	4.52	31.16	3.45	32.51	3.38	31.69	2.42	29.95	2.44	30.33	3.21	30.80	2.77
10	29.70	4.79	31.50	3.38	32.76	3.34	32.55	2.81	30.82	2.01	30.48	3.40	31.02	2.67
11	29.89	4.63	31.77	3.53	32.90	3.49	32.15	2.77	30.39	2.67	30.67	3.63	31.03	2.69
12	30.16	4.86	31.96	3.44	33.44	3.21	32.60	2.97	31.05	2.80	31.13	3.44	31.11	2.75
13	30.40	4.96	31.78	3.63	33.41	3.30	32.37	2.79	30.61	2.65	30.74	3.58	30.76	3.10

Nicotine Hydrogen Tartrate

Body Weight (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	2.0 (1B)	SD	8.0 (2B)	SD	20.0 (3B)	SD	40.0 (4B)	SD
0	27.33	3.40	29.54	1.86	30.29	1.18	28.91	1.09	30.49	1.10
1	27.80	3.51	29.98	1.62	30.80	1.15	29.21	0.99	30.45	1.33
2	28.39	3.55	29.86	1.68	30.70	0.93	28.87	1.06	29.98	1.34
3	28.79	3.78	30.02	1.81	31.03	1.07	29.12	1.13	30.35	1.30
4	28.48	3.88	30.21	1.93	31.46	1.17	29.40	1.21	30.39	1.28
5	28.71	3.88	30.35	1.81	31.53	1.30	29.24	1.15	30.31	1.17
6	29.16	4.10	30.58	1.92	32.27	1.30	29.78	1.07	30.86	1.23
7	29.20	4.27	30.81	2.00	32.45	1.37	29.70	1.27	31.06	1.42
8	29.64	4.37	31.39	1.98	32.94	1.29	30.26	1.08	30.88	1.24
9	29.51	4.52	31.18	2.28	32.96	1.44	30.01	1.35	30.69	1.18
10	29.70	4.79	31.17	2.17	33.12	1.35	30.65	1.26	31.05	1.15
11	29.89	4.63	31.28	2.13	33.28	1.62	30.70	1.26	30.85	1.37
12	30.16	4.86	31.55	2.17	33.44	1.88	30.62	1.26	30.91	1.23
13	30.40	4.96	31.76	2.28	33.93	1.73	31.01	1.39	31.00	1.25

Tobacco Blend

Body Weight Gain (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	0.2 (2A)	SD	2.0 (3A)	SD	4.0 (4A)	SD	8.0 (5A)	SD	20.0 (6A)	SD	40.0 (7A)	SD
0	0.00		0.00		0.00		0.00		0.00		0.00		0.00	
1	0.48	0.27	0.72	0.37	0.40	0.25	0.49	0.23	0.51	0.27	0.50	0.21	0.07	0.28
2	1.07	0.28	1.46	0.47	0.87	0.23	1.08	0.34	0.83	0.45	0.74	0.32	0.24	0.43
3	1.46	0.46	1.74	0.47	1.10	0.50	1.33	0.65	0.93	0.33	1.12	0.35	0.45	0.41
4	1.15	0.62	1.40	0.38	0.93	0.44	1.28	0.63	0.81	0.28	0.79	0.37	0.43	0.47
5	1.38	0.68	1.79	0.62	1.01	0.53	1.97	0.78	1.09	0.14	1.00	0.51	0.40	0.41
6	1.84	0.96	2.31	0.42	1.53	0.53	2.35	0.73	1.52	0.26	1.28	0.57	0.91	0.47
7	1.88	1.16	2.12	0.41	1.31	0.52	2.32	0.95	1.40	0.23	1.21	0.59	0.63	0.66
8	2.31	1.19	2.77	0.57	2.06	0.75	3.02	1.19	1.59	0.29	1.55	0.68	1.10	0.43
9	2.18	1.33	2.59	0.55	1.81	0.72	3.14	1.27	1.54	0.28	1.72	0.67	1.27	0.66
10	2.37	1.57	2.83	0.59	2.03	0.78	3.51	1.37	1.59	0.26	1.80	0.81	1.40	0.72
11	2.56	1.38	3.30	0.74	2.30	0.83	3.59	1.18	1.60	0.33	1.85	0.96	1.20	0.80
12	2.83	1.62	3.54	0.76	2.73	1.05	3.79	1.22	2.03	0.47	2.27	0.91	1.44	0.74
13	3.08	1.69	3.76	0.63	2.82	1.07	3.75	1.24	2.04	0.50	2.20	0.79	1.38	0.57

