

R. J. Reynolds Tobacco Company
Research and Development

Study Number: TOX209

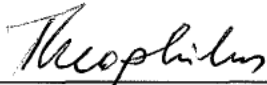
Final Report

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



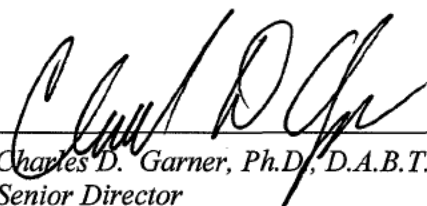
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I FACILITIES AND ADMINISTRATION

1. Sponsor

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

2. Facility

R. J. Reynolds Tobacco Company
Research and Development
Winston-Salem, NC 27102

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:	April 21, 2008
In Life Phase Termination:	May 6, 2008

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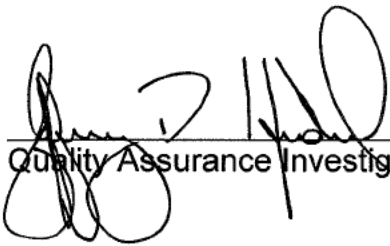
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QUALITY ASSURANCE STATEMENT

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	4/28, 29, 30/09	209QAU01 (4/30/09)


Quality Assurance Investigator11-May-09
Date

V. LIST OF ABBREVIATIONS USED IN REPORT

bw	body weight
C	centigrade
CARB	ciliated associated respiratory bacillus
CFR	Code of Federal Regulations
ECUN	<i>Encephalitozoon cuniculi</i>
FID	flame ionization detector
g	gram
GC	gas chromatography
GDVII	murine encephalomyelitis virus
H-1	H-1 virus
HANT	Hantaan virus
IACUC	Institutional Animal Care and Use Committee
kg	kilogram
KRV	Kilham rat virus
LCMV	lymphocytic choriomeningitis virus
LD ₅₀	lethal dose for 50% of treated animals
MS	mass spectroscopy
MAV	mouse adenovirus
mg	milligram
ml	milliliter
MPV	mouse parvovirus
MTD	maximum tolerated dose
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
RMV	rat minute virus
RPV	rat parvovirus

FINAL REPORT

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

VI. 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 Wistar Hannover rats. 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 rats 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 rats 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 indicates that the formulation methodology developed for this study produced a homogenous 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 investigational 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 homogeneously mixed within the feed to an extent that avoided the occurrence of random areas of either low or higher concentrations different from the anticipated concentrations. 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 rats. The room temperature stability of the test articles and positive control in the feed, measured as nicotine, was determined to assess the required frequency of preparation of the formulated feed. This study revealed the formulated feed was stable for at least ten days. This confirmed the adequacy of the preparation of formulated feed weekly for this study. An additional room temperature stability investigation indicated that the formulated feed was stable for at least one-month. This indicates that future studies would not require weekly feed formulations.

The male, Wistar Hannover rats used in this study were maintained under protocol specified conditions throughout the study and data related to light cycle, room temperature, humidity,

room air exchanges and water quality demonstrated no excursions that could impact the results of the study. In addition, serological and health assessment of sentinel rats indicated no evidence of the presence of contagious disease.

Rats in the control group demonstrated the expected body weight gains for male rats of their age throughout the study, indicating that there were no conditions other than the dosed feed that could unduly affect bodyweight during the study. Rats fed feed formulated with the tobacco blend, tobacco extract or nicotine tartrate demonstrated a strong dose response in respect to body weight. As the dose of nicotine increased, body weight gain decreased and there was a definitive loss of body weight at the 40 mg nicotine/kg body weight/day. Comparison of the body weight data from this rat study to a parallel mouse study (TOX210) using the identical doses indicates that rats are more susceptible to the effects of the test articles and positive control on body weight changes and feed intake than mice.

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. Rats provided feed formulated with the tobacco blend at doses of 0.2-40 mg nicotine/kg bw/day demonstrated 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 demonstrating a loss in body weight gain throughout the study. The final body weights at 40 mg nicotine/kg bw/day were statistically lower than those of the control group. Because the body weight decreases were excessive, the high dose would not be appropriate for use in longer term studies. At 20 mg nicotine/kg bw/day, there was a lack of body weight gain during the first week of the study. However, during the second week of the study body weight demonstrated a slight increase as the rats acclimated somewhat to the dosed feed. At the termination of the feeding period, the rats at this dose demonstrated a statistically significant lower body weight compared to the control group. This decrease was not considered excessive and this dose could be considered as the high dose in a short-term repeated dosing toxicology study. At the 0.2-8.0 mg nicotine/kg bw/day doses, there were dose related decreases in body weight compared to the control. As these doses increased there were dose related delays in the ability of the rats to acclimate to the dosed feed. Doses within this range would be appropriate for use in a short-term repeated dosing toxicology study.

Cumulative percent body weight gain in rats fed feed containing the tobacco extract at doses equivalent to those fed the tobacco blend demonstrated trends almost identical to seen with the tobacco blend in respect to body weight gain. As the dose of nicotine increased, the body weight gain of the rats decreased in a dose dependent manner. However, the decreases seen at the 20 and 40 mg nicotine/kg bw/day were not as depressed as with the tobacco blend. This may indicate the palatability of the feed was greater when formulated with the extract as opposed to the blend. At the termination of the feeding period, the body weights of the rats fed the high dose were statistically lower than the control while the rats fed the 20 mg nicotine/kg bw/day did not statistically differ from the control group. As seen with the data from rats fed the tobacco blend, a dose of 40 mg nicotine/kg bw/day appears excessive for use in a short-term repeated dosing study of longer duration than this palatability study. A high dose of 20 mg nicotine/kg bw/day could be considered for use as a high dose for the tobacco extract in a short-term repeated dose toxicology study with additional doses in the range of 0.2-8.0 or slightly higher.

When rats were fed feed formulated with nicotine hydrogen tartrate, there was a definitive dose response with cumulative body weight gain decreasing as the dose increased. At 40 mg nicotine/kg bw/day body weight gain was negative throughout the 14-day study, indicating actual loss of body weight. Reduction of body weight gain was more depressed with the positive control than with either of the test articles. At 20 mg nicotine/kg bw/day, the trend was similar to that of the tobacco blend but greater than that seen with the tobacco extract. This supports the conclusion that a nicotine dose of 40 mg/kg bw/day would not be appropriate for a longer term study while, 20 mg nicotine/kg bw/day could be used as the high dose in a short-term repeated dose toxicology study. The similarity of trends seen with the nicotine tartrate positive control compared to the two test articles may indicate the lower cumulative body weight gains seen with the test articles are more related to their nicotine content than their content of other tobacco components.

An important finding in this study is that rats are more sensitive to the effects of the test articles and positive control on body weight than are mice. Rats fed feed containing either the tobacco blend, tobacco extract or nicotine hydrogen tartrate at concentrations that yielded nicotine doses between 0.2 and 40.0 mg/kg bw/day demonstrated dose dependent reductions in body weight gain while mice demonstrated a minimal response only at the high dose. This species difference in sensitivity must be taken into consideration when designing longer term toxicology studies.

VII. 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, Wistar Hannover rats. This study was run in parallel with TOX210, a similar study using male, CFW Swiss Webster mice at the same doses.

Short term repeated dosing studies and subchronic toxicity studies using both genders of Wistar Hannover rats 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 maximum 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 because of the chemically complex nature of the test articles. 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 several animal species, including rats and mice ([HSDB, 2008](#)). Nicotine toxicity was considered to be a potential limiting factor in the determination of the doses to be used in this study. These factors support the use of nicotine as the basis for dosing the rats and monitoring the formulation of the rodent feed. Therefore, the dosing of the rats 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 rats. 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 LD₅₀ (a dose that results in death of 50% of the treated animals) of nicotine for rats 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 LD₅₀'s for nicotine, the rats would not receive their nicotine dose as a single bolus but as a feed component. This would result in smaller exposures each time a rat feeds. In addition, nicotine is metabolized by rats 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 was selected to encompass potential nicotine doses to be used in the anticipated toxicology studies and to provide information on the palatability of the diets to the rats without causing undue acute toxicity. These identical doses were also used in a parallel study using mice (TOX210) based upon a similar rationale. 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 the use of both genders of rats or 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 rat to be used in the study. It had been decided that the most appropriate rat strain for the planned toxicology studies was the Wistar Hannover rat strain. This decision was based upon the lower rate of disease in this strain, especially kidney disease, in older rats compared to other common rat strains used in toxicology studies. Supporting this decision was the choice of the Wistar Hannover as the strain of choice for longer term toxicology studies by the National Toxicology Program.

An addition variable was the type of feed to be used in the study. First, a powdered feed would have to be used to allow incorporation of the test articles and positive control into the feed. Second, a feed that allows the rats 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 rodents and has a lower calorie content compared to other possible feeds. The lower caloric content results in a slightly slower body weight gain and has been shown to decrease certain disease conditions, especially kidney disease, in rats on long term studies, as well as increasing survival. Again, this diet has been chosen by the National Toxicology Program for 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 will minimize the risk that rats would find the diets unpalatable during longer term feeding studies while using a minimal number of animals.

VIII. 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 °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 °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 °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 was 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 Use and Care Committee (IACUC) on March 27, 2008 before arrival of the animals into the facility. Ninety male, juvenile Wistar Hannover rats (5-7 weeks of age) from Charles River Laboratories (Raleigh, NC) were received into the facility on April 09, 2008. An additional 10 male, retired breeder Wistar Hannover rats were received for use as sentinel animals. The sentinel rats 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.

b) Animal Identification

Rats were identified by cage card during the pretest period. After allocation to study groups, the rats 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 Doses¹

Group Number	Treatment Group and Nicotine Dose (mg nicotine/kg body weight/day)		Number of Rats	Rat ID Numbers
Control				
1	NTP-2000 feed	(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 rats 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 TOX209A and TOX209B.

The Xybion data collection protocols [TOX209A](#) and [TOX209B](#) were used for body weights and feed consumption of rats 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 dose groups. TOX209A contains Dose Groups 1-13. TOX209B contains the four nicotine tartrate positive control groups (i.e. TOX209B Group 1 is study Group 14; Group 2 is study Group 15; Group 3 is Group 16; and Group 4 is Group 17). The Xybion data collection protocols TOX209A and TOX209B were used for body weights, feed consumption and clinical signs of rats used on this study. Data were input into the Xybion Path/Tox collection protocols under the “A” module, “AINPUT”.

Because the start of the exposure phase was delayed five days for the TOX209A and TOX209B studies, Day 7 of the Xybion data output is study Day 1 (May 21, 2008), etc.

c) Animal Housing

The rats 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 rats 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 18-26°C 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 a backup to the automated system. Rats were individually housed on stainless steel racks in polycarbonate cages 9.25 in. (L) x 10 ½ in. (W) x 8 in. (H) containing Alpha-Dri bedding.

Rats were 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 rats within four days after delivery. Commencement of dosing the rats was dependent upon a favorable review of the health examination, as well as a written statement from the Attending Veterinarian releasing the rats from quarantine. The rats were released from quarantine on April 16, 2008 but continued under quarantine conditions until initiation of feeding the dosed feed.

d) Feed and Water

Rats 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 rats, had *ad libitum* access to NTP-2000 feed (Zeigler Bros., Inc., Gardners, PA) to allow the animals to acclimate to a powdered diet. The sentinel rats 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 rats. Clean feeders were provided weekly. Data for rats 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 rats in glass feed cups with stainless steel lids that minimized spillage but provided the rats 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 rats adequate quantities of feed to ensure *ad libitum* feeding. Because the spacer was below the surface of the feed the rats did not have access to the spacer and there was no evidence of gnawing or biting on the spacers.

Water was provided to the rats 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 rats. 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 that would be anticipated to interfere with the outcome of the study.

e) Allocation of Animals to Study Groups

On April 14, 2008, rats 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, rats 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 risk level. Following allocation into groups, rats 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 into the feed for the rats. This route of administration most appropriately mimics human exposure to smokeless tobacco products within the confines of the study design.

b) Dose Regimen

A total of 17 groups were used along with a sentinel group (Table 1). Each treatment group contained 5 male rats. Rats in Group 1 served as the untreated control group and were fed NTP-2000 feed without addition of tobacco test articles or the positive control. Groups 2-7 were fed NTP-2000 feed with additions of the tobacco blend to yield the following mg nicotine/kg bw/day:

0.2, 2.0, 4.0, 8.0, 20.0 and 40.0, respectively. Nicotine dosing was based upon reported acute short term data toxicity data ([HSDB, 2008](#)) and chosen to be below what was expected to be acutely toxic but believed to be suitable for the determination of palatability. 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 rats in Groups 2-7. The positive controls, 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. Sentinel rats were fed *ad libitum* pelleted Lab Diet, Certified Rodent Diet #5002 (PMI Feeds, Inc.) using cage feeders designed for pelleted feed. Sentinel rats were used to detect any disease or other factors that may influence the study and received no treatment.

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 rats for the duration of the dosing period must be assumed. An assumption of 190 g (0.19 kg) body weight 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 rat strain. Second, the mean feed intake range of the rats for the duration of the feeding period must also be assumed and is related to the mean body weight. An assumption of 20 g feed consumption/day/rat 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 feeding period were based upon estimates of body weight and feed consumption (260 g bw/rat [0.26 kg] and 24 g feed consumption/rat) 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](#).

c) Dosed Diet Formulation

The bulk NTP-2000 unformulated feed was stored at refrigerator temperatures (4°C) 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 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 concentrations 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)	0.07	0.08
3	Dose 2 Tobacco Blend in NTP-2000 feed	(2.0)	0.72	0.83
4	Dose 3 Tobacco Blend in NTP-2000 feed	(4.0)	1.45	1.65
5	Dose 4 Tobacco Blend in NTP-2000 feed	(8.0)	2.89	3.30
6	Dose 5 Tobacco Blend in NTP-2000 feed	(20.0)	7.23	8.25
7	Dose 6 Tobacco Blend in NTP-2000 feed	(40.0)	14.46	16.49
Tobacco Extract				
8	Dose 1 Tobacco Extract in NTP-2000 feed	(0.2)	0.08	0.09
9	Dose 2 Tobacco Extract in NTP-2000 feed	(2.0)	0.83	0.94
10	Dose 3 Tobacco Extract in NTP-2000 feed	(4.0)	1.65	1.88
11	Dose 4 Tobacco Extract in NTP-2000 feed	(8.0)	3.30	3.77
12	Dose 5 Tobacco Extract in NTP-2000 feed	(20.0)	8.26	9.42
13	Dose 6 Tobacco Extract in NTP-2000 feed	(40.0)	16.52	18.84
Positive Control				
14	Dose 1 Nicotine Tartrate in NTP-2000 feed	(2.0)	0.05	0.06
15	Dose 2 Nicotine Tartrate in NTP-2000 feed	(8.0)	0.22	0.25
16	Dose 3 Nicotine Tartrate in NTP-2000 feed	(20.0)	0.54	0.62
17	Dose 4 Nicotine Tartrate in NTP-2000 feed	(40.0)	1.08	1.23

¹ Concentration is expressed as the amount of test article added/g feed. For instance, 0.07 mg of the tobacco blend added per g of feed and 0.08 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 190 g/rat and an estimated feed intake of 20 g/rat/day.

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 260 g/rat and an estimated feed intake of 24 g/rat/day.

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

Samples from the top, middle and bottom regions 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 (trial feed formulation and Series 2 feed formulation) were removed and stored under the conditions of 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 was not only more sensitive but also decreased the sample analysis time. This method did not undergo complete validation during the period of the study and its accuracy and precision was 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

Rats were delivered to the facility on April 9, 2008. The retired Wistar Hannover sentinel rats were handled identical to the study animals and placed in Room 39 with the study animals. Because of the short term nature of the study, prestudy sentinel rats were not employed in this study. At study termination on May 6, 2008 the 10 sentinel rats for health screening were anesthetized with 70% carbon dioxide (CO₂) in air and blood was drawn from the vena cava. While still under anesthesia, the animals were then sacrificed by exsanguination. The health screen rats provided sera appropriate for routine measurement of the following antibodies to disease using the Charles River Laboratory Rat Assessment Plus profile that consisted of the following: pneumonia virus of mice (PVM), Sendai virus, Sialodacryoadenitis virus (SDAV), Kilham rat virus (KRV), H-1 virus (H-1), murine encephalomyelitis virus (GDVII), RIO,

Mycoplasma pulmonis, lymphocytic choriomeningitis virus (LCMV), mouse adenovirus (MAV) 1 & 2, Hantaan virus (HANT), *Encephalitozoon cuniculi* (ECUN), ciliated associated respiratory bacillus (CARB), mouse parvovirus (MPV) or PARV NS1, rat parvovirus (RPV) and rat minute virus (RMV). Serology was performed by Charles River Research Animal Diagnostic Services, Wilmington, MA. In addition, the lungs were removed during the health screen 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 results obtained from the health-screen analysis and a written statement from the Attending Veterinarian releasing the animals from quarantine. The rats were released from quarantine to the study 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 rats. Observations were made five days per week (Monday through Friday, excluding holidays). For weekends and holidays, only one observation per day was performed. Rats whose conditions made it unlikely that they would survive to the next observation period or seemed to be in pain could be euthanized.

c) 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 recorded.

In addition, detailed (scheduled) clinical observations were 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. Both positive and negative findings were recorded. The "A" module of the PATH/TOX system was used for acquisition of clinical signs data.

d) Body Weights

Individual non-fasted body weights were determined the day after delivery and 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 Xyberion PATH/TOX system was used for acquisition of body weight data. Weighing took place at approximately the same time each day (between 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 were made at any time, if deemed necessary by the Attending Veterinarian or Study Director. Rat weights were acquired using Mettler PM2000 balances (Mettler Instrument Corporation, Highstown, NJ).

e) Feed Consumption

After initiation of feeding the dosed feed, the feed was placed into the feed bowl and its weight determined and recorded. The next day, the uneaten feed was weighed and the food consumption calculated. Data were entered into the “A” module of the PATH/TOX computer software. Each rat’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 rat, fresh feed was placed in a bowl and provided to the rat after recording the weight in the PATH/TOX software.

f) Terminal Body Weights

The non-fasted, terminal body weights for the rats in each study group were determined on the 14th day of the feeding period.

4. Statistics

Body Weights

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

IX. RESULTS

A. 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. Feed from the trial run was not fed to the rats. This was followed by Series 1 formulation for the initial 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 of the growth and feed consumption of Wistar Hannover rats. Calculations for Series 2 formulation were based upon data collected during the first week of feeding the dosed feed during the study. The formulated feed from each of these preparations was analyzed 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.

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 initially used gas chromatography (GC) with flame ionization detection (FID) for the high dose but was not suitable for the lower doses because the feed nicotine concentrations were below the limit of quantitation for the method. 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 a potential high dose are presented in Table 3 and at a potential low dose in Table 4. The rationale for determination of homogeneity of the high and low dose only is that if these two doses are homogenous, then the formulation methodology should be adequate at the intermediate doses. Samples were obtained from the top of the formulated feed mixture as well as the middle and bottom of the mixture. Homogeneity data obtained by the GC/FID method at the high dose from the trial run formulation are presented in Table 3.

Table 3: Trial Run Feed Formulation Homogeneity Data¹ and Dose Confirmation at the High Dose

Target Concentration (mg nic/g feed)	Top (mg nic/g feed)	Sample Location Middle (mg nic/g feed)	Bottom (mg nic/g feed)	Determined Average Concentration (mg nic/g feed)
<i>Tobacco Blend</i>				
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
<i>Tobacco Extract</i>				
0.50	0.40 ± 0.04 (20%)	0.43 ± 0.06 (14%)	0.39 ± 0.02 (22%)	0.41 ± 0.02 (18% ± 4%)
<i>Nicotine Tartrate</i>				
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 feed 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. As with the tobacco test articles, there were no visually detectable changes in the color and consistency of the feed after addition of the nicotine hydrogen tartrate salt.

While the GC/FID analytical methodology did not allow the determination of the nicotine concentration in the feed at the low dose, the GC/MS method under development was able to provide estimates of the nicotine concentration. Data from the trial run at the potential low doses for the smokeless tobacco blend and the tobacco extract as well as the nicotine hydrogen tartrate are presented in Table 4.

Table 4: Trial Run Feed Formulation Homogeneity Data and Dose Confirmation for Low Doses¹

Target Concentration (mg nic/g feed)	Top (mg nic/g feed)	Sample Location Middle (mg nic/g feed)	Bottom (mg nic/g feed)	Determined Average Concentration (mg nic/g feed)
<i>Tobacco Blend</i>				
0.003	0.003 (0%)	0.003 (0%)	0.003 (0%)	0.003 ± 0.000^2 (0% \pm 0%) ²
<i>Tobacco Extract</i>				
0.003	NA ³ (%)	NA (%)	NA (%)	NA (%)
<i>Nicotine Tartrate</i>				
0.025	0.022 (12%)	0.021 (16%)	0.020 (20%)	0.021 ± 0.001 (16% \pm 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 \pm the standard deviation of the concentration and percent difference.

³ NA = Data not available.

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 demonstrated adequate homogeneity but was slightly lower than anticipated in concentration. The noted improvements to the feed formulation methodology for the nicotine tartrate salt should improve the ability to obtain nicotine concentrations adequate for use in this investigational study.

Series 1 feed formulations were used during the first week of the study. Analytical data for homogeneity and dose confirmation at the high and low doses are presented in Table 5.

Table 5: Series 1 Feed Formulations Homogeneity Data¹ and Dose Confirmation

Target Concentration (mg nic/g feed)	Top (mg nic/g feed)	Sample Location Middle (mg nic/g feed)	Bottom (mg nic/g feed)	Average Concentration (mg nic/g feed)
40 mg/kg bw/day				
Tobacco Blend				
0.38	0.39 (3%) ³	0.36 (5%)	0.39 (3%)	0.38 ± 0.02 ² (3.6% ± 1.5%)
Tobacco Extract				
0.38	0.49 (29%)	0.33 (13%)	0.37 (3%)	0.40 ± 0.08 (15% ± 13%)
Nicotine Tartrate				
0.38	0.35 (8%)	0.35 (8%)	0.35 (8%)	0.35 ± 0.01 (8% ± 2%)
0.2 mg/kg bw/day				
Tobacco Blend				
0.002	0.001 (50%)	0.001 (50%)	0.001 (50%)	0.001 ± 0.000 (50% ± 0%)
Tobacco Extract				
0.002	0.001 (50%)	0.001 (50%)	0.001 (50%)	0.001 ± 0.000 (50% ± 0%)
2.0 mg/kg bw/day				
Nicotine Tartrate				
0.019	0.018 (5%)	0.015 (21%)	0.014 (26%)	0.016 ± 0.002 (17% ± 11%)

¹ Analytical method uncertainty for nicotine analysis = 5.2%, data represent the mean of duplicate analytical determinations at 40 mg/kg bw/day and a single analytical determination at 0.2 mg/kg bw/day.

² Data represent mean ± standard deviation where appropriate

³ % difference from target concentration

At the 40 mg nicotine/kg bw/day dose, homogeneity data for the tobacco blend indicated that its homogeneity was excellent and appropriate for use in the study. It also indicates that the concentration of nicotine in the feed was equivalent to the anticipated value and adequate for use in the study. The data for the tobacco extract also demonstrated good homogeneity and dose confirmation. The concentration of nicotine was slightly above the anticipated range but should not produce a biologically relevant effect on the data from the study. The nicotine tartrate dosed feed also demonstrated good homogeneity and dose confirmation data and was adequate for use in the study.

At the low dose (0.2 mg nicotine/kg bw/day for the tobacco blend and extract and 2.0 mg nicotine/kg bw/day for nicotine tartrate), the nicotine concentrations were close to the limit of quantitation for the tobacco blend and tobacco extract. The concentration and homogeneity data indicate the feed was appropriate for use in the study.

Data for dose confirmation for the Series 1 feed formulations with the tobacco blend are provided in Table 6.

Table 6: Series 1 Dose Confirmation Data Tobacco Blend

Target Dose (mg of nicotine/kg of bw/day)	Target Concentration (g of nicotine/g of feed)	Determined Feed Nicotine Concentration¹ (mg of nicotine/g of feed)
0.2	0.002	0.002
2.0	0.019	0.015
4.0	0.038	0.036
8.0	0.076	0.065
20.0	0.190	0.142
40.0	0.380	0.302 ²

¹ Data represent the mean of duplicate independent analytical determinations using GC/MS analysis methodology unless otherwise noted.

² Data represent the mean of a single analytical determination using GC/MS analysis methodology.

The dose confirmation data for the tobacco blend indicate increasing nicotine concentrations as the anticipated doses increased. Although there was a trend toward the analytically determined nicotine concentration to be lower than anticipated as the dose increased, comparison of the high dose in Table 6 to that in Table 5 (independent analyses of the same formulation) indicate the high dose was closer to the anticipated value than indicated in Table 6. The reasons for the difference between the two analytical runs on the same sample can not be ascertained from the available data, but could be associated with differences in extraction efficiency between the two analytical determinations.

Dose confirmation data for the Series 1 feed formulation with the tobacco extract are provided in Table 7.

Table 7: Series 1 Dose Confirmation Data Tobacco Extract

Target Dose (mg of nicotine/kg of bw/day)	Target Concentration (g of nicotine/g of feed)	Determined Feed Nicotine Concentration (mg of nicotine/g of feed)
0.2	0.002	0.002
2.0	0.019	0.029
4.0	0.038	0.037
8.0	0.076	0.076
20.0	0.190	0.172
40.0	0.380	0.265 ²

¹ Data represent the mean of duplicate independent analytical determinations using GC/MS analysis methodology unless otherwise noted.

² Data represent the mean of a single analytical determination using GC/MS analysis methodology.

Series 1 feed formulation for the tobacco extract appears adequate for use in the study based upon the increases in nicotine concentrations as the anticipated doses increased. Again, the same trend of greater differences between the anticipated nicotine concentration and the analytically determined values at the higher doses is seen. Also, as seen with the tobacco blend, comparison of the data in Table 5 to the data in Table 7 indicates the values at the high dose in Table 7 may be low.

Data for the analytically determined nicotine concentrations in the Series 1 feed formulation with nicotine tartrate are provided in Table 8.

Table 8: Series 1 Dose Confirmation Data Nicotine Tartrate¹

Target Dose (mg of nicotine/kg of bw/day)	Target Concentration (g of nicotine/g of feed)	Determined Feed Nicotine Concentration (mg of nicotine/g of feed) ²
2.0	0.019	0.018
8.0	0.076	0.063
20.0	0.190	0.153
40.0	0.380	0.296 ²

¹ Data represent the mean of duplicate independent analytical determinations using GC/MS analysis methodology unless otherwise noted.

² Data represent the mean of a single analytical determination using GC/MS analysis methodology.

Feed containing the nicotine tartrate salt demonstrated adequate homogeneity and concentration. As seen with the tobacco blend and tobacco extract, there appears to be a trend toward lower than expected quantities of nicotine at the higher doses. However, this is not seen in Table 5, as previously noted for the tobacco blend and tobacco extract. Whereas the analytically determined feed nicotine concentration in Table 8 at the low nicotine tartrate dose is within the range seen for the identical formulation in Table 5, at the high dose the nicotine concentration is lower than the range seen for this dose in Table 5.

Series 2 feed formulations were used during the last week of the study. Analytical data for homogeneity are presented in Table 9.

Table 9: Series 2 Feed Formulations Homogeneity Data¹ and Dose Confirmation
(40 mg/kg body weight/day)

Target Concentration (mg nic/g feed)	Top (mg nic/g feed)	Sample Location Middle (mg nic/g feed)	Bottom (mg nic/g feed)	Average Concentration (mg nic/g feed)
<i>Tobacco Blend</i>				
0.43	0.41 (5 %) ³	0.41 (5 %)	0.41 (5 %)	0.41 ± 0.00 ² (5 % ± 0 %)
<i>Tobacco Extract</i>				
0.43	0.41 (5 %)	0.39 (9 %)	0.44 (2 %)	0.41 ± 0.03 (5 % + 4%)
<i>Nicotine Tartrate</i>				
0.43	0.40 (7 %)	0.40 (7 %)	0.40 (7 %)	0.40 ± 0.00 (7 %)

¹ Analytical method uncertainty for nicotine analysis = 5.2%, data represent the mean of duplicate analytical runs. ² Data represent mean ± standard deviation where appropriate. ³ % difference from target concentration.

Homogeneity data for the tobacco blend, tobacco extract and nicotine tartrate indicated excellent homogeneity. A comparison of the target concentration to the analytically determined concentration indicates an excellent agreement and is within an acceptable range for use in the study.

Dose confirmations for the Series 2 feed formulation used during the second week of the study are presented in Tables 10-12.

Dose confirmation data for the increasing doses for the tobacco blend are presented in Table 10.

Table 10: Series 2 Dose Confirmation Data Tobacco Blend

Target Dose (mg of nicotine/kg of bw/day)	Target Concentration (g of nicotine/g of feed)	Determined Feed Nicotine Concentration¹ (mg of nicotine/g of feed)
0.2	0.002	0.006
2.0	0.022	0.015
4.0	0.043	0.041
8.0	0.087	0.074
20.0	0.217	0.172
40.0	0.433	0.347

¹ Data represent the mean of duplicate independent analytical determinations using GC/MS analysis methodology unless otherwise noted.

As the anticipated nicotine dose increased so did the analytically determined nicotine concentration. In most cases, the analytically determined nicotine concentration corresponded with the anticipated concentration. However, as seen with the Series 1 feed formulation analytics, at the higher doses the discrepancy between the target nicotine concentration and the analytically determined concentrations appear to increase. As with the Series 1 data, comparison of the high dose data in Table 9 to that in Table 10 indicates that the data in Table 10 at the higher doses may be low. Overall, it appears that the Series 2 feed formulation for the tobacco blend was adequate for use in this investigational study.

Dose confirmation data for the Series 2 feed formulations for the tobacco extract are provided in Table 11.

Table 11: Series 2 Dose Confirmation Data Tobacco Extract

Target Dose (mg of nicotine/kg of bw/day)	Target Concentration (g of nicotine/g of feed)	Determined Feed Nicotine Concentration (mg of nicotine/g of feed)
0.2	0.002	0.001
2.0	0.022	0.027
4.0	0.043	0.032
8.0	0.087	0.087
20.0	0.217	0.138
40.0	0.433	0.341

¹ Data represent the mean of duplicate independent analyses using GC/MS analysis methodology unless otherwise noted.

A comparison of the anticipated nicotine concentration in the Series 2 feed formulation for the tobacco extract with the analytically determined nicotine concentrations indicates that the feed formulation was adequate for use in the study. Again, the higher doses appear to have lower than expected nicotine concentrations. However, comparison of the data for the higher dose in Table 11 to that in Table 9 indicates the data in Table 11 may be low.

Table 12 provides the Series 2 dose confirmation data for feed formulated with increasing concentrations of nicotine tartrate.

Table 12: Series 2 Dose Confirmation Data Nicotine Tartrate

Target Dose (mg of nicotine/kg of bw/day)	Target Concentration (g of nicotine/g of feed)	Determined Feed Nicotine Concentration (mg of nicotine/g of feed)
2.0	0.022	0.018
8.0	0.087	0.070
20.0	0.217	0.167
40.0	0.433	0.331

¹ Data represent the mean of duplicate independent analyses using GC/MS analysis methodology unless otherwise noted.

Comparison of the anticipated nicotine concentration in the feed formulated with the positive control nicotine tartrate to the analytically determined concentrations indicates the Series 2 formulation was adequate for use in this study. As seen with the tobacco blend and tobacco extract, the higher doses appear to demonstrate more discrepancy between the anticipated and analytically determined nicotine concentration. However, comparison to the data in Table 9 indicates the data in Table 12 may be low for the high dose.

Determination of the Stability of the Formulated Feed: 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 and are provided in [Table 13](#). These data compare the nicotine content of freshly formulated feed with that maintained at room temperature for 10-days. There is no indication of a lack of stability of nicotine in these samples. The variability seen with the tobacco blend at the low dose 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.

Taken together, the data on homogeneity and nicotine concentration in the feed formulations indicated the doses in the Series 1 and Series 2 formulations administered to the rats were appropriate for this investigational study.

Table 13: **Ten-Day Stability Data¹ TOX209 Series 2 Feed Formulation**

Dose (mg nic/kg bw/day)	Target Concentration (mg nic/g feed)	"0" day (mg nic/g feed)	"10 th " day (mg nic/g feed)	% Difference
<i>Tobacco Blend</i>				
40.0	0.433	0.347	0.409	15
0.2	0.002	0.007	0.002	71
<i>Tobacco Extract</i>				
40.0	0.433	0.342	0.367	7
0.2	0.002	0.001	0.001	0
<i>Nicotine Tartrate</i>				
40.0	0.433	0.331	0.354	7
2.0	0.022	0.018	0.019	6

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

Overall, these data indicate that the nicotine contained in the tobacco blend, tobacco extract and tartrate salt is stable when blended with the NTP-2000 feed and stored at room temperature for at least 10-days. Although these data do not address the stability of the many other components of these complex natural mixtures, there are currently no data indicating a lack of stability of the test articles when blended with the NTP-2000. These data indicate that it is unnecessary to formulate dosed feed on a weekly basis, which was done for the current study.

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 1-month after initial formulation. The 1-month old samples are compared to the fresh samples in Table 14.

Table 14: **Prestudy Trial Formulations 30-Day Stability Data from the Trial Formulation¹**

Target Concentration (mg nic/g feed)	"0" day (mg nic/g feed)	1-Month (mg nic/g feed)	% Difference
<i>Tobacco Blend</i>			
0.50	0.46 ± 0.01	0.44	4
<i>Tobacco Extract</i>			
0.50	0.41 ± 0.02	0.44	7
<i>Nicotine Tartrate</i>			
0.50	0.41 ± 0.01	0.41	0

¹ The "0" day data are from the mean and standard deviation of the initial nicotine analysis of the Trial Run formulation (see Table 3) by the GC/FID method. The "1-Month" data are from the same feed stored at room temperature for one month and analyzed by the GC/MS method.

These data provide no evidence for a lack of stability of nicotine in the 30 day old feed samples when stored at room temperature. Based upon this initial analysis, it appears that formulated feed can be prepared at least monthly for longer term studies.

B. BIOLOGICAL EVALUATIONS

1. Study Animals

A total of 100 male Wistar Hannover rats, age 5-7 weeks, was received on April 9, 2008 from Charles River Laboratories, Raleigh, NC. The rats were placed in Room 39 and individually housed in polycarbonate cages containing Alpha-Dri bedding. Rats were quarantined for 8 days before being released to the study by the Attending Veterinarian.

Throughout the study the environmental controls of the animal room maintained the following mean daily conditions: temperature $70.7 \pm 0.3^{\circ}\text{F}$ and relative humidity $55.3 \pm 4.5\%$. 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. These variables were within the protocol specified ranges.

There was one occasion when the temperature, relative humidity, air flow and light cycle were not captured during a 30 minute period. This lack of a single data point had no impact on the outcome or interpretation of the results from the study.

On April 14, 2008, 95 rats were assigned to dose groups, by body weight, using the "A" module of the PATH/TOX software. At the discretion of the Study Director, rats 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 21, 2008 and study termination was May 5, 2008. Because of the delay in the start of feeding the formulated feeds, study day one is denoted as study day seven in the Xybion data outputs from this study.

Rats were transferred to clean housing at least once per week. Comprehensive records of the activities within the animal room are maintained as part of the study file.

Prior to initiation of dosing rats were fed NTP-2000 powered feed for one week to acclimate them to the powdered feed. After 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 feeders were provided weekly.

Water was provided to the rats *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 are maintained in the study file.

2. Serology/Health Screens

At the end of the in-life phase of the study 10 sentinel rats were euthanized for serology and necropsy to detect any signs of disease. 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 rats (retired breeders). Histopathological findings in the lungs included congestion, hemorrhage, peribronchiolar/perivascular lymphocytic infiltrations, non-pigmented macrophages, vascular mineralization and chronic inflammation. Chronic inflammation was seen only in one lobe of two rats. The pathologist considered the occurrences of this change as random and nonspecific and not indicative of the presence of contagious disease. The noted congestion and hemorrhage reflect the mode of anesthesia/euthanasia. Nonpigmented macrophages, lymphocytic infiltrations and vascular mineralization were anticipated background changes seen in rats of this age. Overall, there were no indications of the presence of contagious disease. Other than the findings indicating no evidence of contagious disease, the lung histopathology is not relevant to the study animals because the sentinel rats were retired breeders that were older than the study animals. [Appendix IV](#) provides the data from the serology and health screening.

3. Survival

Survival was 100% during the study. This indicates that 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](#). The only reported clinical observations other than “normal” were “thin, emaciated”, “hair loss” and “abrasion”. These changes were reported in the positive control group only and each occurred in only one of the five rats in each group. They were of short duration and reported only in the low dose and the high dose, indicative of a lack of a dose response and are not considered to be related to the test articles or positive control. There were no clinical observations indicating altered behavior or any other evidence of nicotinic effects during the study. This indicates that the doses used in this study were below those that may elicit nicotinic effects in the animals that were detectable through routine clinical observations.

Table 15: Group Incidences and Durations of Clinical Observations

Group	Treatment Group (Doses based on nicotine) (mg nicotine/kg body weight/day)	Observations ¹			
		Normal No visible abnormalities	Thin, emaciated	Hair loss (alopecia)	Abrasion
Control					
1	NTP-2000 Feed	5/5 [100%, 14]	0/5 [0%, 0]	0/5 [0%, 0]	0/5 [0%, 0]
Tobacco Blend					
2	Dose 1 Tobacco in NTP-2000 Feed (0.2)	5/5 [100%, 14]	0/5 [0%, 0]	0/5 [0%, 0]	0/5 [0%, 0]
3	Dose 2 Tobacco in NTP-2000 Feed (2.0)	5/5 [100%, 14]	0/5 [0%, 0]	0/5 [0%, 0]	0/5 [0%, 0]
4	Dose 3 Tobacco in NTP-2000 Feed (4.0)	5/5 [100%, 14]	0/5 [0%, 0]	0/5 [0%, 0]	0/5 [0%, 0]
5	Dose 4 Tobacco in NTP-2000 Feed (8.0)	5/5 [100%, 14]	0/5 [0%, 0]	0/5 [0%, 0]	0/5 [0%, 0]
6	Dose 5 Tobacco in NTP-2000 Feed (20.0)	5/5 [100%, 14]	0/5 [0%, 0]	0/5 [0%, 0]	0/5 [0%, 0]
7	Dose 6 Tobacco in NTP-2000 Feed (40.0)	5/5 [100%, 14]	0/5 [0%, 0]	0/5 [0%, 0]	0/5 [0%, 0]
Tobacco Extract					
8	Dose 1 Tobacco Extract in NTP-2000 Feed (0.2)	5/5 [100%, 14]	0/5 [0%, 0]	0/5 [0%, 0]	0/5 [0%, 0]
9	Dose 2 Tobacco Extract in NTP-2000 Feed (2.0)	5/5 [100%, 14]	0/5 [0%, 0]	0/5 [0%, 0]	0/5 [0%, 0]
10	Dose 3 Tobacco Extract in NTP-2000 Feed (4.0)	5/5 [100%, 14]	0/5 [0%, 0]	0/5 [0%, 0]	0/5 [0%, 0]
11	Dose 4 Tobacco Extract in NTP-2000 Feed (8.0)	5/5 [100%, 14]	0/5 [0%, 0]	0/5 [0%, 0]	0/5 [0%, 0]
12	Dose 5 Tobacco Extract in NTP-2000 Feed (20.0)	5/5 [100%, 14]	0/5 [0%, 0]	0/5 [0%, 0]	0/5 [0%, 0]
13	Dose 6 Tobacco Extract in NTP-2000 Feed (40.0)	5/5 [100%, 14]	0/5 [0%, 0]	0/5 [0%, 0]	0/5 [0%, 0]
Positive Control					
14	Dose 1 Nicotine Tartrate in NTP-2000 Feed (2.0)	4/5 [80%, 14]	1/5 [20%, 4]	1/5 [20%, 1]	0/5 [0%, 0]
15	Dose 2 Nicotine Tartrate in NTP-2000 Feed (8.0)	5/5 [100%, 14]	0/5 [0%, 0]	0/5 [0%, 0]	0/5 [0%, 0]
16	Dose 3 Nicotine Tartrate in NTP-2000 Feed (20.0)	5/5 [100%, 14]	0/5 [0%, 0]	0/5 [0%, 0]	0/5 [0%, 0]
17	Dose 4 Nicotine Tartrate in NTP-2000 Feed (40.0)	4/5 [80%, 14]	1/5 [20%, 1]	0/5 [0%, 0]	1/5 [20%, 4]

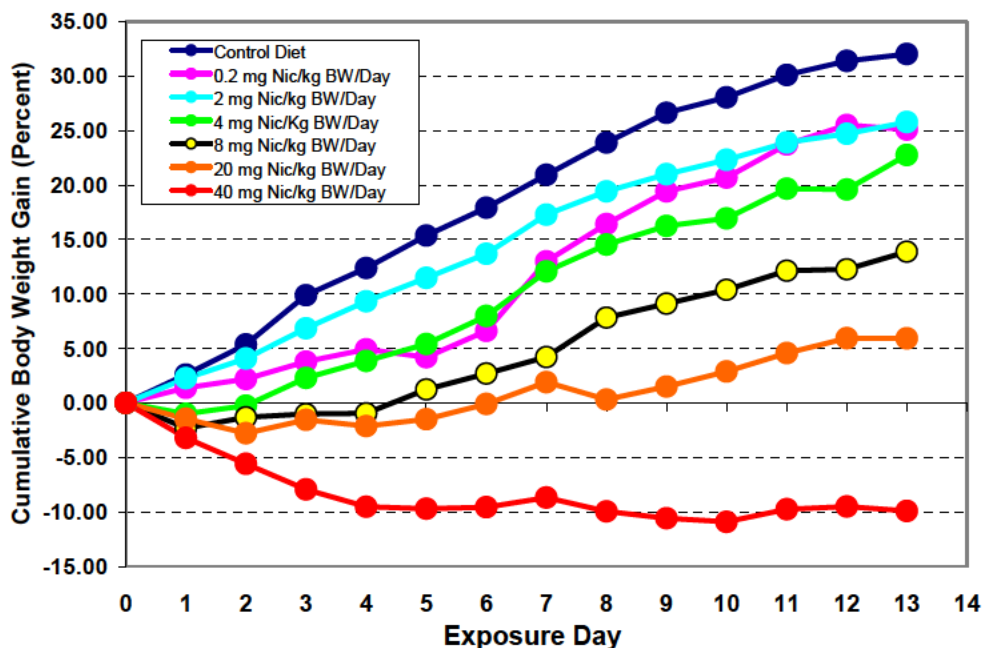
¹Data represent the ratio of the number of animals demonstrating the effect to the initial number of animals in each group. Data in brackets represent the group incidence and number of animal days with the clinical finding.

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](#) provides the body weight data for rats 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.

Figure 1

Study TOX209 Cumulative Percent Body Weight Gain: Test Article Tobacco Blend*

*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 rats 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.

As seen in Figure 1, the control group demonstrated a normal growth curve with the expected increase in percent cumulative body weight gains for male rats of this age. Rats consuming feed formulated with the tobacco blend demonstrate a definitive dose response. As the dose of the tobacco blend increased the percent cumulative body weight gain decreased and there was a definitive loss of body weight at the 40 mg nicotine/kg bw/day. One exception to the dose response is seen in rats fed the lowest dose, 0.2 mg nicotine/kg bw/day. At this dose, body weight gain was similar to that seen at 4 mg nicotine/kg bw/day. Currently, there is no explanation for this exception other than possible animal variability not accounted for because of the small number of rats per group.

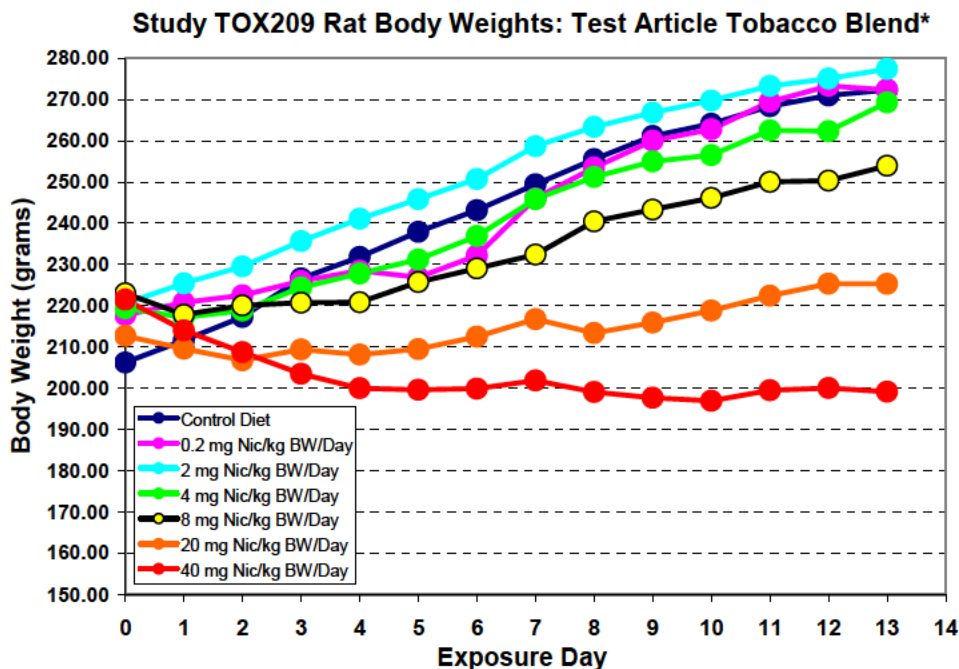
At 20 mg nicotine/kg bw/day there was a slight depression in body weight through day six of the study. After day six, this group began to increase in body weight but body weight gain was less than that of rats exposed to the lower doses. The increases in body weight gain after day six indicate that the rats may have been acclimating to the presence of the tobacco blend in their feed. Rats at the 8 mg nicotine/kg bw/day dose show a similar pattern but begin to increase in body weight at day five of the study. At 4 mg nicotine/kg bw/day, body weight gain began to increase at day three of the study. However, their body weight gain was less than that of rats fed the lower doses. Rats fed 2 mg nicotine/kg bw/day begin to demonstrate body weight gains

slightly less than those of the control by day three of the study and this trend continued for the remainder of the study. As previously noted, rats fed the 0.2 mg nicotine/kg bw/day resembled those fed the 4 mg nicotine/kg bw/day dose. Taken together, these data indicate a dose response in respect to acclimation to the dosed feed. As the nicotine dose increased, so did the period of acclimation to the dosed feed.

These data indicate that the rats could detect the presence of the tobacco blend at all doses used in this study. Either loss of body weight or pronounced depressions in body weight gain were encountered at the 20 and 40 mg nicotine/kg bw/day. These data indicate that a dose 40 mg nicotine/kg bw/day would not be appropriate for use in longer term studies because of the significant loss of body weight with little evidence of acclimation at this dose. At 20 mg/kg bw/day, there is some evidence that the rats were attempting to acclimate to the feed but body weight gain was still highly depressed. However, this dose could be used as the high dose in a 28-day repeated dose toxicology study to acquire useful toxicology data. The current study was a palatability study and did not include most of the toxicological endpoints generally used. Doses between 0.2 and 8 mg nicotine/kg bw/day should also be considered for future studies.

Body weights in grams for each dose group are presented in Table 2. As would be expected, these data follow the trends seen for percent body weight gain after adjustment for the differences in body weight at the initiation of the feeding period.