Tobacco Extract

Body Weight Gain (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	0.2 (8A)	SD	2.0 (9A)	SD	4.0 (10A)	SD	8.0 (11A)	SD	20.0 (12A)	SD	40.0 (13A)	SD
0	0.00		0.00		0.00		0.00		0.00		0.00		0.00	
1	0.48	0.27	0.47	0.12	0.37	0.26	0.16	0.39	0.20	0.14	0.34	0.27	0.14	0.49
2	1.07	0.28	0.99	0.17	0.97	0.18	0.59	0.33	0.69	0.24	0.71	0.33	0.68	0.50
3	1.46	0.46	1.28	0.21	1.34	0.20	0.98	0.29	0.94	0.31	1.14	0.37	0.99	0.61
4	1.15	0.62	1.09	0.52	1.20	0.61	0.80	0.40	0.64	0.17	0.93	0.33	0.70	0.48
5	1.38	0.68	1.34	0.72	1.47	0.54	1.14	0.50	0.85	0.24	1.16	0.46	1.17	0.56
6	1.84	0.96	1.82	0.51	1.91	0.46	1.84	0.57	1.16	0.36	1.59	0.36	1.76	0.52
7	1.88	1.16	1.66	0.36	1.74	0.85	1.59	0.73	0.96	0.29	1.28	0.69	1.46	0.62
8	2.31	1.19	2.06	0.62	2.47	0.73	2.17	1.04	1.60	0.42	1.89	0.90	2.14	0.93
9	2.18	1.33	2.27	0.58	2.53	0.36	2.29	1.03	1.67	0.40	2.03	0.94	2.22	0.91
10	2.37	1.57	2.60	0.55	2.78	0.35	3.15	1.04	2.54	1.08	2.19	0.83	2.45	0.94
11	2.56	1.38	2.87	0.71	2.92	0.44	2.75	1.51	2.12	1.91	2.38	1.11	2.46	0.94
12	2.83	1.62	3.06	0.58	3.46	0.18	3.19	1.65	2.78	2.00	2.84	1.09	2.53	1.11
13	3.08	1.69	2.88	0.78	3.43	0.43	2.97	1.62	2.33	1.76	2.45	1.06	2.19	1.50

Nicotine Hydrogen Tartrate

Body Weight Gain (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	2.0 (1B)	SD	8.0 (2B)	SD	20.0 (3B)	SD	40.0 (4B)	SD
0	0.00		0.00		0.00		0.00		0.00	
1	0.48	0.27	0.44	0.33	0.51	0.24	0.30	0.29	-0.04	0.42
2	1.07	0.28	0.32	0.47	0.41	0.52	-0.04	0.14	-0.51	0.35
3	1.46	0.46	0.48	0.43	0.74	0.58	0.22	0.25	-0.13	0.45
4	1.15	0.62	0.67	0.50	1.17	0.63	0.49	0.34	-0.09	0.32
5	1.38	0.68	0.81	0.66	1.24	0.70	0.33	0.46	-0.17	0.31
6	1.84	0.96	1.04	0.86	1.98	0.78	0.87	0.43	0.37	0.34
7	1.88	1.16	1.27	0.77	2.16	0.83	0.79	0.54	0.58	0.47
8	2.31	1.19	1.85	0.65	2.65	0.82	1.36	0.39	0.40	0.32
9	2.18	1.33	1.64	1.03	2.67	0.91	1.11	0.45	0.21	0.28
10	2.37	1.57	1.63	1.04	2.83	0.97	1.74	0.31	0.57	0.60
11	2.56	1.38	1.74	0.84	2.99	1.17	1.79	0.33	0.36	0.46
12	2.83	1.62	2.02	0.86	3.15	1.05	1.72	0.38	0.42	0.89
13	3.08	1.69	2.23	1.06	3.64	1.21	2.10	0.60	0.52	0.75

Tobacco Blend

Feed Consumption (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	0.2 (2A)	SD	2.0 (3A)	SD	4.0 (4A)	SD	8.0 (5A)	SD	20.0 (6A)	SD	40.0 (7A)	SD
0														
1	5.54	0.84	5.76	0.86	5.66	0.52	5.82	0.36	5.52	0.35	5.92	0.78	5.12	0.42
2	6.33	0.91	6.95	0.79	6.42	0.95	6.45	0.63	6.11	0.56	6.36	0.68	6.50	0.52
3	6.31	1.10	6.96	0.70	6.47	1.01	6.29	0.99	6.03	0.80	7.06	0.63	6.25	0.96
4	6.73	0.93	6.84	0.76	6.62	1.19	6.85	0.89	8.10	1.04	7.30	1.20	7.42	1.34
5	5.87	1.02	5.94	1.40	5.59	0.30	6.18	0.65	6.14	0.90	5.75	0.83	6.40	1.59
6	6.23	0.78	7.00	0.39	7.12	0.85	7.22	0.66	7.32	0.71	6.80	1.26	6.29	0.55
7	6.24	0.83	6.64	0.98	7.10	1.11	6.85	0.64	6.81	1.00	6.75	1.11	6.28	1.44
8	6.22	0.83	6.73	1.46	6.13	0.61	6.89	0.63	5.89	0.76	5.59	0.45	5.88	0.65
9	7.08	0.55	7.72	0.69	6.92	0.32	6.15	0.57	5.37	0.30	5.86	0.72	5.58	0.40
10	7.21	0.78	8.81	2.28	8.96	1.17	6.61	1.33	5.81	0.50	7.12	1.51	6.90	1.59
11	6.05	0.61	6.91	0.59	6.04	0.78	5.65	0.91	5.84	1.05	6.38	1.03	5.65	0.72
12	7.29	0.80	7.59	0.94	8.31	1.32	6.98	1.31	6.99	1.15	6.40	0.51	5.89	0.50
13	6.34	0.62	7.01	1.35	6.82	1.11	7.45	1.34	6.10	0.50	8.26	1.26	6.85	1.20
14	5.77	0.53	7.43	2.05	7.66	1.12	6.51	0.56	7.74	1.12	8.08	1.71	6.82	0.68