Figure 2

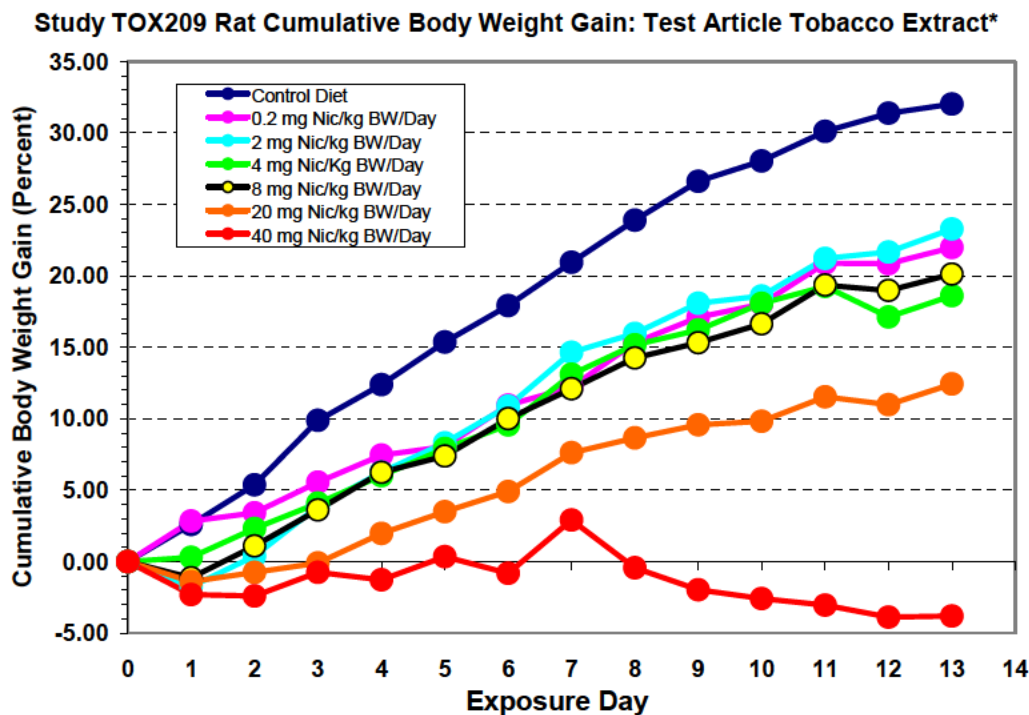


*Data represent the group mean daily body weight. Exposure day zero represents the body weights before exposure to the dosed feed. Exposure day one represents data acquired after one day of exposure to the untreated control feed or feed formulated with the tobacco blend. Body weights were not determined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

At least two possibilities should be considered in respect to explaining these data. First, at an organoleptic level, the rats may consider diets containing the tobacco blend 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 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 in reduced body weight gain.

Percent body weight gain data for rats fed feed formulated with different concentrations of the tobacco extract are presented in Figure 3.

Figure 3



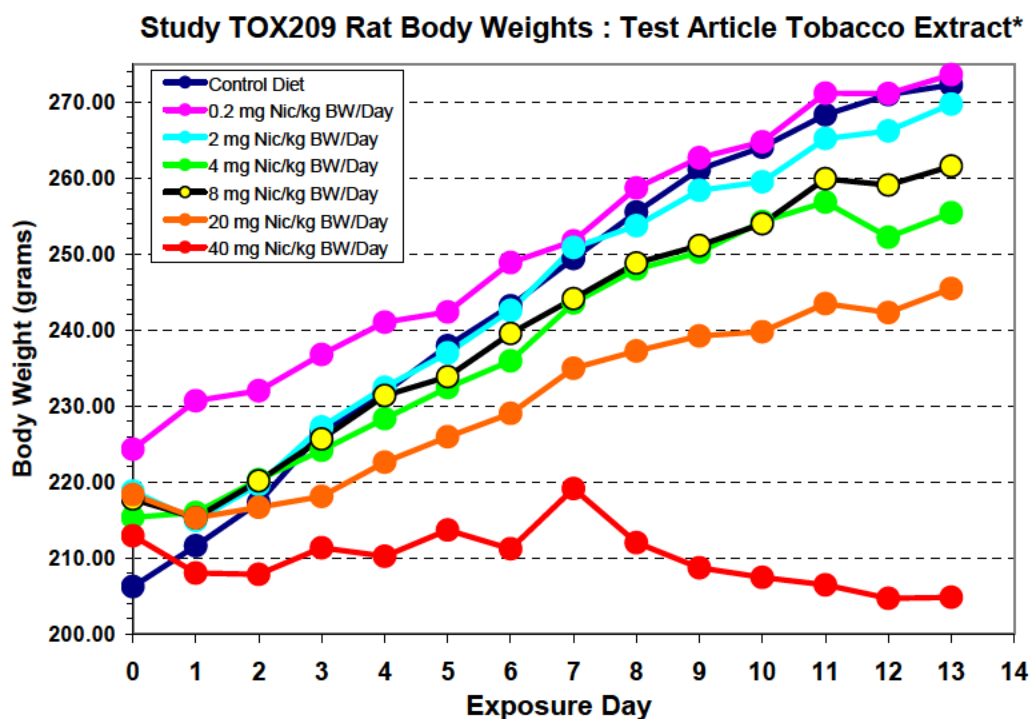
*Data represent the mean cumulative body weight gain expressed as a percent of the initial body weight. Exposure day zero represents the body weight of the rats before being exposed to the dosed feed. Exposure day one represents data acquired after one day of exposure to the untreated control feed or 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.

These data are similar to those from the rats fed feed containing the tobacco blend. All dose groups demonstrated reduced percent cumulative body weight gain when compared to the control. At the high dose, loss of body weight was not reduced to the same degree as with the tobacco blend and did not become constant until after day nine of the study. Similarly, the

decrease in body weight gain at 20 mg nicotine/kg bw/day was not as great as seen with the tobacco blend. At the lower doses, there was not as good a dose response as seen with rats fed feed containing the tobacco blend even though the nicotine concentrations were the same. It may be that the rats found the diets containing the tobacco extract more palatable than those containing the tobacco blend.

Figure 4 provides the data for body weights in grams throughout the study. The trends in body weight follow those seen in the percent cumulative body weight gain data; however, they are shifted because the group mean body weights differed on the first day of the study.

Figure 4

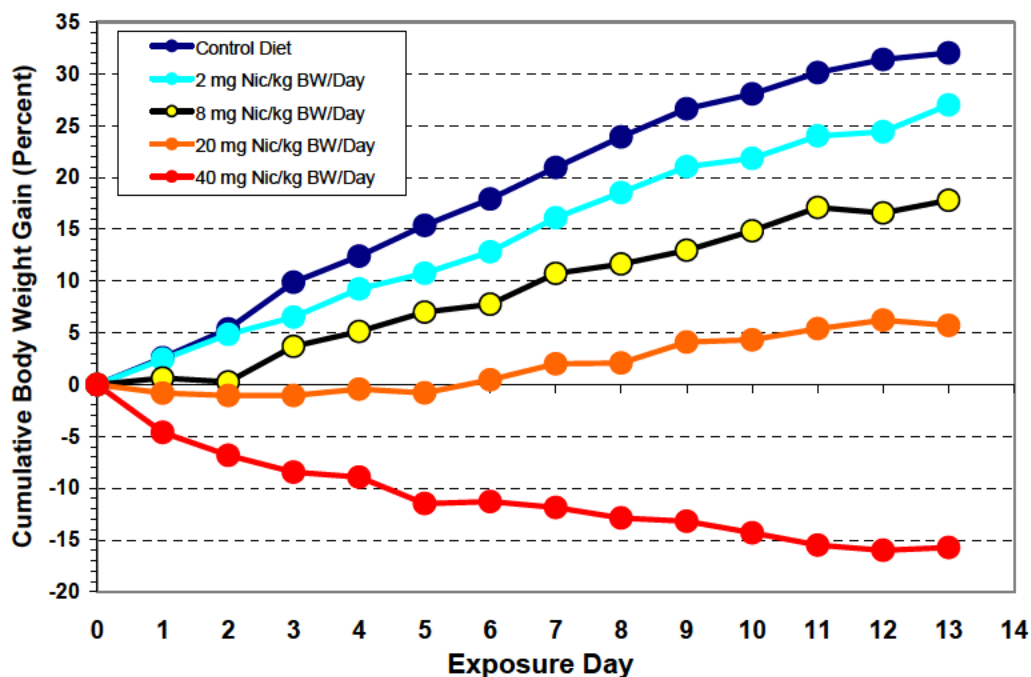


*Data represent the group mean daily body weight. Exposure day zero represents the body weights before exposure to the dosed feed. Exposure day one represents data acquired after one day of exposure to the untreated control feed or feed formulated with the tobacco extract. Body weights were not determined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

These data follow the trends seen when the data is expressed as percent cumulative body gain, as would be expected.

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

Figure 5

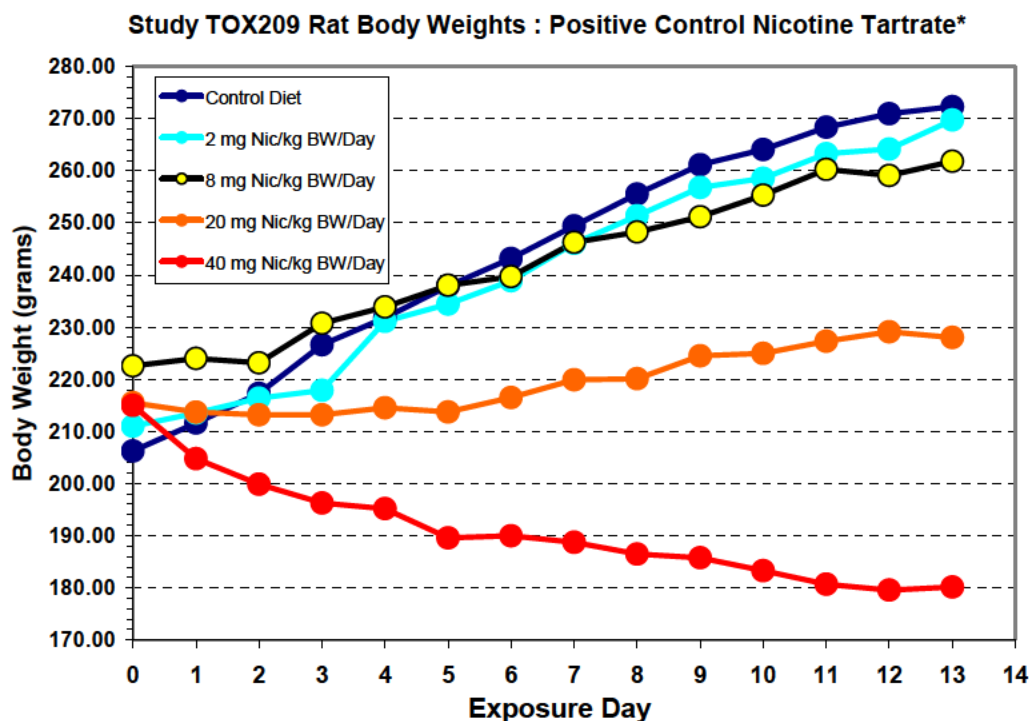
Study TOX209 Cumulative Percent Body Weight Gain : Positive Control Nicotine Tartrate*

*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 rats 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.

Rats fed feed containing nicotine tartrate demonstrated a definitive dose response in percent cumulative body weight gain. As the dose of nicotine tartrate increased body weight gain decreased. At the high dose there was a continued loss in body weight. This loss was higher than those fed equivalent doses of nicotine from the tobacco blend and tobacco extract. At 20 mg/kg bw/day there was a lack of body weight gain until day seven of the study. This was followed by a slight increase in body weight gain until the end of the study. The decrease in body weight gain at 8 mg nicotine/kg bw/day was more than that seen at 2 mg nicotine/kg bw/day. Again, it is obvious a dose of 40 mg nicotine/kg bw/day is too high for use in longer term studies. The overall trends in the data follow those seen with the tobacco and tobacco extract. Whether or not this is due to a lack of palatability of the feed or to a neurophysiological effect of nicotine can not be ascertained from this study. Because of the generally parallel effects between nicotine and the tobacco test articles, these data may indicate that the lack of palatability in the dosed feed may be more associated with its nicotine content than the presence of other tobacco components.

Figure 6 presents the body weights of the rats fed feed containing nicotine hydrogen tartrate on each day of the study. The trends in body weight follow those seen with percent body weight gain. Again, because mean body weights in grams differed on day one of the study these data need to be adjusted to account for these differences.

Figure 6



*Data represent the group mean daily body weight. 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.

As would be expected these data follow the trends seen when the data is expressed as percent cumulative body weight gain and clearly indicate a dose response.

6. Terminal Body Weights

Group mean body weights at study termination are provided in [Table 16](#). Individual animal Terminal Body Weights are provided in [Appendix IX](#).

Table 16: **Terminal Body Weights**

Group	Treatment	Terminal Body Weight (g) \pm SD
1	NTP-2000 Feed	281.7 \pm 16.5
	<i>Smokeless Tobacco Blend</i>	
2	Dose 1 Tobacco in NTP-2000 Feed (0.2) ¹	282.9 \pm 20.9
3	Dose 2 Tobacco in NTP-2000 Feed (2.0)	283.3 \pm 23.7
4	Dose 3 Tobacco in NTP-2000 Feed (4.0)	275.0 \pm 21.6
5	Dose 4 Tobacco in NTP-2000 Feed (8.0)	259.0 \pm 20.9
6	Dose 5 Tobacco in NTP-2000 Feed (20.0)	227.0 \pm 18.7*
7	Dose 6 Tobacco in NTP-2000 Feed (40.0)	204.1 \pm 13.7*
	<i>Tobacco Extract</i>	
8	Dose 1 Tobacco Extract in NTP-2000 Feed (0.2)	276.8 \pm 31.3
9	Dose 2 Tobacco Extract in NTP-2000 Feed (2.0)	276.6 \pm 19.0
10	Dose 3 Tobacco Extract in NTP-2000 Feed (4.0)	265.9 \pm 14.6
11	Dose 4 Tobacco Extract in NTP-2000 Feed (8.0)	266.3 \pm 18.7
12	Dose 5 Tobacco Extract in NTP-2000 Feed (20.0)	255.6 \pm 17.8
13	Dose 6 Tobacco Extract in NTP-2000 Feed (40.0)	206.3 \pm 9.9*
	<i>Positive Control</i>	
14	Dose 1 Nicotine Tartrate in NTP-2000 Feed (2.0)	276.5 \pm 21.1
15	Dose 2 Nicotine Tartrate in NTP-2000 Feed (8.0)	266.0 \pm 25.0
16	Dose 3 Nicotine Tartrate in NTP-2000 Feed (20.0)	233.7 \pm 21.7*
17	Dose 4 Nicotine Tartrate in NTP-2000 Feed (40.0)	179.9 \pm 32.8*

¹Nicotine doses in mg nicotine/kg body weight/day are provided in parentheses.

*Statistically significant difference from Group 1 NTP-2000 Feed ($p \leq 0.05$)

Terminal body weights determined for the groups fed feed containing the tobacco blend on the last day of the study were significantly lower than the control group for the 20 and 40 mg nicotine/kg body weight/day dose groups. The data for the rats fed feed containing the tobacco extract were significantly reduced only at the 40 mg nicotine/kg body weight/day. This may indicate, again, that the feed containing the tobacco extract appears to be more palatable than that containing the tobacco blend. Terminal body weights for rats fed feed containing nicotine hydrogen tartrate were significantly reduced when compared to the control at 20 and 40 mg nicotine/kg body weight/day as seen with the tobacco blend. It would be assumed that the nicotine hydrogen tartrate containing feed would not have the same organoleptic characteristics as those containing tobacco. This may indicate that the lower body weights may be more associated with the effects of nicotine than the organoleptic characteristics of feed contain tobacco or tobacco extract. Additional studies may clarify this hypothesis.

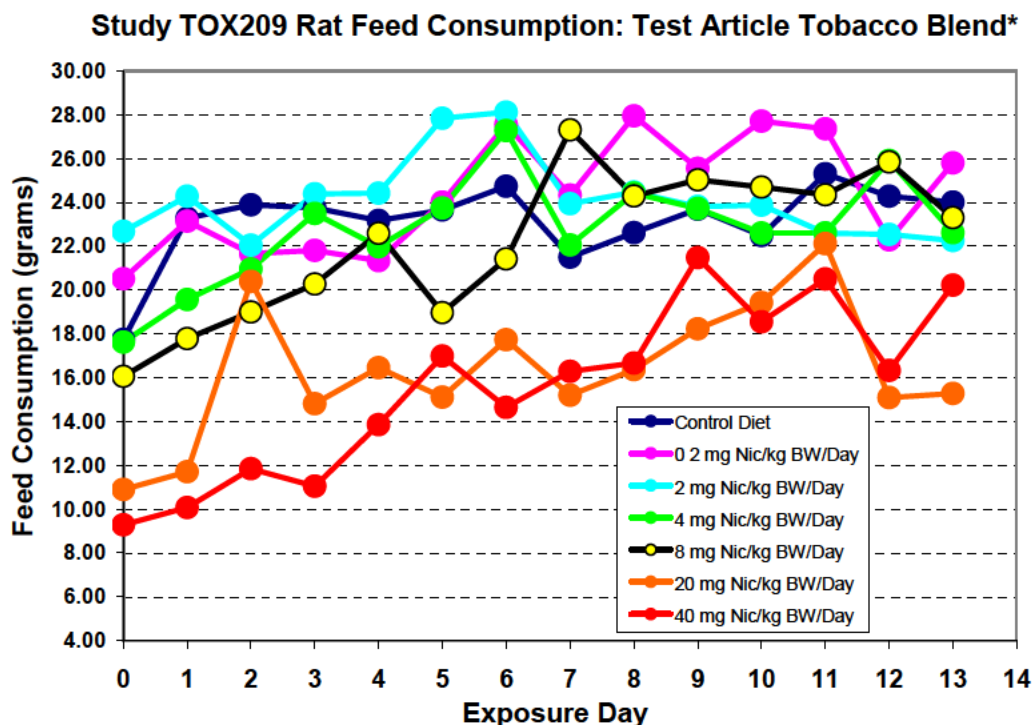
7. Feed Consumption

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 rats fed feed containing the tobacco blend, tobacco extract or nicotine hydrogen tartrate are shown in Figures 7-9.

Feed consumption data for rats fed feed containing the tobacco blend are shown in Figure 7

Figure 7



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

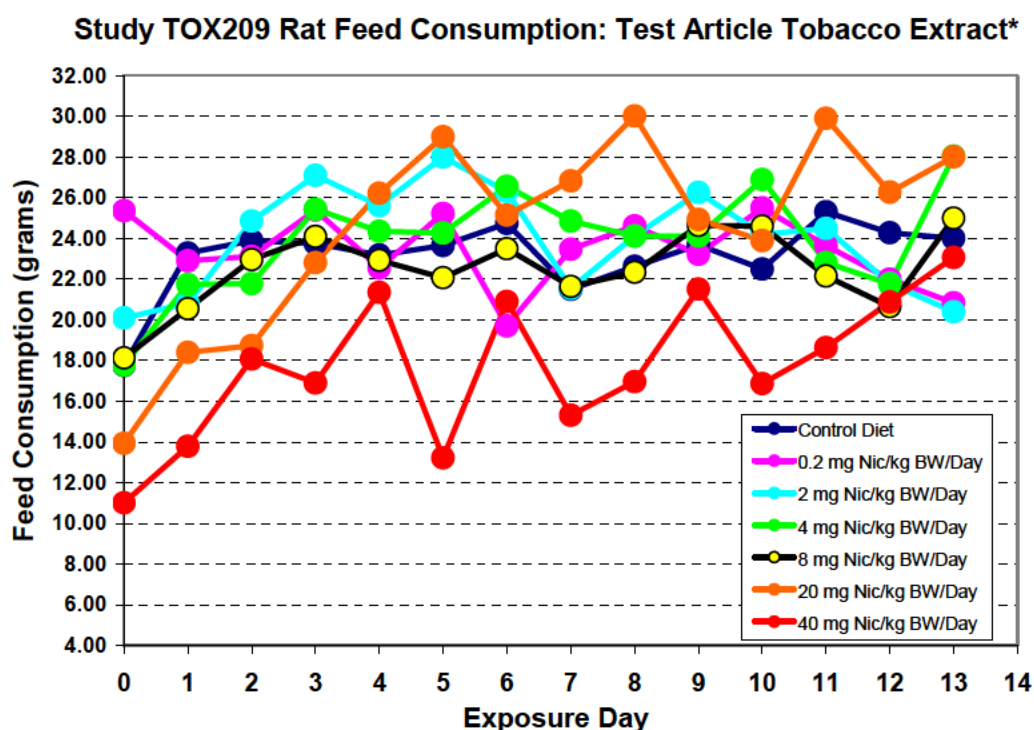
Data for feed consumption for the rats in this study appear erratic on a daily basis irrespective of which feed they were provided, including the control group. The rats fed the control diets maintained a relatively constant feed consumption during the study. The lowest feed consumption was seen in the groups fed 20 and 40 mg nicotine/kg bw/day. These data parallel those seen for body weights. Although the rats fed the 40 mg nicotine/kg bw/day increased their feed intake after the severe depression seen during the first day of the study, they never reached that of the control group. Rats fed 20 mg nicotine/kg bw/day also demonstrated a large decrease in feed consumption during the first day of the study. The 20 mg nicotine/kg bw/day group followed the trend seen at 40 mg nicotine/kg bw/day after day five. Rats fed feed containing 8 mg nicotine/kg body weight/day also showed a decline in feed consumption during the first day of the study but then increased their feed consumption until they reached near control values by

day 7-8, indicating acclimation to the dosed feed. After day eight, their feed consumption was similar to that of the controls. The lower doses generally followed the trends seen with the control group in respect to feed consumption, although their body weights were less than those of the controls during the study.

Overall, the feed consumption at the two highest doses was generally lower than the feed consumption seen at the lower doses and paralleled the body weight data. Again, the 40 mg/kg bw/day dose appears too high for use in future studies, while the 20 mg/kg bw/day may be useful for toxicological assessment. At the lower doses, the feed consumption appears to be similar to that of the control even though body weights were decreased. Whether this was related to a neurophysiological effect of nicotine, such as appetite suppression, cannot be ascertained from this study.

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

Figure 8



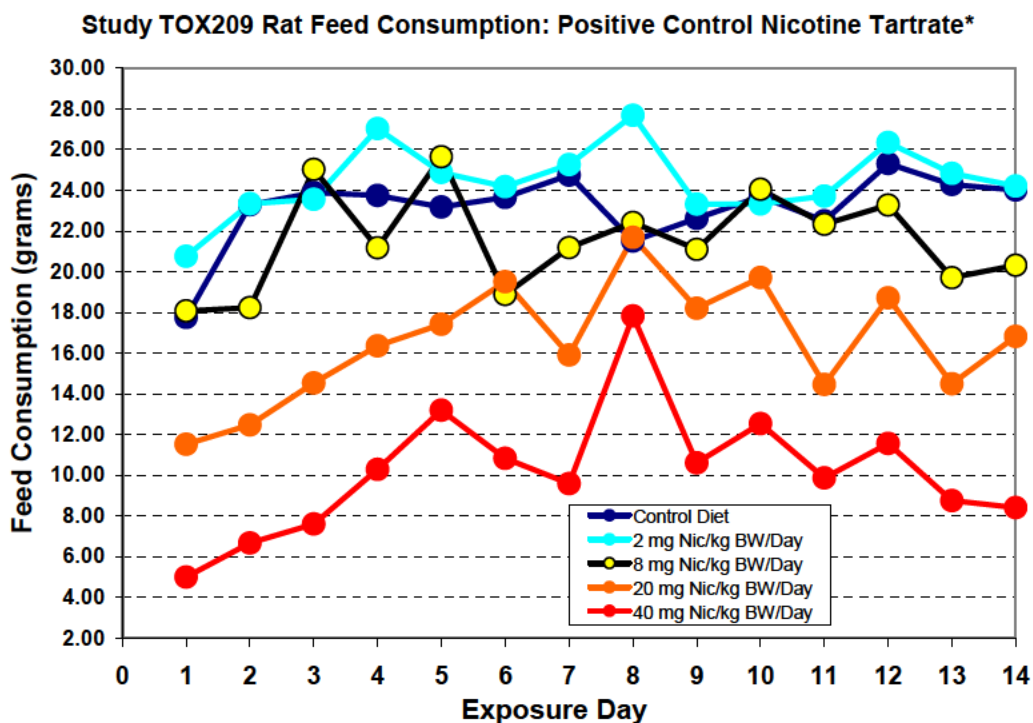
* Data represent the group mean feed consumption for rats fed different doses of nicotine in feed formulated with the tobacco extract.

Rats fed feed containing the tobacco extract at 40 mg nicotine/kg bw/day demonstrated a reduction in feed consumption compared to the control during the first day of the study. Subsequently, they increased their feed consumption but it was always below that of the control except for the last day of the study. At 20 mg nicotine/kg bw/day, there was also a drop in feed

consumption compared to the control during the first day of study. However, by day four their food consumption reached that of the control and remained near control values during the remaining duration of the study. This indicates the rats were acclimating to the dosed feed. In contrast, body weight of this group was less than that of the control. At the lower doses, there does not appear to be a trend toward feed consumption being different from that of the control and feed consumption did not parallel the body weight data because body weights were lower than the control for these groups.

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

Figure 9



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

At the two highest doses, consumption of feed containing nicotine hydrogen tartrate followed a dose response. At 40 mg nicotine/kg bw/day there was an immediate drop in feed consumption during the first day of the study. Although feed consumption increased during the first five days of the study, it did not reach that seen at the 20 mg nicotine/kg bw/day. The decreased feed consumption paralleled the decrease in body weight seen at this dose. At 20 mg nicotine/kg bw/day, there was also a sharp drop in feed consumption during the first day of the study when compared to the control. This drop in feed consumption was not as large as that seen at the highest dose. During the first six days of the study the 20 mg nicotine/kg bw/day dosed animals

demonstrated increased feed intake; this seems to stabilize during the remainder of the study. However, the feed intake of this group never reaches that of the control. These feed consumption data parallel the reductions in body weight gain seen in this group. Feed consumption at the two lower doses appears to follow the trends in feed consumption seen with the controls even though their body weight gains were decreased compared to the controls.

The changes in feed consumption seen with feed containing nicotine tartrate at doses of nicotine identical to those in feed containing tobacco and tobacco extract paralleled those seen with the tobacco and tobacco extract containing feed. This may indicate that nicotine could be the most critical component of the tobacco blend and extract that alters the palatability and affects the body weights of rats fed the dosed feed.

X. DISCUSSION

Review of the feed formulation nicotine analysis data indicates that the formulation methodology developed for this study produced a homogenous 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 investigational 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 homogeneously mixed within the feed to an extent that avoided the occurrence of random areas of either low or higher concentrations different from the anticipated concentrations. This indicates the rats 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 rats. The room temperature stability of the test articles and positive control in the feed, measured as nicotine, were determined to assess the required frequency of preparation of the formulated feed. This study revealed the formulated feed was stable for at least ten-days. This confirmed the adequacy of the preparation of formulated feed weekly for this study. An additional room temperature stability investigation indicated that the formulated feed was stable for at least one-month. This indicates that future studies would not require weekly feed formulations (monthly formulations would be adequate).

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, Wistar Hannover rats used in this study were maintained under protocol specified conditions throughout the study and data related to light cycle, room temperature, humidity, room air exchanges and water quality demonstrated no excursions that could impact the results of the study. In addition, serological and health assessment of sentinel rats indicated no evidence of the presence of contagious disease.

Rats in the control group demonstrated the expected body weight gains for male rats of their age throughout the study, indicating that there were no conditions other than the dosed feed that

could unduly affect body weight during the study. Rats fed feed formulated with the tobacco blend, tobacco extract or nicotine tartrate demonstrated a strong dose response in respect to body weight. As the dose of nicotine increased, body weight gain decreased and there was a definitive loss of body weight at the 40 mg nicotine/kg body weight/day. Comparison of the body weight data from this rat study to a parallel mouse study (TOX210) using identical doses indicates that rats are more susceptible to the effects of the test articles and positive control on body weight changes and feed intake than mice.

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. Rats provided feed formulated with the tobacco blend at doses of 0.2-40 mg nicotine/kg bw/day demonstrated 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 demonstrating a loss in body weight gain throughout the study. The final body weights at 40 mg nicotine/kg bw/day were statistically significantly lower than those of the control group. Because this decrease is considered excessive, it clearly indicates that the high dose would not be appropriate for use in longer term studies. At 20 mg nicotine/kg bw/day, there was a lack of body weight gain during the first week of the study. However, during the second week of the study weight demonstrated a slight increase as the rats acclimated somewhat to the dosed feed. At the termination of the feeding period, the rats at this dose demonstrated a statistically lower body weight compared to the control group. This dose could be considered as the high dose in a short-term repeated dosing toxicology study. At the 0.2-8.0 mg nicotine/kg bw/day doses, there were dose related decreases in body weights compared to the control. As these doses increased there were dose related delays in the ability of the rats to acclimate to the dosed feed. Doses within this range would be appropriate for use in a short-term repeated dosing toxicology study.

Cumulative percent body weight gain in rats fed feed containing the tobacco extract at doses equivalent to those fed the tobacco blend demonstrated trends almost identical to those seen with the tobacco blend in respect to body weight gain. As the dose of nicotine increased, the body weight gain of the rats decreased in a dose dependent manner. However, the decreases seen at the 20 and 40 mg nicotine/kg bw/day were not as depressed as with the tobacco blend. This may indicate the palatability of the feed was greater when formulated with the extract as opposed to the blend. At the termination of the feeding period, the body weights of the rats fed the high dose were statistically significantly lower than the control while the rats fed the 20 mg nicotine/kg bw/day did not statistically differ from the control group. As seen with the data from rats fed the tobacco blend, a dose of 40 mg nicotine/kg bw/day appears excessive for use in a short-term repeated dosing study of longer duration than this palatability study. A high dose of 20 mg nicotine/kg bw/day could be considered for use as a high dose for the tobacco extract in a short-term repeated dose toxicology study with additional doses in the range of 0.2-8.0 or slightly higher.

When rats were fed feed formulated with nicotine hydrogen tartrate, there was a definitive dose response with cumulative body weight gain decreasing as the dose increased. At 40 mg nicotine/kg bw/day body weight gain was negative throughout the 14-day study, indicating actual loss of body weight. Reduction of body weight gain was more pronounced with the

positive control than with either of the test articles. At 20 mg nicotine/kg bw/day, the trend was similar to that of the tobacco blend but greater than that seen with the tobacco extract. This supports the conclusion that a nicotine dose of 40 mg/kg bw/day would not be appropriate for a longer term study while 20 mg nicotine/kg bw/day could be used as the high dose in a short-term repeated dose toxicology study. The similarity of trends seen with the nicotine tartrate positive control compared to the two test articles may indicate the lower cumulative body weight gains seen with the test articles are more related to their nicotine content than their content of other tobacco components.

An important finding in this study is that rats are more sensitive to the effects of the test articles and positive control on body weight than are mice. Rats fed feed containing either the tobacco blend, tobacco extract or nicotine hydrogen tartrate at concentrations that yielded nicotine doses between 0.2 and 40.0 mg/kg bw/day demonstrated dose dependent reductions in body weight gain while mice demonstrated a minimal response only at the high dose. This species difference in sensitivity must be taken into consideration when designing longer term toxicology studies.

XI. 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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- TOX210 Two Week Investigational Study of the Palatability of Smokeless Tobacco Blend and Extract Formulated in NTP-2000 Diets for Mice.

Appendix I

Study Protocol, Amendments to Protocol, Xybion Protocol, Notes to Study File,
Preliminary Report

RJReynolds

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

Protocol Identifier: TOX209

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

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Jenny L. Smith, B.S.

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Date: 3-31-08

Attending Veterinarian:

Preclinical Models of Disease

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Date: 3 Apr 08

Vice-President,
Product Integrity:

Christopher J. Cook
Christopher J. Cook, Ph.D.

Date: 4/4/08

Anticipated Rat Delivery Date: April 9, 2008

Anticipated Final Report Date: November 5, 2008

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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

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Research Resources of North Carolina, Inc.
 On-site

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Investigational Study of the Rat 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 rats is oral through a formulation of the test articles into NTP-2000 feed to be provided to the rats. This raises a fundamental question concerning the acceptance of the formulated feed by the rats. Addition of the test articles to the feed may make it unpalatable to the rats. 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 Wistar Hanover rats (the strain to be used in the toxicology studies) that are closely matched to the age of the rats 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 rats. 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 Wistar Hanover rats.

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 rat'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 rats. If the feed is less palatable than the control diet, the rats 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 rats. 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 rats fed the standard NTP-2000 diet (control group) to the feed intake of rats 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. Feed intake will be measured daily during the 14 day study. In addition, the body weights of the rats fed the control NTP-2000 diet will also be determined daily. Twice daily mortality and morbidity observations will be conducted on all study rats 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 rats per group are provided in the following table:

Group Number	Treatment Group (Doses based on Nicotine) (mg/kg body weight/day)	Number of Rats	Rat 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 Rats

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 search 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 animal model was the mouse, 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 rats; therefore, rat 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

Rats have been classically used in toxicology studies and rat studies are required and accepted by U.S. regulatory agencies as well as international regulatory agencies. Specifically, rats 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 Wistar Hanover Rat

The Wistar Hanover rat has recently been chosen to replace the standard rat (Fisher 334) that has been used by the National Toxicology Program. The reasons for this change include the greater survivability of the Wistar Hanover rat in 104-week oncogenicity studies and its tendency to have fewer spontaneous tumors than other commonly used rat strains. This rat 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 Wistar Hanover rat 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 rats in these studies. This presents the possibility that they will alter the palatability of the diets to the extent that the rats 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 rats to be used is the minimum associated with meaningful statistical analyses of the data. A minimum of 90 (male) Wistar Hanover, juvenile rats (5-7 weeks of age) will be received from Charles River Laboratories (Raleigh, NC) for conduct of this study. Assigned to the TOX209 Xybin protocol will be 85 male rats for the experimental groups, as well as 10 male rats (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 rats/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 rats each. Extra rats (five males) 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 rats 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 (CO₂) in air [*SOP TOX057*]. The final fate of each animal will be documented.

Quarantine/Acclimation and Serological Evaluation

Rats 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 rats will be assigned a pre-allocation identification number, and that number will be indicated on the corresponding cage card.

At the termination of the 14-day feeding period, the 10 sentinel rats 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), Rat Minute virus (RMV), Parvovirus (RPV), Reovirus (REO), *Mycoplasma pulmonis* (MPUL), Lymphocytic choriomeningitis virus (LCMV), Mouse adenovirus 1&2 (MAV), Hantaan virus (HANT), *Encephalitozoon cuniculi* (ECUN), Cilia Associated Respiratory Bacillus (CARB), Sialodacryoadenitis virus (SDAV), Kilham rat virus (KRV), H-1 virus (H-1), GDVII (Murine Encephalomyelitis Virus), and Rat PARV NS1.

Rats euthanized for serological evaluation will then be necropsied to determine any evidence of disease. The carcasses of these rats 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 rats within four days after delivery. Commencement of rat dosing is dependent upon a favorable review of the health examination, as well as a written statement from the Attending Veterinarian releasing the rats from quarantine.

Allocation of Animals to Study Groups

On the fourth 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. At the discretion of the Study Director, rats 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. Rats not selected during the allocation process will either be transferred to another IACUC-approved protocol, or euthanized using 70% CO₂ in air (*SOP TOX057*). The final fate of each rat 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, rats will be uniquely identified with their permanent identification number by tail tattoo [*SOP TOX041*]. Rats 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 TOX067*).

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 rats 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 (18-26°C, or 64.4-78.8°F), 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*).

Rats will be individually housed in polycarbonate cages 19 in. (L) x 10 ½ in. (W) x 8 in. (H) with Alpha-Dri bedding. Rack and cage maintenance will be conducted according to *SOPs TOX016, TOX018, TOX019, TOX020, TOX021, TOX022, EQP002, EQP026, EQP027, EQP035, and EQP072*.

Rats will have *ad libitum* access to NTP-2000 feed, with the exception of the sentinel rats, 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*). Feeders will be placed within a glass dish with vertical sides to catch spilled feed. Spilled feed will be weighted or estimated and accounted for when determining feed consumption. Water will be provided to rats 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 rat 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 rats, including tremors, lethargy and increased sensory sensitivity; while unlikely, in some instances, the rats 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, rats 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 rats will be anesthetized with 70% CO₂ in air and euthanized by exsanguination during serological evaluation (*SOPs TOX002, TOX004, TOX010*). Euthanasia by 70% CO₂ in air is used at terminal sacrifice and for rats 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 hood (*SOP EQP056*), the existence of a room ventilation system, and the use of a container-within-a-container system for transport 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 rats, 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 rats. 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 ($\leq 0^{\circ}\text{C}$). 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 rat diets will be maintained frozen ($\leq 0^{\circ}\text{C}$).

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 ($\leq 0^{\circ}\text{C}$). 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 ($\leq 0^{\circ}\text{C}$).

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). Rat 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 4°C) 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 rats. 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 rats, with the exception of the sentinels, 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 rats, once in the morning and once in the afternoon (at least 6 hours apart) will be performed to identify dead or moribund rats (*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.

Rats 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 rat, 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 rat 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 rats euthanized at study completion. In addition, terminal weights will be taken for rats 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 rats found dead.

Feed Consumption

The day before the start of the 14 day study period, each rat’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 rat’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 rat, weighed fresh feed will be placed in a clean tared feed cup and provided to the rat.

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. Rats 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
- Rat ordering, receipt and quarantine records
- Health screening data
- Records of allocation of rats 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
- Rat 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 rats 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

References

- 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. Swedish 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: TOX209

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

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 rats 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 rats will have *ad libitum* access to NTP-2000 feed. The sentinel rats 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: TOX209

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

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 Rats 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: TOX209

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

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 Rats 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: TOX209

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

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 rats 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: TOX209

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

Study Director: Jenny L. Smith

Protocol Amendment:

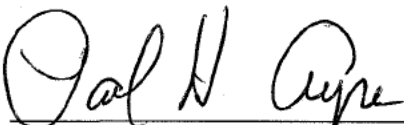
The protocol for Study TOX209 is amended as follows:

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, PhD, DBAT

3-23-2009
Date

Xybion Protocol

R.J.R. Tobacco
Toxicology Division
Building 630/2
Winston-Salem, North Carolina

Protocol RJR-084
Study number: TOX209A

Printed: 22-Apr-08
Page: 1

1.0 STUDY TITLE

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

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 Wistar Hanover rats.

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
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.
QA Investigator	Jeffrey Dwayne Hedrick
Animal Care Technician:	Abraham Doby
Animal Care Technician:	ANDRE BRYANT
Animal Care Technician:	PATRICIA BATCHELOR

R.J.R. Tobacco
Toxicology Division
Building 630/2
Winston-Salem, North Carolina

Protocol RJR-084
Study number: TOX209A

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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

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 - - - - - RAT
Strain - - - - - WISTAR HANOVER
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

R.J.R. Tobacco
Toxicology Division
Building 630/2
Winston-Salem, North Carolina

Protocol RJR-084
Study number: TOX209A

Printed: 22-Apr-08
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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

Abv	Parameter	Units	Parameter range limits				# Dec Pts
			Male		Female		
			Low	High	Low	High	
BW	BODY WEIGHTS	GRAMS	100.00	200.00	100.00	200.00	2

10.1.2 CLINICAL SIGNS 2 /day

Scheduled Days: 3 6

Abv	Parameter
CS	CLINICAL SIGNS

10.1.3 FULL FEEDER WEIGHT FUNCTIONS

Scheduled Days: 6

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

10.1.4 EMPTY FEEDER WEIGHT FUNCTIONS

Scheduled Days: 7

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

10.2 ANIMAL ROOM FUNCTIONS (Exposure phase)

10.2.1 BODY WEIGHT FUNCTIONS

Starting on day 1 every day through day 14

Abv	Parameter	Units	Parameter range limits				# Dec Pts
			Male		Female		
			Low	High	Low	High	
BW	BODY WEIGHTS	grams	150.00	275.00	0.0000	200.00	2

10.2.2 FULL FEEDER WEIGHT FUNCTIONS

Starting on day 1 every day through day 14

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

10.2.3 EMPTY FEEDER WEIGHT FUNCTIONS

Starting on day 2 every day through day 15

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

10.2.4 CLINICAL SIGNS

2 /day

Scheduled Days: 6 10 13 17

Abv	Parameter
CS	CLINICAL SIGNS

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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	- - - - -	Carbon dioxide
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

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Toxicology Division
Building 630/2
Winston-Salem, North Carolina

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1.0 STUDY TITLE

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

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 Wistar Hanover rats.

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
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.
QA Investigator	Jeffrey Dwayne Hedrick
Animal Care Technician:	Abraham Doby
Animal Care Technician:	ANDRE BRYANT
Animal Care Technician:	PATRICIA BATCHELOR

R.J.R. Tobacco
Toxicology Division
Building 630/2
Winston-Salem, North Carolina

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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

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 - - - - - RAT
Strain - - - - - WISTAR HANOVER
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

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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

Abv	Parameter	Units	Parameter range limits				# Dec Pts
			Male		Female		
			Low	High	Low	High	
BW	BODY WEIGHTS	GRAMS	100.00	200.00	100.00	200.00	2

10.1.2 CLINICAL SIGNS 2 /day

Scheduled Days: 3 6

Abv Parameter

CS CLINICAL SIGNS

10.1.3 FULL FEEDER WEIGHT FUNCTIONS

Scheduled Days: 6

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

10.1.4 EMPTY FEEDER WEIGHT FUNCTIONS

Scheduled Days: 7

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

10.2 ANIMAL ROOM FUNCTIONS (Exposure phase)

10.2.1 BODY WEIGHT FUNCTIONS

Starting on day 1 every day through day 14

Abv	Parameter	Units	Parameter range limits				# Dec Pts
			Male		Female		
			Low	High	Low	High	
BW	BODY WEIGHTS	grams	150.00	275.00	0.0000	200.00	2

10.2.2 FULL FEEDER WEIGHT FUNCTIONS

Starting on day 1 every day through day 14

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

10.2.3 EMPTY FEEDER WEIGHT FUNCTIONS

Starting on day 2 every day through day 15

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

10.2.4 CLINICAL SIGNS

2 /day

Scheduled Days: 6 10 13 17

Abv	Parameter
CS	CLINICAL SIGNS

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Winston-Salem, North Carolina

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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	- - - - -	Carbon dioxide
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

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Toxicology Division
Building 630/2
Winston-Salem, North Carolina

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Study number: TOX209B

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1.0 STUDY TITLE

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

Positive Control and Sentinel 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 Wistar Hanover rats.

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
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

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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 - - - - - RAT
Strain - - - - - WISTAR HANOVER
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

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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

Abv	Parameter	Units	Parameter range limits				# Dec Pts
			Male		Female		
			Low	High	Low	High	
BW	BODY WEIGHTS	GRAMS	100.00	200.00	100.00	200.00	2

10.1.2 FULL FEEDER WEIGHT FUNCTIONS

Scheduled Days: 6

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

10.1.3 EMPTY FEEDER WEIGHT FUNCTIONS

Scheduled Days: 7

Abv	Parameter	Units	Parameter range limits				# Dec Pts
			Male		Female		
			Low	High	Low	High	
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

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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 14

Abv	Parameter	Units	Parameter range limits				# Dec Pts	
			Male		Female			
			Low	High	Low	High		
BW	BODY WEIGHTS	GRAMS	150.00	250.00	100.00	200.00	2	

10.2.2 FULL FEEDER WEIGHT FUNCTIONS

Starting on day 1 every day through day 14

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

10.2.3 EMPTY FEEDER WEIGHT FUNCTIONS

Starting on day 2 every day through day 15

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

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
-----	-----	-----	---

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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

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Toxicology Division
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Winston-Salem, North Carolina

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11.0 Protocol amendments:

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

Time: 10:48

Reason: reflect same schedule as 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: 15:50

Corrected schedule for clinical signs.

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

Time: 15:51

Amended schedule to accomodate body weight collection.

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

Time: 14:04

Reason: entry error

o PROPOSED DATES

Dosing initiation date - - - - - Wed. 16-Apr-08

Study completion date - - - - -

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

Time: 14:05

Reason: entry error

o PROPOSED DATES

Dosing initiation date - - - - - Wed. 16-Apr-08

Study completion date - - - - -

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

Time: 14:06

R.J.R. Tobacco
Toxicology Division
Building 630/2
Winston-Salem, North Carolina

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Reason: Delayed exposure ending JS 4-18-08

o PROPOSED DATES

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

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

Time: 14:10

Reason: Extended exposure end date js 4-18-08

Edited phase description

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

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

Time: 14:15

Reason: Delayed exposure start date js 4-18-08

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o CLINICAL SIGNS

2 /day

Scheduled Days: 1 5 8 12

11.9 Date: 20-Apr-08 Approved by: JENNY SMITH

Time: 14:01

Reason: Updated bwt targets

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o BODY WEIGHT FUNCTIONS

Abv	Parameter	Units	Parameter range limits				#
			Male		Female		
			Low	High	Low	High	Pts
BW	BODY WEIGHTS	5	100.00	200.00	0.0000	200.00	2

11.10 Date: 20-Apr-08 Approved by: JENNY SMITH

Time: 14:05

Reason: Updated feeder weights.

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Toxicology Division
Building 630/2
Winston-Salem, North Carolina

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o ANIMAL ROOM FUNCTIONS (Exposure phase)

o FULL FEEDER WEIGHT FUNCTIONS

Abv	Parameter	Units	Parameter range limits				# Dec Pts
			Male		Female		
			Low	High	Low	High	
FF	FULL FEEDER WEIGHTS	30	300.00	375.00	380.00	420.00	1

11.11 Date: 20-Apr-08 Approved by: JENNY SMITH

Time: 14:13

Reason: Updated empty feeder weight

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o EMPTY FEEDER WEIGHT FUNCTIONS

Abv	Parameter	Units	Parameter range limits				# Dec Pts
			Male		Female		
			Low	High	Low	High	
EF	EMPTY FEEDER/FEED CONSUMED	GRAMS	275.00	300.00	10.000	35.000	1

11.12 Date: 20-Apr-08 Approved by: JENNY SMITH

Time: 14:14

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

Time: 08:29

Reason: Updated body weight

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o BODY WEIGHT FUNCTIONS

Abv	Parameter	Units	Parameter range limits				# Dec Pts
			Male		Female		
			Low	High	Low	High	
BW	BODY WEIGHTS	5	150.00	250.00	0.0000	200.00	2

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

Time: 09:05

Reason: Updated body weight range limits

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Toxicology Division
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o ANIMAL ROOM FUNCTIONS (Exposure phase)

o BODY WEIGHT FUNCTIONS

Abv	Parameter	Units	Parameter range limits				# Dec Pts
			Male		Female		
			Low	High	Low	High	
BW	BODY WEIGHTS	grams	150.00	275.00	0.0000	200.00	2

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

Time: 09:07

Reason: Updated decimal parameters

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o FULL FEEDER WEIGHT FUNCTIONS

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

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

Time: 09:09

Reason: Updated full feeder weight parameter limits

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o EMPTY FEEDER WEIGHT FUNCTIONS

Abv	Parameter	Units	Parameter range limits				# Dec Pts
			Male		Female		
			Low	High	Low	High	
EF	EMPTY FEEDER/FEED CONSUMED	GRAMS	300.00	370.00	10.000	35.000	1

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

Time: 09:11

Reason: Updated empty feeder weight parameters

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o ANIMAL ROOM FUNCTIONS (Exposure phase)

o EMPTY FEEDER WEIGHT FUNCTIONS

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

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

Time: 09:39

Reason: Updated schedule for clinical observaions

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o CLINICAL SIGNS 2 /day

Scheduled Days: 6 10 13 17

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

Time: 20:37

Reason: Updated body weight limitations

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o BODY WEIGHT FUNCTIONS

Abv	Parameter	Units	Parameter range limits				# Dec Pts
			Male		Female		
			Low	High	Low	High	
BW	BODY WEIGHTS	grams	150.00	275.00	150.00	275.00	2

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

Time: 20:39

Reason: Updated feeder weights

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o FULL FEEDER WEIGHT FUNCTIONS

R.J.R. Tobacco
Toxicology Division
Building 630/2
Winston-Salem, North Carolina

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Abv	Parameter	Units	Parameter range limits				# Dec Pts
			Male		Female		
			Low	High	Low	High	
FF	FULL FEEDER WEIGHTS	grams	275.00	420.00	275.00	420.00	1

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

Time: 20:58

Reason: Updated body weight targets

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o BODY WEIGHT FUNCTIONS

Abv	Parameter	Units	Parameter range limits				# Dec Pts
			Male		Female		
			Low	High	Low	High	
BW	BODY WEIGHTS	grams	160.00	300.00	160.00	300.00	2

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

Time: 21:00

Reason: Updated full feeder weight targets

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o FULL FEEDER WEIGHT FUNCTIONS

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

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

Time: 21:01

Reason: Updated empty feeder weight targets

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o EMPTY FEEDER WEIGHT FUNCTIONS

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Toxicology Division
Building 630/2
Winston-Salem, North Carolina

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

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

Time: 16:25

Reason: Updated full feeder weight limitations

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o FULL FEEDER WEIGHT FUNCTIONS

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

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

Time: 16:26

Reason: Updated empty feeder weight limitations

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o EMPTY FEEDER WEIGHT FUNCTIONS

Abv	Parameter	Units	Parameter range limits				# Dec Pts
			Male		Female		
			Low	High	Low	High	
EF	EMPTY FEEDER/FEED CONSUMED	GRAMS	275.00	375.00	275.00	375.00	1

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

Time: 16:35

Reason: changed decimal place requirements

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o FULL FEEDER WEIGHT FUNCTIONS

R.J.R. Tobacco
Toxicology Division
Building 630/2
Winston-Salem, North Carolina

Protocol RJR-084
Study number: TOX209A

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

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

Time: 16:35

Reason: updated decimal place limitations

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o EMPTY FEEDER WEIGHT FUNCTIONS

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

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

Time: 10:25

Reason: Updated empty feeder limitations

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o EMPTY FEEDER WEIGHT FUNCTIONS

Abv	Parameter	Units	Parameter range limits				# Dec Pts
			Male		Female		
			Low	High	Low	High	
EF	EMPTY FEEDER/FEED CONSUMED	GRAMS	15.000	25.000	15.000	25.000	2

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

Time: 07:28

Corrected schedule due to change in starting date.

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

Time: 07:29

Corrected schedule due to change in starting date.

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

Time: 07:30

R.J.R. Tobacco
Toxicology Division
Building 630/2
Winston-Salem, North Carolina

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Corrected schedule due to change in starting date.

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

Time: 13:11

Reason: Updated body weight schedule

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o BODY WEIGHT FUNCTIONS

Starting on day 1 every day through day 19

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

Time: 13:12

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

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

Time: 13:13

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

R.J.R. Tobacco
Toxicology Division
Building 630/2
Winston-Salem, North Carolina

Protocol RJR-085
Study number: TOX209B

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1.0 STUDY TITLE

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

Positive Control and Sentinel 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 Wistar Hanover rats.

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
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

R.J.R. Tobacco
Toxicology Division
Building 630/2
Winston-Salem, North Carolina

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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 - - - - - RAT
Strain - - - - - WISTAR HANOVER
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

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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

Abv	Parameter	Units	Parameter range limits				# Dec Pts
			Male		Female		
			Low	High	Low	High	
BW	BODY WEIGHTS	GRAMS	100.00	200.00	100.00	200.00	2

10.1.2 FULL FEEDER WEIGHT FUNCTIONS

Scheduled Days: 6

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

10.1.3 EMPTY FEEDER WEIGHT FUNCTIONS

Scheduled Days: 7

Abv	Parameter	Units	Parameter range limits				# Dec Pts
			Male		Female		
			Low	High	Low	High	
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

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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 14

Abv	Parameter	Units	Parameter range limits				# Dec Pts	
			Male		Female			
			Low	High	Low	High		
BW	BODY WEIGHTS	GRAMS	150.00	250.00	100.00	200.00	2	

10.2.2 FULL FEEDER WEIGHT FUNCTIONS

Starting on day 1 every day through day 14

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

10.2.3 EMPTY FEEDER WEIGHT FUNCTIONS

Starting on day 2 every day through day 15

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

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
-----	-----	-----	---

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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

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Building 630/2
Winston-Salem, North Carolina

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11.0 Protocol amendments:

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

Time: 10:51

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: 16:27

Reason: changed body weight to accomodate new 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: 16:28

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: 14:21

Reason: Delayed exposure phase js 4-18-08

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: 14:22

R.J.R. Tobacco
Toxicology Division
Building 630/2
Winston-Salem, North Carolina

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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: 14:24

Reason: Delayed exposure phase

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o CLINICAL SIGNS 2 /day

Scheduled Days: 1 5 8 12

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

Time: 14:37

Reason: Delayed study start

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o CLINICAL SIGNS 2 /day

Scheduled Days: 1 5 8 12

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

Time: 08:33

Reason: Updated body weight targets

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o BODY WEIGHT FUNCTIONS

Abv	Parameter	Units	Parameter range limits				#
			Male		Female		
			Low	High	Low	High	Pts
BW	BODY WEIGHTS	GRAMS	150.00	250.00	100.00	200.00	2

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

Time: 08:37

Reason: Updated empty feeder weight range limits

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Toxicology Division
Building 630/2
Winston-Salem, North Carolina

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o ANIMAL ROOM FUNCTIONS (Exposure phase)

o EMPTY FEEDER WEIGHT FUNCTIONS

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

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

Time: 09:42

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: 21:06

Reason: Updated Body weight targets

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o BODY WEIGHT FUNCTIONS

Abv	Parameter	Units	Parameter range limits				# Dec Pts
			Male		Female		
			Low	High	Low	High	
BW	BODY WEIGHTS	GRAMS	160.00	300.00	160.00	300.00	2

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

Time: 21:07

Reason: Updated Full Feeder Weight targets

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o FULL FEEDER WEIGHT FUNCTIONS

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Toxicology Division
Building 630/2
Winston-Salem, North Carolina

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

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

Time: 21:09

Reason: Updated empty feeder weight targets

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o EMPTY FEEDER WEIGHT FUNCTIONS

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

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

Time: 16:37

Reason: updated decimal points limitations

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o FULL FEEDER WEIGHT FUNCTIONS

Abv	Parameter	Units	Parameter range limits				# Dec Pts
			Male		Female		
			Low	High	Low	High	
FF	FULL FEEDER WEIGHTS	GRAMS	340.00	375.00	340.00	375.00	2

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

Time: 16:38

Reason: updated decimal place limitations

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o EMPTY FEEDER WEIGHT FUNCTIONS

R.J.R. Tobacco
Toxicology Division
Building 630/2
Winston-Salem, North Carolina

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Abv	Parameter	Units	Parameter range limits				# Dec Pts
			Male		Female		
			Low	High	Low	High	
EF	EMPTY FEEDER/FEED CONSUMED	GRAMS	310.00	375.00	310.00	375.00	2

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

Time: 10:26

Reason: Updated empty feeder limitations

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o EMPTY FEEDER WEIGHT FUNCTIONS

Abv	Parameter	Units	Parameter range limits				# Dec Pts
			Male		Female		
			Low	High	Low	High	
EF	EMPTY FEEDER/FEED CONSUMED	GRAMS	15.000	25.000	15.000	25.000	2

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

Time: 07:31

Corrected schedule due to change in starting date.

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

Time: 07:32

Corrected schedule due to change in starting date.

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

Time: 07:32

Corrected schedule due to change in starting date.

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

Time: 13:17

Reason: Updated body weight schedule

R.J.R. Tobacco
Toxicology Division
Building 630/2
Winston-Salem, North Carolina

Protocol RJR-085
Study number: TOX209B

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-
- o ANIMAL ROOM FUNCTIONS (Exposure phase)
 - o BODY WEIGHT FUNCTIONS
 - Starting on day 1 every day through day 19
 - 11.21 Date: 30-Apr-08 Approved by: JENNY SMITH
 - Time: 13:18
 - 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
 - 11.22 Date: 30-Apr-08 Approved by: JENNY SMITH
 - Time: 13:19
 - Reason: Updated empty feeder weight function
 - o ANIMAL ROOM FUNCTIONS (Exposure phase)
 - o EMPTY FEEDER WEIGHT FUNCTIONS
 - Starting on day 2 every day through day 20

Notes to the Study File

TOX209-210 NOTES TO STUDY FILE

- 1) The exposure phase started on 4-21-08 instead of the proposed 4-16-08. JS 4-21-08
- 2) The clinicals were updated to be performed on the 6, 10, 13, and 17 days of exposure. JS 4-21-08
- 3) Rat #67 has lost a significant amount of weight 213.85 on 4-17-08 to 175.38 on 4-21-08, checked with Deborah Trail (animal care tech). Rat 67 was eating well today 4-21-08, will fill out vet request if rat continues to lose weight. Rat 67 (checked by Andre Bryant) lixit and teeth were checked and are fine. JS 4-21-08

TOX209 Preliminary Report

Preliminary Data Report for TOX209

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

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 8, 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
Attending Veterinarian, Preclinical Models of Disease
Program Manager, Research Resources of N.C., Inc.
Director, Product Integrity, In Vivo Toxicology
Senior Director, Product Integrity
Preclinical Models of Disease
Vice-President, Product Integrity

Jenny L. Smith, B.S.
Chandra D. Williams, D.V.M.
Jessica Baker, B.S., L.A.T
Paul H. Ayres, Ph.D., D.A.B.T.
Natalie Takenaka, Ph.D.

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 Wistar Hanover rats. 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 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 rat's feed. There is the possibility that incorporation of the test articles and positive control in the feed may alter its palatability to rats. If the feed is less palatable than the control diet, the rats 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 rats.

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

The control demonstrated normal body weights for male rats of the age used in the study. Rats fed feed formulated with the tobacco blend, tobacco extract or nicotine tartrate demonstrated a strong dose response in respect to body weight. As the dose of nicotine increased, body weight gain decreased and there was a definitive loss of body weight at the 40 mg nicotine/kg body weight/day.

These data indicate that the rats could detect the presence of the tobacco blend, tobacco extract or nicotine tartrate at all doses used in this study. Either loss of body weight or severe depressions in body weight gain were encountered at the 20 and 40 mg nicotine/kg body weight/day. These data indicate that these doses would not be appropriate for use in future studies. Doses between 0.2 and 8 mg nicotine/kg body weight/day may be considered for future studies.

The changes in feed consumption and body weight seen with feed containing nicotine tartrate, at doses of nicotine identical to that in feed containing tobacco and tobacco extract, paralleled those seen with the tobacco and tobacco extract containing feed. This may indicate that nicotine could be the most critical component of the tobacco that alters the palatability of the diets.

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 Wistar Hanover rats.

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 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 rat's feed (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 rats. If the feed is less palatable than the control diet, the rats 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 rats.

Palatability was assessed by comparing the feed intake of rats fed the standard NTP-2000 diet (control group) to the feed intake of rats 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. Feed intake was measured daily during the 14 day study. In addition, the body weights of the rats fed the control NTP-2000 diet were also determined daily. Twice daily mortality and morbidity observations were conducted on all study rats as well as twice weekly standard clinical observations. No additional data were collected. The duration of the feeding and data collection period was 14 days.

The doses used for the study were 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 planned short-term repeated dosing study. No undue toxicity was expected at these doses.

The experimental groups and the number of rats per group are provided in [Table 1](#).

Table 1: Treatment Groups and Doses¹

Group Number	Treatment Group (mg nicotine/kg body weight/day)	Number of Rats	Rat 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 test article was stored at 4 °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.

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. 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 °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, and then refrozen. The extraction water was analyzed for a series of components and the results are on file with the sponsor. 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 (IAUCC) before arrival of the animals into the facility. Ninety male, juvenile Wistar Hanover rats (5-7 weeks of age) from Charles River Laboratories (Raleigh, NC) were received into the facility on April 09, 2008. An additional 10 male, retired breeder Wistar Hanover rats were received for use as sentinel animals. These rats 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 rats 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).

The rats 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 18-26°C 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.

Rats were individually housed in polycarbonate cages 9.25 in. (L) x 10 ½ in. (W) x 8 in. (H) with Alpha-Dri bedding. Rats 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 rats 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 rats 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 rats. 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.

Rats 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, rats 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, rats were uniquely identified with their permanent identification number by tail tattoo and assigned to cages with permanent cage cards attached, recording 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 rats within four days after delivery. Commencement of rat dosing was dependent upon a favorable review of the health examination, as well as a written statement from the Attending Veterinarian releasing the rats from quarantine.

After allocation to study groups during the acclimation period, it was noted that some animals demonstrated excessive spillage, manipulated the feed and in some instances overturned their feed bowls. 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 (4°C) 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) 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. 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 for the high dose only. The nicotine concentration in the low doses were 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 analysis time.

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 rats, once in the morning and once in the afternoon (at least 6 hours apart) were performed to identify dead or moribund rats. Observations were made five days per week, during weekends only one observation per day was performed.

Rats 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 rat, 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 the day after delivery, 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. Rat weights were acquired using Mettler PM2000 balances (Mettler Instrument Corporation, Highstown, NJ). A non-fasted, terminal body weight was obtained from rats euthanized at study completion.

Feed Consumption: Each day of the study feed was placed into the feed bowl and its weight determined and recorded. The next day, the uneaten feed was weighed and the food consumption calculated. Data were entered into the “A” module of the PATH/TOX computer software. Each rat’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 rat, fresh feed was placed in a bowl and provided to the rat 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 day 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. Feed from the trial run was not fed to the rats. This was followed by Series 1 formulation for the initial phase 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 the growth of the rats. 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 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. These data are from the high dose preparation (40 mg nicotine/kg body weight/day). 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-IFD analysis method.

Table 2: Feed Formulation Trial Run
(40 mg nicotine/kg body weight/day)

Homogeneity Data ¹				
Target Concentration (mg nic/g feed)	<u>Top</u>	Sample Location <u>Middle</u> (mg nic/g feed)	<u>Bottom</u>	Average Concentration (mg nic/g feed)
<u>Tobacco Blend</u>				
0.50	0.46 ± 0.02 (8%) ²	0.47 ± 0.01 (6%)	0.45 ± 0.02 (10%)	0.46 ± 0.01 (8% ± 2%)
<u>Tobacco Extract</u>				
0.50	0.40 ± 0.04 (20%)	0.43 ± 0.06 (14%)	0.39 ± 0.02 (22%)	0.41 ± 0.02 (18% ± 4%)
<u>Nicotine Tartrate</u>				
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

The feed formulated with the tobacco blend demonstrated good homogeneity with the samples being within $\pm 10\%$ of each other. The mean concentration of nicotine in the feed indicated that it was within $\pm 10\%$ 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 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 of the diet in a mortar and pestle to which the nicotine salt is added. Lumps of the salt are 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.

Series 1 feed formulations were used during the first seven days of study. Analytical data for homogeneity are presented in Table 3.

Table 3: **Series 1 Feed Formulations and Dose Confirmation**
(40 mg/kg body weight/day)

Target Concentration (mg nic/g feed)	Homogeneity Data ¹			Average Concentration (mg nic/g feed)
	<u>Top</u>	Sample Location	<u>Bottom</u>	
		<u>Middle</u> (mg nic/g feed)		
		<u>Tobacco Blend</u>		
0.38	0.39 (3%) ³	0.36 (5%)	0.39 (3%)	0.38 ± 0.07 ² (0% ± 1.0%)
		<u>Tobacco Extract</u>		
0.38	0.49 (29%)	0.36 (5%)	0.43 (13%)	0.43 ± 0.07 (13% ± 12%)
		<u>Nicotine Tartrate</u>		
0.38	0.35 (8%)	0.35 (8%)	0.34 (11%)	0.35 ± 0.01 (8% ± 2%)

¹ Analytical method uncertainty for nicotine analysis = $\pm 5.2\%$, data represent the mean of duplicate analytical runs.

² Data represent mean \pm standard deviation where appropriate

³ % difference from target concentration

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 greater than expected range. However, it is believed that the homogeneity was adequate for this preliminary study and would not have an adverse effect on the data. The concentration of nicotine was slightly above the anticipated $\pm 10\%$ range but should not produce a biologically relevant effect on the data from the study. This again, demonstrates that diets 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.

Series 2 feed formulations were used during the last seven days of study. Analytical data for homogeneity are presented in Table 4.

Table 4: **Series 2 Feed Formulations and Dose**
(40 mg/kg body weight/day)

Target Concentration (mg nic/g feed)	Homogeneity Data ¹			Average Concentration (mg nic/g feed)
	<u>Top</u>	Sample Location <u>Middle</u> (mg nic/g feed)	<u>Bottom</u>	
		<u>Tobacco Blend</u>		
0.43	0.41 (5 %)³	0.41 (5 %)	0.41 (5 %)	0.41 ± 0.00² (5 % ± 0 %)
		<u>Tobacco Extract</u>		
0.43	0.41 (5 %)	0.39 (9 %)	0.44 (2 %)	0.41 + 0.03 (5 % + 4%)
		<u>Nicotine Tartrate</u>		
0.43	0.40 (7 %)	0.40 (7 %)	0.40 (7 %)	0.40 + 0.00 (7 %)

¹ Analytical method uncertainty for nicotine analysis = $\pm 5.2\%$, data represent the mean of duplicate analytical runs

² Data represent mean \pm standard deviation where appropriate

³ % difference from target concentration

Homogeneity data for the tobacco blend indicated acceptable homogeneity. The concentration of nicotine in the high dose group was also within an acceptable range.

The tobacco extract demonstrated acceptable homogeneity and the concentration of nicotine was also within an acceptable range. The feed formulated with nicotine tartrate was acceptable in respect to homogeneity and concentration of nicotine.

Dose Confirmation Data: Dose confirmation data for Series 1 and Series 2 formulations are presented in Tables 5 – 10, 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 rats.

Table 5: Series 1 Dose Confirmation Data Tobacco Blend

Target Dose (mg of nicotine/kg of bw/day)	Target Concentration (g of nicotine/g of feed)	Feed Nicotine Concentration (mg of nicotine/g of feed)
0.2	0.0019	DCNA ¹
2.0	0.0190	DCNA
4.0	0.0380	DCNA
8.0	0.0760	DCNA
20.0	0.1900	DCNA
40.0	0.3800	DCNA
		DCNA

¹ DCNA = Data currently not available

Table 6: Series 1 Dose Confirmation Data Tobacco Extract

Target Dose (mg of nicotine/kg of bw/day)	Target Concentration (g of nicotine/g of feed)	Feed Nicotine Concentration (mg of nicotine/g of feed)
0.2	0.0019	DCNA ¹
2.0	0.0190	DCNA
4.0	0.0380	DCNA
8.0	0.0760	DCNA
20.0	0.1900	DCNA
40.0	0.3800	DCNA

¹ DCNA = Data currently not available

Table 7: Series 1 Dose Confirmation Data Nicotine Tartrate

Target Dose (mg of nicotine/kg of bw/day)	Target Concentration (g of nicotine/g of feed)	Feed Nicotine Concentration (mg of nicotine/g of feed)
2.0	0.0190	DCNA ¹
8.0	0.0760	DCNA
20.0	0.1900	DCNA
40.0	0.3800	DCNA

¹ DCNA = Data currently not available

Table 8: Series 2 Dose Confirmation Data Tobacco Blend

Target Dose (mg of nicotine/kg of bw/day)	Target Concentration (g of nicotine/g of feed)	Feed Nicotine Concentration (mg of nicotine/g of feed)
0.2	0.002	0.004
2.0	0.022	0.014
4.0	0.043	0.042
8.0	0.087	0.072
20.0	0.217	0.19
40.0	0.433	0.36

Table 9: Series 2 Dose Confirmation Data Tobacco Extract

Target Dose (mg of nicotine/kg of bw/day)	Target Concentration (g of nicotine/g of feed)	Feed Nicotine Concentration (mg of nicotine/g of feed)
0.2	0.002	0.001
2.0	0.022	0.041
4.0	0.043	0.044
8.0	0.087	0.12
20.0	0.217	0.14
40.0	0.433	0.31

Table 10: Series 2 Dose Confirmation Data Nicotine Tartrate

Target Dose (mg of nicotine/kg of bw/day)	Target Concentration (g of nicotine/g of feed)	Feed Nicotine Concentration (mg of nicotine/g of feed)
2.0	0.022	0.019
8.0	0.087	0.075
20.0	0.217	0.18
40.0	0.433	0.36

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

Table 11: Prestudy Trial Formulations 30 Day Stability Data¹

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
<u>Tobacco Blend</u>				
40	0.50	0.47 ± 0.01	0.44	-6.4
<u>Tobacco Extract</u>				
40	0.50	0.43 ± 0.06	0.44	+2.3
<u>Nic. Tartrate</u>				
40	0.50	0.41 ± 0.01	0.42	+2.4

Preliminary stability data are also available for nicotine from low and high dose of each test article and the positive control for the Series 2 feed 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 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 12: Series 2 Formulations One Week Stability Data¹

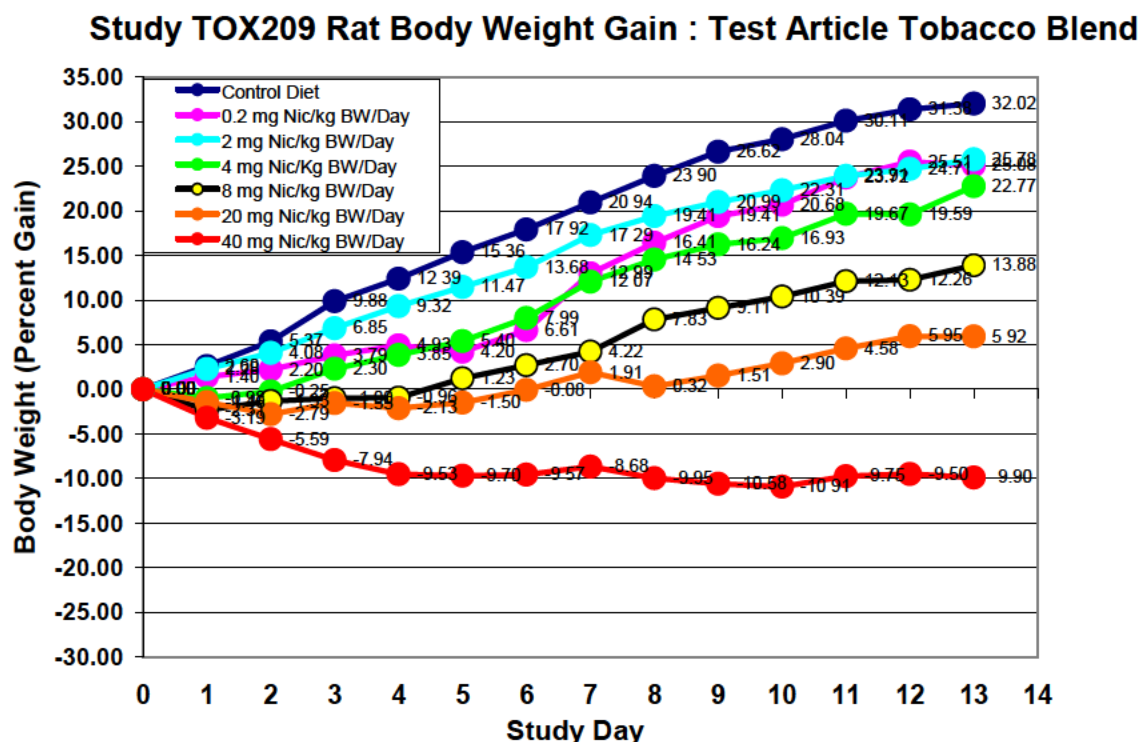
Dose (mg nic/kg bw/day)	Target Concentration (mg nic/g feed)	"0" day (mg nic/g feed)	One Week (mg nic/g feed)	% Difference
<u>Tobacco Blend</u>				
0.2	0.002	0.004	0.002	-50
40	0.433	0.36	0.41	+14
<u>Tobacco Extract</u>				
0.2	0.002	0.001	0.001	0
40	0.433	0.31	0.37	+19
<u>Nic. Tartrate</u>				
2.0	0.021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

¹ Formulated feed in animal room for one week.

Body Weight Data:

Body weight data for rats fed the tobacco blend at different doses of nicotine are provided in Figure 1. These data 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 are presented in the Appendix to this report.

Figure 1



The control demonstrated normal body weight gains for male rats of this age. There is a strong dose response demonstrated in the data. As the dose of the tobacco blend increased body weight gain decreased and there was a definitive loss of body weight at the 40 mg nicotine/kg body/day. One exception to the dose response is seen in rats fed the lowest dose, 0.2 mg nicotine/kg body weight/day. At this dose, body weight gain was similar to that seen at 4 mg nicotine/kg body weight/day. Currently, there is no explanation for this exception. At 20 mg nicotine/kg body weight/day there was a slight depression in body weight through day six of the study. After day six this group began to increase in body weight but body weight gain was less than that of the lower doses. Rats at the 8 mg nicotine/kg body weight/day dose showed a similar pattern but begin to increase in body weight at day five of the study. At 4 mg nicotine/kg body weight/day body weight gain began to increase at day three of the study. However, their body weight gain was less than that of rats fed the lower doses. Rats fed 2 mg nicotine/kg body weight/day begin to demonstrate body weight gains less than those of the control by day three of the study and this trend continued for the remainder of the study. As previously noted, rats fed the 0.2

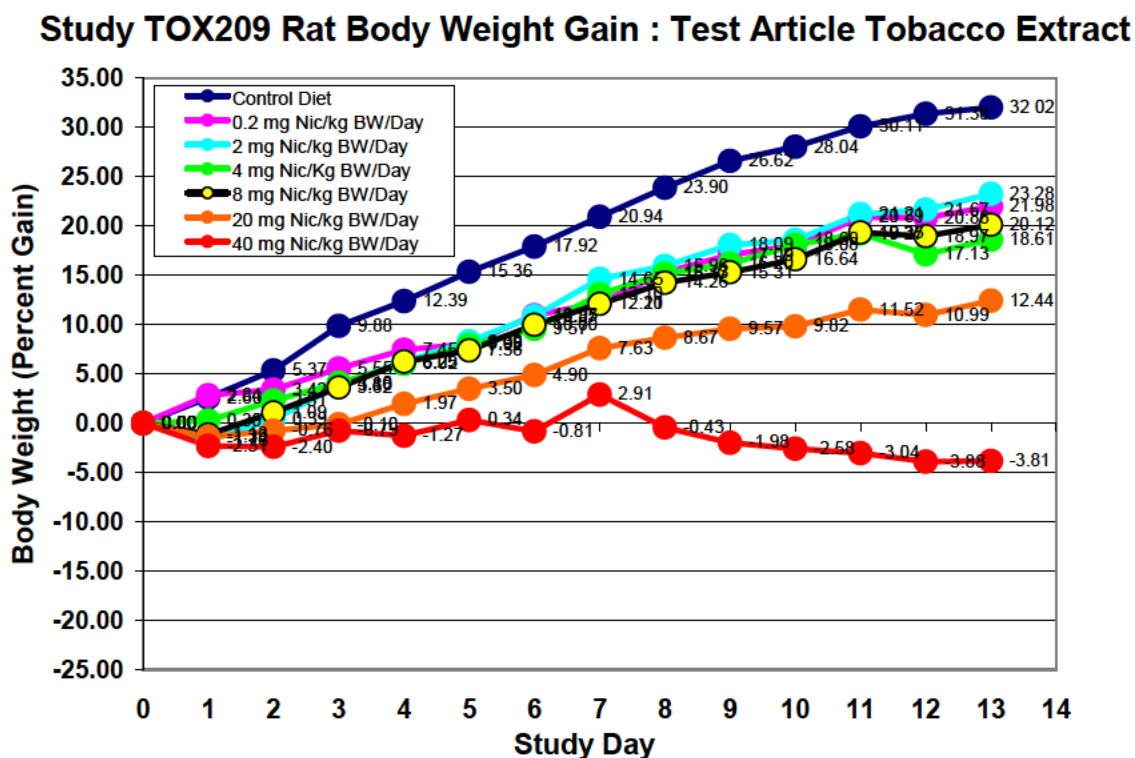
mg nicotine/kg body weight/day resembled those fed the 4 mg nicotine/kg body weight/day dose.

These data indicate that the rats could detect the presence of the tobacco blend at all doses used in this study. Either loss of body weight or severe depressions in body weight gain were encountered at the 20 and 40 mg nicotine/kg body weight/day. These data indicate that these doses would not be appropriate for use in future studies. Doses between 0.2 and 8 mg nicotine/kg body weight/day may be considered for future studies.

At least two possibilities should be considered in respect to explaining these data. First, the rats 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, it is possible that the nicotine in the tobacco blend produced physiological changes undetected in this palatability study that produced an appetite depression or other effect that may alter feed intake and body weight.

Percent body weight gain data for rats fed the feed formulated with the tobacco extract are presented in Figure 2.

Figure 2



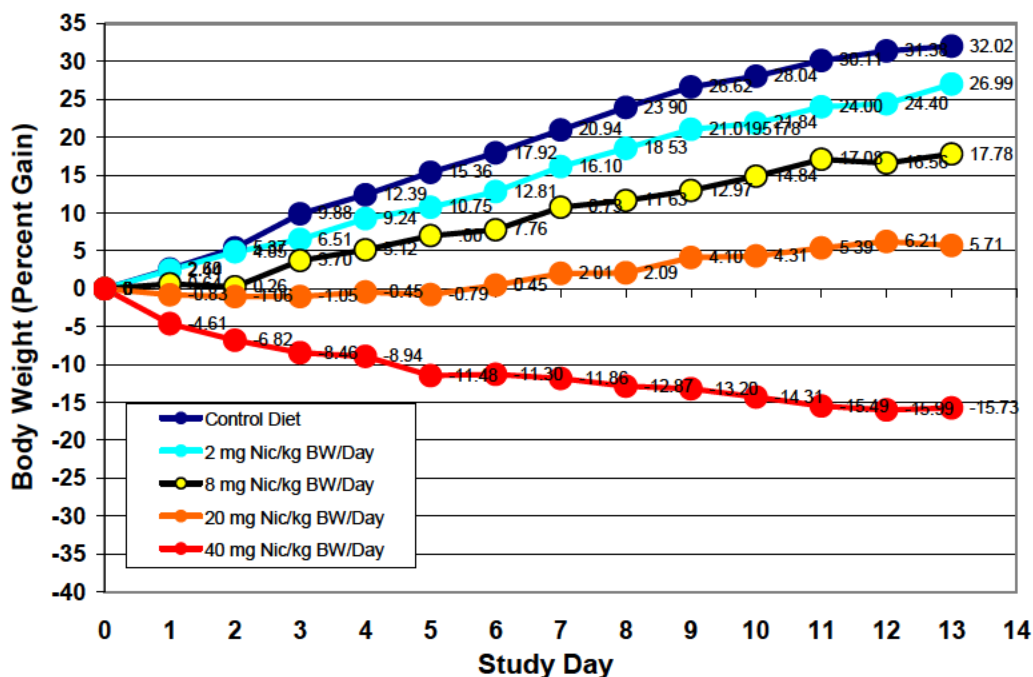
These data are similar to that from the rats fed feed containing the tobacco blend. All dose groups demonstrated reduced percent body weight gain when compared to the control. At

the high dose, loss of body weight was not as severe as with the tobacco blend and did not become constant until after day nine of the study. Similarly, the decrease in body weight gain at 20 mg nicotine/kg body weight/day was not as great as seen with the tobacco blend. At the lower doses, there was not as good a dose response as seen with rats fed feed containing the tobacco blend even though the nicotine concentrations were the same. It may be that the rats found the diets containing the tobacco extract more palatable than those containing the tobacco blend.

Figure 3 provides the percent body weight gain data for rats fed diets containing the positive control, nicotine hydrogen tartrate.

Figure 3

Study TOX209 Rat Body Weight Gain : Test Article Nicotine Tartrate



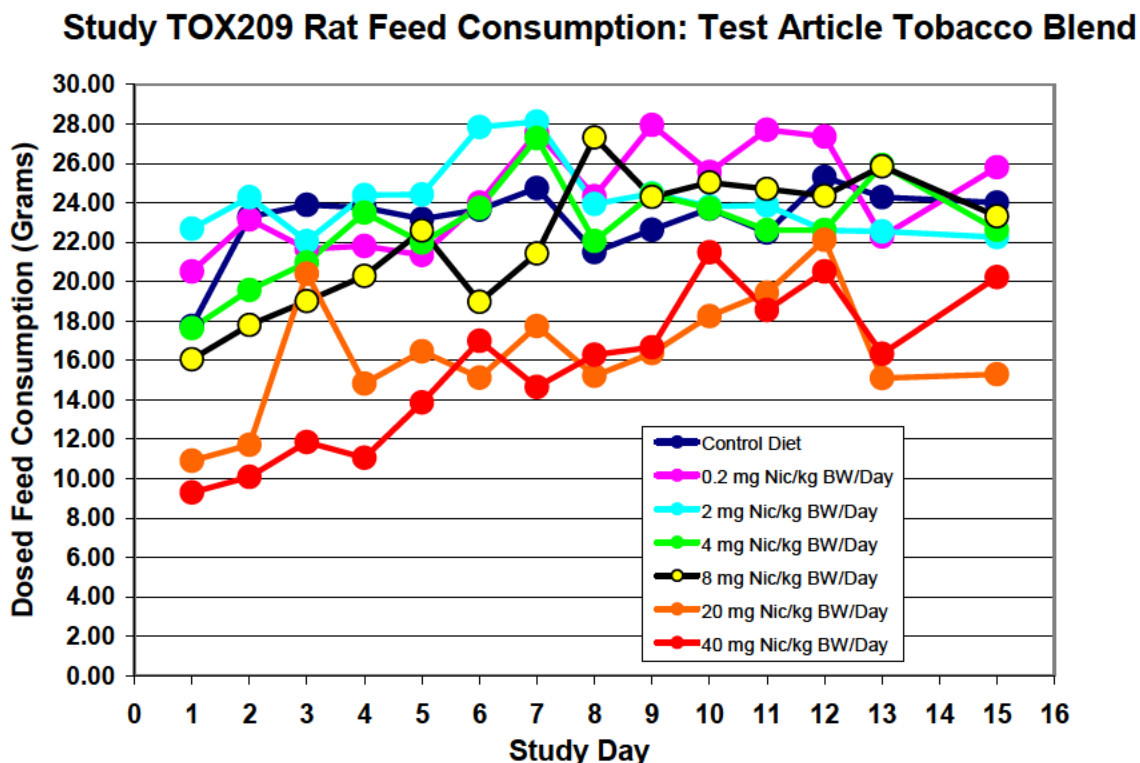
Rats fed feed containing nicotine tartrate demonstrated a definitive dose response in percent body weight gain. As the dose of nicotine tartrate increased body weight gain decreased. At the high dose there was a continued loss in body weight. This loss was higher than those fed equivalent doses of nicotine from the tobacco blend and tobacco extract. At 20 mg/kg body weight/day there was a lack of body weight gain until day seven of the study. This was followed by a slight increase in body weight gain until the end of the study. The decrease in body weight gain at 8 mg nicotine/kg body weight/day was more than that seen at 2 mg nicotine/kg body weight/day. Again, it is obvious that doses of 20 and 40 mg nicotine/kg body weight/day are too high for use in longer term studies. The overall trends in the data follow those seen with the tobacco and 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. These data may indicate that the lack of palatability

in the dosed feed may be associated with its nicotine content more than the presence of other tobacco components.

Feed Consumption:

Feed consumption data for rats fed feed containing the tobacco blend are shown in Figure 4

Figure 4

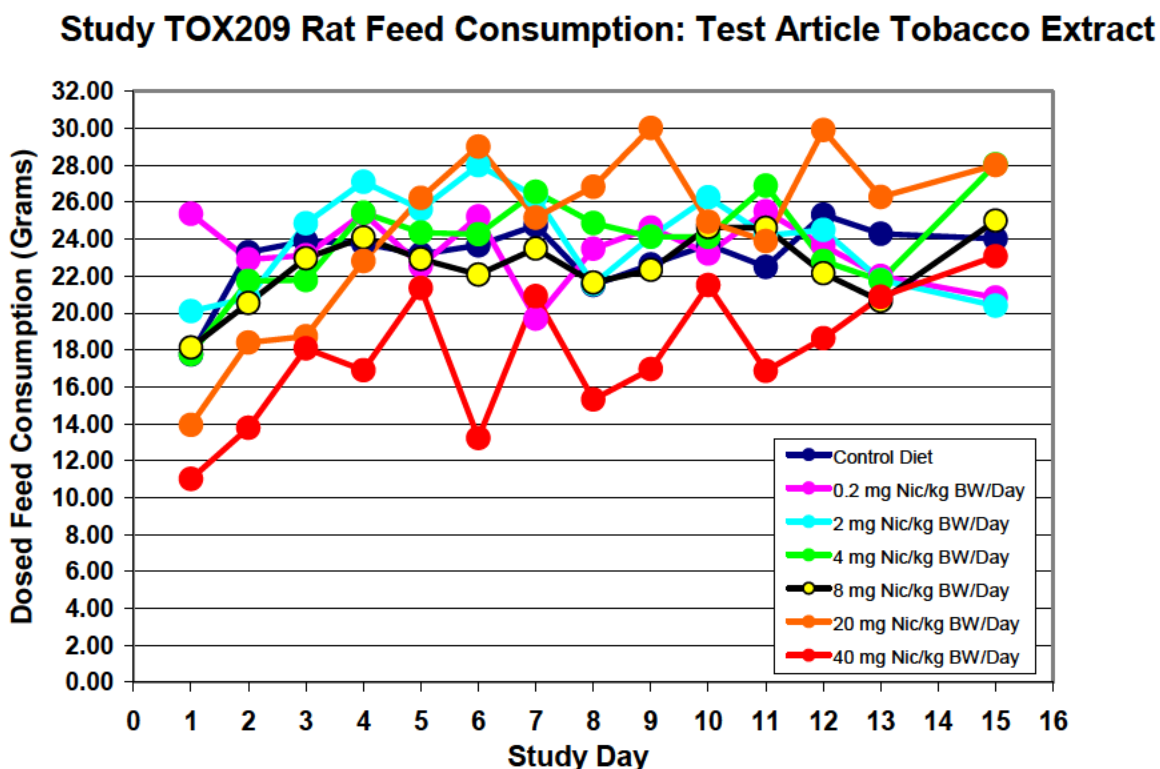


The rats fed the control diets maintained a relatively constant feed consumption during the study. The lowest feed consumption was seen in the groups fed 20 and 40 mg nicotine/kg body weight/day. These data parallel those seen for body weights. Although the rats fed the 40 mg nicotine/kg body weight/day increased their feed intake after the severe depression seen during the first day of the study, they never reached that of the control group. Rats fed 20 mg nicotine/kg body weight/day also demonstrated a large decrease in feed consumption during the first day of the study. The 20 mg nicotine group followed the trend seen at 40 mg nicotine after day five. Rats fed feed containing 8 mg nicotine/kg body weight/day also showed a decline in feed consumption during the first day of the study but then increased their feed consumption until they reached near control values by day 7-8. After day eight, their feed consumption was similar to that of the controls. The lower doses generally followed the trends seen with the control group in respect to feed consumption, although their body weights were less than that of the controls during the study.

Overall, the feed consumption at the two highest doses were lower than those seen at the lower doses and paralleled the body weight data. Again, these doses appear too high for use in future studies. At the lower doses the feed consumption appears to be similar to that of the control even though body weights were decreased. Whether or not this was related to a physiological effect of nicotine, such as appetite suppression, cannot be ascertained from this study.

Feed consumption data for rats fed feed containing the tobacco extract are shown in Figure 5.

Figure 5

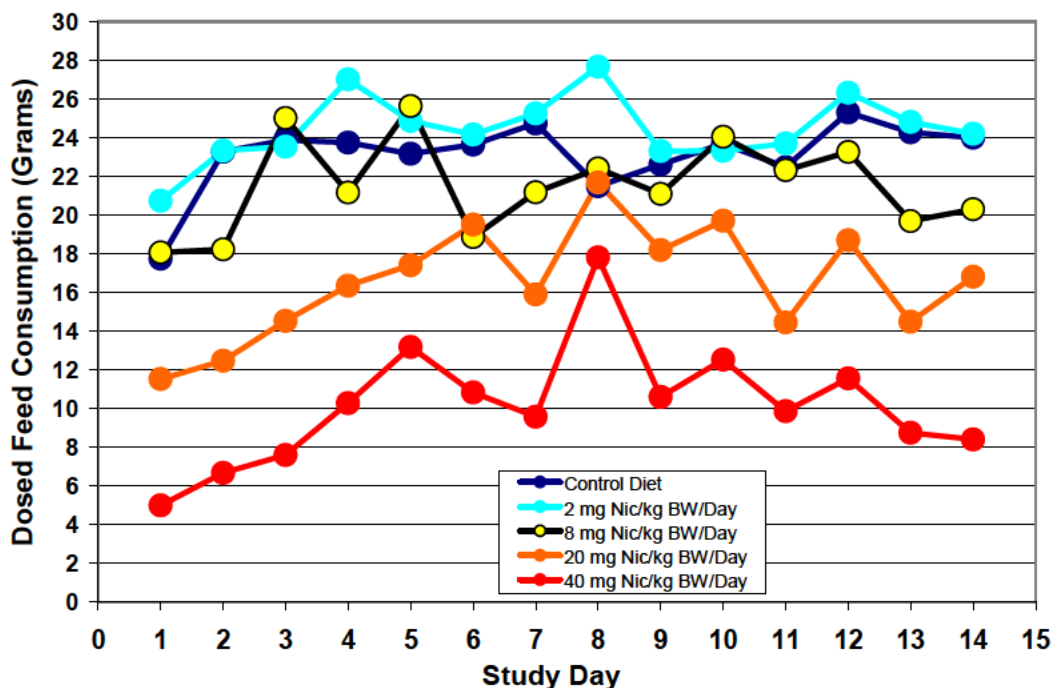


Rats fed feed containing the tobacco extract at a nicotine dose of 40 mg nicotine/kg body weight/day demonstrated a reduction in feed consumption compared to the control during the first day of the study. Subsequently, they increased their feed consumption but it was always below that of the control except for the last day of the study. At 20 mg nicotine/kg body weight/day, there was also a drop in feed consumption compared to the control during the first day of study. However, by day four their food consumption reached that of the control and remained near control values during the duration of the study. In contrast, body weight of this group was less than that of the control. At the lower doses, there does not appear to be a trend toward feed consumption being different from that of the control and feed consumption did not parallel body weight because body weights were lower than the control for these groups.

Feed consumption data for the rats fed feed containing nicotine hydrogen tartrate are shown in Figure 6.

Figure 6

Study TOX209 Rat Feed Consumption: Test Article Nicotine Tartrate



At the two highest doses, consumption of feed containing nicotine hydrogen tartrate followed a dose response. At 40 mg nicotine/kg body weight/day there was an immediate drop in feed consumption during the first day of the study. Although feed consumption increased during the first five days of the study, it did not reach that seen at the 20 mg nicotine/kg body weight/day. The decreased feed consumption paralleled the decrease in body weight seen at this dose. At 20 mg nicotine/kg body weight/day, there was also a sharp drop in feed consumption during the first day of the study when compared to the control. This drop in feed consumption was not as large as that seen at the higher dose. During the first six days of the study the 20 mg nicotine dosed animals demonstrated increased feed intake, this seems to stabilize during the remainder of the study. However, the feed intake of this group never reaches that of the control. These feed consumption data parallel the reductions in body weight gain seen in this group. Feed consumption at the two lower doses appears to follow the trends in feed consumption seen with the controls even though their body weight gains were decreased compared to the controls.

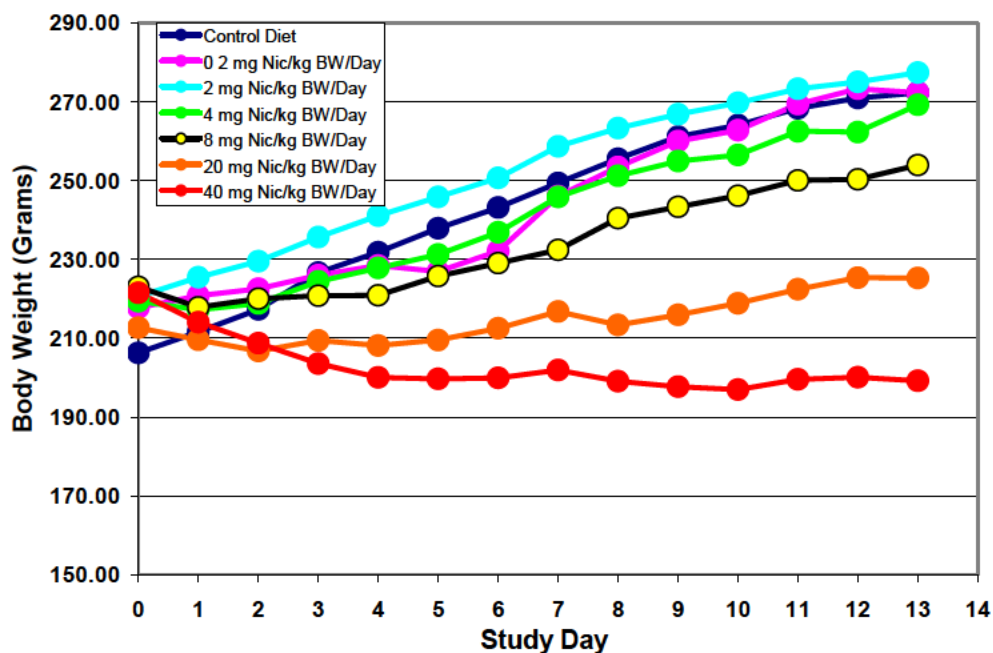
The changes in feed consumption seen with feed containing nicotine tartrate at doses of nicotine identical to that in feed containing tobacco and tobacco extract paralleled those seen with the tobacco and tobacco extract containing feed. This may indicate that nicotine could be the most critical component of the tobacco that alters the palatability of the diets.

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 that could be detected by clinical observations.