Tobacco Extract

Feed Consumption (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	0.2 (8A)	SD	2.0 (9A)	SD	4.0 (10A)	SD	8.0 (11A)	SD	20.0 (12A)	SD	40.0 (13A)	SD
0														
1	5.54	0.84	5.56	0.50	6.48	1.20	5.68	0.51	5.43	0.54	6.02	0.36	5.74	0.60
2	6.33	0.91	6.39	0.50	6.47	0.65	6.24	0.61	5.50	0.40	5.66	0.55	5.72	0.63
3	6.31	1.10	6.05	0.62	7.00	1.03	6.77	1.66	6.18	0.67	6.29	0.36	7.13	0.33
4	6.73	0.93	6.85	0.70	7.50	1.26	7.30	0.70	6.71	0.74	7.96	0.55	6.96	0.35
5	5.87	1.02	6.43	1.32	6.58	1.17	6.14	0.54	5.22	0.96	5.45	1.06	6.29	0.43
6	6.23	0.78	6.50	0.30	7.12	0.77	6.92	0.89	5.99	0.75	6.61	0.91	7.04	0.46
7	6.24	0.83	6.85	0.93	5.85	0.93	5.83	0.54	5.44	0.49	5.85	1.03	6.25	0.38
8	6.22	0.83	6.41	0.97	7.02	0.39	6.80	0.94	6.47	0.44	6.23	0.61	6.67	0.45
9	7.08	0.55	5.87	0.55	6.49	0.79	6.11	0.60	5.97	0.42	6.37	0.70	5.92	0.32
10	7.21	0.78	6.29	0.50	6.77	0.43	6.52	0.65	7.05	1.07	6.63	0.28	7.30	1.43
11	6.05	0.61	6.34	0.65	6.76	1.53	6.05	0.94	6.15	0.85	6.46	1.13	6.09	0.68
12	7.29	0.80	5.77	0.74	6.43	0.46	6.60	0.75	6.57	0.95	6.34	1.06	7.17	1.56
13	6.34	0.62	6.86	0.55	7.28	0.45	7.65	0.92	8.03	1.70	7.13	1.00	6.26	0.99
14	5.77	0.53	7.09	0.97	7.20	1.66	6.87	1.01	7.89	2.53	6.71	0.92	6.88	1.31

Nicotine Hydrogen Tartrate

Feed Consumption (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	2.0 (1B)	SD	8.0 (2B)	SD	20.0 (3B)	SD	40.0 (4B)	SD
0										
1	5.54	0.84	5.55	0.44	5.64	0.33	5.46	0.82	4.96	0.57
2	6.33	0.91	6.97	0.64	7.27	1.09	6.35	0.89	4.99	2.83
3	6.31	1.10	7.47	1.14	6.80	1.09	6.31	0.55	6.79	0.87
4	6.73	0.93	6.73	1.85	5.74	0.76	5.18	0.53	5.60	0.64
5	5.87	1.02	6.94	0.47	7.86	1.47	7.00	1.83	6.88	1.02
6	6.23	0.78	5.42	1.84	7.57	1.34	6.12	0.73	5.62	1.60
7	6.24	0.83	5.80	0.67	6.35	0.34	5.94	0.68	6.03	0.72
8	6.22	0.83	5.81	0.50	6.06	0.82	6.23	4.27	4.71	0.59
9	7.08	0.55	6.24	0.73	7.00	0.87	7.12	1.45	6.26	0.78
10	7.21	0.78	6.71	1.05	7.42	1.04	6.70	0.59	5.68	0.80
11	6.05	0.61	6.05	0.47	6.54	1.01	6.87	0.85	5.76	1.23
12	7.29	0.80	7.31	0.31	8.29	1.23	9.42	0.68	6.55	0.98
13	6.34	0.62	5.73	0.37	6.34	0.63	6.30	1.85	6.03	0.96
14	5.77	0.53	6.49	1.00	6.70	0.32	6.15	1.12	6.23	1.15

Tobacco Blend

Percent Body Weight Gain (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	0.2 (2A)	SD	2.0 (3A)	SD	4.0 (4A)	SD	8.0 (5A)	SD	20.0 (6A)	SD	40.0 (7A)	SD
0	0.00		0.00		0.00		0.00		0.00		0.00		0.00	
1	1.76	0.27	2.71	0.37	1.41	0.25	1.76	0.23	1.83	0.27	1.77	0.21	0.25	0.28
2	3.92	0.28	5.49	0.47	3.07	0.23	3.88	0.34	2.98	0.45	2.62	0.32	0.84	0.43
3	5.34	0.46	6.54	0.47	3.89	0.50	4.78	0.65	3.34	0.33	3.97	0.35	1.58	0.41
4	4.21	0.62	5.26	0.38	3.29	0.44	4.60	0.63	2.91	0.28	2.80	0.37	1.51	0.47
5	5.05	0.68	6.73	0.62	3.57	0.53	7.07	0.78	3.92	0.14	3.55	0.51	1.40	0.41
6	6.73	0.96	8.68	0.42	5.40	0.53	8.44	0.73	5.47	0.26	4.54	0.57	3.19	0.47
7	6.88	1.03	7.97	0.52	4.63	0.64	8.33	0.94	5.03	0.19	4.29	0.63	2.21	0.51
8	8.45	1.16	10.41	0.53	7.28	0.70	10.84	1.05	5.72	0.16	5.50	0.70	3.85	0.53
9	7.98	1.30	9.73	0.55	6.39	0.77	11.27	1.16	5.54	0.13	6.10	0.76	4.45	0.56
10	8.67	1.44	10.64	0.57	7.17	0.83	12.60	1.27	5.72	0.10	6.38	0.83	4.90	0.59
11	9.37	1.58	12.40	0.59	8.12	0.89	12.89	1.37	5.75	0.07	6.56	0.90	4.20	0.62
12	10.35	1.71	13.30	0.60	9.64	0.96	13.61	1.48	7.30	0.04	8.05	0.97	5.04	0.64
13	11.27	1.85	14.13	0.62	9.96	1.02	13.46	1.59	7.34	0.01	7.80	1.04	4.83	0.67