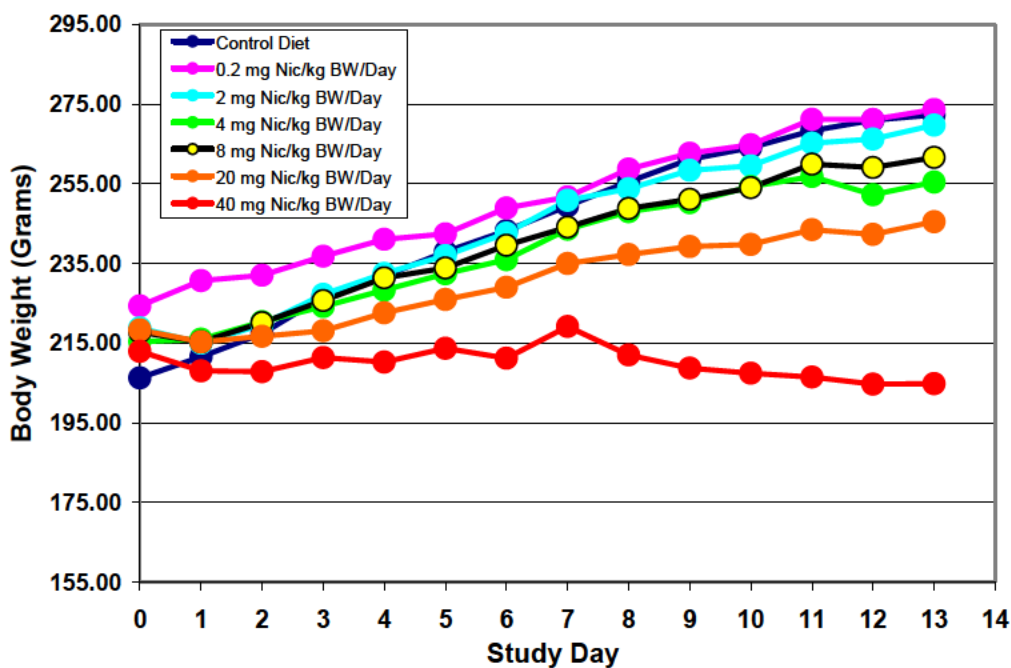
Appendix

Additional Graphs of Data

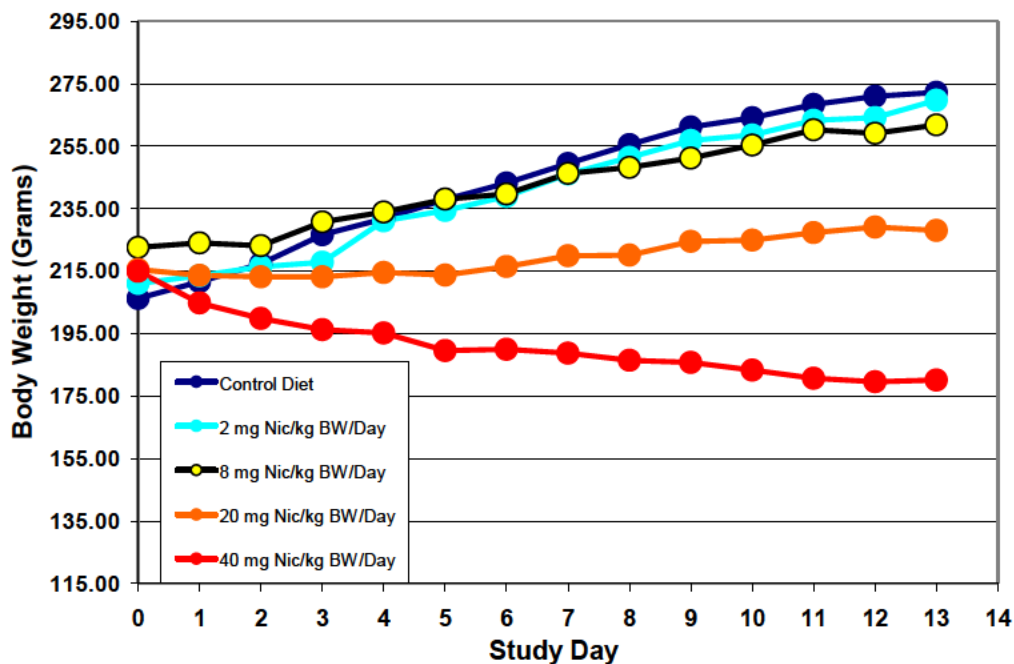
Study TOX209 Rat Body Weights: Test Article Tobacco Blend



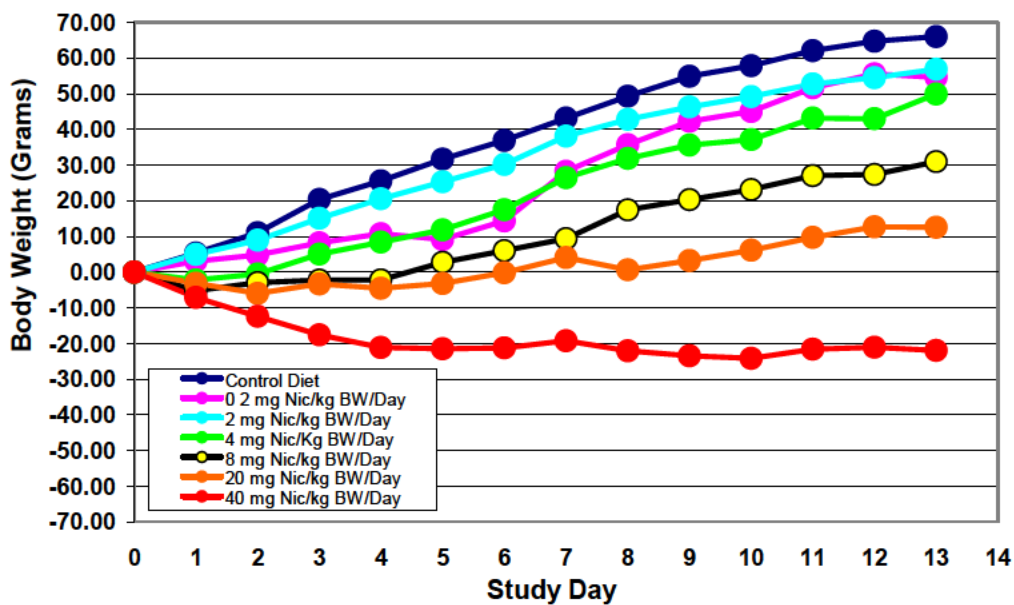
Study TOX209 Rat Body Weights : Test Article Tobacco Extract



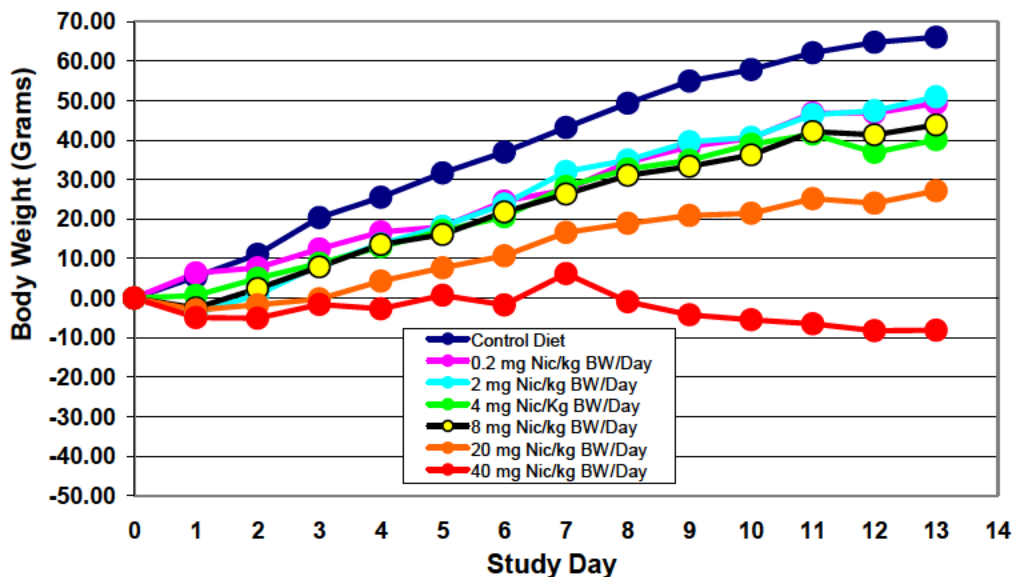
Study TOX209 Rat Body Weights : Test Article Nicotine Tartrate



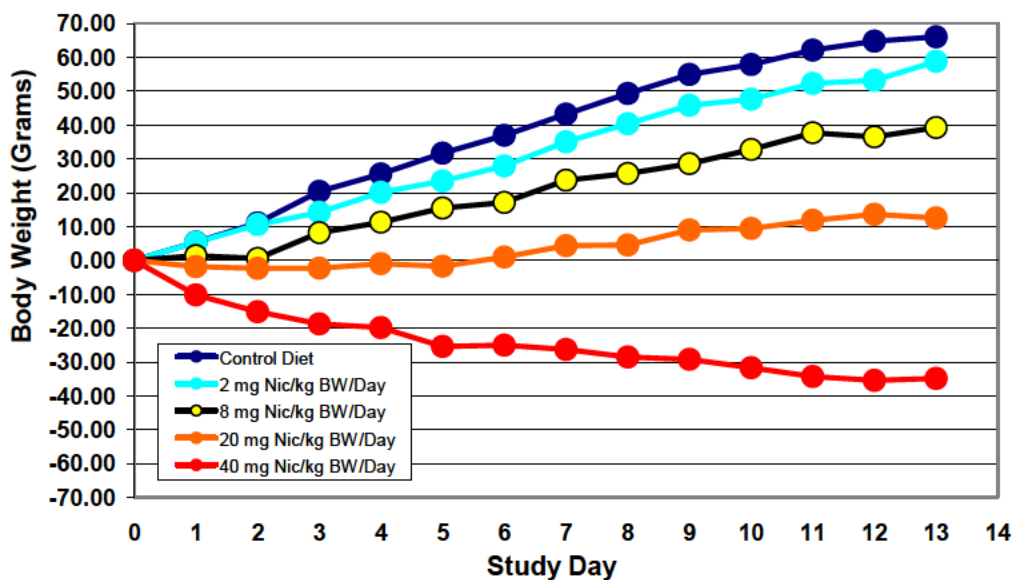
Study TOX209 Rat Body Weights: Test Article Tobacco Blend Absolute Gain in Body Weight (Grams)



**Study TOX209 Rat Body Weights: Test Article Tobacco Extract
Absolute Gain in Body Weight (Grams)**



**Study TOX209 Rat Body Weights: Test Article Nicotine Tartrate
Absolute Gain in Body Weight (Grams)**



Comments Concerning Path/Tox Data Outputs

Data associated with the use of rats 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 TOX209A and TOX209B.

The Xybion data collection protocols TOX209A and TOX209B were used for body weights and feed consumption of rats 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. TOX209A contains Dose Groups 1-13. TOX209B contains the four Nicotine Tartrate Positive Control Groups (i.e. TOX209B Group 1 is Group 14; Group 2 is Group 15; Group 3 is Group 16; and Group 4 is Group 17).

<i>Group Number</i>	<i>Treatment Group (Doses based on Nicotine) (mg/kg body weight/day)</i>	<i>Number of Rats</i>	<i>Rat ID Numbers</i>
<i>Control</i>			
1	NTP-2000 feed	5	1-5
<i>Smokeless Tobacco Blend</i>			
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
<i>Tobacco Extract</i>			
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
<i>Positive Control</i>			
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
<i>Sentinels</i>			
	Sentinels (no treatment)	10	86-95

Because the start of the exposure phase was delayed five days for both TOX209A and TOX209B studies, Day 6 of the Xybion data output is study Day 1 (April 21, 2008), etc.

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RAT/WISTAR HANOVER

Animal body weights in (g)
Study number: TOX209A

PRINTED: 08-May-08
Page: 1

Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

		D a y o f P h a s e												
Animal	Group	6	7	8	9	10	11	12	13	14	15	16	17	18
		Male Animals												
1	1	197.56	204.12	208.65	213.33	220.15	224.31	227.79	235.27	244.26	249.88	255.83	257.10	257.37
2		210.84	214.47	215.89	228.18	236.56	242.97	250.96	257.39	263.57	268.21	272.11	271.53	278.01
3		233.27	227.97	243.39	250.32	254.12	261.20	267.59	271.32	275.37	285.14	284.80	293.49	292.06
4		186.89	221.87	228.85	233.59	234.74	240.80	243.26	249.70	254.54	260.60	262.01	270.26	268.29
5		202.66	189.57	189.79	207.67	213.40	220.34	226.36	233.49	239.93	241.89	245.64	249.30	259.10
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	206.24	211.60	217.31	226.62	231.79	237.92	243.19	249.43	255.53	261.14	264.08	268.34	270.97
	Sdevs	17.42	15.19	20.30	16.94	15.84	16.36	17.15	15.79	14.40	16.76	15.05	16.85	14.38
6	2	202.76	201.65	197.94	200.77	202.63	201.89	204.40	211.88	230.24	237.89	246.44	252.80	260.84
7		243.53	237.74	235.78	234.40	233.93	233.00	250.06	265.52	275.16	279.49	284.24	290.77	292.87
8		230.00	234.12	238.66	244.31	248.07	250.60	255.54	263.16	270.88	275.04	277.96	283.88	285.73
9		196.08	203.29	210.28	215.99	219.80	224.46	230.16	238.74	244.11	248.99	248.27	256.25	255.82
10		216.36	227.12	230.00	234.57	237.99	224.49	220.57	250.86	247.02	258.63	256.98	263.35	271.21
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	217.75	220.78	222.53	226.01	228.48	226.89	232.15	246.03	253.48	260.01	262.78	269.41	273.29
	Sdevs	19.44	17.16	17.65	17.44	17.66	17.58	21.07	21.89	18.99	17.45	17.34	16.97	15.83
11	3	220.37	217.54	225.73	228.89	233.07	232.79	241.55	248.97	252.00	257.73	259.89	262.83	262.42
12		238.65	241.01	247.22	254.10	256.16	263.69	266.61	276.77	282.18	283.55	289.73	293.22	294.91
13		194.10	213.33	217.99	228.36	233.78	239.16	241.86	251.77	254.97	259.15	258.94	265.64	263.16
14		259.03	260.58	264.16	269.79	270.71	274.56	276.91	286.27	291.50	295.14	298.28	299.98	303.82
15		190.59	195.37	192.60	197.11	211.81	219.04	226.69	229.63	236.12	238.64	241.92	244.78	250.87
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	220.55	225.57	229.54	235.65	241.11	245.85	250.72	258.68	263.35	266.84	269.75	273.29	275.04
	Sdevs	29.18	25.45	27.49	27.79	22.80	22.79	20.48	22.77	22.84	22.46	23.46	22.86	22.95
16	4	220.26	218.91	221.27	222.43	220.85	221.53	233.64	241.65	250.26	251.64	253.21	260.61	257.30
17		239.40	240.60	244.55	256.66	256.63	259.17	267.43	274.82	274.64	280.36	284.67	286.73	289.79
18		233.59	233.52	235.50	240.27	244.19	250.48	249.77	257.55	266.01	267.56	266.50	274.97	275.18
19		195.37	184.19	182.40	181.88	191.94	195.96	202.76	218.18	222.17	225.24	226.43	236.18	236.23
20		208.18	208.79	210.37	220.83	225.37	228.92	230.83	236.99	243.06	250.11	251.71	254.04	253.22
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	219.36	217.20	218.82	224.41	227.80	231.21	236.89	245.84	251.23	254.98	256.50	262.51	262.34
	Sdevs	18.07	22.23	24.21	27.92	24.70	24.97	24.04	21.44	20.49	20.75	21.40	19.43	20.67
21	5	247.24	240.14	241.80	245.20	245.35	250.24	251.67	254.96	255.32	259.38	260.15	265.58	260.76
22		208.70	198.65	199.89	196.72	193.48	192.20	200.36	201.72	222.64	222.87	230.51	238.39	244.28
23		234.22	232.28	234.05	240.09	245.75	248.49	258.39	261.83	266.24	271.03	275.08	278.16	277.90
24		219.20	216.97	220.07	221.65	221.94	227.05	229.74	236.08	241.15	243.96	243.18	247.66	246.39

Note: Data for Exposure phase

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Building 630/2
RAT/WISTAR HANOVER

Animal body weights in (g)
Study number: TOX209A

PRINTED: 08-May-08
Page: 2

Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

+

		D a y o f P h a s e												
Animal	Group	6	7	8	9	10	11	12	13	14	15	16	17	18

		M a l e						A n i m a l s						
25	5	205.62	201.21	204.37	200.10	197.77	210.74	204.97	207.37	216.94	219.35	221.85	220.40	222.36
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	223.00	217.85	220.04	220.75	220.86	225.74	229.03	232.39	240.46	243.32	246.15	250.04	250.34
	Sdevs	17.57	18.38	18.18	22.23	25.02	24.85	26.34	27.19	20.96	22.47	21.66	22.65	20.64
26	6	235.27	233.83	228.68	234.50	236.55	234.18	233.30	234.23	236.44	238.10	241.31	246.11	242.89
27		181.70	178.75	180.73	181.47	181.15	185.21	186.79	194.11	194.16	195.58	193.73	198.33	197.56
28		212.95	205.32	203.33	202.87	203.45	202.44	205.68	210.46	206.06	208.20	203.03	212.00	208.94
29		195.78	198.11	195.08	192.42	192.13	193.87	196.47	201.99	204.82	203.58	209.47	212.00	220.67
30		237.74	231.92	225.98	235.73	227.53	231.80	240.37	242.95	225.40	234.07	246.68	243.69	256.70
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	212.69	209.59	206.76	209.40	208.16	209.50	212.52	216.75	213.38	215.91	218.84	222.43	225.35
	Sdevs	24.41	23.38	20.47	24.67	23.40	22.31	23.31	20.99	17.12	19.02	23.71	21.28	24.26
31	7	242.29	227.19	219.04	214.22	213.25	214.35	215.31	213.58	217.66	216.13	218.42	217.19	212.62
32		209.41	200.75	198.01	195.78	195.28	192.60	192.47	196.50	193.07	190.63	191.37	189.57	195.86
33		252.10	239.78	234.23	226.70	221.52	222.31	222.94	226.73	218.50	215.83	211.36	216.94	219.22
34		205.20	204.88	197.13	193.34	187.78	188.69	185.40	186.60	181.81	181.22	179.23	183.93	182.19
35		196.56	197.62	195.25	187.64	182.24	180.18	183.45	186.05	184.28	184.59	184.33	189.96	190.47
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	221.11	214.04	208.73	203.54	200.01	199.63	199.91	201.89	199.06	197.68	196.94	199.52	200.07
	Sdevs	24.50	18.48	17.23	16.34	16.78	17.88	18.06	17.80	17.86	17.04	17.12	16.20	15.44
36	8	217.16	220.99	222.76	217.98	225.42	220.48	232.26	236.12	240.31	244.91	242.95	248.70	248.60
37		252.41	269.36	260.97	271.47	272.37	281.36	283.95	287.78	295.83	299.65	298.15	306.14	304.79
38		186.78	192.01	194.40	198.48	201.14	204.07	206.44	211.81	216.35	219.67	218.92	226.03	226.03
39		238.93	245.46	250.42	255.66	260.68	260.63	269.00	264.57	275.19	282.43	287.18	292.48	295.82
40		226.34	225.68	231.49	240.35	245.61	245.36	252.81	258.31	265.86	266.66	276.69	282.56	280.31
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	224.32	230.70	232.01	236.79	241.04	242.38	248.89	251.72	258.71	262.66	264.78	271.18	271.11
	Sdevs	24.83	28.85	25.87	29.12	28.39	30.86	30.53	28.92	30.96	31.38	32.93	32.97	33.05
41	9	177.83	186.85	189.13	190.84	199.89	205.99	209.10	220.49	221.85	228.68	227.97	236.91	236.69
42		244.12	226.73	224.90	240.46	248.67	253.97	262.63	270.46	275.63	277.99	275.01	283.45	285.91
43		229.79	225.24	233.17	242.41	247.25	251.10	255.17	262.67	267.86	273.34	273.75	277.19	277.03
44		215.96	213.36	221.33	227.23	229.82	233.43	240.42	249.30	251.15	253.95	259.71	262.05	264.11
45		226.32	222.56	229.77	235.32	236.76	240.40	245.62	251.38	252.20	257.96	261.05	266.47	267.33
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	218.80	214.95	219.66	227.25	232.48	236.98	242.59	250.86	253.74	258.38	259.50	265.21	266.21
	Sdevs	25.03	16.54	17.66	21.18	19.80	19.19	20.59	19.03	20.64	19.43	18.98	17.95	18.59

Note: Data for Exposure phase

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TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Animal body weights in (g)
Study number: TOX209A

PRINTED: 08-May-08
Page: 3

Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

		D a y o f P h a s e												
Animal	Group	6	7	8	9	10	11	12	13	14	15	16	17	18

		M a l e						A n i m a l s						
46	10	228.21	227.69	229.91	238.27	238.22	243.75	245.44	255.24	257.82	261.71	262.85	268.79	268.73
47		224.93	222.43	223.54	235.26	236.11	240.74	245.64	250.67	254.76	257.68	265.62	268.70	267.82
48		198.63	203.65	215.67	212.26	225.58	226.62	232.02	244.37	249.86	252.52	257.52	249.69	231.23
49		194.48	198.05	203.63	205.39	209.79	214.33	216.82	226.11	229.55	232.28	234.58	239.83	237.21
50		230.49	228.12	228.91	229.64	231.88	236.55	239.82	241.45	248.07	247.02	250.82	257.18	256.13
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	215.35	215.99	220.33	224.16	228.32	232.40	235.95	243.57	248.01	250.24	254.28	256.84	252.22
	Sdevs	17.33	14.14	10.91	14.55	11.43	12.00	12.05	11.14	11.02	11.46	12.38	12.49	17.30

51	11	248.59	241.92	242.18	248.54	245.12	246.09	260.69	262.77	267.02	272.14	273.32	284.37	282.33
52		187.01	188.23	199.85	205.78	214.43	221.19	224.16	227.77	232.94	233.85	238.93	244.67	244.92
53		229.00	224.92	223.50	233.12	241.31	240.54	246.13	252.96	258.33	258.29	258.42	263.89	261.72
54		207.49	205.37	211.70	215.71	225.57	226.86	229.81	235.05	240.04	243.49	247.20	252.01	251.50
55		216.75	216.04	223.50	225.10	230.42	234.58	236.93	242.11	245.74	247.84	252.11	254.62	254.91
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	217.77	215.30	220.15	225.65	231.37	233.85	239.54	244.13	248.81	251.12	254.00	259.91	259.08
	Sdevs	23.07	20.21	15.74	16.38	12.34	10.05	14.39	13.96	13.79	14.67	12.94	15.30	14.34

56	12	220.20	215.40	215.46	214.93	216.88	220.05	225.01	228.12	225.10	224.99	225.46	225.10	227.51
57		202.47	206.16	205.45	209.18	218.28	223.03	223.93	231.15	237.33	239.38	241.29	245.41	245.31
58		205.37	203.04	204.15	205.04	210.19	212.43	216.99	225.04	225.52	228.36	225.68	231.61	227.15
59		227.54	224.32	224.93	226.18	231.60	235.96	236.37	241.80	244.59	247.32	251.11	251.43	253.30
60		236.02	227.47	233.34	235.18	236.16	238.33	242.79	248.76	253.64	256.07	255.28	263.87	258.29
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	218.32	215.28	216.67	218.10	222.62	225.96	229.02	234.97	237.24	239.22	239.76	243.48	242.31
	Sdevs	14.32	10.76	12.55	12.42	10.84	10.95	10.37	9.96	12.33	12.94	13.92	15.50	14.44

61	13	196.73	197.49	195.68	204.96	202.78	207.53	203.75	213.91	206.81	209.79	208.58	209.18	203.97
62		205.99	198.44	197.45	200.33	204.42	207.40	203.34	212.85	205.70	202.84	198.25	192.64	192.52
63		224.62	225.37	224.63	228.21	221.05	224.88	219.91	233.44	222.89	212.64	213.66	212.13	212.86
64		242.75	228.85	231.60	231.59	229.20	235.43	230.95	237.06	227.03	221.92	221.78	223.25	220.92
65		194.61	190.00	189.86	191.61	193.73	193.11	198.12	198.45	197.69	196.41	194.94	195.09	193.14
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	212.94	208.03	207.84	211.34	210.24	213.67	211.21	219.14	212.02	208.72	207.44	206.46	204.68
	Sdevs	20.45	17.76	18.88	17.65	14.48	16.57	13.73	15.97	12.41	9.71	11.03	12.67	12.37

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Animal body weights in (g)
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FEEDING STUDY/PALATABILITY

Animal Group		Day of Phase
		19
		Male Animals
1	1	259.94
2		281.94
3		294.66
4		268.16
5		256.69
	(n)	5
	Means	272.28
	Sdevs	15.86
6	2	252.53
7		294.83
8		286.34
9		259.72
10		268.37
	(n)	5
	Means	272.36
	Sdevs	17.81
11	3	267.54
12		298.51
13		263.36
14		306.99
15		250.62
	(n)	5
	Means	277.40
	Sdevs	24.15
16	4	264.55
17		297.09
18		283.06
19		243.99
20		257.67
	(n)	5
	Means	269.27
	Sdevs	20.98
21	5	264.97
22		243.87
23		279.46
24		253.52

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Animal body weights in (g)
Study number: TOX209A

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FEEDING STUDY/PALATABILITY

Animal Group		Day of Phase	
		19	
		Male	Animals
25	5		227.91
	(n)		5
	Means		253.95
	Sdevs		19.70
26	6		244.76
27			199.70
28			214.25
29			214.28
30			253.38
	(n)		5
	Means		225.27
	Sdevs		22.73
31	7		207.65
32			194.10
33			216.40
34			182.40
35			195.38
	(n)		5
	Means		199.19
	Sdevs		13.13
36	8		252.16
37			305.50
38			228.22
39			298.84
40			283.42
	(n)		5
	Means		273.63
	Sdevs		32.67
41	9		239.49
42			290.78
43			276.94
44			270.03
45			271.43
	(n)		5
	Means		269.73
	Sdevs		18.79

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Animal body weights in (g)
Study number: TOX209A

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FEEDING STUDY/PALATABILITY

Animal Group		Day of Phase
		19
		Male Animals
46	10	273.72
47		269.30
48		235.57
49		239.16
50		259.35
	(n)	5
	Means	255.42
	Sdevs	17.33
51	11	283.58
52		245.36
53		266.47
54		255.71
55		256.81
	(n)	5
	Means	261.59
	Sdevs	14.39
56	12	230.25
57		249.10
58		229.41
59		254.92
60		263.69
	(n)	5
	Means	245.47
	Sdevs	15.20
61	13	204.92
62		194.50
63		213.83
64		217.99
65		192.85
	(n)	5
	Means	204.82
	Sdevs	11.23

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Animal body weights in (g)
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FEEDING STUDY/PALATABILITY

		D a y o f P h a s e												
Animal	Group	6	7	8	9	10	11	12	13	14	15	16	17	18

		M a l e						A n i m a l s						
66	1	199.15	203.23	207.82	213.46	220.10	223.36	228.39	233.21	234.60	243.90	245.22	251.18	251.44
67		175.38				193.32	204.93	211.91	221.64	225.17	232.43	239.32	246.53	245.42
68		197.51	203.60	214.38	213.72	222.56	223.68	224.19	235.97	243.01	246.85	240.98	241.78	247.17
69		235.76	244.35	247.01	252.83	257.52	258.06	262.21	266.86	272.41	275.73	279.51	285.34	280.89
70		247.29	249.76	252.78	256.40	262.12	262.13	267.91	272.66	281.58	285.02	287.82	291.63	295.88
	(n)	5	4	4	4	5	5	5	5	5	5	5	5	5
	Means	211.02	225.24	230.50	234.10	231.12	234.43	238.92	246.07	251.35	256.79	258.57	263.29	264.16
	Sdevs	29.67	25.29	22.68	23.73	28.64	24.67	24.70	22.38	24.46	22.44	23.20	23.34	22.85

71	2	209.13	224.64	214.34	225.99	222.32	231.78	234.86	241.87	237.12	235.98	249.24	253.34	256.76
72		266.34	264.59	267.86	271.98	277.65	279.65	278.03	285.84	292.00	295.28	296.91	301.51	295.74
73		236.09	230.88	232.85	239.59	240.90	242.94	245.41	251.97	255.10	259.47	264.56	267.36	264.26
74		191.22	190.85	187.04	201.74	208.49	213.07	213.75	220.89	223.44	228.58	229.66	233.99	232.82
75		210.18	209.05	213.73	214.40	220.08	222.69	226.53	230.72	233.56	236.66	236.26	245.15	245.97
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	222.59	224.00	223.16	230.74	233.89	238.03	239.72	246.26	248.24	251.19	255.33	260.27	259.11
	Sdevs	29.22	27.47	29.85	26.97	27.08	25.76	24.35	25.02	27.00	27.23	26.80	26.07	23.65

76	3	207.94	203.05	203.14	202.79	204.33	204.35	203.13	204.15	203.89	205.00	207.21	202.78	209.18
77		200.56	205.41	199.83	199.08	201.09	198.33	205.65	206.69	207.48	205.22	210.04	208.98	213.22
78		211.54	209.27	209.18	211.12	215.75	218.32	221.71	228.67	228.34	235.53	234.87	241.54	238.66
79		215.36	211.40	215.10	216.17	216.13	212.75	216.98	220.48	220.34	227.82	229.07	234.39	234.20
80		242.07	239.31	238.61	236.81	235.29	235.01	234.95	239.47	240.38	248.88	243.52	248.88	250.29
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	215.49	213.69	213.17	213.19	214.52	213.75	216.48	219.89	220.09	224.49	224.94	227.31	229.11
	Sdevs	15.83	14.69	15.37	14.82	13.41	14.14	12.89	14.86	15.01	19.23	15.79	20.34	17.43

81	4	230.59	222.41	219.53	215.24	212.77	205.29	206.79	205.18	205.61	207.62	204.40	204.72	202.52
82		247.11	232.54	229.98	224.82	226.49	223.97	225.50	222.58	216.47	220.06	219.78	212.16	218.54
83		175.52	167.05	162.47	160.35	155.84	149.80	146.12	145.65	143.57	141.40	137.85	136.21	131.28
84		193.02	184.32	178.73	176.47	178.12	170.97	171.63	172.26	169.28	169.19	167.20	164.61	163.26
85		228.70	217.57	208.67	204.38	202.68	197.77	199.81	197.96	197.46	190.51	187.20	185.73	182.26
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	214.99	204.78	199.88	196.25	195.18	189.56	189.97	188.73	186.48	185.76	183.29	180.69	179.57
	Sdevs	29.60	27.78	28.36	27.03	28.22	29.26	31.23	30.12	29.68	31.30	32.08	30.94	34.10

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Animal body weights in (g)
Study number: TOX209B

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FEEDING STUDY/PALATABILITY

+

Animal Group		Day of Phase	
		19	
		Male	Animals
66	1		255.69
67			252.32
68			252.35
69			287.50
70			300.86
	(n)		5
	Means		269.74
	Sdevs		22.84
71	2		255.01
72			299.82
73			267.15
74			239.44
75			247.62
	(n)		5
	Means		261.81
	Sdevs		23.57
76	3		206.02
77			212.18
78			242.22
79			236.02
80			243.62
	(n)		5
	Means		228.01
	Sdevs		17.63
81	4		201.11
82			217.78
83			134.97
84			161.30
85			185.57
	(n)		5
	Means		180.15
	Sdevs		32.72

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Individual Animal Feed Consumed/day (g)
Study number: TOX209A

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Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

		D a y o f P h a s e												
Animal	Group	7	8	9	10	11	12	13	14	15	16	17	18	19

		M a l e						A n i m a l s						
1	1	21.77	25.08	24.03	28.85	22.35	24.06	22.95	25.31	26.23	28.36	26.71	28.26	31.16
2		16.28	18.85	24.61	24.99	22.19	23.93	25.58	21.00	23.95	23.68	24.03	26.16	25.59
3		13.59	30.38	22.11	20.48	22.86	22.84	24.27	16.20	18.13	22.96	21.89	21.61	22.32
4		23.65	27.30	23.78	21.02	25.32	22.60	28.61	23.61	25.41	24.29	23.16	25.35	24.14
5		13.48	14.79	24.94	23.41	23.19	24.82	22.30	21.37	19.36	19.17	16.66	25.17	18.18
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	17.75	23.28	23.89	23.75	23.18	23.65	24.74	21.50	22.62	23.69	22.49	25.31	24.28
	Sdevs	4.71	6.35	1.10	3.38	1.26	0.92	2.50	3.44	3.65	3.28	3.71	2.41	4.75
6	2	11.57	12.66	14.22	17.09	14.42	12.83	16.90	21.95	23.46	26.20	25.44	28.91	20.25
7		13.20		14.33	14.67	15.10	29.09	31.53	26.12	29.85	27.76	28.81	27.31	24.48
8		22.98	26.81	24.96	25.60	23.95	25.84	28.06	26.69	26.92	26.67	29.29	27.92	25.49
9		28.43	29.46	32.38	28.98	31.89	28.28	31.59	26.62	31.39	25.31	30.41	27.86	24.34
10		26.40	23.66	22.41	22.72			29.73	20.29	28.12	21.84	24.58	24.78	16.88
	(n)	5	4	5	5	4	4	5	5	5	5	5	5	5
	Means	20.52	23.15	21.66	21.81	21.34	24.01	27.56	24.33	27.95	25.56	27.71	27.36	22.29
	Sdevs	7.70	7.38	7.67	5.91	8.27	7.58	6.14	3.00	3.03	2.26	2.55	1.55	3.63
11	3	18.35	24.03	21.42	21.41	21.83	23.86	26.44		25.49	23.86	22.79	19.89	21.25
12		22.76	23.77	28.61	21.32	23.30	22.59	25.26	24.81	24.17	27.74	24.63	24.59	25.99
13		31.67	30.85		31.47	24.19	37.32	37.68		19.45	16.78	22.96	21.34	17.53
14		21.48	25.00	21.10	21.08	24.21	29.08	26.51	25.66	26.59	24.22	23.98	23.40	24.82
15		19.12	17.73	17.06	26.66	28.59	26.34	24.70	21.35	26.65	26.43	24.99	23.80	23.10
	(n)	5	5	4	5	5	5	5	3	5	5	5	5	5
	Means	22.68	24.28	22.05	24.39	24.42	27.84	28.12	23.94	24.47	23.81	23.87	22.60	22.54
	Sdevs	5.33	4.66	4.80	4.60	2.52	5.85	5.40	2.28	2.98	4.24	0.98	1.93	3.32
16	4	15.61	17.14	15.45	16.04	15.72	21.62	22.38	20.18	20.41	20.51	20.17	15.82	22.25
17		21.09	23.07	28.90	22.51	26.05	29.27	24.22	17.73	24.67	25.52	22.93	27.30	27.02
18		22.21	26.94	24.18	32.03	28.29		35.20	27.78	27.18	27.24	25.47	25.24	29.52
19		10.66	10.94	10.15	21.01	19.79	19.31	30.24	19.68	23.84	21.06	23.22	22.52	24.56
20		18.60	19.78	26.14	25.93	20.06	24.75	24.34	24.92	25.99	24.27	21.28	22.18	26.24
	(n)	5	5	5	5	5	4	5	5	5	5	5	5	5
	Means	17.63	19.57	20.96	23.50	21.98	23.74	27.28	22.06	24.42	23.72	22.61	22.61	25.92
	Sdevs	4.65	6.06	7.87	5.95	5.10	4.31	5.33	4.15	2.58	2.89	2.02	4.34	2.72
21	5	16.30	19.32	23.19	22.66	27.41	21.11	23.24	28.96	22.85	25.95	28.82	21.91	20.34
22		10.54	10.77	10.25	11.52	12.02	17.71	12.02	25.62	25.89	21.72	23.09	24.06	19.86
23		17.98	20.79	20.53	26.29	25.07	23.03	23.69	27.45	22.52	27.99	21.85	23.48	22.71
24		17.45	19.07	28.34	26.53	25.93	20.07	31.86	31.71		31.04	35.35	33.43	34.89

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Individual Animal Feed Consumed/day (g)
Study number: TOX209A

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FEEDING STUDY/PALATABILITY

		D a y o f P h a s e												
Animal	Group	7	8	9	10	11	12	13	14	15	16	17	18	19

		M a l e						A n i m a l s						
25	5	18.03	19.00	12.68	14.39	22.52	12.94	16.28	22.79	25.90	18.43	14.39	18.85	31.45
	(n)	5	5	5	5	5	5	5	5	4	5	5	5	5
	Means	16.06	17.79	19.00	20.28	22.59	18.97	21.42	27.31	24.29	25.03	24.70	24.35	25.85
	Sdevs	3.16	3.99	7.48	6.93	6.17	3.88	7.62	3.37	1.86	5.01	7.86	5.47	6.88
26	6	11.10	10.80			14.65	12.30	14.44	15.23	16.52	17.00	17.72	17.57	
27		10.72	13.76	22.45	18.12	22.20	14.78	19.66	20.73	14.56	15.34	22.31	22.25	15.02
29		11.00	11.25	12.18	13.87	13.35	12.97	15.97	16.42	16.13	17.46	18.79	21.39	11.42
30		10.76	11.01	26.58	12.46	15.65	20.42	20.88	8.47	18.26	23.17	18.90	27.26	18.85
	(n)	4	4	3	3	4	4	4	4	4	4	4	4	3
	Means	10.90	11.71	20.40	14.82	16.46	15.12	17.74	15.21	16.37	18.24	19.43	22.12	15.10
	Sdevs	0.18	1.38	7.41	2.95	3.94	3.69	3.03	5.08	1.52	3.41	1.99	3.99	3.72
31	7	12.75		16.85	13.06	18.63	20.94	16.52	17.39	14.39	23.59	20.64		11.55
32		7.39	6.45	6.59	8.50	9.88	11.78	12.28	10.47	10.17	18.98	9.48	17.21	13.63
33		6.68	7.39	8.70	8.90	9.94	11.84	13.47	9.11	9.03	11.01	12.24	13.02	
34		10.90	15.58	16.13	14.12	19.45	27.93	17.92	32.28	32.16	39.64	36.84	38.20	26.16
35		8.72	10.88	10.98	10.68	11.41	12.47	13.08	12.18	17.61	14.16	13.57	13.59	13.96
	(n)	5	4	5	5	5	5	5	5	5	5	5	4	4
	Means	9.29	10.08	11.85	11.05	13.86	16.99	14.65	16.29	16.67	21.48	18.55	20.51	16.33
	Sdevs	2.52	4.14	4.52	2.49	4.78	7.24	2.43	9.48	9.31	11.22	11.02	11.94	6.64
36	8	20.35	19.57	10.81	21.31	11.99	25.31	21.87	20.11	24.05	17.65	19.55	18.78	16.85
37		29.05	21.28	28.37	24.79	26.56	24.01	22.68	26.52	24.55	24.05	27.73	24.02	22.72
38		25.33	23.78	23.76	24.58	24.28	23.97	17.88	23.01	20.21	24.44	28.34	27.16	23.72
39		30.33	28.65	26.97	31.95	25.94		10.38	29.10	31.73	25.81	26.91	28.45	27.66
40		21.75	21.29	25.67	24.34	23.97	27.60	25.66	18.59	22.55	24.11	24.99	19.77	19.04
	(n)	5	5	5	5	5	4	5	5	5	5	5	5	5
	Means	25.36	22.91	23.12	25.39	22.55	25.22	19.69	23.47	24.62	23.21	25.50	23.64	22.00
	Sdevs	4.37	3.54	7.09	3.93	6.00	1.70	5.90	4.37	4.32	3.19	3.56	4.31	4.21
41	9	22.46	19.54	16.74	22.80	18.98	21.39	18.87	15.52	19.11	21.55	20.01	19.89	17.94
42		10.82	11.56	25.38	25.16	25.08	27.26	27.89	23.86	19.15	21.37	25.18	22.34	14.22
43		20.88	26.45	31.80	31.06	30.18	29.17	32.44	28.67	33.95	33.04	29.73	28.19	27.88
44		22.09	22.52	26.46	26.79	27.04	30.79	25.73	20.66	21.62	26.15	24.69	26.31	27.01
45		24.19	23.98	23.85	29.68	26.75	31.37	26.57	19.04	26.79	29.20	21.33	25.72	21.87
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	20.09	20.81	24.85	27.10	25.61	28.00	26.30	21.55	24.12	26.26	24.19	24.49	21.78
	Sdevs	5.31	5.74	5.43	3.34	4.14	4.02	4.89	4.99	6.32	5.02	3.79	3.33	5.84

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Individual Animal Feed Consumed/day (g)
Study number: TOX209A

PRINTED: 08-May-08
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Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

		D a y o f P h a s e												
Animal	Group	7	8	9	10	11	12	13	14	15	16	17	18	19

		M a l e						A n i m a l s						
46	10	20.59	23.12	26.34	22.95	30.33	27.27	27.06	24.74	29.37	28.25	30.35	29.12	
47		15.46	18.21	27.74	23.96	24.63	28.05	25.18	25.52	20.25	27.53	27.89	25.59	22.73
48		18.49	28.56	16.96	31.63	22.01	24.71	29.57	23.44	25.85	24.73			19.72
49		16.30	19.90	18.18	20.87	20.54	19.78	24.60	21.45	21.02	21.69	23.92	19.66	25.64
50		18.00	18.86	19.66	27.75	24.23	21.47	26.37	29.16		18.34	25.38	16.85	18.92
	(n)	5	5	5	5	5	5	5	5	4	5	4	4	4
	Means	17.77	21.73	21.78	25.43	24.35	24.26	26.56	24.86	24.12	24.11	26.89	22.81	21.75
	Sdevs	2.00	4.26	4.92	4.27	3.74	3.59	1.94	2.85	4.29	4.13	2.83	5.57	3.07

51	11	19.85	17.48	27.34		22.05	26.45	20.91	20.96	23.10	36.31	31.65	25.72	
52		13.43	24.62	22.56	22.99	22.33	18.02	22.39	19.58	20.31	21.87	20.89	20.24	18.73
53		16.99	17.00	22.29	21.87	19.34	18.90	21.15	18.70	18.25	18.15	20.41	20.38	19.41
54		19.05	22.38	22.34	27.04	26.20	20.57	27.57	26.49	25.78	22.80	25.68	22.98	21.88
55		21.31	21.21	20.29	24.44	24.66	26.42	25.44	22.49	24.22	24.06	24.36	21.43	22.57
	(n)	5	5	5	4	5	5	5	5	5	5	5	5	4
	Means	18.13	20.54	22.96	24.09	22.92	22.07	23.49	21.64	22.33	24.64	24.60	22.15	20.65
	Sdevs	3.05	3.25	2.61	2.23	2.63	4.09	2.91	3.07	3.03	6.89	4.53	2.28	1.86

56	12	14.99	16.43	17.56	16.85	26.70	35.12	24.27	20.27	31.25	18.41	13.53	20.98	18.54
57		14.20	14.33	15.81	23.98	17.58	22.19	21.61	20.94	19.84	18.24	21.63	22.57	23.06
58		12.87	15.32	14.23	18.51	20.38	20.39	19.37	16.04	17.05	16.03	17.60	17.05	15.44
59		14.32	19.74	21.64	30.32	27.67	25.62	24.91	34.23	31.42	32.37	20.32	35.18	28.59
60		13.28	26.16	24.40	24.37	38.79	41.66	35.60	42.65	50.48	39.59	46.35	53.74	45.70
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	13.93	18.40	18.73	22.81	26.22	29.00	25.15	26.83	30.01	24.93	23.89	29.90	26.27
	Sdevs	0.85	4.79	4.21	5.34	8.20	9.08	6.24	11.17	13.17	10.45	12.93	14.96	11.94

61	13	7.93	9.38	17.43	12.41	20.89	14.36	20.76	10.45	11.73		17.76	15.21	16.86
62		11.49	14.41	14.17	18.31	19.26	13.50	18.20	9.26	9.56	9.93	9.37	12.27	12.14
63		11.74	12.27	18.79	11.86	20.13	13.27	26.02			21.42	11.46	16.30	18.86
64		9.84	16.50	17.23	17.62	23.11	16.50	16.15	15.72	22.19	18.64	11.51	14.36	
65		14.04	16.32	22.80	24.28	23.36	8.49	23.35	25.79	24.36	36.04	34.20	35.01	35.60
	(n)	5	5	5	5	5	5	5	4	4	4	5	5	4
	Means	11.01	13.78	18.08	16.90	21.35	13.22	20.90	15.31	16.96	21.51	16.86	18.63	20.87
	Sdevs	2.28	3.00	3.13	5.06	1.82	2.94	3.94	7.53	7.40	10.85	10.19	9.28	10.22

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Individual Animal Feed Consumed/day (g)
Study number: TOX209A

PRINTED: 08-May-08
Page: 4

Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Animal Group		Day of Phase	20
		Male	Animals
1	1		18.43
2			25.63
4			33.06
5			18.93
	(n)		4
	Means		24.01
	Sdevs		6.87
6	2		35.49
7			30.04
9			24.07
10			13.65
	(n)		4
	Means		25.81
	Sdevs		9.35
11	3		18.81
12			21.86
13			21.67
14			23.67
15			25.31
	(n)		5
	Means		22.26
	Sdevs		2.43
16	4		19.41
17			22.91
18			28.65
19			17.81
20			24.34
	(n)		5
	Means		22.62
	Sdevs		4.27
21	5		18.02
22			22.38
23			23.62
24			29.22
	(n)		4
	Means		23.31
	Sdevs		4.61

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Individual Animal Feed Consumed/day (g)
Study number: TOX209A

PRINTED: 08-May-08
Page: 5

Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Animal Group		Day of Phase	
		20	
		Male	Animals
26	6		13.07
27			16.08
29			16.71
	(n)		3
	Means		15.29
	Sdevs		1.95
31	7		22.71
32			14.82
34			31.00
35			12.39
	(n)		4
	Means		20.23
	Sdevs		8.42
36	8		16.18
37			21.05
38			20.16
39			25.51
40			21.24
	(n)		5
	Means		20.83
	Sdevs		3.32
41	9		15.08
42			15.70
43			29.27
44			18.06
45			23.83
	(n)		5
	Means		20.39
	Sdevs		6.05
46	10		30.90
47			29.76
48			33.38
49			24.46
50			21.72
	(n)		5
	Means		28.04
	Sdevs		4.81

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Individual Animal Feed Consumed/day (g)
Study number: TOX209A

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Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Animal Group		Day of Phase	
		20	
		Male	Animals
51	11		43.49
52			13.89
53			14.89
54			21.24
55			31.43
	(n)		5
	Means		24.99
	Sdevs		12.48
56	12		25.77
57			25.07
58			21.64
59			22.78
60			44.78
	(n)		5
	Means		28.01
	Sdevs		9.52
61	13		19.64
62			11.14
63			24.12
65			37.33
	(n)		4
	Means		23.06
	Sdevs		10.93

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Individual Animal Feed Consumed/day (g)
Study number: TOX209B

PRINTED: 08-May-08
Page: 1

Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

		D a y o f P h a s e												
Animal	Group	7	8	9	10	11	12	13	14	15	16	17	18	19

		M a l e						A n i m a l s						
66	1	17.40	19.28	20.91	22.37	21.63	23.01	21.04	20.60	21.40	24.71	23.55	22.79	22.68
67		18.79	22.07	30.49	31.65	33.58	29.29	27.78	29.18	20.87	25.25	21.90	29.40	32.47
68		22.33	27.05	18.47	27.03	22.12	21.26	26.89	28.79	25.12	13.40	21.50	27.41	26.69
69		24.17	22.73	23.53	23.98	24.11	23.42	22.61	28.08	23.46	26.30	21.36	26.22	19.39
70		21.07	25.58	24.34	30.13	22.92	23.89	27.97	31.76	25.74	26.99	30.25	25.90	22.92
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	20.75	23.34	23.55	27.03	24.87	24.17	25.26	27.68	23.32	23.33	23.71	26.34	24.83
	Sdevs	2.71	3.05	4.52	3.94	4.96	3.03	3.21	4.20	2.17	5.62	3.76	2.41	4.99

71	2	23.70	14.47	25.08	15.71	25.97	19.66	20.70	16.98	17.42	28.62	21.46	30.93	16.79
72		20.59	23.36	27.09	24.78	34.43	12.91	24.53	31.06	20.75	25.17	28.41	32.08	24.55
73		17.06	20.72	24.21	26.22	23.61	21.46	22.40	20.61	25.85	20.48	27.14	13.51	21.26
74		13.95	10.90	27.22	20.98	22.23	16.84	18.60	19.04	20.45	19.49	15.77	22.84	21.12
75		15.03	21.68	21.50	18.18	21.95	23.48	19.67	24.41	21.02	26.48	18.87	17.00	14.80
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	18.07	18.23	25.02	21.17	25.64	18.87	21.18	22.42	21.10	24.05	22.33	23.27	19.70
	Sdevs	4.04	5.30	2.35	4.40	5.17	4.13	2.34	5.54	3.03	3.92	5.38	8.23	3.89

76	3	9.34	13.67	14.32	15.38	13.57	19.13	13.01	19.45		17.93	10.61	20.26	8.96
77		13.35	11.72	13.20	14.74	13.67	18.81	12.67	13.40	13.97	16.81	13.63	15.89	12.99
78		14.96	14.34	14.72	23.15	27.11	19.85	21.72	22.95	24.67	25.81	17.11	21.95	19.83
79		6.68	12.20	16.59	12.50	16.23	20.21	14.52	30.63		22.77	10.96	17.45	12.88
80		13.26	10.42	13.80	15.98	16.52	19.55	17.60	22.03	15.97	15.24	19.93	18.00	17.80
	(n)	5	5	5	5	5	5	5	5	3	5	5	5	5
	Means	11.52	12.47	14.53	16.35	17.42	19.51	15.90	21.69	18.20	19.71	14.45	18.71	14.49
	Sdevs	3.41	1.56	1.29	4.02	5.59	0.56	3.79	6.23	5.69	4.42	4.02	2.40	4.33

81	4	7.02	8.93	8.86	11.54	12.42	18.39	12.30	16.25	11.65	13.96	11.55	11.63	10.94
82			7.26		2.81			9.29	23.44	2.23	9.66	8.66	12.77	10.22
83		4.91	6.24	7.10	7.80	7.66	8.69	8.33	9.25	8.33	9.44	6.34		10.69
84		4.72	7.90	8.52	18.47			1.24	18.82		9.96	7.35	2.05	7.87
85		3.29	3.00	5.93	10.80	19.49	5.40	16.74	21.32	20.18	19.64	15.39	19.77	4.01
	(n)	4	5	4	5	3	3	5	5	4	5	5	4	5
	Means	4.99	6.67	7.60	10.28	13.19	10.83	9.58	17.82	10.60	12.53	9.86	11.56	8.75
	Sdevs	1.54	2.27	1.35	5.72	5.95	6.75	5.70	5.49	7.49	4.39	3.66	7.29	2.91

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Individual Animal Feed Consumed/day (g)
Study number: TOX209B

PRINTED: 08-May-08
Page: 2

Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Animal Group		Day	of	Phase
		20		
		Male Animals		
66	1			21.06
67				25.77
68				27.27
69				20.06
70				26.89
	(n)			5
	Means			24.21
	Sdevs			3.40
71	2			17.53
72				26.78
73				25.12
74				19.81
75				12.31
	(n)			5
	Means			20.31
	Sdevs			5.85
76	3			12.14
77				13.25
78				18.28
79				18.40
80				22.01
	(n)			5
	Means			16.82
	Sdevs			4.07
81	4			11.76
82				8.79
83				6.42
84				6.40
85				8.62
	(n)			5
	Means			8.40
	Sdevs			2.20

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	mg/ml	
density 2:	1.2028	mg/ml	
density 3:	1.2036	mg/ml	
Average:	1.2032	mg/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

TOX209 Series 1

TOX209 TEST ARTICLE NEEDED FOR UPCOMING PALATABILITY STUDY FOR **RAT**

Series 1

	Tobacco Blend (mg nic/kg bw/day)	Assumed Average BW kg	mg of NIC	g of tob	Assumed g of feed consumed	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	0.190	0.038	0.00145	20.000	0.000072	1200	0.0868	0.0072	0.0019
Group 3	2.0	0.190	0.38	0.01446	20.000	0.000723	1200	0.8677	0.0723	0.0190
Group 4	4.0	0.190	0.76	0.02892	20.000	0.001446	1200	1.7354	0.1446	0.0380
Group 5	8.0	0.190	1.52	0.05785	20.000	0.002892	1200	3.4708	0.2892	0.0760
Group 6	20.0	0.190	3.8	0.14462	20.000	0.007231	1200	8.6770	0.7231	0.1900
Group 7	40.0	0.190	7.6	0.28923	20.000	0.014462	1200	17.3539	1.4462	0.3800

26.3 mg of nic per g of tobacco
blend Chemistry Lab

	Tobacco Extract (mg nic/kg bw/day)	Assumed Average BW kg	mg of Nic	g of tob	Assumed g of feed consumed	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	0.190	0.038	0.00165	20.000	0.00008	1200	0.0991	0.0083	0.0019
Group 9	2.0	0.190	0.38	0.01652	20.000	0.00083	1200	0.9913	0.0826	0.0190
Group 10	4.0	0.190	0.76	0.03304	20.000	0.00165	1200	1.9826	0.1652	0.0380
Group 11	8.0	0.190	1.52	0.06609	20.000	0.00330	1200	3.9652	0.3304	0.0760
Group 12	20.0	0.190	3.8	0.16522	20.000	0.00826	1200	9.9130	0.8261	0.1900
Group 13	40.0	0.190	7.6	0.33043	20.000	0.01652	1200	19.8261	1.6522	0.3800

23.0 mg of nic per g of tobacco
extract Chemistry Lab

	Nicotine Hydrogen Tartrate (mg nic/kg bw/day)	Assumed Average BW kg	mg of Nic	mg of nic tartrate	Assumed g of feed consumed	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	0.190	0.38	1.083	20.000	0.0541	1200	64.957	0.06496	0.0190
Group 15	8.0	0.190	1.52	4.330	20.000	0.2165	1200	259.829	0.25983	0.0760
Group 16	20.0	0.190	3.8	10.826	20.000	0.5413	1200	649.573	0.64957	0.1900
Group 17	40.0	0.190	7.6	21.652	20.000	1.0826	1200	1299.145	1.29915	0.3800

1 g of NIC in 2.85 g of NICOTINE TARTRATE

0.351 Proportion of Nicotine in Nicotine
Tartrate

Sigma Aldrich

TOX209 Series 2

TOX209 TEST ARTICLE NEEDED FOR UPCOMING PALATABILITY STUDY FOR RAT

Series 2

	Tobacco Blend (mg nic/kg bw/day)	Assumed Average BW kg	mg of NIC	g of tob	Assumed g of feed consumed	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	0.260	0.052	0.00198	24.0	0.000082	1200	0.0989	0.0082	0.0022	26.3 mg of nic per g of tobacco blend Chemistry Lab
Group 3	2.0	0.260	0.52	0.01979	24.0	0.000825	1200	0.9895	0.0825	0.0217	
Group 4	4.0	0.260	1.04	0.03958	24.0	0.001649	1200	1.9790	0.1649	0.0433	
Group 5	8.0	0.260	2.08	0.07916	24.0	0.003298	1200	3.9579	0.3298	0.0867	
Group 6	20.0	0.260	5.2	0.19790	24.0	0.008246	1200	9.8948	0.8246	0.2167	
Group 7	40.0	0.260	10.4	0.39579	24.0	0.016491	1200	19.7895	1.6491	0.4333	
	Tobacco Extract (mg nic/kg bw/day)	Assumed Average BW kg	mg of Nic	g of tob	Assumed g of feed consumed	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	0.260	0.052	0.00226	24.0	0.00009	1200	0.1130	0.0094	0.0022	23.0 mg of nic per g of tobacco extract Chemistry Lab
Group 9	2.0	0.260	0.52	0.02261	24.0	0.00094	1200	1.1304	0.0942	0.0217	
Group 10	4.0	0.260	1.04	0.04522	24.0	0.00188	1200	2.2609	0.1884	0.0433	
Group 11	8.0	0.260	2.08	0.09043	24.0	0.00377	1200	4.5217	0.3768	0.0867	
Group 12	20.0	0.260	5.2	0.22609	24.0	0.00942	1200	11.3043	0.9420	0.2167	
Group 13	40.0	0.260	10.4	0.45217	24.0	0.01884	1200	22.6087	1.8841	0.4333	
	Nicotine Hydrogen Tartrate (mg nic/kg bw/day)	Assumed Average BW kg	mg of Nic	mg of nic tartrate	Assumed g of feed consumed	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	0.260	0.52	1.481	24.0	0.0617	1500	92.593	0.09259	0.0217	0.351 1 g of NIC in 2.85 g of NICOTINE TARTRATE Proportion of Nicotine in Nicotine Tartrate Sigma Aldrich
Group 15	8.0	0.260	2.08	5.926	24.0	0.2469	1500	370.370	0.37037	0.0867	
Group 16	20.0	0.260	5.2	14.815	24.0	0.6173	1500	925.926	0.92593	0.2167	
Group 17	40.0	0.260	10.4	29.630	24.0	1.2346	1500	1851.852	1.85185	0.4333	

Analytical Chemistry Summary

TOX209 Analytical Chemistry Summary

Trial Run						
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

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

Sub. Date	GN#	Test Article	Dose (mg/kg/day)	Initial Data@	Initial Data@	Final Data	Expected (mg/g)
				Nicotine (FID)* (mg/g)	Nicotine (MS)# (mg/g)	Nicotine (MS)# (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

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

Sub. Date	GN#	Test Article	Dose (mg/kg/day)	Initial Data@	Final Data	Expected (mg/g)
				Nicotine (FID)* (mg/g)	Nicotine (FID)* (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 trial run formulation

TOX209 Analytical Chemistry Summary

Series 1 Feed Formulation

Sub. Date	GN#	Test Article	Dose (mg/kg/day)	Nicotine (FID)* (mg/g)	Nicotine (MS)# (mg/g)	Expected (mg/g)
4/18/2008	76363AA	Blend Top	40 mg/kg	0.39		
	76363AB	Blend Mid	40 mg/kg	0.36		
	76363AC	Blend Bot	40 mg/kg	0.39		
	Mean			0.38		0.38
	76363AD	Extract Top	40 mg/kg	0.49		
	76363AE	Extract Mid	40 mg/kg	0.33		
	76363AF	Extract Bot	40 mg/kg	0.37		
	Mean			0.40		0.38
	76363AG	Tartrate Top	40 mg/kg	0.35		
	76363AH	Tartrate Mid	40 mg/kg	0.35		
	76363AI	Tartrate Bot	40 mg/kg	0.35		
	Mean			0.35		0.38
6/12/2008	77615AA	Control Feed	0		0.00	0.000
	77615AB	Blend	0.2		0.002	0.002
	77615AC	Blend	2.0		0.014	0.019
	77615AD	Blend	4.0		0.035	0.038
	77615AE	Blend	8.0		0.062	0.076
	77615AF	Blend	20.0		0.138	0.190
	77615AG	Blend	40.0		0.302	0.380
	77615AH	Extract	0.2		0.002	0.002
	77615AI	Extract	2.0		0.050	0.019
	77615AJ	Extract	4.0		0.053	0.038
	77615AK	Extract	8.0		0.060	0.076
	77615AL	Extract	20.0		0.178	0.190
	77615AM	Extract	40.0		0.265	0.380
	77615AN	Tartrate	2.0		0.018	0.019
	77615AO	Tartrate	8.0		0.063	0.076
	77615AP	Tartrate	20.0		0.153	0.190
	77615AQ	Tartrate	40.0		0.296	0.380

TOX209 Analytical Chemistry Summary

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)
4/16/2008	76269AA	Blend Top	0.2		0.001	0.002
	76269AB	Blend Mid	0.2		0.001	0.002
	76269AC	Blend Bot	0.2		0.001	0.002
	76269AD	Blend Top	40.0		\	
	76269AE	Blend Mid	40.0		\	
	76269AF	Blend Bot	40.0		\	
	76269AG	Blend	2.0		0.017	0.019
	76269AH	Blend	4.0		0.037	0.038
	76269AI	Blend	8.0		0.068	0.076
	76269AJ	Blend	20.0		0.147	0.190
	76269AK	Extract Top	0.2		0.001	0.002
	76269AL	Extract Mid	0.2		0.001	0.002
	76269AM	Extract Bot	0.2		0.001	0.002
	76269AN	Tartrate Top	2.0		0.018	0.019
	76269AO	Tartrate Mid	2.0		0.015	0.019
	76269AP	Tartrate Bot	2.0		0.014	0.019
	76269AQ	Extract Top	40.0		\	
	76269AR	Extract Mid	40.0		\	
	76269AS	Extract Bot	40.0		\	
	76269AT	Tartrate Top	40.0		\	
	76269AU	Tartrate Mid	40.0		\	
	76269AV	Tartrate Bot	40.0		\	
	76269AX	Tartrate	20.0		0.153	0.190
	76269AY	Extract	2.0		0.008	0.019
	76269AZ	Extract	4.0		0.022	0.038
	76269BA	Extract	8.0		0.093	0.076
	76269BB	Extract	20.0		0.166	0.190
	76269BC	Control	0.00		0.000	0.000
	76269BE	Tartrate	8.0		0.068	0.076

TOX209 Analytical Chemistry Summary

Series 2 Feed Formulation

Sub. Date	GN#	Test Article	Dose (mg/kg/day)	Nicotine (FID)* (mg/g)	Nicotine (MS)# (mg/g)	Expected (mg/g)
4/29/2008	76562AA	Blend Top	40.0	0.41		0.43
	76562AB	Blend Mid	40.0	0.41		0.43
	76562AC	Blend Bot	40.0	0.41		0.43
	Mean			0.41		
	76562AD	Extract Top	40.0	0.41		0.43
	76562AE	Extract Mid	40.0	0.39		0.43
	76562AF	Extract Bot	40.0	0.44		0.43
	Mean			0.41		
	76562AG	Tartrate Top	40.0	0.40		0.43
	76562AH	Tartrate Mid	40.0	0.40		0.43
	76562AI	Tartrate Bot	40.0	0.40		0.43
	Mean			0.40		

Series 2 Feed Formulation

Sub. Date	GN#	Test Article	Dose (mg/kg/day)	Nicotine (FID)* (mg/g)	Nicotine (MS)# (mg/g)	Expected (mg/g)
6/12/2008	77620AA	Control Feed			0.001	0.000
	77620AB	Blend	0.2		0.009	0.002
	77620AC	Blend	2.0		0.016	0.022
	77620AD	Blend	8.0		0.077	0.087
	77620AE	Blend	20.0		0.155	0.217
	77620AF	Blend	40.0		0.338	0.433
	77620AG	Extract	0.2		0.001	0.002
	77620AH	Extract	2.0		0.014	0.022
	77620AI	Extract	4.0		0.021	0.043
	77620AJ	Extract	8.0		0.053	0.087
	77620AK	Extract	20.0		0.142	0.217
	77620AL	Extract	40.0		0.374	0.433
	77620AM	Tartrate	2.0		0.017	0.022
	77620AN	Tartrate	8.0		0.065	0.087
	77620AO	Tartrate	20.0		0.154	0.217
	77620AP	Tartrate	40.0		0.306	0.433
	77620AQ	Blend	4.0		0.042	0.043 sample out of order

Series 2 Feed Formulation: Second Submission

Sub. Date	GN#	Test Article	Dose (mg/kg/day)	Nicotine (FID)* (mg/g)	Nicotine (MS)# (mg/g)	Expected (mg/g)
5/2/2008	76746AF	Blend	0.2		0.004	0.002
	76746AE	Blend	2.0		0.014	0.022
	76746AD	Blend	4.0		0.041	0.042
	76746AC	Blend	8.0		0.071	0.087
	76746AB	Blend	20.0		0.190	0.217
	76746AA	Blend	40.0		0.357	0.433
	76746AL	Extract	0.2		0.001	0.002
	76746AK	Extract	2.0		0.040	0.022
	76746AJ	Extract	4.0		0.043	0.042
	76746AI	Extract	8.0		0.122	0.087
	76746AH	Extract	20.0		0.134	0.217
	76746AG	Extract	40.0		0.309	0.433
	76746AM	Tartrate	2.0		0.019	0.022
	76746AN	Tartrate	8.0		0.075	0.087
	76746AO	Tartrate	20.0		0.181	0.217
	76746AP	Tartrate	40.0		0.356	0.433

Series 2 Feed Formulation: 10-Day Stability Data Feed Prepared on 4-24 & 4-24

Sub. Date	GN#	Test Article	Dose (mg/kg/day)	Nicotine (FID)* (mg/g)	Initial Data@	Final Data	Expected (mg/g)
					Nicotine (MS)# (mg/g)	Nicotine (MS)# (mg/g)	
5/2/2008	76750AA	Blend	40.0		0.347	0.409	0.433
	76750AB	Blend	0.2		0.007	0.002	0.002
	76750AC	Extract	40.0		0.342	0.367	0.433
	76750AD	Extract	0.2		0.001	0.001	0.002
	76750AE	Tartrate	40.0		0.331	0.354	0.433
	76750AF	Tartrate	2.0		0.018	0.019	0.022

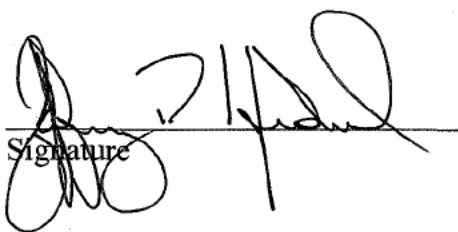
@ Data represent the mean of the first and second submission for the Series 2 formulation

Analytical Chemistry Data

TOX209

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

The following Tables list the GN Numbers assigned to Nicotine Dose Articles (Control, Tobacco Blend, Tobacco Extract and Nicotine Tartrate). The GN Numbers were located on sample cups submitted to Analytical Chemistry for analysis for Study TOX209.


Signature

9-Feb-09
Date

Study Number	GN Number	Description
TOX209A	GN76269AA	Article NTP-2000/Tobacco Blend Dose 0.2 mg of nic/kg of bwt (Top)
TOX209A	GN76269AB	Article NTP-2000/Tobacco Blend Dose 0.2 mg of nic/kg of bwt (Middle)
TOX209A	GN76269AC	Article NTP-2000/Tobacco Blend Dose 0.2 mg of nic/kg of bwt (Bottom)
TOX209A	GN76269AG	Article NTP-2000/Tobacco Blend Dose 2.0 mg of nic/kg of bwt
TOX209A	GN76269AH	Article NTP-2000/Tobacco Blend Dose 4.0 mg of nic/kg of bwt
TOX209A	GN76269AI	Article NTP-2000/Tobacco Blend Dose 8.0 mg of nic/kg of bwt (Top)
TOX209A	GN76269AJ	Article NTP-2000/Tobacco Blend Dose 20.0 mg of nic/kg of bwt
TOX209A	GN76269AK	Article NTP-2000/Tobacco Extract Dose 0.2 mg of nic/kg of bwt (Top)
TOX209A	GN76269AL	Article NTP-2000/Tobacco Extract Dose 0.2 mg of nic/kg of bwt (Middle)
TOX209A	GN76269AM	Article NTP-2000/Tobacco Extract Dose 0.2 mg of nic/kg of bwt (Bottom)
TOX209B	GN76269AN	Article NTP-2000/Nicotine Tartrate Dose 2.0 mg of nic/kg of bwt (Top)
TOX209B	GN76269AO	Article NTP-2000/Nicotine Tartrate Dose 2.0 mg of nic/kg of bwt (Middle)
TOX209B	GN76269AP	Article NTP-2000/Nicotine Tartrate Dose 2.0 mg of nic/kg of bwt (Bottom)
TOX209B	GN76269AX	Article NTP-2000/Nicotine Tartrate Dose 20.0 mg of nic/kg of bwt
TOX209A	GN76269AY	Article NTP-2000/Tobacco Extract Dose 2.0 mg of nic/kg of bwt
TOX209A	GN76269AZ	Article NTP-2000/Tobacco Extract Dose 4.0 mg of nic/kg of bwt
TOX209A	GN76269BA	Article NTP-2000/Tobacco Extract Dose 8.0 mg of nic/kg of bwt
TOX209A	GN76269BB	Article NTP-2000/Tobacco Extract Dose 20.0 mg of nic/kg of bwt
TOX209A	GN76269BC	Article NTP-2000/ Dose 0.0 mg of nic/kg of bwt
TOX209B	GN76269BE	Article NTP-2000/Nicotine Tartrate Dose 8.0 mg of nic/kg of bwt
TOX209A	GN77615AB	Article NTP-2000/Tobacco Blend Dose 0.2 mg of nic/kg of bwt
TOX209A	GN77615AC	Article NTP-2000/Tobacco Blend Dose 2.0 mg of nic/kg of bwt
TOX209A	GN77615AD	Article NTP-2000/Tobacco Blend Dose 4.0 mg of nic/kg of bwt
TOX209A	GN77615AE	Article NTP-2000/Tobacco Blend Dose 8.0 mg of nic/kg of bwt
TOX209A	GN77615AF	Article NTP-2000/Tobacco Blend Dose 20.0 mg of nic/kg of bwt
TOX209A	GN77615AG	Article NTP-2000/Tobacco Blend Dose 40.0 mg of nic/kg of bwt
TOX209A	GN77615AH	Article NTP-2000/Tobacco Extract Dose 0.2 mg of nic/kg of bwt
TOX209A	GN77615AI	Article NTP-2000/Tobacco Extract Dose 2.0 mg of nic/kg of bwt
TOX209A	GN77615AJ	Article NTP-2000/Tobacco Extract Dose 4.0 mg of nic/kg of bwt
TOX209A	GN77615AK	Article NTP-2000/Tobacco Extract Dose 8.0 mg of nic/kg of bwt
TOX209A	GN77615AL	Article NTP-2000/Tobacco Extract Dose 20.0 mg of nic/kg of bwt
TOX209A	GN77615AM	Article NTP-2000/Tobacco Extract Dose 40.0 mg of nic/kg of bwt
TOX209B	GN77615AN	Article NTP-2000/Nicotine Tartrate Dose 2.0 mg of nic/kg of bwt
TOX209B	GN77615AO	Article NTP-2000/Nicotine Tartrate Dose 8.0 mg of nic/kg of bwt
TOX209B	GN77615AP	Article NTP-2000/Nicotine Tartrate Dose 20.0 mg of nic/kg of bwt
TOX209B	GN77615AQ	Article NTP-2000/Nicotine Tartrate Dose 40.0 mg of nic/kg of bwt
TOX209A	GN77620AA	Article NTP-2000/ Dose 0.0 mg of nic/kg of bwt
TOX209A	GN77620AB	Article NTP-2000/Tobacco Blend Dose 0.2 mg of nic/kg of bwt
TOX209A	GN77620AC	Article NTP-2000/Tobacco Blend Dose 2.0 mg of nic/kg of bwt
TOX209A	GN77620AD	Article NTP-2000/Tobacco Blend Dose 8.0 mg of nic/kg of bwt
TOX209A	GN77620AE	Article NTP-2000/Tobacco Blend Dose 20.0 mg of nic/kg of bwt
TOX209A	GN77620AF	Article NTP-2000/Tobacco Blend Dose 40.0 mg of nic/kg of bwt
TOX209A	GN77620AG	Article NTP-2000/Tobacco Extract Dose 0.2 mg of nic/kg of bwt
TOX209A	GN77620AH	Article NTP-2000/Tobacco Extract Dose 2.0 mg of nic/kg of bwt
TOX209A	GN77620AI	Article NTP-2000/Tobacco Extract Dose 4.0 mg of nic/kg of bwt
TOX209A	GN77620AJ	Article NTP-2000/Tobacco Extract Dose 8.0 mg of nic/kg of bwt
TOX209A	GN77620AK	Article NTP-2000/Tobacco Extract Dose 20.0 mg of nic/kg of bwt
TOX209A	GN77620AL	Article NTP-2000/Tobacco Extract Dose 40.0 mg of nic/kg of bwt
TOX209B	GN77620AM	Article NTP-2000/Nicotine Tartrate Dose 2.0 mg of nic/kg of bwt
TOX209B	GN77620AN	Article NTP-2000/Nicotine Tartrate Dose 8.0 mg of nic/kg of bwt
TOX209B	GN77620AO	Article NTP-2000/Nicotine Tartrate Dose 20.0 mg of nic/kg of bwt
TOX209B	GN77620AP	Article NTP-2000/Nicotine Tartrate Dose 40.0 mg of nic/kg of bwt
TOX209A	GN77620AQ	Article NTP-2000/Tobacco Blend Dose 4.0 mg of nic/kg of bwt

TOX209		
Study Number	GN Number	Description
TOX209A	GN76276BD	Article NTP-2000/Tobacco Extract Dose 40.0 mg of nic/kg of bwt
TOX209A	GN76276BE	Article NTP-2000/Tobacco Extract Dose 0.2 mg of nic/kg of bwt
TOX209B	GN76276BF	Article NTP-2000/Nicotine Tartrate Dose 40.0 mg of nic/kg of bwt
TOX209B	GN76276BG	Article NTP-2000/Nicotine Tartrate Dose 2.0 mg of nic/kg of bwt
TOX209A	GN76276BH	Article NTP-2000/Tobacco Blend Dose 40.0 mg of nic/kg of bwt
TOX209A	GN76276BI	Article NTP-2000/Tobacco Blend Dose 0.2 mg of nic/kg of bwt
TOX209	GN75963AA	Article NTP-2000/Tobacco Blend Dose 40.0 mg of nic/kg of bwt (Top)
TOX209	GN75963AB	Article NTP-2000/Tobacco Blend Dose 40.0 mg of nic/kg of bwt (Middle)
TOX209	GN75963AC	Article NTP-2000/Tobacco Blend Dose 40.0 mg of nic/kg of bwt (Bottom)
TOX209	GN75963AD	Article NTP-2000/Tobacco Blend Dose 0.2 mg of nic/kg of bwt (Top)
TOX209	GN75963AE	Article NTP-2000/Tobacco Blend Dose 0.2 mg of nic/kg of bwt (Middle)
TOX209	GN75963AF	Article NTP-2000/Tobacco Blend Dose 0.2 mg of nic/kg of bwt (Bottom)
TOX209	GN75963AG	Article NTP-2000/Nicotine Tartrate Dose 40.0 mg of nic/kg of bwt (Top)
TOX209	GN75963AH	Article NTP-2000/Nicotine Tartrate Dose 40.0 mg of nic/kg of bwt (Middle)
TOX209	GN75963AI	Article NTP-2000/Nicotine Tartrate Dose 40.0 mg of nic/kg of bwt (Bottom)
TOX209	GN75963AJ	Article NTP-2000/Nicotine Tartrate Dose 2.0 mg of nic/kg of bwt (Top)
TOX209	GN75963AK	Article NTP-2000/Nicotine Tartrate Dose 2.0 mg of nic/kg of bwt (Middle)
TOX209	GN75963AL	Article NTP-2000/Nicotine Tartrate Dose 2.0 mg of nic/kg of bwt (Bottom)
TOX209	GN76002A	Article NTP-2000/Test Article 2 Extract Dose 40.0 mg of nic/kg of bwt (Top)
TOX209	GN76002AB	Article NTP-2000/Test Article 2 Extract Dose 40.0 mg of nic/kg of bwt (Middle)
TOX209	GN76002AC	Article NTP-2000/Test Article 2 Dose 40.0 mg of nic/kg of bwt
TOX209	GN76002AD	Article NTP-2000 Diet
TOX209	GN76002AE	Article NTP-2000/Test Article 2 Extract Dose 0.2 mg of nic/kg of bwt (Top)
TOX209	GN76002AF	Article NTP-2000/Test Article 2 Extract Dose 0.2 mg of nic/kg of bwt (Middle)
TOX209	GN76002AG	Article NTP-2000/Test Article 2 Extract Dose 0.2 mg of nic/kg of bwt (Bottom)
TOX209A	GN76750AA	Article NTP-2000/Tobacco Blend Dose 40.0 mg of nic/kg of bwt
TOX209A	GN76750AB	Article NTP-2000/Tobacco Blend Dose 0.2 mg of nic/kg of bwt
TOX209A	GN76750AC	Article NTP-2000/Tobacco Extract Dose 40.0 mg of nic/kg of bwt
TOX209A	GN76750AD	Article NTP-2000/Tobacco Extract Dose 0.2 mg of nic/kg of bwt
TOX209B	GN76750AE	Article NTP-2000/Nicotine Tartrate Dose 40.0 mg of nic/kg of bwt
TOX209B	GN76750AF	Article NTP-2000/Nicotine Tartrate Dose 2.0 mg of nic/kg of bwt

TOX209 Trail Run Feed Formulation

Homogeneity Data

Tobacco Blend

SAMPLE SUBMISSION RECORD

Study Number:

TOX209

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

Submitted by:

Geray Smith

Submitted on:

4-3-08

Sample Identification (GN number)	Analysis	Sample Identification (GN number)	Analysis
GN75963AA	46	GN75963AL	46
GN75963AB	46		
GN75963AC	46		
GN75963AD	46		
GN75963AE	46		
GN75963AF	46		
GN75963AG	46		
GN75963AH	46		
GN75963AI	46		
GN75963AJ	46		
GN75963AK	46		

Comments:

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

Sample(s) Received By:

[Signature]

Received On:

4-3-08

TOX215.003.091406

Number: GN75963 Date Requested: 04/03/08 Date Completed: 04/11/08
Project Description: Feed Study

Status: COMPLETED Protocol: GENERAL Prog Code: 900 Related #:

Requestor Name: SMITH, JENNY L

Req. Phone: 741-0125

Req. Location: 630-2

Req. Room:

Backup Name: PIKE, SUSAN

Backup Phone: 741-0921

Laboratory Notes:

User: WAYNE SCOTT Date: 04/11/08 Time: 08:44:09

For samples AA through AI, monitor was accepted with anatabine std
dev out of limits, customer requested nicotine data only.

Wayne Scott
0932

05/01/08

TRIAL Run

TOX209 Final Report Adobe File Page 186 of 443

Testno: GN75963 Prog no: 900

Protocol: GENERAL

Needed: 04/04/08

Requester: SMITH, JENNY L

Phone: 741-0125

Description: Feed Study

Number: TOX209

Part: GN75963AA Points: 1 Butt Len: 0 Part Name: NTP/TEST ART1 40.0 T
Date: 20080403 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN75963AB Points: 1 Butt Len: 0 Part Name: NTP/Test Art 1 40.0 M
Date: 20080403 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN75963AC Points: 1 Butt Len: 0 Part Name: NTP/Test Art 1 40.0 B
Date: 20080403 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN75963AG Points: 1 Butt Len: 0 Part Name: NTP/ NIC TAR 40.0 T
Date: 20080403 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN75963AH Points: 1 Butt Len: 0 Part Name: NTP/ NIC TAR 40.0 M
Date: 20080403 Shift: Comments:

42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN75963AI Points: 1 Butt Len: 0 Part Name: NTP/ NIC TAR 40.0 B
Date: 20080403 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Number: GN75963 Date Requested: 04/03/08 Date Completed: 04/11/08

Project Description: Feed Study

Status: COMPLETED Protocol: GENERAL Prog Code: 900 Related #:

Requestor Name: SMITH, JENNY L

Req. Phone: 741-0125

Req. Location: 630-2

Req. Room:

Backup Name: PIKE, SUSAN

Backup Phone: 741-0921

Laboratory Notes:

User: WAYNE SCOTT Date: 04/11/08 Time: 08:44:09

For samples AA through AI, monitor was accepted with anatabine std
dev out of limits, customer requested nicotine data only.

Wayne Scott
0932

Page 2

PART SUMMARY RESULTS

4/11/2008 10:02:53 AM

Test Part	GN 75963 AA	GN 75963 AB	GN 75963 AC
PART_NAME	NTP/TEST ART1 40.0	NTP/Test Art 1 40.	NTP/Test Art 1 40.
BUTT_LENGTH	0	0	0
GN_DATE	20080403	20080403	20080403
GN_SHIFT			
GN_COMMENTS			
GN_TIME			

SPECIFIC ALKALOIDS

	MISSING	MISSING	MISSING
TOTAL ALKALOIDS %	MISSING	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	0.
2ND TOTAL ALKALOIDS%	MISSING	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	0.
NICOTINE %	0.0460	0.0472	0.0447
Std. Deviation	0.00201	0.00119	0.00182
Count	3.	3.	3.
NORNICOTINE %	MISSING	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	0.
MYOSMINE %	<0.0003	<0.0003	<0.0003
Std. Deviation	0.00000	0.00000	0.00000
Count	3.	3.	3.
ANABASINE %	<0.0003	<0.0003	<0.0003
Std. Deviation	N/A	0.00000	N/A
Count	1.	2.	1.
ANATABINE %	0.0010	0.0012	0.0011
Std. Deviation	0.00025	0.00015	0.00012
Count	3.	3.	3.
NICOTINE MG/G	0.46	0.47	0.45
Std. Deviation	0.020	0.012	0.017
Count	3.	3.	3.

Test Part	GN 75963 AA	GN 75963 AB	GN 75963 AC
T PART_NAME	NTP/TEST ART1 40.0	NTP/Test Art 1 40.	NTP/Test Art 1 40.
T BUTT_LENGTH	0	0	0
GN DATE	20080403	20080403	20080403
GN SHIFT			
GN COMMENTS			
GN TIME			

SPECIFIC ALKALOIDS

TOTAL ALKALOIDS %

	MISSING	MISSING	MISSING
Averages Point 1			
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	0.	0.

2ND TOTAL ALKALOIDS%

	MISSING	MISSING	MISSING
Averages Point 1			
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	0.	0.

NICOTINE %

Averages Point 1	0.0460	0.0472	0.0447
Std. Dev. Point 1	0.00201	0.00119	0.00182
Count Point 1	3.	3.	3.

NORNICOTINE %

	MISSING	MISSING	MISSING
Averages Point 1			
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	0.	0.

MYOSMINE %

Averages Point 1	<0.0003	<0.0003	<0.0003
Std. Dev. Point 1	0.00000	0.00000	0.00000
Count Point 1	3.	3.	3.

ANABASINE %

Averages Point 1	<0.0003	<0.0003	<0.0003
Std. Dev. Point 1	N/A	0.00000	N/A
Count Point 1	1.	2.	1.

ANATABINE %

Averages Point 1	0.0010	0.0012	0.0011
Std. Dev. Point 1	0.00025	0.00015	0.00012
Count Point 1	3.	3.	3.

NICOTINE MG/G

Averages Point 1	0.46	0.47	0.45
Std. Dev. Point 1	0.020	0.012	0.017
Count Point 1	3.	3.	3.

Test Part	GN 75963 AA	GN 75963 AB	GN 75963 AC
TEST PART_NAME	NTP/TEST ART1 40.0	NTP/Test Art 1 40.	NTP/Test Art 1 40.
TEST BUTT_LENGTH	0	0	0
GN_DATE	20080403	20080403	20080403
GN_SHIFT			
GN_COMMENTS			
GN_TIME			

SPECIFIC ALKALOIDS

TOTAL ALKALOIDS %	MISSING	MISSING	MISSING
2ND TOTAL ALKALOIDS%	MISSING	MISSING	MISSING
NICOTINE %	0.0460	0.0472	0.0447
NORNICOTINE %	MISSING	MISSING	MISSING
MYOSMINE %	<0.0003	<0.0003	<0.0003
ANABASINE %	<0.0003	<0.0003	<0.0003
ANATABINE %	0.0010	0.0012	0.0011
NICOTINE MG/G	0.46	0.47	0.45

Test Part	GN 75963 AA	GN 75963 AB	GN 75963 AC
T PART NAME	NTP/TEST ART1 40.0	NTP/Test Art 1 40.	NTP/Test Art 1 40.
T BUTT LENGTH	0	0	0
G DATE	20080403	20080403	20080403
GN SHIFT			
GN COMMENTS			
GN TIME			

SPECIFIC ALKALOIDS

TOTAL ALKALOIDS %

	GN 75963 AA	GN 75963 AB	GN 75963 AC
Point 1 Rep 1	MISSING	MISSING	MISSING
Point 1 Rep 2	MISSING	MISSING	MISSING
Point 1 Rep 3	MISSING	MISSING	MISSING

2ND TOTAL ALKALOIDS%

	GN 75963 AA	GN 75963 AB	GN 75963 AC
Point 1 Rep 1	MISSING	MISSING	MISSING
Point 1 Rep 2	MISSING	MISSING	MISSING
Point 1 Rep 3	MISSING	MISSING	MISSING

NICOTINE %

	GN 75963 AA	GN 75963 AB	GN 75963 AC
Point 1 Rep 1	0.0481	0.0476	0.0459
Point 1 Rep 2	0.0457	0.0459	0.0426
Point 1 Rep 3	0.0441	0.0482	0.0456

NORNICOTINE %

	GN 75963 AA	GN 75963 AB	GN 75963 AC
Point 1 Rep 1	MISSING	MISSING	MISSING
Point 1 Rep 2	MISSING	MISSING	MISSING
Point 1 Rep 3	MISSING	MISSING	MISSING

MYOSMINE %

	GN 75963 AA	GN 75963 AB	GN 75963 AC
Point 1 Rep 1	<0.0003	<0.0003	<0.0003
Point 1 Rep 2	<0.0003	<0.0003	<0.0003
Point 1 Rep 3	<0.0003	<0.0003	<0.0003

ANABASINE %

	GN 75963 AA	GN 75963 AB	GN 75963 AC
Point 1 Rep 1	MISSING	MISSING	<0.0003
Point 1 Rep 2	<0.0003	<0.0003	MISSING
Point 1 Rep 3	MISSING	<0.0003	MISSING

ANATABINE %

	GN 75963 AA	GN 75963 AB	GN 75963 AC
Point 1 Rep 1	0.0013	0.0010	0.0012
Point 1 Rep 2	0.0010	0.0012	0.0010
Point 1 Rep 3	0.0008	0.0013	0.0012

NICOTINE MG/G

	GN 75963 AA	GN 75963 AB	GN 75963 AC
Point 1 Rep 1	0.48	0.48	0.46
Point 1 Rep 2	0.46	0.46	0.43
Point 1 Rep 3	0.44	0.48	0.46

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POINT SUMMARY RESULTS

4/30/2008 8:43:42 AM

Test Part	GN 75963 AG	GN 75963 AH	GN 75963 AI
T PART NAME	NTP/ NIC TAR 40.0 T	NTP/ NIC TAR 40.0 M	NTP/ NIC TAR 40.0 B
TEST BUTT LENGTH	0	0	0
GN DATE	20080403	20080403	20080403
GN SHIFT			
GN COMMENTS			
GN TIME			

SPECIFIC ALKALOIDS

TOTAL ALKALOIDS %

	MISSING	MISSING	MISSING
Averages Point 1			
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	0.	0.

2ND TOTAL ALKALOIDS%

	MISSING	MISSING	MISSING
Averages Point 1			
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	0.	0.

NICOTINE %

	0.0419	0.0414	0.0406
Averages Point 1			
Std. Dev. Point 1	0.00145	0.00100	0.00071
Count Point 1	3.	3.	3.

NORNICOTINE %

	MISSING	MISSING	MISSING
Averages Point 1			
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	0.	0.

MYOSMINE %

	<0.0003	MISSING	<0.0003
Averages Point 1			
Std. Dev. Point 1	0.00000	N/A	0.00000
Count Point 1	2.	0.	2.

ANABASINE %

	MISSING	<0.0003	MISSING
Averages Point 1			
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	1.	0.

ANATABINE %

	MISSING	0.0002	0.0002
Averages Point 1			
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	1.	1.

NICOTINE MG/G

	0.42	0.41	0.40
Averages Point 1			
Std. Dev. Point 1	0.017	0.006	0.006
Count Point 1	3.	3.	3.

Test Part	GN 75963 AG	GN 75963 AH	GN 75963 AI
PART_NAME	NTP/ NIC TAR 40.0 T	NTP/ NIC TAR 40.0 M	NTP/ NIC TAR 40.0 B
T_BUTT_LENGTH	0	0	0
GN_DATE	20080403	20080403	20080403
GN_SHIFT			
GN_COMMENTS			
GN_TIME			

SPECIFIC ALKALOIDS

	MISSING	MISSING	MISSING
TOTAL ALKALOIDS %	N/A	N/A	N/A
Std. Deviation	0.	0.	0.
Count	MISSING	MISSING	MISSING
2ND TOTAL ALKALOIDS%	N/A	N/A	N/A
Std. Deviation	0.	0.	0.
Count	0.0419	0.0414	0.0406
NICOTINE %	0.00145	0.00100	0.00071
Std. Deviation	3.	3.	3.
Count	MISSING	MISSING	MISSING
NORNICOTINE %	N/A	N/A	N/A
Std. Deviation	0.	0.	0.
Count	<0.0003	MISSING	<0.0003
MYOSMINE %	0.00000	N/A	0.00000
Std. Deviation	2.	0.	2.
Count	MISSING	<0.0003	MISSING
ANABASINE %	N/A	N/A	N/A
Std. Deviation	0.	1.	0.
Count	MISSING	0.0002	0.0002
ANATABINE %	N/A	N/A	N/A
Std. Deviation	0.	1.	1.
Count	0.42	0.41	0.40
NICOTINE MG/G	0.017	0.006	0.006
Std. Deviation	3.	3.	3.
Count			

0.41

Tartar

Test Part	GN 75963 AG	GN 75963 AH	GN 75963 AI
T PART_NAME	NTP/ NIC TAR 40.0 T	NTP/ NIC TAR 40.0 M	NTP/ NIC TAR 40.0 B
T BUTT_LENGTH	0	0	0
DATE	20080403	20080403	20080403
GN_SHIFT			
GN_COMMENTS			
GN_TIME			

SPECIFIC ALKALOIDS

TOTAL ALKALOIDS %

Averages Point 1	MISSING	MISSING	MISSING
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	0.	0.

2ND TOTAL ALKALOIDS%

Averages Point 1	MISSING	MISSING	MISSING
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	0.	0.

NICOTINE %

Averages Point 1	0.0419	0.0414	0.0406
Std. Dev. Point 1	0.00145	0.00100	0.00071
Count Point 1	3.	3.	3.

NORNICOTINE %

Averages Point 1	MISSING	MISSING	MISSING
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	0.	0.

MYOSMINE %

Averages Point 1	<0.0003	MISSING	<0.0003
Std. Dev. Point 1	0.00000	N/A	0.00000
Count Point 1	2.	0.	2.

ANABASINE %

Averages Point 1	MISSING	<0.0003	MISSING
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	1.	0.

ANATABINE %

Averages Point 1	MISSING	0.0002	0.0002
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	1.	1.

NICOTINE MG/G

Averages Point 1	0.42	0.41	0.40
Std. Dev. Point 1	0.017	0.006	0.006
Count Point 1	3.	3.	3.

Test Part	GN 75963 AG	GN 75963 AH	GN 75963 AI
T PART_NAME	NTP/ NIC TAR 40.0 T	NTP/ NIC TAR 40.0 M	NTP/ NIC TAR 40.0 B
T BUTT_LENGTH	0	0	0
DATE	20080403	20080403	20080403
GN_SHIFT			
GN_COMMENTS			
GN_TIME			

SPECIFIC ALKALOIDS

TOTAL ALKALOIDS %

	GN 75963 AG	GN 75963 AH	GN 75963 AI
Point 1 Rep 1	MISSING	MISSING	MISSING
Point 1 Rep 2	MISSING	MISSING	MISSING
Point 1 Rep 3	MISSING	MISSING	MISSING

2ND TOTAL ALKALOIDS%

	GN 75963 AG	GN 75963 AH	GN 75963 AI
Point 1 Rep 1	MISSING	MISSING	MISSING
Point 1 Rep 2	MISSING	MISSING	MISSING
Point 1 Rep 3	MISSING	MISSING	MISSING

NICOTINE %

	GN 75963 AG	GN 75963 AH	GN 75963 AI
Point 1 Rep 1	0.0428	0.0410	0.0405
Point 1 Rep 2	0.0402	0.0406	0.0400
Point 1 Rep 3	0.0426	0.0425	0.0414

NORNICOTINE %

	GN 75963 AG	GN 75963 AH	GN 75963 AI
Point 1 Rep 1	MISSING	MISSING	MISSING
Point 1 Rep 2	MISSING	MISSING	MISSING
Point 1 Rep 3	MISSING	MISSING	MISSING

MYOSMINE %

	GN 75963 AG	GN 75963 AH	GN 75963 AI
Point 1 Rep 1	MISSING	MISSING	<0.0003
Point 1 Rep 2	<0.0003	MISSING	<0.0003
Point 1 Rep 3	<0.0003	MISSING	MISSING

ANABASINE %

	GN 75963 AG	GN 75963 AH	GN 75963 AI
Point 1 Rep 1	MISSING	MISSING	MISSING
Point 1 Rep 2	MISSING	<0.0003	MISSING
Point 1 Rep 3	MISSING	MISSING	MISSING

ANATABINE %

	GN 75963 AG	GN 75963 AH	GN 75963 AI
Point 1 Rep 1	MISSING	0.0002	MISSING
Point 1 Rep 2	MISSING	MISSING	MISSING
Point 1 Rep 3	MISSING	MISSING	0.0002

NICOTINE MG/G

	GN 75963 AG	GN 75963 AH	GN 75963 AI
Point 1 Rep 1	0.43	0.41	0.40
Point 1 Rep 2	0.40	0.41	0.40
Point 1 Rep 3	0.43	0.42	0.41

Test Part	GN 75963 AG	GN 75963 AH	GN 75963 AI
TEST PART_NAME	NTP/ NIC TAR 40.0 T	NTP/ NIC TAR 40.0 M	NTP/ NIC TAR 40.0 B
TEST BUTT_LENGTH	0	0	0
GN_DATE	20080403	20080403	20080403
GN_SHIFT			
GN_COMMENTS			
GN_TIME			

SPECIFIC ALKALOIDS

TOTAL ALKALOIDS %	MISSING	MISSING	MISSING
2ND TOTAL ALKALOIDS%	MISSING	MISSING	MISSING
NICOTINE %	0.0419	0.0414	0.0406
NORNICOTINE %	MISSING	MISSING	MISSING
MYOSMINE %	<0.0003	MISSING	<0.0003
ANABASINE %	MISSING	<0.0003	MISSING
ANATABINE %	MISSING	0.0002	0.0002
NICOTINE MG/G	0.42	0.41	0.40

Legacy Test Number Part	GN75963AA	GN75963AB	GN75963AC	GN75963AG	GN75963AH	GN75963AI
Legacy Part Name	NTP/TEST ART1 4	NTP/Test Art 1 40. M	NTP/Test Art 1 40. B	NTP/ NIC TAR 40.0 T	NTP/ NIC TAR 40.0 M	NTP/ NIC TAR 40.0 B
Print Code						
Test Tipping Id						
Test Filter Id						
Test Paper Id						
Test Blend Id						
Legacy Butt Length	0	0	0	0	0	0

Order	Legacy An:	Legacy Result Name	Legacy Result Avg	Legacy Result Avg	Legacy Result Avg	Legacy Result Avg	Legacy Result Avg	Legacy Result Avg
50,000	SPECIFIC 2ND TOTAL ALKALOIDS%	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING
50,000	SPECIFIC ANABASINE %	<0.0003	<0.0003	<0.0003	MISSING	<0.0003	MISSING	MISSING
50,000	SPECIFIC ANATABINE %	0.0010	0.0012	0.0011	MISSING	0.0002	0.0002	0.0002
50,000	SPECIFIC MYOSMINE %	<0.0003	<0.0003	<0.0003	<0.0003	MISSING	<0.0003	<0.0003
50,000	SPECIFIC NICOTINE %	0.0460	0.0472	0.0447	0.0419	0.0414	0.0406	0.0406
50,000	SPECIFIC NICOTINE MG/G	0.46	0.47	0.45	0.42	0.41	0.40	0.40
50,000	SPECIFIC NORNICOTINE %	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING
50,000	SPECIFIC TOTAL ALKALOIDS %	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING

gn75963		GC/MS Method mg/g	Expected mg/g	HVGC-Specific Alkaloid GC Method mg/g
Top AA	Tobacco Blend 40 dose	0.487	0.5	0.460
Mid AB		0.463	0.5	0.470
Bottom AC		0.406	0.5	0.450
Top AD	Tobacco Blend 0.2 dose	0.003	0.0025	bdl
Mid AE		0.003	0.0025	bdl
Bottom AF		0.003	0.0025	bdl
Top AG	Nicotine Tartrate 40 dose	0.413	0.5	0.420
Mid AH		0.424	0.5	0.410
Bottom AI		0.411	0.5	0.400
Top AJ	Nicotine Tartrate 2.0 dose	0.022	0.025	bdl
Mid AK		0.021	0.025	bdl
Bottom AL		0.020	0.025	bdl

Samples were prepared and analyzed in duplicate for both the GC/MS and HCGC Specific Alkaloid GC methods. The values reported are the average of the two replicates.

These data are from the
Trial Run Feed Formulation
in support of TOX209/TOX210.
They represent the test high
dose and the test low dose.
This data sheet compares the
GC/MS and GC/FID analytical
methods

Annotated by
JRM 02/05/09

TOX209 Trail Run Feed Formulation

Homogeneity Data

Tobacco Extract

SAMPLE SUBMISSION RECORD

Study Number: TOX209

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

Submitted by: C. Smith

Submitted on: 4-4-08

[illegible]

Comments:

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

Sample(s) Received By:

Received On:

TOX215.003.091406

Tobacco extract 40mg nic/kg bw homogeneity

Te ☐ Number: GN76002 Date Requested: 04/04/08 Date Completed: 04/11/08
Project Description: Feed Study Test

Status: COPIED Protocol: GENERAL Prog Code: 900 Related #:

Requestor Name: SMITH, JENNY L

Req. Phone: 741-0125

Req. Location: 630-2

Req. Room:

Backup Name: PIKE, SUSAN

Backup Phone: 741-0921

Laboratory Notes:

User: WAYNE SCOTT Date: 04/11/08 Time: 08:44:46

For samples AA through AC, monitor was accepted with anatabine std
dev out of limits, customer requested nicotine data only.

☐ Wayne Scott
0932

☐

Number: GN76002 Date Requested: 04/04/08 Date Completed: 04/11/08
Project Description: Feed Study Test

Status: COMPLETED Protocol: GENERAL Prog Code: 900 Related #:

Requestor Name: SMITH, JENNY L

Req. Phone: 741-0125

Req. Location: 630-2

Req. Room:

Backup Name: PIKE, SUSAN

Backup Phone: 741-0921

Laboratory Notes:

User: WAYNE SCOTT Date: 04/11/08 Time: 08:44:46

For samples AA through AC, monitor was accepted with anatabine std
dev out of limits, customer requested nicotine data only.

Wayne Scott

0932

04/04/08

TOX209 Final Report Adobe File Page 203 of 443

Testno: GN76002 Prog no: 900

Protocol: GENERAL

Needed: 04/07/08

Requester: SMITH, JENNY L

Phone: 741-0125

Description: Feed Study Test

R Number: TOX209

Part: GN76002AA Points: 3 Butt Len: 0 Part Name: NTP/ART2 40.0 mg T
Date: 20080404 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76002AB Points: 3 Butt Len: 0 Part Name: NTP/ART2 40.0 mg M
Date: 20080404 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76002AC Points: 3 Butt Len: 0 Part Name: NTP/ ART2 40.0mg B
Date: 20080404 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76002AD Points: 3 Butt Len: 0 Part Name: NTP2000 DIET
Date: 20080404 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76002AE Points: 1 Butt Len: 0 Part Name: NTP/ART2 0.2 mg T
Date: 20080404 Shift: Comments:

Part: GN76002AF Points: 1 Butt Len: 0 Part Name: NTP/ART2 0.2 mg M
Date: 20080404 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76002AG Points: 1 Butt Len: 0 Part Name: NTP/ART2 0.20 mg B
Date: 20080404 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Test Part
 T PART_NAME
 T BUTT_LENGTH
 GN DATE
 GN SHIFT
 GN COMMENTS
 GN TIME

GN 76002 AA	GN 76002 AB	GN 76002 AC
NTP/ART2 40.0 mg T	NTP/ART2 40.0 mg M	NTP/ART2 40.0mg B
0	0	0
20080404	20080404	20080404

SPECIFIC ALKALOIDS

	MISSING	<0.0517	MISSING
TOTAL ALKALOIDS %	MISSING	<0.0517	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	1.	0.
2ND TOTAL ALKALOIDS%	MISSING	<0.0053	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	1.	0.
NICOTINE %	0.0397	0.0434	0.0388
Std. Deviation	0.00401	0.00632	0.00165
Count	3.	3.	3.
NORNICOTINE %	<0.0035	<0.0035	MISSING
Std. Deviation	N/A	N/A	N/A
Count	1.	1.	0.
MYOSMINE %	<0.0003	<0.0003	<0.0003
Std. Deviation	0.00000	0.00000	0.00000
Count	3.	3.	3.
ANABASINE %	MISSING	<0.0003	<0.0003
Std. Deviation	N/A	N/A	N/A
Count	0.	1.	1.
ANABASINE %	0.0008	0.0010	0.0010
Std. Deviation	0.00006	0.00020	0.00020
Count	3.	3.	3.
NICOTINE MG/G	0.40	0.43	0.39
Std. Deviation	0.038	0.064	0.021
Count	3.	3.	3.