Tobacco Extract

Percent Body Weight Gain (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	0.2 (8A)	SD	2.0 (9A)	SD	4.0 (10A)	SD	8.0 (11A)	SD	20.0 (12A)	SD	40.0 (13A)	SD
0	0.00		0.00		0.00		0.00		0.00		0.00		0.00	
1	1.76	0.27	1.63	0.12	1.23	0.26	0.54	0.39	0.71	0.14	1.20	0.27	0.49	0.49
2	3.92	0.28	3.43	0.17	3.24	0.18	2.01	0.33	2.44	0.24	2.51	0.33	2.38	0.50
3	5.34	0.46	4.43	0.21	4.47	0.20	3.33	0.29	3.33	0.31	4.03	0.37	3.47	0.61
4	4.21	0.62	3.77	0.52	4.00	0.61	2.72	0.40	2.26	0.17	3.29	0.33	2.45	0.48
5	5.05	0.68	4.64	0.72	4.90	0.54	3.88	0.50	3.01	0.24	4.10	0.46	4.10	0.56
6	6.73	0.96	6.30	0.51	6.37	0.46	6.26	0.57	4.10	0.36	5.62	0.36	6.16	0.52
7	6.88	1.16	5.74	0.36	5.80	0.85	5.41	0.73	3.40	0.29	4.52	0.69	5.11	0.62
8	8.45	1.19	7.13	0.62	8.24	0.73	7.38	1.04	5.66	0.42	6.68	0.90	7.49	0.93
9	7.98	1.37	7.85	0.70	8.44	0.87	7.79	0.93	5.91	0.40	7.18	0.80	7.77	0.78
10	8.67	1.51	9.00	0.77	9.27	0.96	10.71	1.02	8.98	0.43	7.74	0.88	8.58	0.82
11	9.37	1.66	9.93	0.83	9.74	1.05	9.35	1.11	7.50	0.46	8.41	0.95	8.61	0.86
12	10.35	1.81	10.59	0.90	11.54	1.14	10.85	1.20	9.83	0.49	10.04	1.03	8.86	0.90
13	11.27	1.96	9.97	0.97	11.44	1.22	10.10	1.29	8.24	0.52	8.66	1.10	7.67	0.94

Nicotine Hydrogen Tartrate

Percent Body Weight Gain (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	2.0 (1B)	SD	8.0 (2B)	SD	20.0 (3B)	SD	40.0 (4B)	SD
0	0.00		0.00		0.00		0.00		0.00	
1	1.76	0.27	1.49	0.33	1.68	0.24	1.04	0.29	-0.13	0.42
2	3.92	0.28	1.08	0.47	1.35	0.52	-0.14	0.14	-1.67	0.35
3	5.34	0.46	1.62	0.43	2.44	0.58	0.76	0.25	-0.43	0.45
4	4.21	0.62	2.27	0.50	3.86	0.63	1.69	0.34	-0.30	0.32
5	5.05	0.68	2.74	0.66	4.09	0.70	1.14	0.46	-0.56	0.31
6	6.73	0.96	3.52	0.86	6.54	0.78	3.01	0.43	1.21	0.34
7	6.88	1.03	4.30	0.87	7.13	0.90	2.73	0.49	1.90	0.30
8	8.45	1.16	6.26	0.96	8.75	1.00	4.70	0.54	1.31	0.28
9	7.98	1.30	5.55	1.06	8.81	1.09	3.84	0.59	0.69	0.26
10	8.67	1.44	5.52	1.15	9.34	1.19	6.02	0.64	1.87	0.24
11	9.37	1.58	5.89	1.25	9.87	1.28	6.19	0.69	1.18	0.23
12	10.35	1.71	6.84	1.34	10.40	1.37	5.95	0.74	1.38	0.21
13	11.27	1.85	7.55	1.43	12.02	1.47	7.26	0.79	1.71	0.19

Appendix IX

Terminal Body Weights

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
MOUSE/SWISS WEBSTER

Mean Animal Body Weights in (g)
Study number: TOX210A

Dosing start date: 16-Apr-08

PRINTED: 21-Oct-08
Page: 1

FEEDING STUDY/PALATABILITY

Group(s)		Day of Phase 19
		Male Animals
1	(N)	5
	Means	30.40
	Sdevs	4.96
2	(N)	5
	Means	30.38
	Sdevs	2.63
3	(N)	5
	Means	31.13
	Sdevs	4.08
4	(N)	5
	Means	31.60
	Sdevs	3.12
5	(N)	5
	Means	29.85
	Sdevs	1.46
6	(N)	5
	Means	30.40
	Sdevs	3.07
7	(N)	5
	Means	29.95
	Sdevs	2.12
8	(N)	5
	Means	31.78
	Sdevs	3.63
9	(N)	5
	Means	33.41
	Sdevs	3.30
10	(N)	5
	Means	32.37
	Sdevs	2.79
11	(N)	5
	Means	30.61
	Sdevs	2.65

R.J.R. TOBACCO | Mean Animal Body Weights in (g) | PRINTED: 21-Oct-08
TOXICOLOGY DIVISION | Study number: TOX210A | Page: 2
Building 630/2
MOUSE/SWISS WEBSTER | Dosing start date: 16-Apr-08 | FEEDING STUDY/PALATABILITY