Dose 40 mg/kg/day
 Tobacco Extract
 Top

Dose 40 mg/kg/day
 Tobacco Extract
 Middle

Dose 40 mg/kg/day
 Tobacco Extract
 Bottom

Test Part	GN 76002 AA	GN 76002 AB	GN 76002 AC
T PART_NAME	NTP/ART2 40.0 mg T	NTP/ART2 40.0 mg M	NTP/ART2 40.0mg B
T BUTT_LENGTH	0	0	0
GN_DATE	20080404	20080404	20080404
GN_SHIFT			
GN_COMMENTS			
GN_TIME			

SPECIFIC ALKALOIDS

	GN 76002 AA	GN 76002 AB	GN 76002 AC
TOTAL ALKALOIDS %			
Averages Point 1	MISSING	<0.0517	MISSING
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	1.	0.
2ND TOTAL ALKALOIDS%			
Averages Point 1	MISSING	<0.0053	MISSING
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	1.	0.
NICOTINE %			
Averages Point 1	0.0397	0.0434	0.0388
Std. Dev. Point 1	0.00401	0.00632	0.00165
Count Point 1	3.	3.	3.
NORNICOTINE %			
Averages Point 1	<0.0035	<0.0035	MISSING
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	1.	1.	0.
MYOSMINE %			
Averages Point 1	<0.0003	<0.0003	<0.0003
Std. Dev. Point 1	0.00000	0.00000	0.00000
Count Point 1	3.	3.	3.
ANABASINE %			
Averages Point 1	MISSING	<0.0003	<0.0003
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	1.	1.
ANATABINE %			
Averages Point 1	0.0008	0.0010	0.0010
Std. Dev. Point 1	0.00006	0.00020	0.00020
Count Point 1	3.	3.	3.
NICOTINE MG/G			
Averages Point 1	0.40	0.43	0.39
Std. Dev. Point 1	0.038	0.064	0.021
Count Point 1	3.	3.	3.

Test Part	GN 76002 AA	GN 76002 AB	GN 76002 AC
T PART_NAME	NTP/ART2 40.0 mg T	NTP/ART2 40.0 mg M	NTP/ART2 40.0mg B
T BUTT_LENGTH	0	0	0
GN_DATE	20080404	20080404	20080404
GN_SHIFT			
GN_COMMENTS			
GN_TIME			

SPECIFIC ALKALOIDS

TOTAL ALKALOIDS %

Point 1 Rep 1	MISSING	<0.0517	MISSING
Point 1 Rep 2	MISSING	MISSING	MISSING
Point 1 Rep 3	MISSING	MISSING	MISSING

2ND TOTAL ALKALOIDS%

Point 1 Rep 1	MISSING	<0.0053	MISSING
Point 1 Rep 2	MISSING	MISSING	MISSING
Point 1 Rep 3	MISSING	MISSING	MISSING

NICOTINE %

Point 1 Rep 1	0.0443	0.0464	0.0374
Point 1 Rep 2	0.0381	0.0476	0.0383
Point 1 Rep 3	0.0368	0.0361	0.0406

NORNICOTINE %

Point 1 Rep 1	<0.0035	<0.0035	MISSING
Point 1 Rep 2	MISSING	MISSING	MISSING
Point 1 Rep 3	MISSING	MISSING	MISSING

MYOSMINE %

Point 1 Rep 1	<0.0003	<0.0003	<0.0003
Point 1 Rep 2	<0.0003	<0.0003	<0.0003
Point 1 Rep 3	<0.0003	<0.0003	<0.0003

ANABASINE %

Point 1 Rep 1	MISSING	<0.0003	<0.0003
Point 1 Rep 2	MISSING	MISSING	MISSING
Point 1 Rep 3	MISSING	MISSING	MISSING

ANATABINE %

Point 1 Rep 1	0.0008	0.0012	0.0008
Point 1 Rep 2	0.0007	0.0010	0.0010
Point 1 Rep 3	0.0008	0.0008	0.0012

NICOTINE MG/G

Point 1 Rep 1	0.44	0.46	0.37
Point 1 Rep 2	0.38	0.48	0.38
Point 1 Rep 3	0.37	0.36	0.41

10K209

PART SUMMARY RESULTS

4/11/2008 10:03:48 AM

Test Part	GN 76002 AA	GN 76002 AB	GN 76002 AC
T PART_NAME	NTP/ART2 40.0 mg T	NTP/ART2 40.0 mg M	NTP/ART2 40.0mg B
T BUTT_LENGTH	0	0	0
GN_DATE	20080404	20080404	20080404
GN_SHIFT			
GN_COMMENTS			
GN_TIME			

SPECIFIC ALKALOIDS

	MISSING	<0.0517	MISSING
TOTAL ALKALOIDS %	MISSING	<0.0517	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	1.	0.
2ND TOTAL ALKALOIDS%	MISSING	<0.0053	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	1.	0.
NICOTINE %	0.0397	0.0434	0.0388
Std. Deviation	0.00401	0.00632	0.00165
Count	3.	3.	3.
NORNICOTINE %	<0.0035	<0.0035	MISSING
Std. Deviation	N/A	N/A	N/A
Count	1.	1.	0.
MYOSMINE %	<0.0003	<0.0003	<0.0003
Std. Deviation	0.00000	0.00000	0.00000
Count	3.	3.	3.
ANABASINE %	MISSING	<0.0003	<0.0003
Std. Deviation	N/A	N/A	N/A
Count	0.	1.	1.
ANATABINE %	0.0008	0.0010	0.0010
Std. Deviation	0.00006	0.00020	0.00020
Count	3.	3.	3.
NICOTINE MG/G	0.40	0.43	0.39
Std. Deviation	0.038	0.064	0.021
Count	3.	3.	3.

Test Part	GN 76002 AA	GN 76002 AB	GN 76002 AC
TEST PART_NAME	NTP/ART2 40.0 mg T	NTP/ART2 40.0 mg M	NTP/ART2 40.0mg B
TEST BUTT_LENGTH	0	0	0
GN DATE	20080404	20080404	20080404
GN_SHIFT			
GN_COMMENTS			
GN_TIME			

SPECIFIC ALKALOIDS

TOTAL ALKALOIDS %	MISSING	<0.0517	MISSING
2ND TOTAL ALKALOIDS%	MISSING	<0.0053	MISSING
NICOTINE %	0.0397	0.0434	0.0388
NORNICOTINE %	<0.0035	<0.0035	MISSING
MYOSMINE %	<0.0003	<0.0003	<0.0003
ANABASINE %	MISSING	<0.0003	<0.0003
ANATABINE %	0.0008	0.0010	0.0010
NICOTINE MG/G	0.40	0.43	0.39

extract
Tobacco ~~blend~~
40 mg nic/kg bw
Top

Tobacco extract
40 mg nic/kg bw
middle

Tobacco extract
40 mg nic/kg bw
bottom

TOX209 Trail Run Feed Formulation

Homogeneity Data

Nicotine Hydrogen Tartrate

SAMPLE SUBMISSION RECORD

Study Number: TOX209

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

Submitted by: Geray SmithSubmitted on: 4-3-08

Sample Identification (GN number)	Analysis	Sample Identification (GN number)	Analysis
GN75963AA	46	GN75963AL	46
GN75963AB	46		
GN75963AC	46		
GN75963AD	46		
GN75963AE	46		
GN75963AF	46		
GN75963AG	46		
GN75963AH	46		
GN75963AI	46		
GN75963AJ	46		
GN75963AK	46		

Comments:

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

Sample(s) Received By: [Signature]Received On: 4-3-08

TOX215.003.091406

Number: GN75963 Date Requested: 04/03/08 Date Completed: 04/11/08

Project Description: Feed Study

Status: COMPLETED Protocol: GENERAL Prog Code: 900 Related #:

Requestor Name: SMITH, JENNY L

Req. Phone: 741-0125

Req. Location: 630-2

Req. Room:

Backup Name: PIKE, SUSAN

Backup Phone: 741-0921

Laboratory Notes:

User: WAYNE SCOTT Date: 04/11/08 Time: 08:44:09

For samples AA through AI, monitor was accepted with anatabine std
dev out of limits, customer requested nicotine data only.

Wayne Scott

0932

05/01/08

TRIAL Run

TOX209 Final Report Adobe File Page 212 of 443

Testno: GN75963 Prog no: 900

Protocol: GENERAL

Needed: 04/04/08

Requester: SMITH, JENNY L

Phone: 741-0125

Description: Feed Study

Number: TOX209

Part: GN75963AA Points: 1 Butt Len: 0 Part Name: NTP/TEST ART1 40.0 T
Date: 20080403 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN75963AB Points: 1 Butt Len: 0 Part Name: NTP/Test Art 1 40.0 M
Date: 20080403 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN75963AC Points: 1 Butt Len: 0 Part Name: NTP/Test Art 1 40.0 B
Date: 20080403 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN75963AG Points: 1 Butt Len: 0 Part Name: NTP/ NIC TAR 40.0 T
Date: 20080403 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN75963AH Points: 1 Butt Len: 0 Part Name: NTP/ NIC TAR 40.0 M
Date: 20080403 Shift: Comments:

42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN75963AI Points: 1 Butt Len: 0 Part Name: NTP/ NIC TAR 40.0 B
Date: 20080403 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Number: GN75963 Date Requested: 04/03/08 Date Completed: 04/11/08

Project Description: Feed Study

Status: COMPLETED Protocol: GENERAL Prog Code: 900 Related #:

Requestor Name: SMITH, JENNY L

Req. Phone: 741-0125

Req. Location: 630-2

Req. Room:

Backup Name: PIKE, SUSAN

Backup Phone: 741-0921

Laboratory Notes:

User: WAYNE SCOTT Date: 04/11/08 Time: 08:44:09

For samples AA through AI, monitor was accepted with anatabine std
dev out of limits, customer requested nicotine data only.

Wayne Scott
0932

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POINT SUMMARY RESULTS

4/30/2008 8:43:42 AM

Test Part	GN 75963 AG	GN 75963 AH	GN 75963 AI
T PART NAME	NTP/ NIC TAR 40.0 T	NTP/ NIC TAR 40.0 M	NTP/ NIC TAR 40.0 B
TEST BUTT LENGTH	0	0	0
GN DATE	20080403	20080403	20080403
GN SHIFT			
GN COMMENTS			
GN TIME			

SPECIFIC ALKALOIDS

TOTAL ALKALOIDS %

	MISSING	MISSING	MISSING
Averages Point 1			
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	0.	0.

2ND TOTAL ALKALOIDS%

	MISSING	MISSING	MISSING
Averages Point 1			
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	0.	0.

NICOTINE %

	0.0419	0.0414	0.0406
Averages Point 1			
Std. Dev. Point 1	0.00145	0.00100	0.00071
Count Point 1	3.	3.	3.

NORNICOTINE %

	MISSING	MISSING	MISSING
Averages Point 1			
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	0.	0.

MYOSMINE %

	<0.0003	MISSING	<0.0003
Averages Point 1			
Std. Dev. Point 1	0.00000	N/A	0.00000
Count Point 1	2.	0.	2.

ANABASINE %

	MISSING	<0.0003	MISSING
Averages Point 1			
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	1.	0.

ANATABINE %

	MISSING	0.0002	0.0002
Averages Point 1			
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	1.	1.

NICOTINE MG/G

	0.42	0.41	0.40
Averages Point 1			
Std. Dev. Point 1	0.017	0.006	0.006
Count Point 1	3.	3.	3.

Test Part	GN 75963 AG	GN 75963 AH	GN 75963 AI
PART_NAME	NTP/ NIC TAR 40.0 T	NTP/ NIC TAR 40.0 M	NTP/ NIC TAR 40.0 B
T_BUTT_LENGTH	0	0	0
GN_DATE	20080403	20080403	20080403
GN_SHIFT			
GN_COMMENTS			
GN_TIME			

SPECIFIC ALKALOIDS

	MISSING	MISSING	MISSING
TOTAL ALKALOIDS %	N/A	N/A	N/A
Std. Deviation	0.	0.	0.
Count	MISSING	MISSING	MISSING
2ND TOTAL ALKALOIDS%	N/A	N/A	N/A
Std. Deviation	0.	0.	0.
Count	0.0419	0.0414	0.0406
NICOTINE %	0.00145	0.00100	0.00071
Std. Deviation	3.	3.	3.
Count	MISSING	MISSING	MISSING
NORNICOTINE %	N/A	N/A	N/A
Std. Deviation	0.	0.	0.
Count	<0.0003	MISSING	<0.0003
MYOSMINE %	0.00000	N/A	0.00000
Std. Deviation	2.	0.	2.
Count	MISSING	<0.0003	MISSING
ANABASINE %	N/A	N/A	N/A
Std. Deviation	0.	1.	0.
Count	MISSING	0.0002	0.0002
ANATABINE %	N/A	N/A	N/A
Std. Deviation	0.	1.	1.
Count	0.42	0.41	0.40
NICOTINE MG/G	0.017	0.006	0.006
Std. Deviation	3.	3.	3.
Count			

0.41

Tartar

Test Part	GN 75963 AG	GN 75963 AH	GN 75963 AI
T PART_NAME	NTP/ NIC TAR 40.0 T	NTP/ NIC TAR 40.0 M	NTP/ NIC TAR 40.0 B
T BUTT_LENGTH	0	0	0
DATE	20080403	20080403	20080403
GN_SHIFT			
GN_COMMENTS			
GN_TIME			

SPECIFIC ALKALOIDS

TOTAL ALKALOIDS %

Averages Point 1	MISSING	MISSING	MISSING
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	0.	0.

2ND TOTAL ALKALOIDS%

Averages Point 1	MISSING	MISSING	MISSING
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	0.	0.

NICOTINE %

Averages Point 1	0.0419	0.0414	0.0406
Std. Dev. Point 1	0.00145	0.00100	0.00071
Count Point 1	3.	3.	3.

NORNICOTINE %

Averages Point 1	MISSING	MISSING	MISSING
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	0.	0.

MYOSMINE %

Averages Point 1	<0.0003	MISSING	<0.0003
Std. Dev. Point 1	0.00000	N/A	0.00000
Count Point 1	2.	0.	2.

ANABASINE %

Averages Point 1	MISSING	<0.0003	MISSING
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	1.	0.

ANATABINE %

Averages Point 1	MISSING	0.0002	0.0002
Std. Dev. Point 1	N/A	N/A	N/A
Count Point 1	0.	1.	1.

NICOTINE MG/G

Averages Point 1	0.42	0.41	0.40
Std. Dev. Point 1	0.017	0.006	0.006
Count Point 1	3.	3.	3.

Test Part	GN 75963 AG	GN 75963 AH	GN 75963 AI
T PART_NAME	NTP/ NIC TAR 40.0 T	NTP/ NIC TAR 40.0 M	NTP/ NIC TAR 40.0 B
T BUTT_LENGTH	0	0	0
DATE	20080403	20080403	20080403
GN_SHIFT			
GN_COMMENTS			
GN_TIME			

SPECIFIC ALKALOIDS

TOTAL ALKALOIDS %

	GN 75963 AG	GN 75963 AH	GN 75963 AI
Point 1 Rep 1	MISSING	MISSING	MISSING
Point 1 Rep 2	MISSING	MISSING	MISSING
Point 1 Rep 3	MISSING	MISSING	MISSING

2ND TOTAL ALKALOIDS%

	GN 75963 AG	GN 75963 AH	GN 75963 AI
Point 1 Rep 1	MISSING	MISSING	MISSING
Point 1 Rep 2	MISSING	MISSING	MISSING
Point 1 Rep 3	MISSING	MISSING	MISSING

NICOTINE %

	GN 75963 AG	GN 75963 AH	GN 75963 AI
Point 1 Rep 1	0.0428	0.0410	0.0405
Point 1 Rep 2	0.0402	0.0406	0.0400
Point 1 Rep 3	0.0426	0.0425	0.0414

NORNICOTINE %

	GN 75963 AG	GN 75963 AH	GN 75963 AI
Point 1 Rep 1	MISSING	MISSING	MISSING
Point 1 Rep 2	MISSING	MISSING	MISSING
Point 1 Rep 3	MISSING	MISSING	MISSING

MYOSMINE %

	GN 75963 AG	GN 75963 AH	GN 75963 AI
Point 1 Rep 1	MISSING	MISSING	<0.0003
Point 1 Rep 2	<0.0003	MISSING	<0.0003
Point 1 Rep 3	<0.0003	MISSING	MISSING

ANABASINE %

	GN 75963 AG	GN 75963 AH	GN 75963 AI
Point 1 Rep 1	MISSING	MISSING	MISSING
Point 1 Rep 2	MISSING	<0.0003	MISSING
Point 1 Rep 3	MISSING	MISSING	MISSING

ANATABINE %

	GN 75963 AG	GN 75963 AH	GN 75963 AI
Point 1 Rep 1	MISSING	0.0002	MISSING
Point 1 Rep 2	MISSING	MISSING	MISSING
Point 1 Rep 3	MISSING	MISSING	0.0002

NICOTINE MG/G

	GN 75963 AG	GN 75963 AH	GN 75963 AI
Point 1 Rep 1	0.43	0.41	0.40
Point 1 Rep 2	0.40	0.41	0.40
Point 1 Rep 3	0.43	0.42	0.41

Test Part	GN 75963 AG	GN 75963 AH	GN 75963 AI
TEST PART_NAME	NTP/ NIC TAR 40.0 T	NTP/ NIC TAR 40.0 M	NTP/ NIC TAR 40.0 B
TEST BUTT_LENGTH	0	0	0
GN_DATE	20080403	20080403	20080403
GN_SHIFT			
GN_COMMENTS			
GN_TIME			

SPECIFIC ALKALOIDS			
TOTAL ALKALOIDS %	MISSING	MISSING	MISSING
2ND TOTAL ALKALOIDS%	MISSING	MISSING	MISSING
NICOTINE %	0.0419	0.0414	0.0406
NORNICOTINE %	MISSING	MISSING	MISSING
MYOSMINE %	<0.0003	MISSING	<0.0003
ANABASINE %	MISSING	<0.0003	MISSING
ANATABINE %	MISSING	0.0002	0.0002
NICOTINE MG/G	0.42	0.41	0.40

Legacy Test Number Part GN75963AA GN75963AB GN75963AC GN75963AG GN75963AH GN75963AI
 Legacy Part Name NTP/TEST ART1 4 NTP/Test Art 1 40. M NTP/Test Art 1 40. B NTP/ NIC TAR 40.0 T NTP/ NIC TAR 40.0 M NTP/ NIC TAR 40.0 B

Print Code
 Test Tipping Id
 Test Filter Id
 Test Paper Id
 Test Blend Id

Order	Legacy An:	Legacy Result Name	Legacy Result Avg	Legacy Result Avg	Legacy Result Avg	Legacy Result Avg	Legacy Result Avg	Legacy Result Avg
			0	0	0	0	0	0
50,000	SPECIFIC 2ND TOTAL ALKALOIDS%	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING
50,000	SPECIFIC ANABASINE %	<0.0003	<0.0003	<0.0003	MISSING	<0.0003	MISSING	MISSING
50,000	SPECIFIC ANATABINE %	0.0010	0.0012	0.0011	MISSING	0.0002	0.0002	0.0002
50,000	SPECIFIC MYOSMINE %	<0.0003	<0.0003	<0.0003	<0.0003	MISSING	<0.0003	<0.0003
50,000	SPECIFIC NICOTINE %	0.0460	0.0472	0.0447	0.0419	0.0414	0.0406	0.0406
50,000	SPECIFIC NICOTINE MG/G	0.46	0.47	0.45	0.42	0.41	0.40	0.40
50,000	SPECIFIC NORNICOTINE %	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING
50,000	SPECIFIC TOTAL ALKALOIDS %	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING

gn75963		GC/MS Method mg/g	Expected mg/g	HVGC-Specific Alkaloid GC Method mg/g
Top AA	Tobacco Blend 40 dose	0.487	0.5	0.460
Mid AB		0.463	0.5	0.470
Bottom AC		0.406	0.5	0.450
Top AD	Tobacco Blend 0.2 dose	0.003	0.0025	bdl
Mid AE		0.003	0.0025	bdl
Bottom AF		0.003	0.0025	bdl
Top AG	Nicotine Tartrate 40 dose	0.413	0.5	0.420
Mid AH		0.424	0.5	0.410
Bottom AI		0.411	0.5	0.400
Top AJ	Nicotine Tartrate 2.0 dose	0.022	0.025	bdl
Mid AK		0.021	0.025	bdl
Bottom AL		0.020	0.025	bdl

Samples were prepared and analyzed in duplicate for both the GC/MS and HCGC Specific Alkaloid GC methods. The values reported are the average of the two replicates.

These data are from the
Trial Run Feed Formulation
in support of TOX209/TOX210.
They represent the test high
dose and the test low dose.
This data sheet compares the
GC/MS and GC/FID analytical
methods

Annotated by
JRH 02/05/09

TOX209 Series 1 Feed Formulation Analytical Data

TOX209 Series 1 Feed Formulation

40 mg nicotine/kg body weight/day

Homogeneity Data

SAMPLE SUBMISSION RECORD

Study Number: TOX210209^①

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

Submitted by: G. SmithSubmitted on: 4-18-08

Sample Identification (GN number)	Analysis	Sample Identification (GN number)	Analysis
GN76363 AA	46		
GN76363 AB	46		
GN76363 AC	46		
GN76363 AD	46		
GN76363 AE	46		
GN76363 AF	46		
GN76363 AG	46		
GN76363 AH	46		
GN76363 AI	46		

Comments: ① Entry Error GS 4-18-08

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

Sample(s) Received By: Sue BurtReceived On: 4-18-08

TOX215.004 041808

04/18/08

10:31:18

TOX209 Final Report Adobe File Page 224 of 443

Testno: GN76363 Prog no: 900

Protocol: GENERAL

Needed: //

Requester: SMITH, JENNY L

Phone: 741-0125

Description: TOX209

Lab Number: TOX209

Lab Instructions:

Please run duplicates

Part: GN76363AA Points: 1 Butt Len: 0 Part Name: TOB BLEN 40.0 top
Date: 20080418 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76363AB Points: 1 Butt Len: 0 Part Name: TOB BLEN 40.0 mid
Date: 20080418 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76363AC Points: 1 Butt Len: 0 Part Name: TOB BLEN 40.0 bot
Date: 20080418 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76363AD Points: 1 Butt Len: 0 Part Name: TOB EXT 40.0 top
Date: 20080418 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76363AE Points: 1 Butt Len: 0 Part Name: TOB EXT 40.0 mid
Date: 20080418 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76363AF Points: 1 Butt Len: 0 Part Name: TOB EXT 40.0 bot
Date: 20080418 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76363AG Points: 1 Butt Len: 0 Part Name: NIC TAR 40.0 top
Date: 20080418 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76363AH Points: 1 Butt Len: 0 Part Name: NIC TAR 40.0 mid
Date: 20080418 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76363AI Points: 1 Butt Len: 0 Part Name: NIC TAR 40.0 bot
Date: 20080418 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

TO Number: GN76363 Date Requested: 04/18/08 Date Completed: 04/23/08

Project Description: TOX209

Status: COMPLETED Protocol: GENERAL Prog Code: 900 Related #:

Requestor Name: SMITH, JENNY L

Req. Phone: 741-0125

Req. Location: 630-2

Req. Room:

Backup Name: PIKE, SUSAN

Backup Phone: 741-0921

Lab Instructions:

Please run duplicates

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PART SUMMARY RESULTS

4/30/2008 9:41:44 AM

Test Part	GN 76363 AA	GN 76363 AB	GN 76363 AC
TEST PART_NAME	TOB BLEN 40.0 top	TOB BLEN 40.0 mid	TOB BLEN 40.0 bot
TEST BUTT_LENGTH	0	0	0
GN_DATE	20080418	20080418	20080418
GN_SHIFT			
GN_COMMENTS			
GN_TIME			

SPECIFIC ALKALOIDS

	MISSING	MISSING	MISSING
TOTAL ALKALOIDS %	MISSING	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	0.
2ND TOTAL ALKALOIDS%	MISSING	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	0.
NICOTINE %	0.0385	0.0355	0.0392
Std. Deviation	0.00198	0.00156	0.00021
Count	2.	2.	2.
NORNICOTINE %	<0.0035	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	1.	0.	0.
MYOSMINE %	MISSING	MISSING	<0.0003
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	1.
ANABASINE %	MISSING	<0.0003	<0.0003
Std. Deviation	N/A	N/A	N/A
Count	0.	1.	1.
ANATABINE %	0.0009	0.0007	0.0010
Std. Deviation	0.00021	0.00000	0.00000
Count	2.	2.	2.
NICOTINE MG/G	0.39	0.36	0.39
Std. Deviation	0.021	0.021	0.000
Count	2.	2.	2.

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PART SUMMARY RESULTS

4/30/2008 9:41:44 AM

Test Part	GN 76363 AD	GN 76363 AE	GN 76363 AF
TEST PART NAME	TOB EXT 40.0 top	TOB EXT 40.0 mid	TOB EXT 40.0 bot
TEST BUTT LENGTH	0	0	0
GN DATE	20080418	20080418	20080418
GN SHIFT			
GN COMMENTS			
GN TIME			

SPECIFIC ALKALOIDS

	MISSING	MISSING	MISSING
TOTAL ALKALOIDS %	MISSING	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	0.
2ND TOTAL ALKALOIDS%	MISSING	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	0.
NICOTINE %	0.0485	0.0329*	0.0363*
Std. Deviation	0.00523	0.00460	0.00948
Count	2.	2.	2.
NORNICOTINE %	MISSING	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	0.
MYOSMINE %	<0.0003	MISSING	MISSING
Std. Deviation	0.00000	N/A	N/A
Count	2.	0.	0.
ANABASINE %	<0.0003	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	1.	0.	0.
ANATABINE %	0.0013	0.0009	0.0007
Std. Deviation	0.00035	0.00021	0.00021
Count	2.	2.	2.
NICOTINE MG/G	0.49	0.33*	0.37*
Std. Deviation	0.049	0.042	0.092
Count	2.	2.	2.

Page 4

PART SUMMARY RESULTS

4/30/2008 9:41:44 AM

Test Part	GN 76363 AG	GN 76363 AH	GN 76363 AI
T PART_NAME	NIC TAR 40.0 top	NIC TAR 40.0 mid	NIC TAR 40.0 bot
TEST_BUTT_LENGTH	0	0	0
GN_DATE	20080418	20080418	20080418
GN_SHIFT			
GN_COMMENTS			
GN_TIME			

SPECIFIC ALKALOIDS

	MISSING	MISSING	MISSING
TOTAL ALKALOIDS %	MISSING	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	0.
2ND TOTAL ALKALOIDS%	MISSING	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	0.
NICOTINE %	0.0353	0.0352	0.0354
Std. Deviation	0.00028	0.00014	0.00156
Count	2.	2.	2.
NORNICOTINE %	MISSING	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	0.
MYOSMINE %	MISSING	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	0.
ANABASINE %	MISSING	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	0.
ANATABINE %	MISSING	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	0.
NICOTINE MG/G	0.35	0.35	0.35
Std. Deviation	0.000	0.000	0.014
Count	2.	2.	2.

* Average may not be meaningful. See Individual Replicate Values.

TOX209 Series 1 Feed Formulation

Dose Confirmation Data

SAMPLE SUBMISSION RECORD

Study Number: TOX209 Series 1

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

Submitted by: G. SmithSubmitted on: 6-12-08

Sample Identification (GN number)	Analysis	Sample Identification (GN number)	Analysis
GN77615AAB	46	GN77615AL	46
GN77615AB	46	GN77615AM	46
GN77615AC	46	GN77615AO	46
GN77615AD	46	GN77615AP	46
GN77615AE	46	GN77615AQ	46
GN77615AF	46		
GN77615AG	46		
GN77615AH	.01 to 46		
GN77615AI	0.05 46		
GN77615AJ	46		
GN77615AK	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

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

TOX209 Series 1 Feed Formulation Analytical Data

GC/MS Methodology

Homogeneity and Dose Confirmation

SAMPLE SUBMISSION RECORD

Study Number: TOX209

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

Submitted by: Gerry SmithSubmitted on: 4-16-08

Sample Identification (GN number)	Analysis	Sample Identification (GN number)	Analysis
GN76269 AA	46	GN76269 AL	46
GN76269 AB	46	GN76269 AM	46
GN76269 AC	46	GN76269 AN	46
GN76269 AD	46	GN76269 AO	46
GN76269 AE	46	GN76269 AP	46
GN76269 AF	46	GN76269 AQ	46
GN76269 AG	46	GN76269 AR	46
GN76269 AH	46	GN76269 AS	46
GN76269 AI	46	GN76269 AT	46
GN76269 AJ	46	GN76269 AU	46
GN76269 AK	46	GN76269 AV	46

Comments: Entry Error g8 4-16-08

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

Sample(s) Received By: Gerry BowmanReceived On: 4/16/08

TOX215.003.091406

Submitted on: 4-16-08

Comments:

Sample(s) Received By:

Received On:

04/15/08

15:33:13

TOX209 Final Report Adobe File Page 235 of 443

Testno: GN76269 Prog no: 900 Protocol: GENERAL Needed: 04/16/08
Requester: SMITH, JENNY L Phone: 741-0125
Description: Tobacco blend
PDR Number: TOX209A
Lab Instructions:
Please run duplicate

Part: GN76269AA Points: 1 Butt Len: 0 Part Name: TOB BLEND 0.2mg TOP
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76269AB Points: 1 Butt Len: 0 Part Name: TOB BLEND 0.2 MID
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76269AC Points: 1 Butt Len: 0 Part Name: TOB BLEND 0.2mg BOT
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76269AD Points: 1 Butt Len: 0 Part Name: TOB BLEND 40.0mg TOP
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76269AE Points: 1 Butt Len: 0 Part Name: TOB BLEND 40.0mg MID
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76269AF Points: 1 Butt Len: 0 Part Name: TOB BLEND 40.0mg BOT
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76269AG Points: 1 Butt Len: 0 Part Name: TOB BLEND 2.0mg
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76269AH Points: 1 Butt Len: 0 Part Name: TOB BLEND 4.0mg
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76269AI Points: 1 Butt Len: 0 Part Name: TOB BLEND 8.0mg
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76269AJ Points: 1 Butt Len: 0 Part Name: TOB BLEND 20.0mg
Date: 20080415 Shift: Comments:

04/15/08

15:33:13

TOX209 Final Report Adobe File Page 236 of 443

46(42)	SPECIFIC ALKALOIDS		CHEMICAL
Part: GN76269AK	Points: 1	Butt Len: 0	Part Name: TOB EXTRACT 0.20 TOP
Date: 20080415	Shift:	Comments:	
46(42)	SPECIFIC ALKALOIDS		CHEMICAL
Part: GN76269AL	Points: 1	Butt Len: 0	Part Name: TOB EXTRACT 0.20 MID
Date: 20080415	Shift:	Comments:	
46(42)	SPECIFIC ALKALOIDS		CHEMICAL
Part: GN76269AM	Points: 1	Butt Len: 0	Part Name: TOB EXTRACT 0.20 BOT
Date: 20080415	Shift:	Comments:	
46(42)	SPECIFIC ALKALOIDS		CHEMICAL
Part: GN76269AN	Points: 1	Butt Len: 0	Part Name: NIC TAR 2.0 TOP
Date: 20080415	Shift:	Comments:	
46(42)	SPECIFIC ALKALOIDS		CHEMICAL
Part: GN76269AO	Points: 1	Butt Len: 0	Part Name: NIC TAR 2.0 MID
Date: 20080415	Shift:	Comments:	
46(42)	SPECIFIC ALKALOIDS		CHEMICAL
Part: GN76269AP	Points: 1	Butt Len: 0	Part Name: NIC TAR 2.0 BOT
Date: 20080415	Shift:	Comments:	
46(42)	SPECIFIC ALKALOIDS		CHEMICAL
Part: GN76269AQ	Points: 1	Butt Len: 0	Part Name: NIC EXTRACT 40.0 TOP
Date: 20080415	Shift:	Comments:	
46(42)	SPECIFIC ALKALOIDS		CHEMICAL
Part: GN76269AR	Points: 1	Butt Len: 0	Part Name: NIC EXTRACT 40.0 MID
Date: 20080415	Shift:	Comments:	
46(42)	SPECIFIC ALKALOIDS		CHEMICAL
Part: GN76269AS	Points: 1	Butt Len: 0	Part Name: NIC EXTRACT 40.0 BOT
Date: 20080415	Shift:	Comments:	
46(42)	SPECIFIC ALKALOIDS		CHEMICAL
Part: GN76269AT	Points: 1	Butt Len: 0	Part Name: NIC TAR 40.0 TOP
Date: 20080415	Shift:	Comments:	
46(42)	SPECIFIC ALKALOIDS		CHEMICAL
Part: GN76269AU	Points: 1	Butt Len: 0	Part Name: NIC TAR 40.0 MID

04/15/08

LIMS TESTSAMPLE COVERSHEET

Page: 3

15:33:13

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Date: 20080415 Shift: Comments:

(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76269AV Points: 1 Butt Len: 0 Part Name: NIC TAR 40.0 BOT
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76269AX Points: 1 Butt Len: 0 Part Name: NIC TAR 20.0
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76269AY Points: 1 Butt Len: 0 Part Name: TOBACCO EXTRACT 2.0
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76269AZ Points: 1 Butt Len: 0 Part Name: TOBACCO EXTRACT 4.0
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76269BA Points: 1 Butt Len: 0 Part Name: TOBACCO EXTRACT 8.0
Date: 20080415 Shift: Comments:

(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76269BB Points: 1 Butt Len: 0 Part Name: TOBACCO EXTRACT 20.0
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76269BC Points: 1 Butt Len: 0 Part Name: NTP-2000
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76269BE Points: 1 Butt Len: 0 Part Name: NIC TAR 8.0MG
Date: 20080415 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

TOX 209 RAT FEEDING STUDY

TOX 209 RAT F

Rep 1 Nicotine (mg/g) Rep 2 Nicotine (mg/g) Average

Sample #	Test Article	Dose	Sample #			
GN76269AA	Tobacco Blend	0.2	GN76269AA	0.00124	0.0015	0.00137
GN76269AB	Tobacco Blend	0.2	GN76269AB	<0.0001	0.0011	0.00108
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	0.01463	0.0184	0.0152
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 STUDY TOX 210 MOUSE FEEDING STUDY

Sample #	Test Article	Dose	Sample #			
GN76276AC	Tobacco Blend	0.2	GN76276AC	0.00115	0.0011	0.00115
GN76276AB	Tobacco Blend	0.2	GN76276AB	0.00209	0.0011	0.00209
GN76276AA	Tobacco Blend	0.2	GN76276AA	0.00111	*	0.00111
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
GN76276AE	Tobacco Extract	0.2	GN76276AE	0.00093	*	0.00093
GN76276AF	Tobacco Extract	0.2	GN76276AF	0.00126	*	0.00126
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
GN76276AH	Nicotine Tartrate	2.0	GN76276AH	0.01002	0.0114	0.01070
GN76276AI	Nicotine Tartrate	2.0	GN76276AI	0.01057	0.0094	0.00999
GN76276AJ	Nicotine Tartrate	40.0	GN76276AJ	analyzed by High Volume GC area		
GN76276AK	Nicotine Tartrate	40.0	GN76276AK			
GN76276AL	Nicotine Tartrate	40.0	GN76276AL			

* not average- rep 1 only- see note below

* not average- rep 1 only- see note below

* not average- rep 1 only- see note below

* not average- rep 1 only- see note below

* not average- rep 1 only- see note below

* not average- rep 1 only- see note below

TOX 209 RAT FEEDING STUDY

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 STUDY TOX 210 MOUSE FEEDING STUDY

Sample #	Test Article	Dose	Sample #			
	NTP-2000 Diet	0				
GN76276AS	Tobacco Blend	2.0	GN76276AS	0.00864	0.0097	0.00864

* not average- rep 1 only- see note below

GN76276AT	Tobacco Blend	4.0	GN76276AT
GN76276AU	Tobacco Blend	8.0	GN76276AU
GN76276BC	Tobacco Blend	20.0	GN76276BC
GN76276AV	Tobacco Extract	2.0	GN76276AV
GN76276AW	Tobacco Extract	4.0	GN76276AW
GN76276AX	Tobacco Extract	8.0	GN76276AX
GN76276AY	Tobacco Extract	20.0	GN76276AY
GN76276BA	Nicotine Tartrate	8.0	GN76276BA
GN76276BB	Nicotine Tartrate	20.0	GN76276BB

0.01348
0.03199
0.10260
0.00220
0.01106
0.03376
0.08161
0.03114
0.08844

0.0222
0.0397
0.0824
0.0029
0.0161
0.0267
0.0527
0.0349
0.0885

0.01348
0.03199
0.10260
0.00220
0.01106
0.03376
0.08161
0.03114
0.08844

* not average- rep 1 only- see note below
* not average- rep 1 only- see note below
* not average- rep 1 only- see note below
* not average- rep 1 only- see note below
* not average- rep 1 only- see note below
* not average- rep 1 only- see note below
* not average- rep 1 only- see note below
* not average- rep 1 only- see note below
* not average- rep 1 only- see note below

PLUS

TOX 209 RAT FEEDING STUDY

Sample #	Test Article	Dose	Sample #
GN76276BD	Tobacco Extract	40.0	GN76276BD
GN76276BE	Tobacco Extract	0.2	GN76276BE
GN76276BF	Nicotine Tartrate	40.0	GN76276BF
GN76276BG	Nicotine Tartrate	2.0	GN76276BG
GN76276BH	Tobacco Blend	40.0	GN76276BH
GN76276BI	Tobacco Blend	0.2	GN76276BI

0.46699
0.00160
0.37349
0.02017
0.39827
0.00594

0.3951
0.0021
0.3828
0.0179
0.3805
0.0018

0.46699
0.00160
0.37349
0.02017
0.39827
0.00594

* not average- rep 1 only- see note below
* not average- rep 1 only- see note below
* not average- rep 1 only- see note below
* not average- rep 1 only- see note below
* not average- rep 1 only- see note below
* not average- rep 1 only- see note below

Notes

RESULTS REPORTED BY THIRD FLOOR AREA
(ALSO RAN ON GC/MS AND ALREADY REPORTED
AS PRELIMINARY VALIDATION DATA)

TOX209

Series 2 Feed Formulation Analytics

TOX209 Series 2 Feed Formulation

40 mg nicotine/kg body weight/day

Homogeneity Data using GC/FID Methodology

Submitted on: 4-29-08

Sample Identification (GN number)	Analysis	Sample Identification (GN number)	Analysis
GN 76562AA	46		
GN 76562AB	46		
GN 76562AC	46		
GN 76562AD	46		
GN 76562AE	46		
GN 76562AF	46		
GN 76562AG	46		
GN 76562AH	46		
GN 76562AI	46		

Comments:

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

Sample(s) Received By: Mr. J. J. J. J. J.

Received On: 4/29/08

TOX215.003.091406

04/30/08

09:19:35

TOX209 Final Report Adobe File Page 243 of 443

Testno: GN76562 Prog no: 900
Requester: SMITH, JENNY L
Description: Feed Study Nicotine
PDR Number: TOX209
Lab Instructions:
Please run duplicates. JSmith

Protocol: GENERAL

Needed: //

Phone: 741-0125

Part: GN76562AA Points: 1 Butt Len: 0 Part Name: TOB BLE 40.0mg Top
Date: 20080425 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76562AB Points: 1 Butt Len: 0 Part Name: TOB BLE 40.0 mg MID
Date: 20080425 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76562AC Points: 1 Butt Len: 0 Part Name: TOB BLE 40.0 mg Bot
Date: 20080425 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76562AD Points: 1 Butt Len: 0 Part Name: TOB EXT 40.0 mg TOP
Date: 20080425 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76562AE Points: 1 Butt Len: 0 Part Name: TOB EXT 40.0 mg MID
Date: 20080425 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76562AF Points: 1 Butt Len: 0 Part Name: TOB EXT 40.0 mg BOT
Date: 20080425 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76562AG Points: 1 Butt Len: 0 Part Name: NIC TAR 40.0 mg TOP
Date: 20080425 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76562AH Points: 1 Butt Len: 0 Part Name: NIC TAR 40.0 mg MID
Date: 20080425 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76562AI Points: 1 Butt Len: 0 Part Name: NIC TAR 40.0 mg BOT
Date: 20080425 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

TOX209
SP 5/2/08

TO Number: GN76562 Date Requested: 04/25/08 Date Completed: 05/02/08

Project Description: Feed Study Nicotine

Status: COMPLETED Protocol: GENERAL Prog Code: 900 Related #:

Requestor Name: SMITH, JENNY L

Req. Phone: 741-0125

Req. Location: 630-2

Req. Room:

Backup Name: PIKE, SUSAN

Backup Phone: 741-0921

Lab Instructions:

Please run duplicates. JSmith

5/2/2008 2:01:47 PM

PART SUMMARY RESULTS

TOX209
5/5/2/08

Test Part	GN 76562 AA	GN 76562 AB	GN 76562 AC
TOB PART_NAME	TOB BLE 40.0mg Top	TOB BLE 40.0 mg MID	TOB BLE 40.0 mg Bot
TEST_BUTT_LENGTH	0	0	0
GN_DATE	20080425	20080425	20080425
GN_SHIFT			
GN_COMMENTS			
GN_TIME			

SPECIFIC ALKALOIDS

TOTAL ALKALOIDS %	MISSING	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	0.
2ND TOTAL ALKALOIDS%	MISSING	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	0.
NICOTINE %	0.0407	0.0410	0.0407
Std. Deviation	0.00219	0.00007	0.00410
Count	2.	2.	2.
NORNICOTINE %	<0.0035	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	1.	0.	0.
MYOSMINE %	MISSING	<0.0003	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	1.	0.
ANABASINE %	MISSING	MISSING	<0.0003
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	1.
ANATABINE %	0.0010	0.0010	0.0009
Std. Deviation	0.00000	0.00000	0.00021
Count	2.	2.	2.
NICOTINE MG/G	0.41	0.41	0.41
Std. Deviation	0.021	0.000	0.042
Count	2.	2.	2.

TOX209
01/5/2008

Test Part	GN 76562 AD	GN 76562 AE	GN 76562 AF
TOB PART_NAME	TOB EXT 40.0 mg TOP	TOB EXT 40.0 mg MID	TOB EXT 40.0 mg BOT
TEST_BUTT_LENGTH	0	0	0
GN_DATE	20080425	20080425	20080425
GN_SHIFT			
GN_COMMENTS			
GN_TIME			

SPECIFIC ALKALOIDS

TOTAL ALKALOIDS %	MISSING	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	0.
2ND TOTAL ALKALOIDS%	MISSING	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	0.
NICOTINE %	0.0412	0.0387	0.0436
Std. Deviation	0.00042	0.00269	0.00778
Count	2.	2.	2.
NORNICOTINE %	MISSING	MISSING	<0.0035
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	1.
MYOSMINE %	MISSING	MISSING	<0.0003
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	1.
ANABASINE %	<0.0003	<0.0003	MISSING
Std. Deviation	N/A	N/A	N/A
Count	1.	1.	0.
ANATABINE %	0.0009	0.0008	0.0011
Std. Deviation	0.00014	0.00007	0.00014
Count	2.	2.	2.
NICOTINE MG/G	0.41	0.39	0.44
Std. Deviation	0.000	0.028	0.078
Count	2.	2.	2.

5/2/2008 2:01:47 PM

PART SUMMARY RESULTS

TOX209
5/5/2/08

Test Part	GN 76562 AG	GN 76562 AH	GN 76562 AI
TEST_PART_NAME	NIC TAR 40.0 mg TOP	NIC TAR 40.0 mg MID	NIC TAR 40.0 mg BOT
TEST_BUTT_LENGTH	0	0	0
GN_DATE	20080425	20080425	20080425
GN_SHIFT			
GN_COMMENTS			
GN_TIME			

SPECIFIC ALKALOIDS

TOTAL ALKALOIDS %	MISSING	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	0.
2ND TOTAL ALKALOIDS%	MISSING	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	0.
NICOTINE %	0.0401	0.0396	0.0398
Std. Deviation	0.00078	0.00071	0.00028
Count	2.	2.	2.
NORNICOTINE %	MISSING	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	0.
MYOSMINE %	MISSING	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	0.
ANABASINE %	<0.0003	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	1.	0.	0.
ANATABINE %	MISSING	MISSING	MISSING
Std. Deviation	N/A	N/A	N/A
Count	0.	0.	0.
NICOTINE MG/G	0.40	0.40	0.40
Std. Deviation	0.014	0.007	0.000
Count	2.	2.	2.

TOX209 Series 2 Feed Formulation Analytical Data

GC/MS Methodology

Dose Confirmation

SAMPLE SUBMISSION RECORD

Study Number: TOX209 Series 2

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

Submitted by: AmilaSubmitted on: 6-12-08

Sample Identification (GN number)	Analysis	Sample Identification (GN number)	Analysis
GN77620AA	46	GN77620AL	46
GN77620AB	46	GN77620AM	46
GN77620AC	46	GN77620AN	46
GN77620AD	46	GN77620AO	46
GN77620AE	46	GN77620AP	46
GN77620AF	46	GN77620AQ	46
GN77620AG	46		
GN77620AH	46		
GN77620AI	46		
GN77620AJ	46		
GN77620AK	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

06/12/08

11:12:24

TOX209 Final Report Adobe File Page 250 of 443

Testno: GN77620 Prog no: 900 Protocol: GENERAL Needed: //
Requester: SMITH, JENNY L Phone: 741-0125
Description: TOX209 Series 2
PDR Number: TOX209 2
Lab Instructions:
Please Run duplicate

Part: GN77620AA Date: 20080612	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: NTP-2000 DIET
Part: GN77620AB Date: 20080612	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: GR 2 DOSE1 (0.2)
Part: GN77620AC Date: 20080612	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: GR 3 DOSE2 (2.0)
Part: GN77620AD Date: 20080612	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: GR 5 DOSE4 (8.0)
Part: GN77620AE Date: 20080612	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: GR 6 DOSE 5 20.0MG
Part: GN77620AF Date: 20080612	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: GR 7 DOSE 6 40.0MG
Part: GN77620AG Date: 20080612	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: GR 8 DOSE 1 0.2 MG
Part: GN77620AH Date: 20080612	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: GR 9 DOSE 2 2.0 MG
Part: GN77620AI Date: 20080612	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: GR 10 DOSE 3 4.0 MG
Part: GN77620AJ Date: 20080612	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: GR 11 DOSE 4 8.0 MG
Part: GN77620AK Date: 20080612	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: GR 12 DOSE 5 20.0 MG
Part: GN77620AL Date: 20080612	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: GR 13 DOSE 6 40.0 MG

06/12/08

11:12:24

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Part: GN77620AM Date: 20080612	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: GR 14 DOSE 1 2.0 MG
Part: GN77620AN Date: 20080612	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: GR 15 DOSE 2 8.0 MG
Part: GN77620AO Date: 20080612	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: GR 16 DOSE 3 20.0 MG
Part: GN77620AP Date: 20080612	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: GR 17 DOSE 4 40.0 MG
Part: GN77620AQ Date: 20080612	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: GR 4 DOSE 3 4.0MG

Table 1: Nicotine Results GN77624, GN77620, and GN77622

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

- Data from PAD report
- Reanalysis

IR

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

- * Sample problem - data not used

TOX209 Series 2 Feed Formulation Analytical Data

GC/MS Methodology

Dose Confirmation

Additional Data Submission

05/02/08

10:45:57

TOX209 Final Report Adobe File Page 254 of 443

Testno: GN76746 Prog no: 900 Protocol: GENERAL Needed: 05/05/08
Requester: SMITH, JENNY L Phone: 741-0125
Description: TOX 209 FEED FORMULATED ON 4-24/4-25
R Number: TX T09 RA

Part: GN76746AA✓ Date: 20080502	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: TOB BLE 40.0
Part: GN76746AB✓ Date: 20080502	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: TOB BLE 20.0 MG
Part: GN76746AC✓ Date: 20080502	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: TOB BLE 8.0 MG
Part: GN76746AD✓ Date: 20080502	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: TOB BLE 4.0 MG
Part: GN76746AE✓ Date: 20080502	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: TOB BLE 2.0 MG
Part: GN76746AF✓ Date: 20080502	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: TOB BLE 0.2 MG
Part: GN76746AG✓ Date: 20080502	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: TOB EXT 40.0 MG
Part: GN76746AH✓ Date: 20080502	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: TOB EXT 20.0 MG
Part: GN76746AI✓ Date: 20080502	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: TOB EXT 8.0 MG
Part: GN76746AJ✓ Date: 20080502	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: TOB EXT 4.0 MG
Part: GN76746AK✓ Date: 20080502	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: TOB EXT 2.0 MG
Part: GN76746AL✓ Date: 20080502	Points: 1 Shift:	Butt Len: 0 Comments:	Part Name: TOB EXT 0.2 MG
Part: GN76746AM✓	Points: 1	Butt Len: 0	Part Name: NIC TAR 2.0 MG

05/02/08

LIMS TESTSAMPLE COVERSHEET

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TOX209 Final Report Adobe File Page 255 of 443

Date: 20080502

Shift:

Comments:

Part: GN76746AN✓

Date: 20080502

Points: 1 Butt Len: 0 Part Name: NIC TAR 8.0 MG

Shift: Comments:

Part: GN76746AO✓

Date: 20080502

Points: 1 Butt Len: 0 Part Name: NIC TAR 20.0MG

Shift: Comments:

Part: GN76746AP

Date: 20080502

Points: 1 Butt Len: 0 Part Name: NIC TAR 40.0MG

Shift: Comments:

listed as GN76746
not listed

Table 3: Nicotine Results GN77202, GN76746, and GN76747

GN77202	mg/g	Average		GN76746	mg/g	Dose Average		GN76747	mg/g	Dose Average
2								7		
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		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	AL1	0.001	0.2 mg
AL2	0.016	0.016		AL2	0.001	0.001	NT	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			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		
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.

IR

TOX209

Stability of Nicotine in Formulated Feed

TOX209

10 Day Stability of Nicotine in Formulated Feed

Determined with Series 2 Formulated Feed

05/02/08

LIMS TESTSAMPLE COVERSHEET

Page: 1

11:03:41

TOX209 Final Report Adobe File Page 259 of 443

Testno: GN76750 Prog no: 900 Protocol: GENERAL Needed: 05/06/08
Requester: SMITH, JENNY L Phone: 741-0125
Description: TOX209 FORMULATED ON 4-24/4-25-08
Lab Number: TOX209
Lab Instructions:
PLEASE RUN DUPLICATES

Part: GN76750AA Points: 1 Butt Len: 0 Part Name: TOB BLE 40.0 MG STA
Date: 20080502 Shift: Comments:

Part: GN76750AB Points: 1 Butt Len: 0 Part Name: TOB BLE 0.2 MG STA
Date: 20080502 Shift: Comments:

Part: GN76750AC Points: 1 Butt Len: 0 Part Name: TOB EXT 40.0 MG STA
Date: 20080502 Shift: Comments:

Part: GN76750AD Points: 1 Butt Len: 0 Part Name: TOB EXT 0.2 MG STA
Date: 20080502 Shift: Comments:

Part: GN76750AE Points: 1 Butt Len: 0 Part Name: NIC TAR 40.0 MG STA
Date: 20080502 Shift: Comments:

Part: GN76750AF Points: 1 Butt Len: 0 Part Name: NIC TAR 2.0 MG STA
Date: 20080502 Shift: Comments:

Please Leave at Room Temp. until 5-5-08
JS 5-2-08

Table 4: Nicotine Results GN76749 and GN76750

	GN7674 9	mg/g	Dose (mg) Average		GN7675 0	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)

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.

• 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

• TOX209 Overlook Stability Data (10-day stability)
 • LIMS submission sheet dated 05/02/08

• According to dates on LIMS submission sheet, this would be a 10 day stability.

IR

IR

TOX209

1 Month Stability of Nicotine in Formulated Feed

Determined with Trial Run Formulated Feed

05/02/08

LIMS TESTSAMPLE COVERSHEET

Page: 1
11:00:07

TOX209 Final Report Adobe File Page 262 of 443

Testno: GN76749

Prog no: 900

Protocol: GENERAL

Needed: 05/05/08

Requester: SMITH, JENNY L

Phone: 741-0125

Description: TOX209 FEED FORMULATED ON 4-2/4-3 STABILIEY

LR Number: TOX209

Part: GN76749AA✓ Points: 1 Butt Len: 0 Part Name: TOB BLE 40.0MG STA
Date: 20080502 Shift: Comments:

Part: GN76749AB✓ Points: 1 Butt Len: 0 Part Name: TOB BLE 0.2 MG STA
Date: 20080502 Shift: Comments:

Part: GN76749AC✓ Points: 1 Butt Len: 0 Part Name: TOB EXT 40.0 MG STA
Date: 20080502 Shift: Comments:

Part: GN76749AD✓ Points: 1 Butt Len: 0 Part Name: TOB EXT 0.2 MG STA
Date: 20080502 Shift: Comments:

Part: GN76749AE✓ Points: 1 Butt Len: 0 Part Name: NIC TAR 40.0 MG STA
Date: 20080502 Shift: Comments:

Part: GN76749AF Points: 1 Butt Len: 0 Part Name: NIC TAR 2.0 MG STA
Date: 20080502 Shift: Comments:

Table 4: Nicotine Results GN76749 and GN76750

GN7674 9	mg/g	Average	GN7675 0	mg/g	Average
AA1	0.466	40	AA1	0.416	
AA2	0.411	0.438	AA2	0.402	0.409
AB1	0.002	0.2	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

TOX209 1 month stability

TOX209 stability study ~~1 week~~ 10-day

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.

TOX209 (RAI)

Submitted by:

_____ G. Smith

Submitted on: 4-30-08

[illegible]

Comments:

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

Sample(s) Received By:

Lucy Bt

Received On:

4/30/08

04/30/08

10:31:12

TOX209 Final Report Adobe File Page 265 of 443

Testno: GN76677 Prog no: 900 Protocol: GENERAL Needed: 05/02/08
Requester: SMITH, JENNY L Phone: 741-0125
Description: Rat Feed Study Stability Samples
PDR Number: TX209 RAT
Lab Instructions:
Please run as duplicates

Part: GN76677AA Points: 1 Butt Len: 0 Part Name: TOB BLE RM TEMP 4-2
Date: 20080430 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76677AB Points: 1 Butt Len: 0 Part Name: TOB EXT RM TEMP 4-3
Date: 20080430 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76677AC Points: 1 Butt Len: 0 Part Name: NIC TAR RM TEMP 4-2
Date: 20080430 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76677AD Points: 1 Butt Len: 0 Part Name: TOB BLE REF TEMP 4-2
Date: 20080430 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76677AE Points: 1 Butt Len: 0 Part Name: TOB EXT REF TEMP 4-3
Date: 20080430 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Part: GN76677AF Points: 1 Butt Len: 0 Part Name: NIC TAR REF TEMP 4-2
Date: 20080430 Shift: Comments:

46(42) SPECIFIC ALKALOIDS CHEMICAL

Test Part	GN 76677 AA	GN 76677 AB	GN 76677 AC
TEST PART NAME	TOB BLE RM TEMP 4-2	TOB EXT RM TEMP 4-3	NIC TAR RM TEMP 4-2
TEST BUTT_LENGTH	0	0	0
GN_DATE	20080430	20080430	20080430
GN_SHIFT			
GN_COMMENTS			
GN_TIME			

SPECIFIC ALKALOIDS

TOTAL ALKALOIDS %	MISSING	MISSING	MISSING
2ND TOTAL ALKALOIDS%	MISSING	MISSING	MISSING
NICOTINE %	0.0501	0.0511	0.0453
NORNICOTINE %	<0.0033	<0.0033	MISSING
MYOSMINE %	MISSING	MISSING	MISSING
ANABASINE %	MISSING	<0.0003	MISSING
ANATABINE %	0.0013	0.0011	MISSING
NICOTINE MG/G	0.50	0.51	0.45

Analytical Chemistry PAD Report

PAD TEST MEMORANDUM

AUTHOR: Karen B. Kilby
Timothy A. Ellisor

DATE: August 5, 2008

R016152



RJR R&D
SCIENTIFIC INFORMATION SERVICES LIBRARY

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:

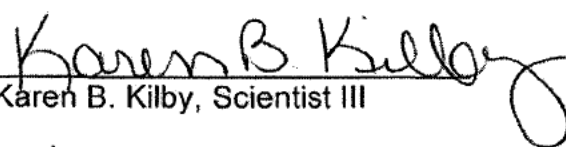
Rat and mouse feed samples with varying dosing levels of nicotine were submitted for analysis. The samples were logged into LIMS under the following identification numbers: GN76749 (AA-AF), GN76750 (AA-AF), GN76747 (AA-AP), GN77202 (AA-AR), GN77172 (AA-AI), GN77522 (AA-AO), GN77615 (AA-AQ), GN77622 (AA-AP),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

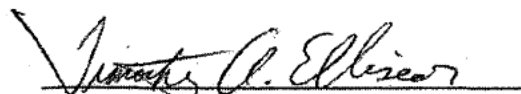
STATUS:

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

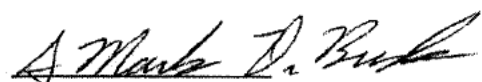
KEYWORDS:

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
CB ?	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	3.0		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
	• TOX210 Series 2				AQ1	0.045					
	• Decoded from sample cup				AQ2	0.038	0.042				

sent to chemistry
• Submission Date 06/12/08

AL

Table 2: Nicotine Results GN77615, GN77522, And GN77172

Table 1. Baseline Results GN7761, GN77522, And GN77172

GN7761			GN77522			GN77172		
5	mg/g	Average		mg/g	Average		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

GN7674 9	mg/g	Average	GN7675 0	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.

Analytical Chemistry PAD Report
Decoded in Respect to Sample Identity

Decode 1

PAD TEST MEMORANDUM

AUTHOR: Karen B. Kilby
Timothy A. Ellisor

DATE: August 5, 2008

R016152



SCIENTIFIC INFORMATION SERVICES LIBRARY

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.

GN76746

listed in report as GN76746, which
correlates with submission sheet.

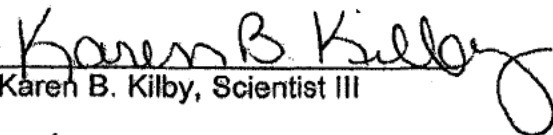
STATUS:

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

KEYWORDS:

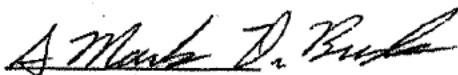
GC/MS, nicotine, smokeless tobacco, SNUS, Feed, Diet

- TB = Tobacco Blend
- TE = Tobacco Extract
- NT = Nicotine Hydrogen Tartrate


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

18

- 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 GN76746~~
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	TB	AA2	0.363	0.357	TB	AA2	0.197	0.182
AB1	0.148		TB	AB1	0.176	20	TB	AB1	0.095	20 mg
AB2	0.171	0.159	TB	AB2	0.204	0.190	TB	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	TB	AC2	0.070	0.071	TB	AC2	0.031	0.029
AD1	0.512		TB	AD1	0.048	40	TB	AD1	0.014	4.0 mg
AD2	0.571	0.541	TB	AD2	0.034	0.041	TB	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	TB	AE2	0.010	0.014	TB	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	TB	AF2	0.005	0.004	TB	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	TE	AG2	0.224	0.203
AH1	1.491		TE	AH1	0.154	20	TE	AH1	0.056	20 mg
AH2	1.596	1.544	TE	AH2	0.115	0.134	TE	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	TE	AI2	0.144	0.122	TE	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	TE	AJ2**	0.019	0.043	TE	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	TE	AK2**	0.069	0.040	TE	AK2	0.003	0.003
AL1	0.017		TE	AL1	0.001	0.2	NT	AL1	0.001	0.2 mg
AL2	0.016	0.016	NT	AL2	0.001	0.001	NT	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	NT	AM2	0.019	0.019	NT	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	NT	AN2	0.075	0.075	NT	AN2	<0.001	<0.001
AO1	0.851		NT	AO1	0.178	20	NT	AO1	0.031	8 mg
AO2	0.868	0.859	NT	AO2	0.183	0.181	NT	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	NT	AP2	0.356	0.356	NT	AP2	0.009	0.008
AQ3	1.483			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		
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.

IR

Table 4: Nicotine Results GN76749 and GN76750

	GN7674 9	mg/g	Dose (mg) Average		GN7675 0	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)

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.

• 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

• TOX209 One-week Stability Data (10-day stability)
• LIMS Submission sheet dated 05/02/08

• According to dates on LIMS submission sheet, this would be a 10 day stability.

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Analytical Chemistry Reports

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 added 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, 1L.
- 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-d₇ (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

GC/MS Parameters

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.

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 0.0500g of Quinoline-d₇ (Internal standard) in 4 liters of MTBE and mix well.

6.1.2 2N NaOH solution: Weigh 80g 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:

$$\left[\frac{0.4000 \text{ g nicotine} \times 1000 \frac{\text{mg}}{\text{g}} \times 0.99(\text{purity})}{100 \text{ mL}} \right] = 3.96 \frac{\text{mg}}{\text{mL}} \text{ nicotine}$$

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:

$$\left(\frac{3.96 \frac{\text{mg}}{\text{mL}} \text{ nicotine} \times 5 \text{ mL}}{100 \text{ mL}} \right) = 0.198 \frac{\text{mg}}{\text{mL}} \text{ nicotine}$$

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:

$$9.3.1 \quad C \text{ (mg/mL)} = ax^2 + bx + c$$

where: C= Concentration of nicotine
a = quadratic term

b = linear term

c = constant term

x = component peak area/internal standard peak area

$$9.3.2 \quad \frac{\text{mg nicotine}}{\text{g feed}} = \frac{C \text{ (mg/mL)} \times 5 \text{ mL}}{\text{Feed Sample wt. (g)}}$$

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.

The Quantitative Determination of Nicotine in Rat/Mouse Feed Samples by GC/MS

Figure 1: Selected Ion 136 Internal Standard Peak (Quinoline-d₇):
Abundance

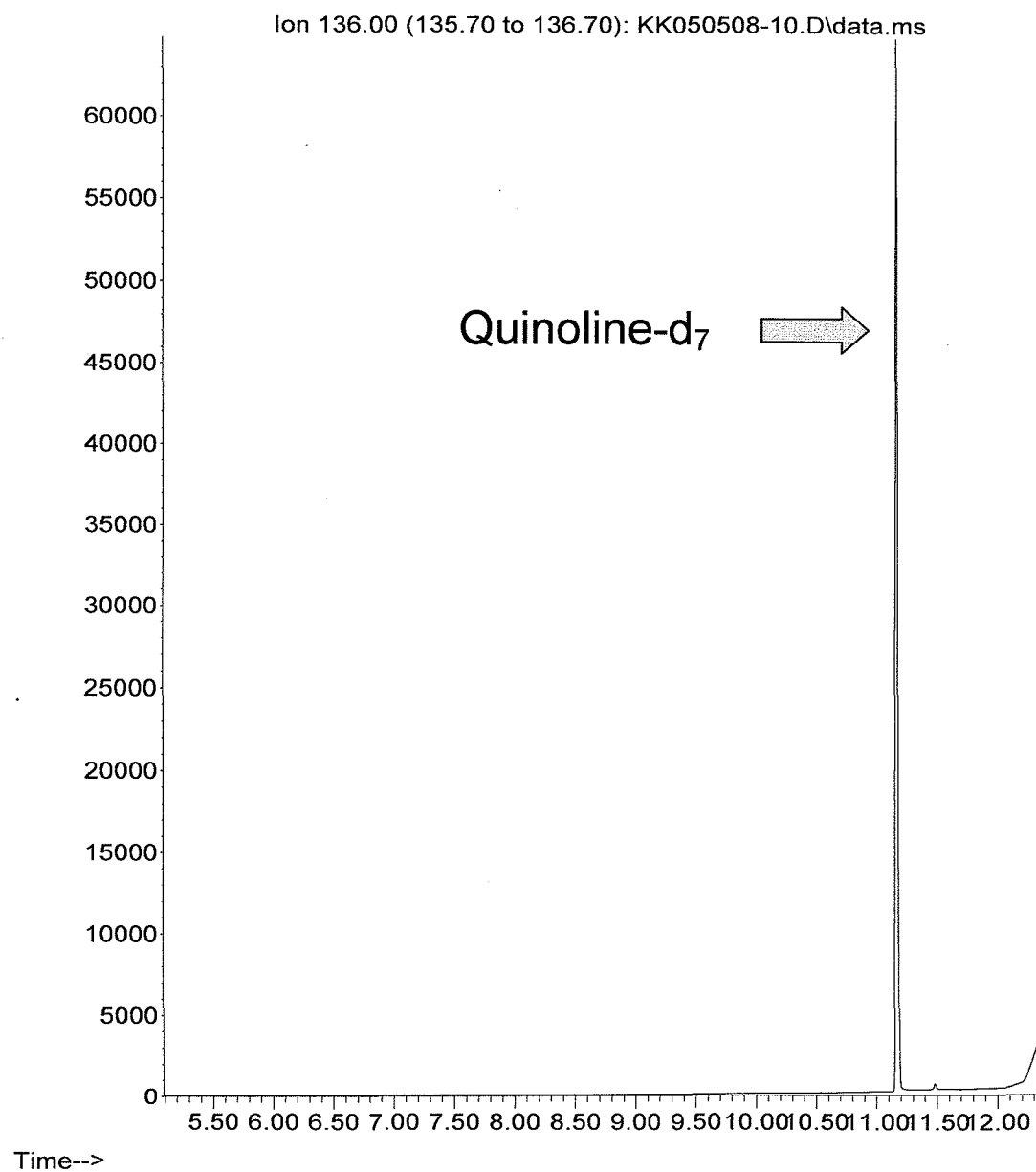
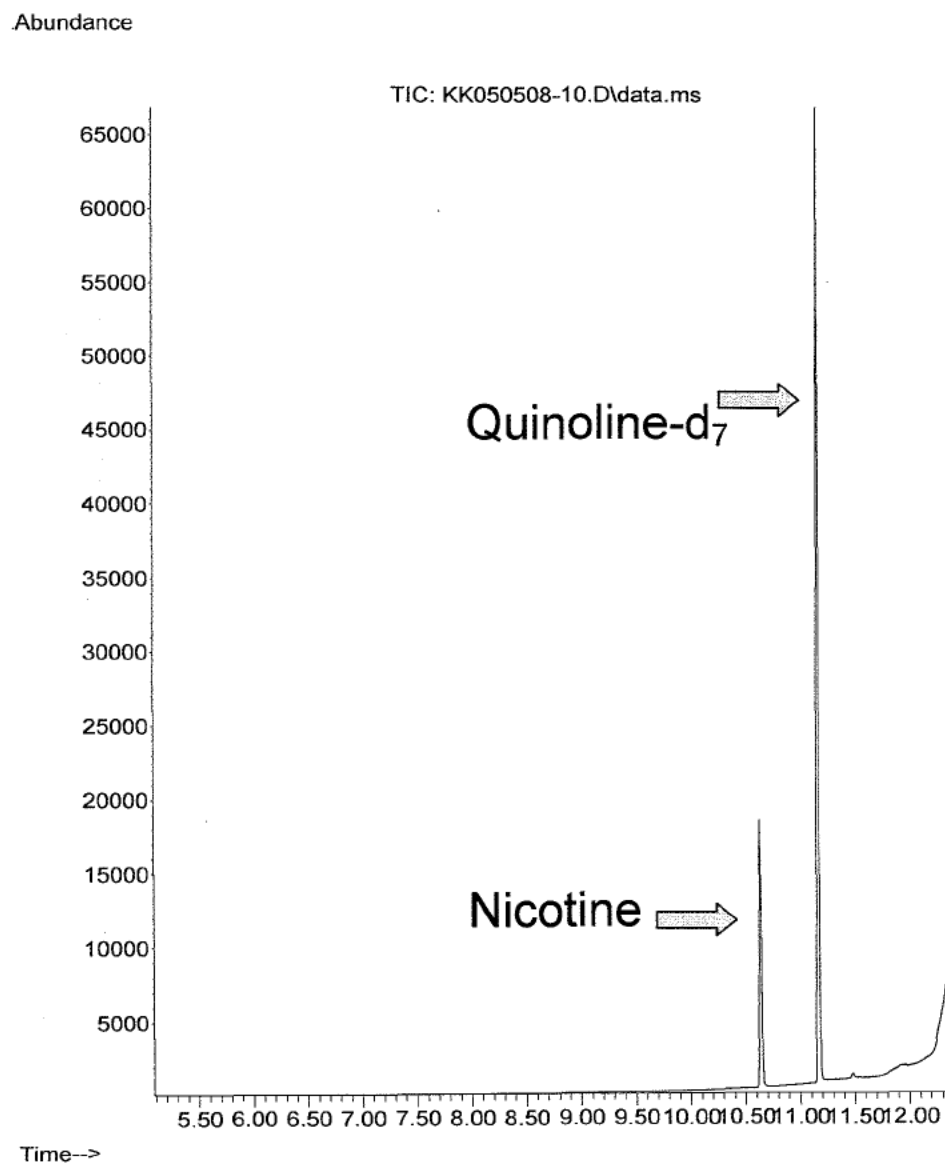


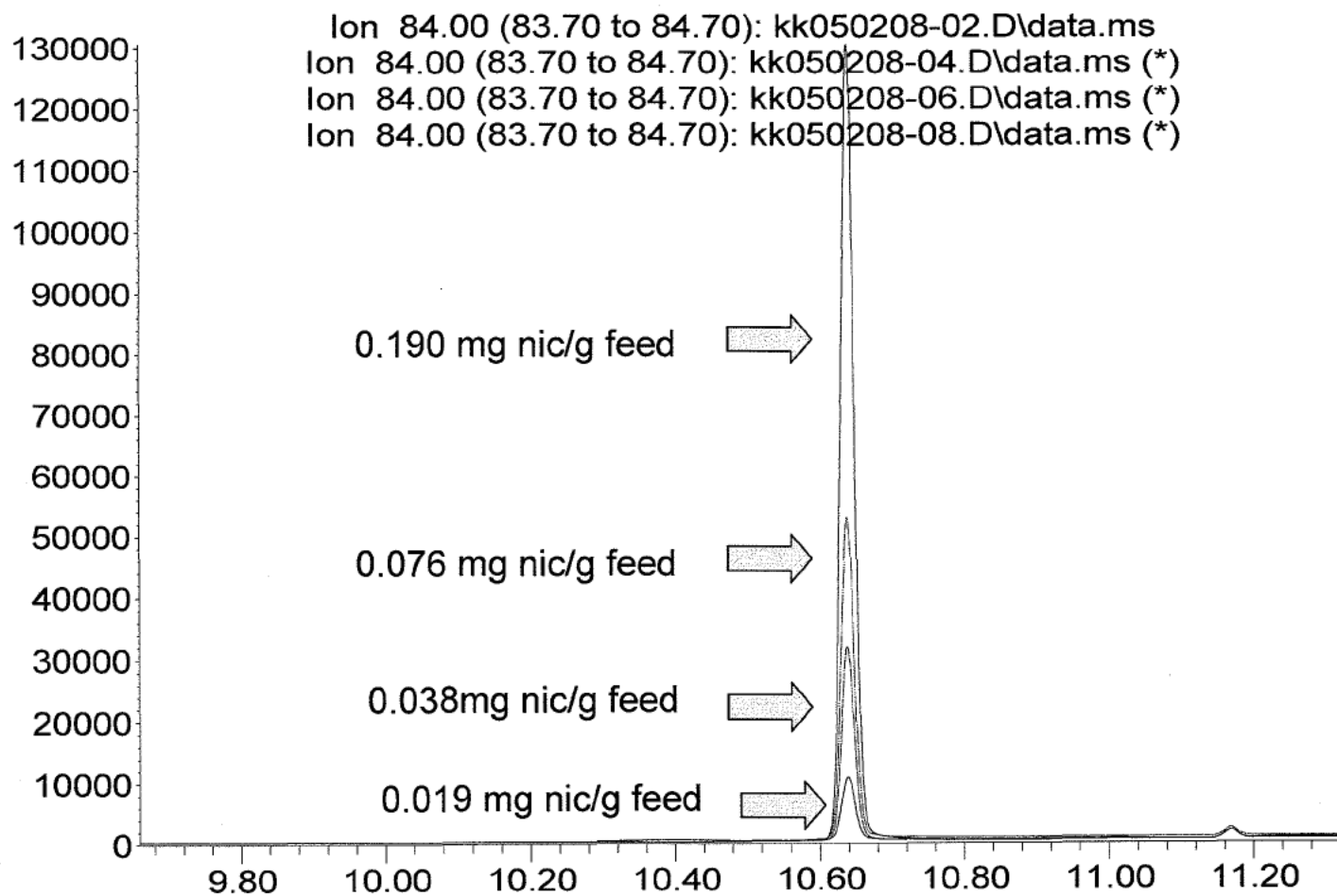
Figure 2: TIC Chromatogram of Calibration Standard (Std 5):



The Quantitative Determination of Nicotine in Rat/Mouse Feed Samples by GC/MS

Figure 3: Overlaid Chromatograms (Selected Ion 84) of four Test Extracts
Ranging from 0.019 to 0.19 mg nicotine per gram rat feed:

Abundance



PAD TEST MEMORANDUM

AUTHOR: Karen B. Kilby
Timothy A. Ellisor

DATE: August 4, 2008

R016153



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.

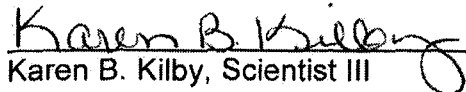
Acceptable results were measured for all aspects of the validation study; therefore, the proposed method shall be used for the determination of nicotine in rat/mouse feed to support the Smokeless Tobacco Stewardship Animal Feed Palatability Project.

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


Karen B. Kilby, Scientist III


Timothy A. Ellisor, Technician III

Reviewed by:


S. Mark DeBusk, Lead Manager Product Quality

Cc:

Bert Gordon
Brad Rhoades
Paul Ayres

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 μ L of the standard solution (0.0145 mg nicotine), the second level contained 500 μ L of the standard solution (0.1445 mg nicotine), and the third level contained 1000 μ L 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-d₇. 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-d₇ (Figures 1 and 2). A regression coefficient of 0.9974 and 1.0000, (r²) 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

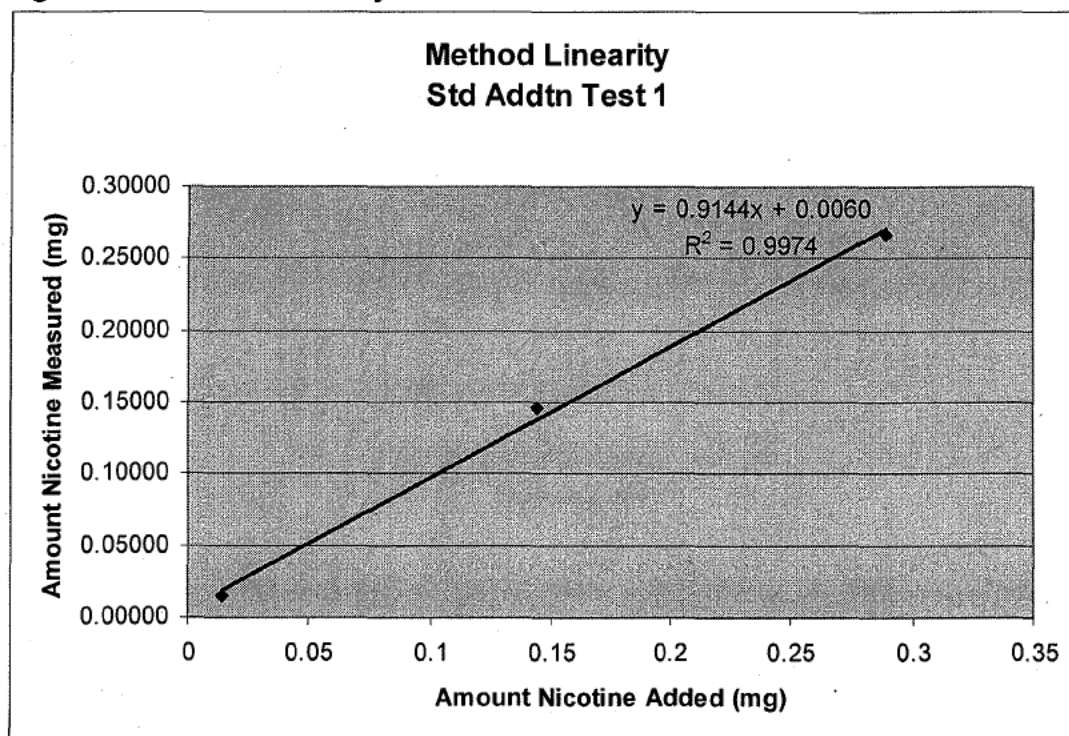
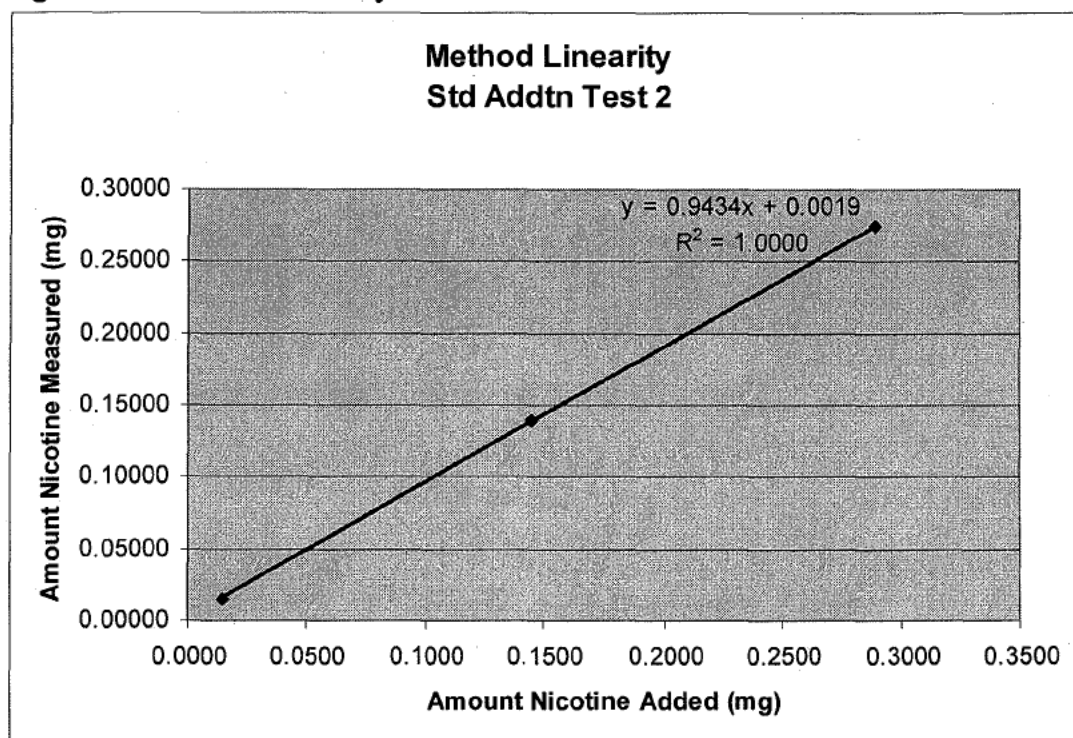


Figure 2: Method Linearity -Standard Addition Test 2**Instrument Precision**

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-d₇ (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-d₇ (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:

$$9.3.1 \quad C \text{ (mg/mL)} = ax^2 + bx + c$$

where: C= Concentration of nicotine

a = quadratic term

b = linear term

c = constant term

x = component peak area/internal standard peak area

$$9.3.2 \quad \frac{\text{mg nicotine}}{\text{g feed}} = C \frac{\text{(mg/mL)}}{\text{Feed Sample wt. (g)}} \times 5 \text{ mL}$$

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
(Quinoline-d₇):
Abundance

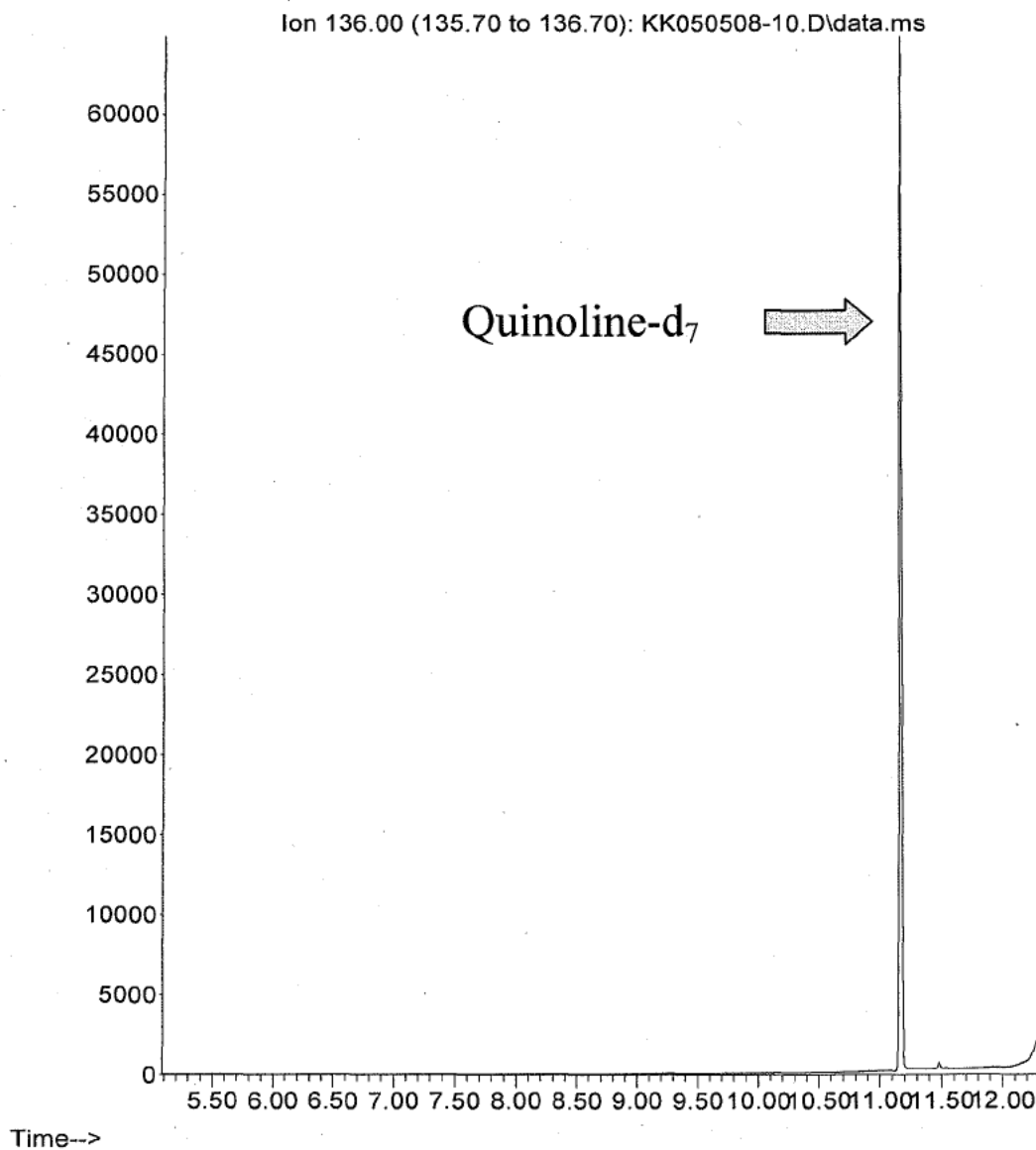
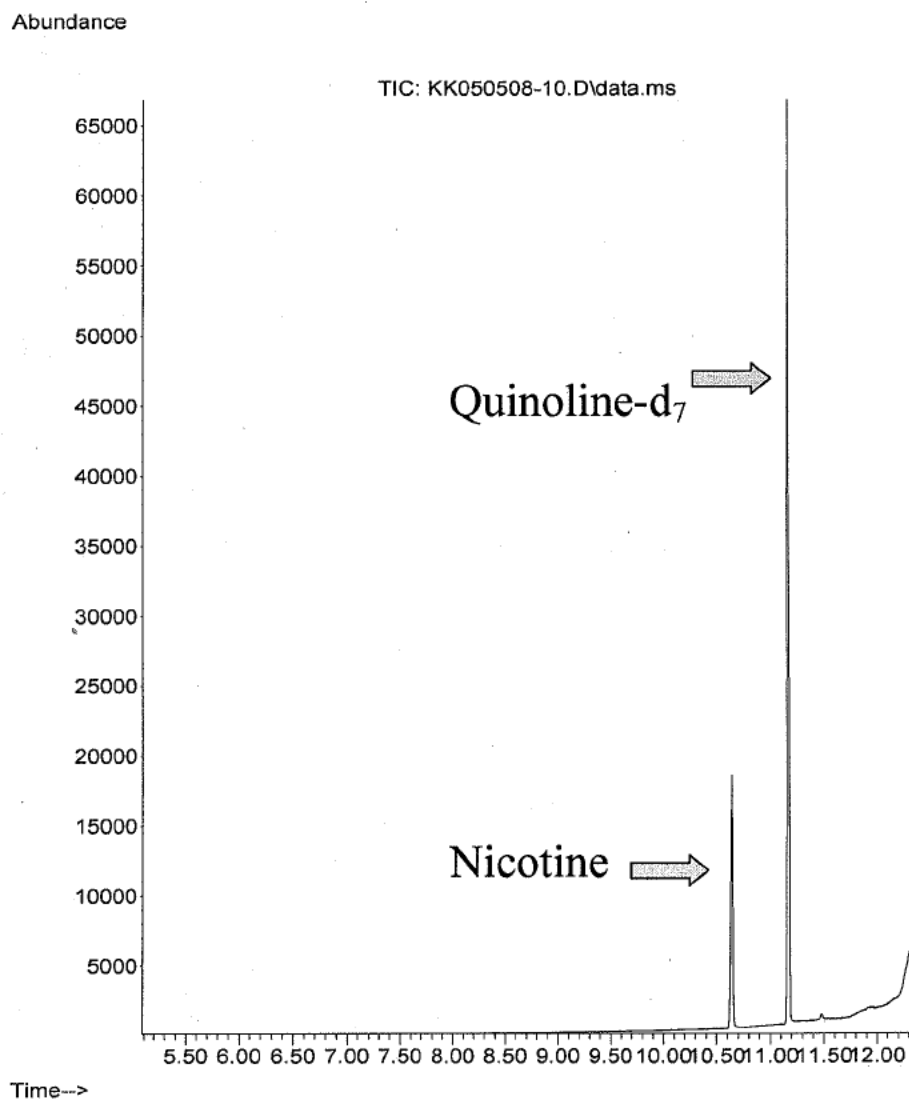
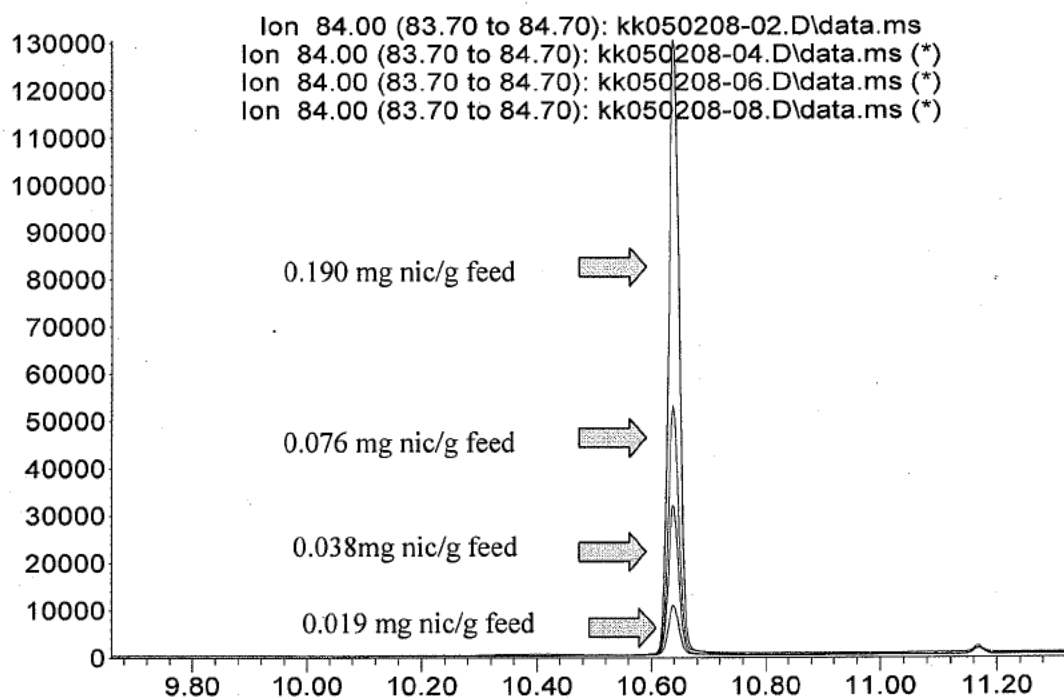


Figure 2: TIC Chromatogram of Calibration Standard (Std 5):

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Page 16 of 16

Figure 3: Overlaid Chromatograms (Selected Ion 84) of four Test Extracts
Ranging from 0.019 to 0.19 mg nicotine per gram rat feed:

Abundance



Appendix IV

Serology and Histopathology of the Lung

RJReynolds

Subject: Serology Results for TOX209

Date: May 9, 2008

To: Jenny Smith

From: Chandra Williams, DVM

Attached are the serology results of serum samples taken from animals on the TOX209 study. The samples were collected from 10 male rats (animal numbers 23-32) rats. Rats were euthanized for whole blood sample collection. Serum was removed from the whole blood samples and submitted on all animals. 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 Rat Assessment Plus profile) listed below.

Sendai Virus	Mouse Adenovirus (MAV) 1 & 2
Pneumonia Virus of Mice (PVM)	Hantaviruses (HANT)
Sialodacryoadenitis Virus (SDAV)	<i>Encephalitozoon cuniculi</i> (ECUN)
Kilham Rat Virus (KRV)	Cilia Associated Respiratory Bacillus (CARB)
H-1 Virus (H-1)	Mouse Parvovirus (MPV)* or PARV NS1
GDVII (Murine Encephalomyelitis Virus)	Rat Parvovirus (RPV)
REO	Rat Minute Virus (RMV)
<i>Mycoplasma pulmonis</i>	
Lymphocytic Choriomeningitis Virus (LCMV)	

*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: Thursday, May 8, 2008 at 14:33

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 #: 2008-025866****Diagnostic Summary Report**PO Box 1236
Winston-Salem, NC 27102 USA

Attn: Dr. Chandra Williams

Tel: 336-741-0121

Received: 07 May 2008
Approved: 08 May 2008, 14:33
Bill Method: PO# 4534185982
Test Specimen: Rat

Sample Set	Service (# Tested)	Profile	Assay	Tested	+	+/-	?
#1	Serology (10)	All Results Negative					

+ = Positive, +/- = Equivocal, ? = Indeterminate

Service Approvals

Service	Approved By*	Date
Serology	Janet M. Wunderlich	08 May 2008, 14:33

**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: Thursday, May 8, 2008 at 14:33

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 #: 2008-025866****Product:** Not Indicated**Test Specimen:** Rat**Received:** 07 May 2008**Serology Results Report****Department Review:** Approved by Janet M. Wunderlich, 08 May 2008, 14:33*

Sample #: Code :	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>
	Tox 209R 23	Tox 209R 24	Tox 209R 25	Tox 209R 26	Tox 209R 27	Tox 209R 28	Tox 209R 29	Tox 209R 30	Tox 209R 31	Tox 209R 32
MFIA SEND	-	-	-	-	-	-	-	-	-	-
MFIA PVM	-	-	-	-	-	-	-	-	-	-
MFIA SDAV	-	-	-	-	-	-	-	-	-	-
MFIA KRV	-	-	-	-	-	-	-	-	-	-
MFIA H-1	-	-	-	-	-	-	-	-	-	-
MFIA RPV	-	-	-	-	-	-	-	-	-	-
MFIA RMV	-	-	-	-	-	-	-	-	-	-
MFIA NS-1	-	-	-	-	-	-	-	-	-	-
MFIA REO	-	-	-	-	-	-	-	-	-	-
MFIA GDVII	-	-	-	-	-	-	-	-	-	-
MFIA LCMV	-	-	-	-	-	-	-	-	-	-
MFIA MAV 1 & 2	-	-	-	-	-	-	-	-	-	-
MFIA HANT	-	-	-	-	-	-	-	-	-	-
MFIA MPUL	-	-	-	-	-	-	-	-	-	-
MFIA ECUN	-	-	-	-	-	-	-	-	-	-
MFIA CARB	-	-	-	-	-	-	-	-	-	-
MFIA Anti-Ig	P	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-209 (Seventh Wave Study No. SW08-0144)

SUBJECT: Scheduled Health Screen, Histopathology, End Of Study


Dear Doctor Williams:

Formalin-preserved samples of infused lungs from ten male Wistar-han rats were processed at Seventh Wave, beginning on May 8, 2008. Microscopic examination was performed on each of the five lung lobes from every rat. 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, vascular mineralization, and chronic inflammation. The chronic inflammation was of minimal intensity and noted in only one lung lobe of two rats (Nos. 29 and 32). The occurrences of this change are regarded as random and nonspecific; they do not indicate the presence of contagious disease.

The congestion and hemorrhage probably reflect the mode of anesthesia/euthanasia via carbon dioxide/exsanguination. The nonpigmented macrophages, lymphocytic infiltrations, and vascular mineralization are anticipated background changes typically seen in rats of this age and strain.

CONCLUSION

Histopathologic examination revealed no evidence of intercurrent infectious disease in any of the rats examined.


John W. Sagartz, D.V.M.
Diplomate, A.C.V.P.

JWS: cjh

Seventh Wave Document No.: 311

cc: Jenny Smith
Paul Ayres
Jessica Baker
Sheri Bowman

SW08-0144 (TOX 209 HS)

QUALITY CONTROL 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.

<u>May 7. 2008</u>	Part 1 of 9 - Project Sheet Review
<u>May 13. 2008</u>	Part 2 of 9 - Master Individual/Multiple Animal Worksheet
<u>May 9 2008</u>	Part 3 of 9 - Histology Setup
<u>May 13. 2008</u>	Part 4 of 9 - Histology Completion
<u>May 13. 2008</u>	Part 5 of 9 - Slide/Block Match (100%)
<u>May 13. 2008</u>	Part 6 of 9 - Slide/Label Check (100%)
<u>May 13. 2008</u>	Part 7 of 9 - Wet Tissue Check (100%)
<u>May 18. 2008</u>	Part 8 of 9 - Rough Draft Report
<u>May 18. 2008</u>	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-209 (SW08-0144) Species: Wistar-han Rats 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		
190 B LUNG, RIGHT APICAL LOBE, H&E		
188 B LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E		
189 B LUNG, RIGHT CARDIAC 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: 23 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

-HEMORRHAGE, MULTIFOCAL, MINIMAL
-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, FOCAL, MINIMAL
-MACROPHAGES, NONPIGMENTED, MULTIFOCAL,
MINIMAL

LUNG, RIGHT APICAL LOBE, H&E

-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, FOCAL, MINIMAL
-MACROPHAGES, NONPIGMENTED, MULTIFOCAL,
MINIMAL

LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E

-HEMORRHAGE, MULTIFOCAL, MINIMAL
-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, MULTIFOCAL, MILD
-MACROPHAGES, NONPIGMENTED, MULTIFOCAL,
MINIMAL

LUNG, RIGHT CARDIAC LOBE, H&E

-MACROPHAGES, NONPIGMENTED, MULTIFOCAL,
MINIMAL

ANIMAL NUMBER: 24 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, MODERATE
-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, MULTIFOCAL, MILD
-MINERALIZATION, VASCULAR, FOCAL, MILD
-MACROPHAGES, NONPIGMENTED, MULTIFOCAL,
MINIMAL

LUNG, INTERMEDIATE LOBE, H&E

-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, MULTIFOCAL, MILD
-HEMORRHAGE, MULTIFOCAL, MINIMAL
-MINERALIZATION, VASCULAR, FOCAL, MILD
-MACROPHAGES, NONPIGMENTED, MULTIFOCAL,
MINIMAL

SPECIES: Wistar-han Rats
PROJECT NUMBER: TOX-209 (SW08-0144)

STAR Page: 1

Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
R. J. Reynolds Tobacco Company
Scheduled Health Screen, Histopathology, End Of Study

ANIMAL NUMBER: 24 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 05-18-2008.

MICROSCOPIC OBSERVATIONS (continued):

LUNG, RIGHT APICAL LOBE, H&E

-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, MULTIFOCAL, MILD

-HEMORRHAGE, MULTIFOCAL, MILD

-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD

LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E

-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, FOCAL, MILD

-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD

LUNG, RIGHT CARDIAC LOBE, H&E

-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, FOCAL, MILD

-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD

ANIMAL NUMBER: 25 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

-MINERALIZATION, VASCULAR, FOCAL, MILD

-HEMORRHAGE, MULTIFOCAL, MILD

-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD

LUNG, INTERMEDIATE LOBE, H&E

-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, FOCAL, MILD

-MACROPHAGES, NONPIGMENTED, MULTIFOCAL,
MINIMAL

LUNG, RIGHT APICAL LOBE, H&E

-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, MULTIFOCAL, MILD

-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD

LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E

-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, FOCAL, MILD

-MINERALIZATION, VASCULAR, FOCAL, MILD

-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD

LUNG, RIGHT CARDIAC LOBE, H&E

-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, FOCAL, MILD

-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD

SPECIES: Wistar-han Rats
PROJECT NUMBER: TOX-209 (SW08-0144)

STAR Page: 2

Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
R. J. Reynolds Tobacco Company
Scheduled Health Screen, Histopathology, End Of Study

ANIMAL NUMBER: 26 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, MINIMAL

-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD

LUNG, INTERMEDIATE LOBE, H&E

-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, MULTIFOCAL, MILD

-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD

LUNG, RIGHT APICAL LOBE, H&E

-CONGESTION, DIFFUSE, MINIMAL

-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD

LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E

-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, FOCAL, MILD

-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD

LUNG, RIGHT CARDIAC LOBE, H&E

-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, MULTIFOCAL, MILD

-HEMORRHAGE, FOCAL, MINIMAL

-MACROPHAGES, NONPIGMENTED, MULTIFOCAL,
MINIMAL

ANIMAL NUMBER: 27 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, MODERATE

-MINERALIZATION, VASCULAR, MULTIFOCAL, MILD

-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, MULTIFOCAL, MILD-MACROPHAGES, NONPIGMENTED, MULTIFOCAL,
MINIMAL

SPECIES: Wistar-han Rats
PROJECT NUMBER: TOX-209 (SW08-0144)

STAR Page: 3

Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
R. J. Reynolds Tobacco Company
Scheduled Health Screen, Histopathology, End Of Study

ANIMAL NUMBER: 27 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 05-18-2008.