Group(s)		Day of Phase 19
12	(N)	5
	Means	30.74
	Sdevs	3.58
13	(N)	5
	Means	30.76
	Sdevs	3.10

Note: Data for Exposure phase
*(+) = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance
%($) = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

Group(s)		Day of Phase 19
		Male Animals
1	(N)	5
	Means	31.76
	Sdevs	2.28
2	(N)	5
	Means	33.93
	Sdevs	1.73
3	(N)	5
	Means	31.01
	Sdevs	1.39
4	(N)	5
	Means	31.00
	Sdevs	1.25
5	(N)	
	Means	
	Sdevs	

Note: Data for Exposure phase
*(+) = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance
%($) = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

Appendix X

Feed Consumption

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
MOUSE/SWISS WEBSTER

Individual Animal Feed Consumed/day (g)
Study number: TOX210A

Dosing start date: 16-Apr-08

PRINTED: 21-Nov-08
Page: 1

FEEDING STUDY/PALATABILITY

Animal	Group		Day of Phase 7	8	9	10	11	12	13	14	15	16	17	18	19
			Male Animals												
1	1		4.85	5.94	6.48	6.99	5.87	5.96	6.45	5.68	6.93	6.33	6.30	6.55	6.24
2			6.35	7.20	7.17	7.17	7.12	7.36	7.31	7.20	7.56	7.30	6.91	7.30	7.34
3			5.38	5.94	6.53	6.57	6.20	6.47	6.22	6.93	7.72	6.98	5.86	6.82	5.66
4			6.47	7.34	6.98	7.68	5.88	6.17	6.21	6.09	6.40		5.93	7.17	6.38
5			4.66	5.22	4.41	5.23	4.28	5.21	4.99	5.22	6.81	8.21	5.25	8.62	6.06
		(n)	5	5	5	5	5	5	5	5	5	4	5	5	5
		Means	5.54	6.33	6.31	6.73	5.87	6.23	6.24	6.22	7.08	7.21	6.05	7.29	6.34
		Sdevs	0.84	0.91	1.10	0.93	1.02	0.78	0.83	0.83	0.55	0.78	0.61	0.80	0.62
6	2		6.28	5.95	7.34	7.58	7.32	6.93	6.73	7.85	7.67	11.78	7.68	8.53	7.15
7			4.49	6.70	7.59	6.21	7.58	7.31	6.04	6.16	7.47	8.88	6.43	6.44	5.96
8			6.29		7.51	7.74	4.99	7.43	7.18	8.68	8.28		7.41		9.18
9			5.96	7.62	6.04	6.22	5.19	6.45	7.89	5.53	8.45	8.33	6.66	8.14	6.97
10				7.54	6.34	6.45	4.62	6.86	5.36	5.44	6.73	6.25	6.39	7.23	5.79
		(n)	4	4	5	5	5	5	5	5	5	4	5	4	5
		Means	5.76	6.95	6.96	6.84	5.94	7.00	6.64	6.73	7.72	8.81	6.91	7.59	7.01
		Sdevs	0.86	0.79	0.72	0.76	1.40	0.39	0.98	1.46	0.69	2.28	0.59	0.94	1.35
11	3		6.40	7.64	7.28	7.39	5.75	8.13	6.59	6.93	7.17	9.78	7.44	10.47	8.53
12				6.49	6.92	8.35	6.03	6.78	6.05	6.05	6.94	8.63	5.68	7.50	6.62
13			5.63	6.67	7.28	5.80	5.42	7.55	6.39	6.37	7.09	7.45	5.78	7.78	6.31
14			5.37	6.31	5.86	5.69	5.51	7.28	8.73	5.26	7.02	9.97	5.57	7.17	7.10
15			5.25	5.00	4.99	5.89	5.25	5.88	7.75	6.02	6.36		5.75	8.62	5.53
		(n)	4	5	5	5	5	5	5	5	5	4	5	5	5
		Means	5.66	6.42	6.47	6.62	5.59	7.12	7.10	6.13	6.92	8.96	6.04	8.31	6.82
		Sdevs	0.52	0.95	1.01	1.19	0.30	0.85	1.11	0.61	0.32	1.17	0.78	1.32	1.11
16	4		5.74	6.15	6.04	7.62	6.86	8.10	7.68	7.58	5.91	5.45	4.52	6.33	6.98
17			6.33	6.65	6.81	6.88	6.50	7.12	6.57	6.95	6.48	6.36	5.90	6.49	6.59
18			5.71	7.42	7.75	7.78	6.45	6.59	6.55	7.40	6.81	8.91	6.71	7.12	9.83
19			5.97	6.30	5.36	6.32	5.88	6.64	6.12	6.16	6.23	6.19	5.46	5.80	6.87
20			5.36	5.73	5.51	5.65	5.21	7.67	7.34	6.35	5.32	6.14		9.16	6.98
		(n)	5	5	5	5	5	5	5	5	5	5	4	5	5
		Means	5.82	6.45	6.29	6.85	6.18	7.22	6.85	6.89	6.15	6.61	5.65	6.98	7.45
		Sdevs	0.36	0.63	0.99	0.89	0.65	0.66	0.64	0.63	0.57	1.33	0.91	1.31	1.34
21	5		5.29	5.78	6.85	9.12	7.44	7.73	7.41	5.70	4.93	5.61	4.63	6.83	6.06
22			5.39	6.54	6.69	8.49	5.90	6.67	7.30	5.19	5.59	5.64	5.50	6.26	5.94
23			5.14	5.30	4.88	8.22	6.52	7.91	5.81	5.34	5.51	5.70	7.47	7.80	6.79
24			5.85	6.64	6.04	8.34	5.04	6.43	5.66	7.09	5.18	6.68	6.09	8.45	11.57

Note: Data for Exposure phase

R.J.R. TOBACCO | Individual Animal Feed Consumed/day (g) | PRINTED: 21-Nov-08
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Animal	Group		7	8	9	10	11	12	13	14	15	16	17	18	19
							Day of Phase								
							Male Animals								
25	5		5.93	6.27	5.71	6.34	5.82	7.86	7.86	6.14	5.64	5.42	5.53	5.61	5.60
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	5.52	6.11	6.03	8.10	6.14	7.32	6.81	5.89	5.37	5.81	5.84	6.99	7.19
		Sdevs	0.35	0.56	0.80	1.04	0.90	0.71	1.00	0.76	0.30	0.50	1.05	1.15	2.49
26	6		7.14	6.96	8.18	9.28	5.82	6.19	6.08	5.38	5.71	6.17	5.77	6.49	6.96
27			5.37	5.83	6.65	6.71	6.62	6.52	7.60	6.17	5.66	6.20	6.98	6.23	7.81
28			5.17	5.50	6.73	6.63	4.64	6.89	5.55	5.55	4.91	9.77	7.39	5.90	
29			6.12	6.52	6.90	7.38	6.46	5.52	8.22	5.84	6.14	6.94	6.89	6.14	9.95
30			5.78	7.01	6.83	6.50	5.23	8.87	6.31	4.99	6.87	6.54	4.89	7.23	8.32
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	4
		Means	5.92	6.36	7.06	7.30	5.75	6.80	6.75	5.59	5.86	7.12	6.38	6.40	8.26
		Sdevs	0.78	0.68	0.63	1.16	0.83	1.26	1.11	0.45	0.72	1.51	1.03	0.51	1.26
31	7		5.56	6.15	5.63	6.16	4.62	5.51	5.35	5.15	5.06	5.08	4.84	5.37	5.00
32			5.12	5.87	5.59	6.94	5.81	6.31	5.87	6.63	6.02	6.82	5.65	5.91	6.43
33			5.11	7.24	6.08	6.97	6.85	7.07	5.96	5.79	5.59	6.08	5.40	5.64	7.07
34			4.44	6.63	6.03	9.70	8.87	6.36	8.81	6.44	5.91	7.15	5.55	6.71	7.95
35			5.36	6.60	7.91	7.33	5.87	6.22	5.40	5.38	5.33	9.37	6.80	5.83	7.81
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	5.12	6.50	6.25	7.42	6.40	6.29	6.28	5.88	5.58	6.90	5.65	5.89	6.85
		Sdevs	0.42	0.52	0.96	1.34	1.59	0.55	1.44	0.65	0.40	1.59	0.72	0.50	1.20
36	8		4.95	5.79	5.86	5.94	8.08	6.29	8.27	5.15	4.97	5.62	6.06	4.77	6.09
37			5.99	7.09	6.07	7.95	5.71	6.43	6.80	6.02	6.23	6.65	6.95	6.50	7.40
38			6.06	6.78	7.11	6.84	5.01	6.53	6.70	7.74	5.75		5.52	5.31	7.00
39			5.65	6.25	5.59	6.48	7.57	6.25	5.65	6.91	6.07	6.20	6.11	5.84	
40			5.15	6.05	5.62	7.04	5.80	6.99	6.85	6.21	6.32	6.68	7.07	6.42	6.94
		(n)	5	5	5	5	5	5	5	5	5	4	5	5	4
		Means	5.56	6.39	6.05	6.85	6.43	6.50	6.85	6.41	5.87	6.29	6.34	5.77	6.86
		Sdevs	0.50	0.53	0.62	0.74	1.32	0.30	0.93	0.97	0.55	0.50	0.65	0.74	0.55
41	9		5.73	5.82	8.75	5.80	5.19	6.24	5.72	7.45	6.15		7.85	6.78	7.77
42			8.42	6.82	6.22	8.85	6.85	8.36	6.01	7.43	5.57	6.81	5.02	5.75	6.75
43			6.82	7.42	6.96	8.61	6.65	7.04	6.21	6.72	6.41	6.74	6.05	6.53	7.09
44			6.00	6.29	6.32	7.31	5.90	7.00	6.93	6.82	6.59	6.24	6.06	6.65	7.50
45			5.42	6.00	6.73	6.92	8.31	6.96	4.39	6.67	7.73	7.28	8.81		
		(n)	5	5	5	5	5	5	5	5	5	4	5	4	4
		Means	6.48	6.47	7.00	7.50	6.58	7.12	5.85	7.02	6.49	6.77	6.76	6.43	7.28
		Sdevs	1.20	0.65	1.03	1.26	1.17	0.77	0.93	0.39	0.79	0.43	1.53	0.46	0.45

Note: Data for Exposure phase

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Animal	Group		Day of Phase 7	8	9	10	11	12	13	14	15	16	17	18	19
							Male		Animals						
46	10		6.14	6.55	6.51	7.56		8.35	6.16	8.30	5.90	6.84	7.37	6.17	7.19
47			5.38	5.83	6.17	8.20	6.90	7.07	5.17	6.14	5.74	5.68	5.17	6.68	8.62
48			5.93	6.40	6.35	6.89	6.08	6.62	6.30	6.96	6.42	6.38	6.06	5.85	6.81
49			4.93	5.44	5.22	6.36	5.62	5.98	5.33	5.88	5.50	6.28	5.15	6.48	6.94
50			6.03	6.98	9.60	7.47	5.97	6.60	6.21	6.73	6.99	7.43	6.49	7.82	8.67
		(n)	5	5	5	5	4	5	5	5	5	5	5	5	5
		Means	5.68	6.24	6.77	7.30	6.14	6.92	5.83	6.80	6.11	6.52	6.05	6.60	7.65
		Sdevs	0.51	0.61	1.66	0.70	0.54	0.89	0.54	0.94	0.60	0.65	0.94	0.75	0.92
51	11		5.01	5.36	5.46	5.79	4.73	5.16	5.23	6.10	5.40	5.72	5.07	5.56	5.94
52			5.37	5.12	5.68	6.32	4.57	5.38	5.49	5.99	5.73	6.55	7.03	5.91	8.89
53			6.05	5.98	6.98	7.50	6.68	6.89		6.73	6.43	8.41	5.54	6.44	9.93
54			4.81	5.15	6.79	7.43	4.43	6.59	4.94	6.45	6.04	7.85	6.89	8.00	8.83
55			5.89	5.87	5.98	6.53	5.71	5.93	6.09	7.06	6.27	6.72	6.20	6.92	6.56
		(n)	5	5	5	5	5	5	4	5	5	5	5	5	5
		Means	5.43	5.50	6.18	6.71	5.22	5.99	5.44	6.47	5.97	7.05	6.15	6.57	8.03
		Sdevs	0.54	0.40	0.67	0.74	0.96	0.75	0.49	0.44	0.42	1.07	0.85	0.95	1.70
56	12		6.27	6.41	5.81	7.63	5.67	7.56	5.40	5.61	5.30	6.86	5.39	6.03	6.59
57			6.38	6.02	6.81	7.76	6.83	5.55	7.37	5.66	6.09	6.91	5.63	4.94	6.12
58			5.65	5.54	6.28	8.58	5.87	5.75	6.15	6.37	6.57	6.23	6.01	6.22	6.63
59			6.17	5.29	6.33	8.48	4.83	6.89	4.56	6.45	6.79	6.67	8.01	7.85	7.71
60			5.62	5.05	6.23	7.33	4.05	7.28	5.79	7.06	7.09	6.48	7.26	6.66	8.59
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	6.02	5.66	6.29	7.96	5.45	6.61	5.85	6.23	6.37	6.63	6.46	6.34	7.13
		Sdevs	0.36	0.55	0.36	0.55	1.06	0.91	1.03	0.61	0.70	0.28	1.13	1.06	1.00
61	13		6.03	5.88	7.39	6.60	5.96	7.67	5.94	6.54	5.57	5.44	5.32	5.79	5.34
62			5.86	5.78	7.37	7.36	6.67	7.00	6.32	6.43	5.97	7.00	7.10	7.97	7.31
63			5.00	4.73	6.68	6.70	6.85	7.28	6.69	6.90	6.39	8.77	5.82	6.69	6.14
64			5.30	5.71	7.08	7.29	5.95	6.45	6.51	6.17	5.70		5.81	9.50	
65			6.50	6.49		6.85	6.04	6.82	5.79	7.33	5.99	7.98	6.42	5.92	
		(n)	5	5	4	5	5	5	5	5	5	4	5	5	3
		Means	5.74	5.72	7.13	6.96	6.29	7.04	6.25	6.67	5.92	7.30	6.09	7.17	6.26
		Sdevs	0.60	0.63	0.33	0.35	0.43	0.46	0.38	0.45	0.32	1.43	0.68	1.56	0.99

Note: Data for Exposure phase

Animal	Group		Day of Phase 20
			Male Animals
1	1		6.17
2			5.93
3			6.22
4			4.93
5			5.62
		(n)	5
		Means	5.77
		Sdevs	0.53
6	2		5.20
7			6.60
8			9.41
9			6.17
10			9.79
		(n)	5
		Means	7.43
		Sdevs	2.05
11	3		8.98
12			7.85
13			5.87
14			7.83
15			7.76
		(n)	5
		Means	7.66
		Sdevs	1.12
16	4		5.99
17			6.19
18			6.65
19			6.31
20			7.41
		(n)	5
		Means	6.51
		Sdevs	0.56
21	5		7.70
22			6.13
23			7.54
24			8.05

Note: Data for Exposure phase

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FEEDING STUDY/PALATABILITY

Animal	Group		Day of Phase 20
			Male Animals
25	5		9.26
		(n)	5
		Means	7.74
		Sdevs	1.12
26	6		5.99
27			8.19
29			10.17
30			7.96
		(n)	4
		Means	8.08
		Sdevs	1.71
31	7		6.13
32			6.33
33			6.64
34			7.19
35			7.79
		(n)	5
		Means	6.82
		Sdevs	0.68
36	8		6.45
37			8.57
38			6.79
39			7.49
40			6.15
		(n)	5
		Means	7.09
		Sdevs	0.97
41	9		6.56
42			5.99
43			5.99
44			7.54
45			9.94
		(n)	5
		Means	7.20
		Sdevs	1.66

Note: Data for Exposure phase

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FEEDING STUDY/PALATABILITY

Animal	Group		Day of Phase 20
			Male Animals
46	10		8.58
47			6.15
48			6.18
49			6.90
50			6.52
		(n)	5
		Means	6.87
		Sdevs	1.01
51	11		6.05
52			5.73
53			10.25
54			11.02
55			6.39
		(n)	5
		Means	7.89
		Sdevs	2.53
56	12		6.65
57			6.59
58			7.39
59			5.28
60			7.64
		(n)	5
		Means	6.71
		Sdevs	0.92
61	13		5.58
62			6.07
63			7.36
64			8.49
		(n)	4
		Means	6.88
		Sdevs	1.31

Note: Data for Exposure phase

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Building 630/2
MOUSE/SWISS WEBSTER

Individual Animal Feed Consumed/day (g)
Study number: TOX210B

Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

Animal	Group		7	8	9	10	11	12	13	14	15	16	17	18	19
							Day of Phase								
							Male Animals								
66	1		5.49	6.06	6.73	4.84	6.38	5.77	5.27	5.42	5.61	5.63	6.57	7.34	5.49
67			5.44	7.01	8.89	7.02		6.50	5.44	5.41	7.19	7.72	6.01		5.52
68			5.55		7.71	9.71	6.90	6.46	5.93	6.59	5.97	7.48	6.18	7.13	5.73
69			6.25	7.49	8.05	6.28	7.54	6.18	6.92	6.03	6.82	5.54	5.28	7.04	5.53
70			5.02	7.31	5.97	5.79	6.95	2.17	5.45	5.60	5.60	7.19	6.19	7.74	6.36
		(n)	5	4	5	5	4	5	5	5	5	5	5	4	5
		Means	5.55	6.97	7.47	6.73	6.94	5.42	5.80	5.81	6.24	6.71	6.05	7.31	5.73
		Sdevs	0.44	0.64	1.14	1.85	0.47	1.84	0.67	0.50	0.73	1.05	0.47	0.31	0.37
71	2		5.94	6.30	6.36	5.85	7.30	6.85	6.11	6.29	7.53	7.35	6.17	8.91	6.05
72			5.81	9.08	7.45	6.02	10.24	9.90	6.09	7.25	8.06	9.15	8.26	9.93	6.68
73			5.26	6.58	5.48	4.95	6.90	6.84	6.36	4.99	5.75	7.06	6.57	8.26	5.44
74			5.88	7.03	6.43	5.07	6.60	7.50	6.25	5.91	6.82	6.34	5.79	6.66	7.08
75			5.29	7.38	8.30	6.82	8.27	6.74	6.92	5.88	6.85	7.19	5.90	7.69	6.43
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	5.64	7.27	6.80	5.74	7.86	7.57	6.35	6.06	7.00	7.42	6.54	8.29	6.34
		Sdevs	0.33	1.09	1.09	0.76	1.47	1.34	0.34	0.82	0.87	1.04	1.01	1.23	0.63
76	3		6.63	6.14	6.77	5.85	7.32	5.95	5.12		6.51	6.19	7.80	9.47	9.02
77			5.94	6.90	6.47	4.76	3.81	6.46	6.63	2.81	9.56	7.10	7.33		5.46
78			5.08	7.45	6.26		7.53	6.06	6.11	12.44	6.84	6.87	7.29	8.71	7.11
79			4.56	5.11	6.67	5.36	8.40	7.07	6.49	5.31	6.99	7.36	6.02	10.07	5.78
80			5.10	6.13	5.40	4.75	7.92	5.07	5.35	4.34	5.70	5.99	5.92		4.14
		(n)	5	5	5	4	5	5	5	4	5	5	5	3	5
		Means	5.46	6.35	6.31	5.18	7.00	6.12	5.94	6.23	7.12	6.70	6.87	9.42	6.30
		Sdevs	0.82	0.89	0.55	0.53	1.83	0.73	0.68	4.27	1.45	0.59	0.85	0.68	1.85
81	4		4.85	5.59	6.74	5.22	6.20	3.15	5.35	4.98	6.59	6.57	5.28	6.91	6.66
82			4.57	0.00		6.02	7.04	7.42	7.16	5.02	7.28	5.69	6.61	7.36	6.81
83			4.28	6.52	7.13	6.37	7.47	5.40	5.95	4.41	6.39	5.87	5.27	6.91	4.45
84			5.53	5.99	5.61	4.74	5.54	5.55	5.47	3.83	5.22	4.38	4.26	4.84	5.83
85			5.56	6.85	7.67	5.63	8.13	6.56	6.22	5.32	5.83	5.91	7.37	6.72	6.38
		(n)	5	5	4	5	5	5	5	5	5	5	5	5	5
		Means	4.96	4.99	6.79	5.60	6.88	5.62	6.03	4.71	6.26	5.68	5.76	6.55	6.03
		Sdevs	0.57	2.83	0.87	0.64	1.02	1.60	0.72	0.59	0.78	0.80	1.23	0.98	0.96

Note: Data for Exposure phase

Animal	Group		Day of Phase 20
			Male Animals
66	1		5.34
67			7.29
68			7.60
69			5.61
70			6.59
		(n)	5
		Means	6.49
		Sdevs	1.00
71	2		6.15
72			6.83
73			6.85
74			6.94
75			6.74
		(n)	5
		Means	6.70
		Sdevs	0.32
76	3		5.57
77			6.92
78			7.69
79			5.63
80			4.96
		(n)	5
		Means	6.15
		Sdevs	1.12
81	4		7.68
82			7.00
83			5.85
84			4.71
85			5.91
		(n)	5
		Means	6.23
		Sdevs	1.15

Note: Data for Exposure phase