MICROSCOPIC OBSERVATIONS (continued):

LUNG, INTERMEDIATE LOBE, H&E

-HEMORRHAGE, MULTIFOCAL, MILD
-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, FOCAL, MILD
-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
-HEMORRHAGE, MULTIFOCAL, MODERATE
-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, MULTIFOCAL, MILD
-MACROPHAGES, NONPIGMENTED, MULTIFOCAL,
MINIMAL
-HEMORRHAGE, MULTIFOCAL, MILD
-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, MULTIFOCAL, MILD
-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
-HEMORRHAGE, MULTIFOCAL, MODERATE
-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, FOCAL, MILD
-MACROPHAGES, NONPIGMENTED, MULTIFOCAL,
MINIMAL

LUNG, RIGHT APICAL LOBE, H&E

LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E

LUNG, RIGHT CARDIAC LOBE, H&E

ANIMAL NUMBER: 28 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
-MINERALIZATION, VASCULAR, FOCAL, MILD
-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
-HEMORRHAGE, MULTIFOCAL, MINIMAL
-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, MULTIFOCAL, MILD
-MACROPHAGES, NONPIGMENTED, MULTIFOCAL,
MINIMAL
-HEMORRHAGE, MULTIFOCAL, MINIMAL

LUNG, INTERMEDIATE LOBE, H&E

SPECIES: Wistar-han Rats
PROJECT NUMBER: TOX-209 (SW08-0144)

STAR Page: 4

Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
R. J. Reynolds Tobacco Company
Scheduled Health Screen, Histopathology, End Of Study

ANIMAL NUMBER: 28 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 05-18-2008.

MICROSCOPIC OBSERVATIONS (continued):

LUNG, RIGHT APICAL LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD
	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
LUNG, RIGHT CARDIAC LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD
	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD

ANIMAL NUMBER: 29 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, MINIMAL
LUNG, INTERMEDIATE LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD
	-MINERALIZATION, VASCULAR, FOCAL, MILD
	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT APICAL LOBE, H&E	-MINERALIZATION, VASCULAR, FOCAL, MILD
	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD
	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD
	-HEMORRHAGE, FOCAL, MINIMAL
	-MINERALIZATION, VASCULAR, FOCAL, MILD
	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT CARDIAC LOBE, H&E	-MINERALIZATION, VASCULAR, FOCAL, MILD
	-INFLAMMATION, CHRONIC, FOCAL, MILD
	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD

SPECIES: Wistar-han Rats
PROJECT NUMBER: TOX-209 (SW08-0144)

STAR Page: 5

Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
R. J. Reynolds Tobacco Company
Scheduled Health Screen, Histopathology, End Of Study

ANIMAL NUMBER: 30 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

LUNG, INTERMEDIATE LOBE, H&E

-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD

LUNG, RIGHT APICAL LOBE, H&E

-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, MULTIFOCAL, MILD

-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD

-HEMORRHAGE, FOCAL, 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, MULTIFOCAL, MILD

-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD

-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, MULTIFOCAL, MILD

-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD

ANIMAL NUMBER: 31 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, MILD

LUNG, INTERMEDIATE LOBE, H&E

-MACROPHAGES, NONPIGMENTED, MULTIFOCAL,
MINIMAL

LUNG, RIGHT APICAL LOBE, H&E

-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, MULTIFOCAL, MILD

-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD

-HEMORRHAGE, MULTIFOCAL, MINIMAL

-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, FOCAL, MILD-MACROPHAGES, NONPIGMENTED, MULTIFOCAL,
MINIMAL

-HEMORRHAGE, MULTIFOCAL, MINIMAL

SPECIES: Wistar-han Rats
PROJECT NUMBER: TOX-209 (SW08-0144)

STAR Page: 6

Single Tabulated Animal Report (continued)
Individual Macroscopic and Microscopic Observations
R. J. Reynolds Tobacco Company
Scheduled Health Screen, Histopathology, End Of Study

ANIMAL NUMBER: 31 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 05-18-2008.

MICROSCOPIC OBSERVATIONS (continued):

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, MULTIFOCAL, MILD

-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD

ANIMAL NUMBER: 32 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, FOCAL, MILD-MACROPHAGES, NONPIGMENTED, MULTIFOCAL,
MINIMAL

LUNG, INTERMEDIATE LOBE, H&E

-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, MULTIFOCAL, MILD-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
-HEMORRHAGE, MULTIFOCAL, MINIMAL

LUNG, RIGHT APICAL LOBE, H&E

-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, MULTIFOCAL, MILD-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
-HEMORRHAGE, MULTIFOCAL, MILD

LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E

-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, MULTIFOCAL, MILD

-MINERALIZATION, VASCULAR, FOCAL, MILD

-MACROPHAGES, NONPIGMENTED, MULTIFOCAL,
MINIMAL

LUNG, RIGHT CARDIAC LOBE, H&E

-INFLAMMATION, CHRONIC, FOCAL, MINIMAL

-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR,
PERIVASCULAR, FOCAL, MILD-MACROPHAGES, NONPIGMENTED, MULTIFOCAL,
MINIMAL

SPECIES: Wistar-han Rats
PROJECT NUMBER: TOX-209 (SW08-0144)

STAR Page: 7

Overall Incidence for Males
R. J. Reynolds Tobacco Company
Scheduled Health Screen, Histopathology, End Of Study

PROJECT NUMBER: TOX-209 (SW08-0144) SPECIES: Wistar-han Rats
Printed on 05-18-2008.

Tissue/ Diagnosis/ Modifier(s)	Health Screen -----
LUNG, LEFT LOBE, H&E	(10)
HEMORRHAGE	8
MULTIFOCAL, MINIMAL	3
MULTIFOCAL, MILD	3
MULTIFOCAL, MODERATE	2
INFILTRATION, LYMPHOCYTIC	9
PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD	1
PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD	8
MACROPHAGES, NONPIGMENTED	10
MULTIFOCAL, MINIMAL	6
MULTIFOCAL, MILD	4
MINERALIZATION, VASCULAR	4
FOCAL, MILD	3
MULTIFOCAL, MILD	1
LUNG, INTERMEDIATE LOBE, H&E	(10)
HEMORRHAGE	5
MULTIFOCAL, MINIMAL	4
MULTIFOCAL, MILD	1
INFILTRATION, LYMPHOCYTIC	10
PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MINIMAL	1
PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD	2
PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD	7
MACROPHAGES, NONPIGMENTED	10
MULTIFOCAL, MINIMAL	5
MULTIFOCAL, MILD	5
MINERALIZATION, VASCULAR	2
FOCAL, MILD	2
LUNG, RIGHT APICAL LOBE, H&E	(10)
CONGESTION	1
DIFFUSE, MINIMAL	1
HEMORRHAGE	5
FOCAL, MILD	1
MULTIFOCAL, MINIMAL	2
MULTIFOCAL, MILD	1
MULTIFOCAL, MODERATE	1
INFILTRATION, LYMPHOCYTIC	8
PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MINIMAL	1
PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD	1
PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD	6
MACROPHAGES, NONPIGMENTED	10
MULTIFOCAL, MINIMAL	3
MULTIFOCAL, MILD	7
MINERALIZATION, VASCULAR	1
FOCAL, MILD	1

() = Number Of Animals Examined For This Tissue

All modifiers are printed.

Microscopic Incidence Page: 1

Overall Incidence for Males (continued)
 R. J. Reynolds Tobacco Company
 Scheduled Health Screen, Histopathology, End Of Study

PROJECT NUMBER: TOX-209 (SW08-0144) SPECIES: Wistar-han Rats
 Printed on 05-18-2008.

Tissue/ Diagnosis/ Modifier(s)	Health Screen -----
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	(10)
HEMORRHAGE	3
FOCAL, MINIMAL	1
MULTIFOCAL, MILD	2
INFILTRATION, LYMPHOCYTIC	10
PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD	5
PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD	5
INFLAMMATION, CHRONIC	1
FOCAL, MINIMAL	1
MACROPHAGES, NONPIGMENTED	10
MULTIFOCAL, MINIMAL	3
MULTIFOCAL, MILD	7
MINERALIZATION, VASCULAR	3
FOCAL, MILD	3
LUNG, RIGHT CARDIAC LOBE, H&E	(10)
HEMORRHAGE	2
FOCAL, MINIMAL	1
MULTIFOCAL, MODERATE	1
INFILTRATION, LYMPHOCYTIC	8
PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD	5
PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD	3
INFLAMMATION, CHRONIC	1
FOCAL, MILD	1
MACROPHAGES, NONPIGMENTED	10
MULTIFOCAL, MINIMAL	4
MULTIFOCAL, MILD	6
MINERALIZATION, VASCULAR	1
FOCAL, MILD	1

() = Number Of Animals Examined For This Tissue

All modifiers are printed.

Microscopic Incidence Page: 2

Appendix V

Survival

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Dead Animal Status List for All Animals
Study number: TOX209A

PRINTED: 14-Oct-08
Page: 1

Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Animal Number	Grp	Sex	Study Phase	Date and Time Data was Entered	Oper. No.	Date of Phase Death	Phase Day	Death Typ	Status	Term. Body Wt. (g)	Ow	GrS
1	1	M	Exposure phase	05-May-08 07:58	203	05-May-08	20	s	Final phase sacrifice	270.3	-	-
2	1	M	Exposure phase	05-May-08 07:59	203	05-May-08	20	s	Final phase sacrifice	295.1	-	-
3	1	M	Exposure phase	05-May-08 08:00	203	05-May-08	20	s	Final phase sacrifice	302.9	-	-
4	1	M	Exposure phase	05-May-08 08:01	203	05-May-08	20	s	Final phase sacrifice	275.5	-	-
5	1	M	Exposure phase	05-May-08 08:01	203	05-May-08	20	s	Final phase sacrifice	264.7	-	-
6	2	M	Exposure phase	05-May-08 08:02	203	05-May-08	20	s	Final phase sacrifice	268.8	-	-
7	2	M	Exposure phase	05-May-08 08:02	203	05-May-08	20	s	Final phase sacrifice	311.5	-	-
8	2	M	Exposure phase	05-May-08 08:03	203	05-May-08	20	s	Final phase sacrifice	299.0	-	-
9	2	M	Exposure phase	05-May-08 08:03	203	05-May-08	20	s	Final phase sacrifice	269.8	-	-
10	2	M	Exposure phase	05-May-08 08:04	203	05-May-08	20	s	Final phase sacrifice	265.4	-	-
11	3	M	Exposure phase	05-May-08 08:04	203	05-May-08	20	s	Final phase sacrifice	270.1	-	-
12	3	M	Exposure phase	05-May-08 08:05	203	05-May-08	20	s	Final phase sacrifice	304.2	-	-
13	3	M	Exposure phase	05-May-08 08:05	203	05-May-08	20	s	Final phase sacrifice	271.2	-	-
14	3	M	Exposure phase	05-May-08 08:06	203	05-May-08	20	s	Final phase sacrifice	312.7	-	-
15	3	M	Exposure phase	05-May-08 08:06	203	05-May-08	20	s	Final phase sacrifice	258.3	-	-
16	4	M	Exposure phase	05-May-08 08:07	203	05-May-08	20	s	Final phase sacrifice	268.1	-	-
17	4	M	Exposure phase	05-May-08 08:08	203	05-May-08	20	s	Final phase sacrifice	302.8	-	-
18	4	M	Exposure phase	05-May-08 08:08	203	05-May-08	20	s	Final phase sacrifice	291.2	-	-
19	4	M	Exposure phase	05-May-08 08:09	203	05-May-08	20	s	Final phase sacrifice	249.6	-	-
20	4	M	Exposure phase	05-May-08 08:09	203	05-May-08	20	s	Final phase sacrifice	263.1	-	-
21	5	M	Exposure phase	05-May-08 08:10	203	05-May-08	20	s	Final phase sacrifice	265.4	-	-
22	5	M	Exposure phase	05-May-08 08:11	203	05-May-08	20	s	Final phase sacrifice	255.6	-	-
23	5	M	Exposure phase	05-May-08 08:11	203	05-May-08	20	s	Final phase sacrifice	287.5	-	-
24	5	M	Exposure phase	05-May-08 08:12	203	05-May-08	20	s	Final phase sacrifice	257.1	-	-
25	5	M	Exposure phase	05-May-08 08:12	203	05-May-08	20	s	Final phase sacrifice	229.3	-	-
26	6	M	Exposure phase	05-May-08 08:13	203	05-May-08	20	s	Final phase sacrifice	247.7	-	-
27	6	M	Exposure phase	05-May-08 08:13	203	05-May-08	20	s	Final phase sacrifice	206.5	-	-
28	6	M	Exposure phase	05-May-08 08:14	203	05-May-08	20	s	Final phase sacrifice	214.4	-	-
29	6	M	Exposure phase	05-May-08 08:14	203	05-May-08	20	s	Final phase sacrifice	220.9	-	-
30	6	M	Exposure phase	05-May-08 08:15	203	05-May-08	20	s	Final phase sacrifice	246.0	-	-
31	7	M	Exposure phase	05-May-08 08:16	203	05-May-08	20	s	Final phase sacrifice	220.1	-	-
32	7	M	Exposure phase	05-May-08 08:16	203	05-May-08	20	s	Final phase sacrifice	198.7	-	-
33	7	M	Exposure phase	05-May-08 08:17	203	05-May-08	20	s	Final phase sacrifice	216.0	-	-
34	7	M	Exposure phase	05-May-08 08:18	203	05-May-08	20	s	Final phase sacrifice	186.7	-	-
35	7	M	Exposure phase	05-May-08 08:18	203	05-May-08	20	s	Final phase sacrifice	199.1	-	-
36	8	M	Exposure phase	05-May-08 08:19	203	05-May-08	20	s	Final phase sacrifice	257.0	-	-
37	8	M	Exposure phase	05-May-08 08:19	203	05-May-08	20	s	Final phase sacrifice	300.1	-	-
38	8	M	Exposure phase	05-May-08 08:19	203	05-May-08	20	s	Final phase sacrifice	231.6	-	-
39	8	M	Exposure phase	05-May-08 08:20	203	05-May-08	20	s	Final phase sacrifice	303.4	-	-
40	8	M	Exposure phase	05-May-08 08:20	203	05-May-08	20	s	Final phase sacrifice	291.8	-	-
41	9	M	Exposure phase	05-May-08 08:21	203	05-May-08	20	s	Final phase sacrifice	246.1	-	-
42	9	M	Exposure phase	05-May-08 08:22	203	05-May-08	20	s	Final phase sacrifice	296.0	-	-
43	9	M	Exposure phase	05-May-08 08:22	203	05-May-08	20	s	Final phase sacrifice	288.3	-	-
44	9	M	Exposure phase	05-May-08 08:23	203	05-May-08	20	s	Final phase sacrifice	275.8	-	-
45	9	M	Exposure phase	05-May-08 08:23	203	05-May-08	20	s	Final phase sacrifice	277.0	-	-

Note: * = pretest animal no. P = partial data. C = complete data. - = no data.

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Dead Animal Status List for All Animals
Study number: TOX209A

PRINTED: 14-Oct-08
Page: 2

Dosing start date: 16-Apr-08

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
46	10	M	Exposure phase	05-May-08 08:23	203	05-May-08	20	s	Final phase sacrifice	280.3	-	-
47	10	M	Exposure phase	05-May-08 08:24	203	05-May-08	20	s	Final phase sacrifice	280.5	-	-
48	10	M	Exposure phase	05-May-08 08:24	203	05-May-08	20	s	Final phase sacrifice	253.4	-	-
49	10	M	Exposure phase	05-May-08 08:25	203	05-May-08	20	s	Final phase sacrifice	249.4	-	-
50	10	M	Exposure phase	05-May-08 08:26	203	05-May-08	20	s	Final phase sacrifice	266.0	-	-
51	11	M	Exposure phase	05-May-08 08:27	203	05-May-08	20	s	Final phase sacrifice	296.0	-	-
52	11	M	Exposure phase	05-May-08 08:27	203	05-May-08	20	s	Final phase sacrifice	244.1	-	-
53	11	M	Exposure phase	05-May-08 08:28	203	05-May-08	20	s	Final phase sacrifice	266.2	-	-
54	11	M	Exposure phase	05-May-08 08:28	203	05-May-08	20	s	Final phase sacrifice	262.7	-	-
55	11	M	Exposure phase	05-May-08 08:29	203	05-May-08	20	s	Final phase sacrifice	262.5	-	-
56	12	M	Exposure phase	05-May-08 08:29	218	05-May-08	20	s	Final phase sacrifice	239.9	-	-
57	12	M	Exposure phase	05-May-08 08:30	218	05-May-08	20	s	Final phase sacrifice	255.2	-	-
58	12	M	Exposure phase	05-May-08 08:31	218	05-May-08	20	s	Final phase sacrifice	238.5	-	-
59	12	M	Exposure phase	05-May-08 08:32	218	05-May-08	20	s	Final phase sacrifice	262.8	-	-
60	12	M	Exposure phase	05-May-08 08:33	218	05-May-08	20	s	Final phase sacrifice	281.6	-	-
61	13	M	Exposure phase	05-May-08 08:34	218	05-May-08	20	s	Final phase sacrifice	209.6	-	-
62	13	M	Exposure phase	05-May-08 08:35	218	05-May-08	20	s	Final phase sacrifice	197.7	-	-
63	13	M	Exposure phase	05-May-08 08:36	218	05-May-08	20	s	Final phase sacrifice	216.0	-	-
64	13	M	Exposure phase	05-May-08 08:36	218	05-May-08	20	s	Final phase sacrifice	214.1	-	-
65	13	M	Exposure phase	05-May-08 08:37	218	05-May-08	20	s	Final phase sacrifice	193.9	-	-

Note: * = pretest animal no. P = partial data. C = complete data. - = no data.

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Group Survival and Mortality Table - Males

Study number: TOX209A

Deaths: All

Dosing start date: 16-Apr-08

PRINTED: 14-Oct-08

Page: 1

FEEDING STUDY/PALATABILITY

		D a y o f P h a s e																						
mg/kg		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
0.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
0.2	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
2.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
4.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
8.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
20.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
40.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
0.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
0.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
0.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
0.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0

0.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0

+
 Key a = number animals alive at the start of each study day
 b = number of mortalities during each study day
 Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Group Survival and Mortality Table - Males

Study number: TOX209A

Deaths: All

Dosing start date: 16-Apr-08

PRINTED: 14-Oct-08

Page: 2

FEEDING STUDY/PALATABILITY

		D a y o f P h a s e																						
mg/kg		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
0.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
0.2	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
2.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
4.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
8.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
20.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
40.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
0.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
0.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
0.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
0.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0

0.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0

+
 Key a = number animals alive at the start of each study day
 b = number of mortalities during each study day
 Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Group Survival and Mortality Table - Males

Study number: TOX209A

Deaths: All

Dosing start date: 16-Apr-08

PRINTED: 14-Oct-08

Page: 3

FEEDING STUDY/PALATABILITY

		D a y o f P h a s e																						
mg/kg		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
0.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
0.2	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
2.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
4.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
8.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
20.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
40.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
0.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
0.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
0.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
0.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0

0.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0

+

Key a = number animals alive at the start of each study day
b = number of mortalities during each study day
Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Dead Animal Status List for All Animals
Study number: TOX209B

PRINTED: 14-Oct-08
Page: 1

Dosing start date: 16-Apr-08

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
66	1	M	Exposure phase	05-May-08 08:09	218	05-May-08	20	s	Final phase sacrifice	260.2	-	-
67	1	M	Exposure phase	05-May-08 08:10	218	05-May-08	20	s	Final phase sacrifice	263.4	-	-
68	1	M	Exposure phase	05-May-08 08:11	218	05-May-08	20	s	Final phase sacrifice	260.1	-	-
69	1	M	Exposure phase	05-May-08 08:12	218	05-May-08	20	s	Final phase sacrifice	295.3	-	-
70	1	M	Exposure phase	05-May-08 08:13	218	05-May-08	20	s	Final phase sacrifice	303.5	-	-
71	2	M	Exposure phase	05-May-08 08:14	218	05-May-08	20	s	Final phase sacrifice	262.1	-	-
72	2	M	Exposure phase	05-May-08 08:15	218	05-May-08	20	s	Final phase sacrifice	305.6	-	-
73	2	M	Exposure phase	05-May-08 08:16	218	05-May-08	20	s	Final phase sacrifice	272.0	-	-
74	2	M	Exposure phase	05-May-08 08:17	218	05-May-08	20	s	Final phase sacrifice	243.0	-	-
75	2	M	Exposure phase	05-May-08 08:18	218	05-May-08	20	s	Final phase sacrifice	247.4	-	-
76	3	M	Exposure phase	05-May-08 08:18	218	05-May-08	20	s	Final phase sacrifice	208.9	-	-
77	3	M	Exposure phase	05-May-08 08:19	218	05-May-08	20	s	Final phase sacrifice	214.6	-	-
78	3	M	Exposure phase	05-May-08 08:20	218	05-May-08	20	s	Final phase sacrifice	244.7	-	-
79	3	M	Exposure phase	05-May-08 08:21	218	05-May-08	20	s	Final phase sacrifice	239.0	-	-
80	3	M	Exposure phase	05-May-08 08:22	218	05-May-08	20	s	Final phase sacrifice	261.2	-	-
81	4	M	Exposure phase	05-May-08 08:22	218	05-May-08	20	s	Final phase sacrifice	203.3	-	-
82	4	M	Exposure phase	05-May-08 08:23	218	05-May-08	20	s	Final phase sacrifice	216.9	-	-
83	4	M	Exposure phase	05-May-08 08:24	218	05-May-08	20	s	Final phase sacrifice	134.6	-	-
84	4	M	Exposure phase	05-May-08 08:25	218	05-May-08	20	s	Final phase sacrifice	161.9	-	-
85	4	M	Exposure phase	05-May-08 08:26	218	05-May-08	20	s	Final phase sacrifice	179.6	-	-

Note: * = pretest animal no. P = partial data. C = complete data. - = no data.

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Building 630/2
RAT/WISTAR HANOVER

Group Survival and Mortality Table - Males

Study number: TOX209B

Deaths: All

Dosing start date: 16-Apr-08

PRINTED: 14-Oct-08

Page: 1

FEEDING STUDY/PALATABILITY

		D a y o f P h a s e																						
mg/kg		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
2.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
8.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
20.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
40.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
0.0	a	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Key a = number animals alive at the start of each study day

b = number of mortalities during each study day

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Group Survival and Mortality Table - Males

Study number: TOX209B

Deaths: All

Dosing start date: 16-Apr-08

PRINTED: 14-Oct-08

Page: 2

FEEDING STUDY/PALATABILITY

		D a y o f P h a s e																						
mg/kg		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
2.0		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
8.0		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
20.0		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
40.0		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
0.0		10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	a	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Key a = number animals alive at the start of each study day

b = number of mortalities during each study day

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Group Survival and Mortality Table - Males
Study number: TOX209B
Deaths: All
Dosing start date: 16-Apr-08

PRINTED: 14-Oct-08
Page: 3

FEEDING STUDY/PALATABILITY

		D a y o f P h a s e																							
mg/kg		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
+																									
	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0	
2.0	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	
	c	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	5	5	
+																									
	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
8.0	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	
	c	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	5	5	
+																									
	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
20.0	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	
	c	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	5	5	
+																									
	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	0
40.0	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	
	c	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	5	5	
+																									
	a	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
0.0	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	c	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
+																									

Key a = number animals alive at the start of each study day
b = number of mortalities during each study day
c = cumulative number of animals dead at start of each study day
Note: Data for Exposure phase

Appendix VI

Clinical Observations

Interval: 3! - 17" Days														
Group	1		2		3		Males 4		5		6		7	
Observation	(5)		(5)		(5)		(5)		(5)		(5)		(5)	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Normal														
Normal/no visible abnormalities	5	22.0	5	22.0	5	22.0	5	22.0	5	22.0	5	22.0	5	22.0

Key: () = Number of animals alive at start of interval
a = Number animals affected
b = Mean number of animal days with clinical sign
Note: ! = Quarantine/Acclimation; " = Exposure phase

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Building 630/2
RAT/WISTAR HANOVER

Summary of Clinical Signs
Study number: TOX209A

PRINTED: 02-Oct-08
Page: 2

Dosing start date: 16-Apr-08

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	22.0	5	21.2	5	22.0	5	22.0	5	22.0	5	22.0

Key: () = Number of animals alive at start of interval
a = Number animals affected
b = Mean number of animal days with clinical sign
Note: ! = Quarantine/Acclimation; " = Exposure phase

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TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Summary of Clinical Signs
Study number: TOX209B

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Page: 1

Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

+ Interval: 3! - 19" Days										
Group	1		2		Males 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	100.0	5	100.0	5	100.0	5	100.0	0	0.0
Body surface										
Thin/emaciated	1	20.0	0	0.0	0	0.0	1	20.0	0	0.0
Hair loss (alopecia)	1	20.0	0	0.0	0	0.0	0	0.0	0	0.0
Abrasion	1	20.0	0	0.0	0	0.0	0	0.0	0	0.0

Key: () = Number of animals alive at start of interval

a = Number animals affected

b = Percent of animals with observation during interval

Note: ! = Quarantine/Acclimation; " = Exposure phase

Appendix VII

Body Weights

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RAT/WISTAR HANOVER

Animal body weights in (g)
Study number: TOX209A

PRINTED: 11-Nov-08
Page: 1

Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Animal Group		Day of Phase													
		6!	1"	2	6	7	8	9	10	11	12	13	14	15	
Male Animals															
1	1	165.57	166.69	170.72	197.56	204.12	208.65	213.33	220.15	224.31	227.79	235.27	244.26	249.88	
2		192.60	202.79	205.19	210.84	214.47	215.89	228.18	236.56	242.97	250.96	257.39	263.57	268.21	
3		220.29	212.80	214.20	233.27	227.97	243.39	250.32	254.12	261.20	267.59	271.32	275.37	285.14	
4		174.50	185.13	190.96	186.89	221.87	228.85	233.59	234.74	240.80	243.26	249.70	254.54	260.60	
5		178.23	181.47	189.80	202.66	189.57	189.79	207.67	213.40	220.34	226.36	233.49	239.93	241.89	
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5	
	Means	186.24	189.78	194.17	206.24	211.60	217.31	226.62	231.79	237.92	243.19	249.43	255.53	261.14	
	Sdevs	21.38	18.19	16.60	17.42	15.19	20.30	16.94	15.84	16.36	17.15	15.79	14.40	16.76	
6	2	181.96	187.21	191.31	202.76	201.65	197.94	200.77	202.63	201.89	204.40	211.88	230.24	237.89	
7		211.72	215.69	216.22	243.53	237.74	235.78	234.40	233.93	233.00	250.06	265.52	275.16	279.49	
8		195.91	203.89	206.24	230.00	234.12	238.66	244.31	248.07	250.60	255.54	263.16	270.88	275.04	
9		157.96	163.43	167.69	196.08	203.29	210.28	215.99	219.80	224.46	230.16	238.74	244.11	248.99	
10		173.37	182.36	185.63	216.36	227.12	230.00	234.57	237.99	224.49	220.57	250.86	247.02	258.63	
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5	
	Means	184.18	190.52	193.42	217.75	220.78	222.53	226.01	228.48	226.89	232.15	246.03	253.48	260.01	
	Sdevs	20.65	20.15	18.78	19.44	17.16	17.65	17.44	17.66	17.58	21.07	21.89	18.99	17.45	
11	3	181.58	187.59	185.71	220.37	217.54	225.73	228.89	233.07	232.79	241.55	248.97	252.00	257.73	
12		193.41	201.42	208.69	238.65	241.01	247.22	254.10	256.16	263.69	266.61	276.77	282.18	283.55	
13		177.46	183.44	181.71	194.10	213.33	217.99	228.36	233.78	239.16	241.86	251.77	254.97	259.15	
14		218.54	224.70	223.73	259.03	260.58	264.16	269.79	270.71	274.56	276.91	286.27	291.50	295.14	
15		160.85	168.85	176.03	190.59	195.37	192.60	197.11	211.81	219.04	226.69	229.63	236.12	238.64	
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5	
	Means	186.37	193.20	195.17	220.55	225.57	229.54	235.65	241.11	245.85	250.72	258.68	263.35	266.84	
	Sdevs	21.44	21.09	20.22	29.18	25.45	27.49	27.79	22.80	22.79	20.48	22.77	22.84	22.46	
16	4	188.62	194.42	199.82	220.26	218.91	221.27	222.43	220.85	221.53	233.64	241.65	250.26	251.64	
17		212.79	217.55	224.93	239.40	240.60	244.55	256.66	256.63	259.17	267.43	274.82	274.64	280.36	
18		191.73	198.71	200.23	233.59	233.52	235.50	240.27	244.19	250.48	249.77	257.55	266.01	267.56	
19		155.79	164.01	169.28	195.37	184.19	182.40	181.88	191.94	195.96	202.76	218.18	222.17	225.24	
20		173.56	179.50	186.76	208.18	208.79	210.37	220.83	225.37	228.92	230.83	236.99	243.06	250.11	
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5	
	Means	184.50	190.84	196.20	219.36	217.20	218.82	224.41	227.80	231.21	236.89	245.84	251.23	254.98	
	Sdevs	21.29	20.23	20.42	18.07	22.23	24.21	27.92	24.70	24.97	24.04	21.44	20.49	20.75	
21	5	213.51	217.83	223.80	247.24	240.14	241.80	245.20	245.35	250.24	251.67	254.96	255.32	259.38	
22		168.87	175.76	174.50	208.70	198.65	199.89	196.72	193.48	192.20	200.36	201.72	222.64	222.87	
23		200.66	205.38	207.83	234.22	232.28	234.05	240.09	245.75	248.49	258.39	261.83	266.24	271.03	
24		180.12	185.85	183.13	219.20	216.97	220.07	221.65	221.94	227.05	229.74	236.08	241.15	243.96	

Note: ! = Quarantine/Acclimation; " = Exposure phase

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Building 630/2
RAT/WISTAR HANOVER

Animal body weights in (g)
Study number: TOX209A

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Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Animal	Group	D a y o f P h a s e													
		6!	1"	2	6	7	8	9	10	11	12	13	14	15	
25	5		172.18	179.11	177.87	205.62	201.21	204.37	200.10	197.77	210.74	204.97	207.37	216.94	219.35
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	
		Means	187.07	192.79	193.43	223.00	217.85	220.04	220.75	220.86	225.74	229.03	232.39	240.46	243.32
		Sdevs	19.27	18.10	21.43	17.57	18.38	18.18	22.23	25.02	24.85	26.34	27.19	20.96	22.47
26	6		214.35	218.49	220.42	235.27	233.83	228.68	234.50	236.55	234.18	233.30	234.23	236.44	238.10
27			149.25	154.41	157.42	181.70	178.75	180.73	181.47	181.15	185.21	186.79	194.11	194.16	195.58
28			174.53	179.27	178.65	212.95	205.32	203.33	202.87	203.45	202.44	205.68	210.46	206.06	208.20
29			181.59	191.29	197.77	195.78	198.11	195.08	192.42	192.13	193.87	196.47	201.99	204.82	203.58
30		203.39	206.58	203.80	237.74	231.92	225.98	235.73	227.53	231.80	240.37	242.95	225.40	234.07	
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5	
	Means	184.62	190.01	191.61	212.69	209.59	206.76	209.40	208.16	209.50	212.52	216.75	213.38	215.91	
	Sdevs	25.49	24.85	24.26	24.41	23.38	20.47	24.67	23.40	22.31	23.31	20.99	17.12	19.02	
31	7		198.96	203.88	206.43	242.29	227.19	219.04	214.22	213.25	214.35	215.31	213.58	217.66	216.13
32			175.20	181.01	181.61	209.41	200.75	198.01	195.78	195.28	192.60	192.47	196.50	193.07	190.63
33			214.46	216.75	217.75	252.10	239.78	234.23	226.70	221.52	222.31	222.94	226.73	218.50	215.83
34			184.25	194.48	187.08	205.20	204.88	197.13	193.34	187.78	188.69	185.40	186.60	181.81	181.22
35		161.76	173.35	174.03	196.56	197.62	195.25	187.64	182.24	180.18	183.45	186.05	184.28	184.59	
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5	
	Means	186.93	193.89	193.38	221.11	214.04	208.73	203.54	200.01	199.63	199.91	201.89	199.06	197.68	
	Sdevs	20.50	17.40	18.14	24.50	18.48	17.23	16.34	16.78	17.88	18.06	17.80	17.86	17.04	
36	8		177.49	184.27	187.85	217.16	220.99	222.76	217.98	225.42	220.48	232.26	236.12	240.31	244.91
37			219.97	229.30	224.64	252.41	269.36	260.97	271.47	272.37	281.36	283.95	287.78	295.83	299.65
38			158.38	163.63	160.77	186.78	192.01	194.40	198.48	201.14	204.07	206.44	211.81	216.35	219.67
39			198.02	204.55	211.46	238.93	245.46	250.42	255.66	260.68	260.63	269.00	264.57	275.19	282.43
40		183.30	192.45	194.65	226.34	225.68	231.49	240.35	245.61	245.36	252.81	258.31	265.86	266.66	
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5	
	Means	187.43	194.84	195.87	224.32	230.70	232.01	236.79	241.04	242.38	248.89	251.72	258.71	262.66	
	Sdevs	23.08	24.36	24.33	24.83	28.85	25.87	29.12	28.39	30.86	30.53	28.92	30.96	31.38	
41	9		148.90	157.45	163.35	177.83	186.85	189.13	190.84	199.89	205.99	209.10	220.49	221.85	228.68
42			204.41	209.34	212.19	244.12	226.73	224.90	240.46	248.67	253.97	262.63	270.46	275.63	277.99
43			182.38	189.64	188.75	229.79	225.24	233.17	242.41	247.25	251.10	255.17	262.67	267.86	273.34
44			172.76	181.09	188.01	215.96	213.36	221.33	227.23	229.82	233.43	240.42	249.30	251.15	253.95
45		192.53	195.00	203.10	226.32	222.56	229.77	235.32	236.76	240.40	245.62	251.38	252.20	257.96	
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5	
	Means	180.20	186.50	191.08	218.80	214.95	219.66	227.25	232.48	236.98	242.59	250.86	253.74	258.38	
	Sdevs	21.08	19.22	18.53	25.03	16.54	17.66	21.18	19.80	19.19	20.59	19.03	20.64	19.43	

Note: ! = Quarantine/Acclimation; " = Exposure phase

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Building 630/2
RAT/WISTAR HANOVER

Animal body weights in (g)
Study number: TOX209A

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Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Animal	Group	D a y o f P h a s e												
		6!	1"	2	6	7	8	9	10	11	12	13	14	15

		M a l e						A n i m a l s						
46	10	187.31	193.28	194.09	228.21	227.69	229.91	238.27	238.22	243.75	245.44	255.24	257.82	261.71
47		192.79	195.16	197.94	224.93	222.43	223.54	235.26	236.11	240.74	245.64	250.67	254.76	257.68
48		217.16	180.97	183.48	198.63	203.65	215.67	212.26	225.58	226.62	232.02	244.37	249.86	252.52
49		157.12	161.34	166.14	194.48	198.05	203.63	205.39	209.79	214.33	216.82	226.11	229.55	232.28
50		172.27	212.04	208.79	230.49	228.12	228.91	229.64	231.88	236.55	239.82	241.45	248.07	247.02
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	185.33	188.56	190.09	215.35	215.99	220.33	224.16	228.32	232.40	235.95	243.57	248.01	250.24
	Sdevs	22.58	18.81	16.16	17.33	14.14	10.91	14.55	11.43	12.00	12.05	11.14	11.02	11.46
51	11	214.59	211.24	219.41	248.59	241.92	242.18	248.54	245.12	246.09	260.69	262.77	267.02	272.14
52		160.59	167.06	173.24	187.01	188.23	199.85	205.78	214.43	221.19	224.16	227.77	232.94	233.85
53		200.85	204.94	212.67	229.00	224.92	223.50	233.12	241.31	240.54	246.13	252.96	258.33	258.29
54		169.87	175.08	180.75	207.49	205.37	211.70	215.71	225.57	226.86	229.81	235.05	240.04	243.49
55		181.02	184.04	192.09	216.75	216.04	223.50	225.10	230.42	234.58	236.93	242.11	245.74	247.84
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	185.38	188.47	195.63	217.77	215.30	220.15	225.65	231.37	233.85	239.54	244.13	248.81	251.12
	Sdevs	22.17	19.02	19.94	23.07	20.21	15.74	16.38	12.34	10.05	14.39	13.96	13.79	14.67
56	12	208.26	204.60	205.98	220.20	215.40	215.46	214.93	216.88	220.05	225.01	228.12	225.10	224.99
57		172.99	180.74	182.98	202.47	206.16	205.45	209.18	218.28	223.03	223.93	231.15	237.33	239.38
58		161.85	172.85	174.57	205.37	203.04	204.15	205.04	210.19	212.43	216.99	225.04	225.52	228.36
59		191.85	197.81	193.85	227.54	224.32	224.93	226.18	231.60	235.96	236.37	241.80	244.59	247.32
60		190.07	193.65	199.52	236.02	227.47	233.34	235.18	236.16	238.33	242.79	248.76	253.64	256.07
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	185.00	189.93	191.38	218.32	215.28	216.67	218.10	222.62	225.96	229.02	234.97	237.24	239.22
	Sdevs	17.98	12.92	12.64	14.32	10.76	12.55	12.42	10.84	10.95	10.37	9.96	12.33	12.94
61	13	180.87	185.82	187.11	196.73	197.49	195.68	204.96	202.78	207.53	203.75	213.91	206.81	209.79
62		169.58	171.22	180.50	205.99	198.44	197.45	200.33	204.42	207.40	203.34	212.85	205.70	202.84
63		200.04	206.20	200.88	224.62	225.37	224.63	228.21	221.05	224.88	219.91	233.44	222.89	212.64
64		205.88	209.41	208.66	242.75	228.85	231.60	231.59	229.20	235.43	230.95	237.06	227.03	221.92
65		155.93	160.81	161.70	194.61	190.00	189.86	191.61	193.73	193.11	198.12	198.45	197.69	196.41
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	182.46	186.69	187.77	212.94	208.03	207.84	211.34	210.24	213.67	211.21	219.14	212.02	208.72
	Sdevs	20.80	21.25	18.31	20.45	17.76	18.88	17.65	14.48	16.57	13.73	15.97	12.41	9.71

Note: ! = Quarantine/Acclimation; " = Exposure phase

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TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Animal body weights in (g)
Study number: TOX209A

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Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Animal Group		Day of Phase			
		16	17	18	19
Male Animals					
1	1	255.83	257.10	257.37	259.94
2		272.11	271.53	278.01	281.94
3		284.80	293.49	292.06	294.66
4		262.01	270.26	268.29	268.16
5		245.64	249.30	259.10	256.69
	(n)	5	5	5	5
	Means	264.08	268.34	270.97	272.28
	Sdevs	15.05	16.85	14.38	15.86
6	2	246.44	252.80	260.84	252.53
7		284.24	290.77	292.87	294.83
8		277.96	283.88	285.73	286.34
9		248.27	256.25	255.82	259.72
10		256.98	263.35	271.21	268.37
	(n)	5	5	5	5
	Means	262.78	269.41	273.29	272.36
	Sdevs	17.34	16.97	15.83	17.81
11	3	259.89	262.83	262.42	267.54
12		289.73	293.22	294.91	298.51
13		258.94	265.64	263.16	263.36
14		298.28	299.98	303.82	306.99
15		241.92	244.78	250.87	250.62
	(n)	5	5	5	5
	Means	269.75	273.29	275.04	277.40
	Sdevs	23.46	22.86	22.95	24.15
16	4	253.21	260.61	257.30	264.55
17		284.67	286.73	289.79	297.09
18		266.50	274.97	275.18	283.06
19		226.43	236.18	236.23	243.99
20		251.71	254.04	253.22	257.67
	(n)	5	5	5	5
	Means	256.50	262.51	262.34	269.27
	Sdevs	21.40	19.43	20.67	20.98
21	5	260.15	265.58	260.76	264.97
22		230.51	238.39	244.28	243.87
23		275.08	278.16	277.90	279.46
24		243.18	247.66	246.39	253.52

Note: Data for Exposure phase

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Building 630/2
RAT/WISTAR HANOVER

Animal body weights in (g)
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Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Animal Group		Day of Phase			
		16	17	18	19
Male Animals					
25	5	221.85	220.40	222.36	227.91
	(n)	5	5	5	5
	Means	246.15	250.04	250.34	253.95
	Sdevs	21.66	22.65	20.64	19.70
26	6	241.31	246.11	242.89	244.76
27		193.73	198.33	197.56	199.70
28		203.03	212.00	208.94	214.25
29		209.47	212.00	220.67	214.28
30		246.68	243.69	256.70	253.38
	(n)	5	5	5	5
	Means	218.84	222.43	225.35	225.27
	Sdevs	23.71	21.28	24.26	22.73
31	7	218.42	217.19	212.62	207.65
32		191.37	189.57	195.86	194.10
33		211.36	216.94	219.22	216.40
34		179.23	183.93	182.19	182.40
35		184.33	189.96	190.47	195.38
	(n)	5	5	5	5
	Means	196.94	199.52	200.07	199.19
	Sdevs	17.12	16.20	15.44	13.13
36	8	242.95	248.70	248.60	252.16
37		298.15	306.14	304.79	305.50
38		218.92	226.03	226.03	228.22
39		287.18	292.48	295.82	298.84
40		276.69	282.56	280.31	283.42
	(n)	5	5	5	5
	Means	264.78	271.18	271.11	273.63
	Sdevs	32.93	32.97	33.05	32.67
41	9	227.97	236.91	236.69	239.49
42		275.01	283.45	285.91	290.78
43		273.75	277.19	277.03	276.94
44		259.71	262.05	264.11	270.03
45		261.05	266.47	267.33	271.43
	(n)	5	5	5	5
	Means	259.50	265.21	266.21	269.73
	Sdevs	18.98	17.95	18.59	18.79

Note: Data for Exposure phase

R.J.R. TOBACCO
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Building 630/2
RAT/WISTAR HANOVER

Animal body weights in (g)
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FEEDING STUDY/PALATABILITY

Animal Group		D a y o f P h a s e			
		16	17	18	19
Male Animals					
46	10	262.85	268.79	268.73	273.72
47		265.62	268.70	267.82	269.30
48		257.52	249.69	231.23	235.57
49		234.58	239.83	237.21	239.16
50		250.82	257.18	256.13	259.35
	(n)	5	5	5	5
	Means	254.28	256.84	252.22	255.42
	Sdevs	12.38	12.49	17.30	17.33
51	11	273.32	284.37	282.33	283.58
52		238.93	244.67	244.92	245.36
53		258.42	263.89	261.72	266.47
54		247.20	252.01	251.50	255.71
55		252.11	254.62	254.91	256.81
	(n)	5	5	5	5
	Means	254.00	259.91	259.08	261.59
	Sdevs	12.94	15.30	14.34	14.39
56	12	225.46	225.10	227.51	230.25
57		241.29	245.41	245.31	249.10
58		225.68	231.61	227.15	229.41
59		251.11	251.43	253.30	254.92
60		255.28	263.87	258.29	263.69
	(n)	5	5	5	5
	Means	239.76	243.48	242.31	245.47
	Sdevs	13.92	15.50	14.44	15.20
61	13	208.58	209.18	203.97	204.92
62		198.25	192.64	192.52	194.50
63		213.66	212.13	212.86	213.83
64		221.78	223.25	220.92	217.99
65		194.94	195.09	193.14	192.85
	(n)	5	5	5	5
	Means	207.44	206.46	204.68	204.82
	Sdevs	11.03	12.67	12.37	11.23

Note: Data for Exposure phase

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RAT/WISTAR HANOVER

Animal body weights in (g)
Study number: TOX209B

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Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Animal	Group	D a y o f P h a s e												
		6!	1"	2	6	7	8	9	10	11	12	13	14	15

		M a l e A n i m a l s												
66	1	161.33	168.13	175.72	199.15	203.23	207.82	213.46	220.10	223.36	228.39	233.21	234.60	243.90
67		209.05	213.85		175.38				193.32	204.93	211.91	221.64	225.17	232.43
68		172.06	177.36	180.49	197.51	203.60	214.38	213.72	222.56	223.68	224.19	235.97	243.01	246.85
69		201.53	206.95	210.32	235.76	244.35	247.01	252.83	257.52	258.06	262.21	266.86	272.41	275.73
70		203.36	212.48	207.52	247.29	249.76	252.78	256.40	262.12	262.13	267.91	272.66	281.58	285.02
	(n)	5	5	4	5	4	4	4	5	5	5	5	5	5
	Means	189.47	195.75	193.51	211.02	225.24	230.50	234.10	231.12	234.43	238.92	246.07	251.35	256.79
	Sdevs	21.31	21.41	17.93	29.67	25.29	22.68	23.73	28.64	24.67	24.70	22.38	24.46	22.44
71	2	196.63	203.76		209.13	224.64	214.34	225.99	222.32	231.78	234.86	241.87	237.12	235.98
72		207.19	229.89	225.09	266.34	264.59	267.86	271.98	277.65	279.65	278.03	285.84	292.00	295.28
73		197.68	201.40	209.58	236.09	230.88	232.85	239.59	240.90	242.94	245.41	251.97	255.10	259.47
74		155.11	160.23	163.65	191.22	190.85	187.04	201.74	208.49	213.07	213.75	220.89	223.44	228.58
75		183.19	181.37	182.92	210.18	209.05	213.73	214.40	220.08	222.69	226.53	230.72	233.56	236.66
	(n)	5	5	4	5	5	5	5	5	5	5	5	5	5
	Means	187.96	195.33	195.31	222.59	224.00	223.16	230.74	233.89	238.03	239.72	246.26	248.24	251.19
	Sdevs	20.26	26.12	27.36	29.22	27.47	29.85	26.97	27.08	25.76	24.35	25.02	27.00	27.23
76	3	173.00	177.58	177.41	207.94	203.05	203.14	202.79	204.33	204.35	203.13	204.15	203.89	205.00
77		191.69	199.02	184.39	200.56	205.41	199.83	199.08	201.09	198.33	205.65	206.69	207.48	205.22
78		180.16	185.38	189.95	211.54	209.27	209.18	211.12	215.75	218.32	221.71	228.67	228.34	235.53
79		180.70	188.90	184.81	215.36	211.40	215.10	216.17	216.13	212.75	216.98	220.48	220.34	227.82
80		204.48	211.78	215.59	242.07	239.31	238.61	236.81	235.29	235.01	234.95	239.47	240.38	248.88
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	186.01	192.53	190.43	215.49	213.69	213.17	213.19	214.52	213.75	216.48	219.89	220.09	224.49
	Sdevs	12.30	13.23	14.76	15.83	14.69	15.37	14.82	13.41	14.14	12.89	14.86	15.01	19.23
81	4	189.72	197.82	195.45	230.59	222.41	219.53	215.24	212.77	205.29	206.79	205.18	205.61	207.62
82		203.71	214.10	219.43	247.11	232.54	229.98	224.82	226.49	223.97	225.50	222.58	216.47	220.06
83		205.04	150.41	153.53	175.52	167.05	162.47	160.35	155.84	149.80	146.12	145.65	143.57	141.40
84		170.36	174.96	172.02	193.02	184.32	178.73	176.47	178.12	170.97	171.63	172.26	169.28	169.19
85		189.94	194.59	199.35	228.70	217.57	208.67	204.38	202.68	197.77	199.81	197.96	197.46	190.51
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	191.75	186.38	187.96	214.99	204.78	199.88	196.25	195.18	189.56	189.97	188.73	186.48	185.76
	Sdevs	14.01	24.45	25.57	29.60	27.78	28.36	27.03	28.22	29.26	31.23	30.12	29.68	31.30

Note: ! = Quarantine/Acclimation; " = Exposure phase

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Building 630/2
RAT/WISTAR HANOVER

Animal body weights in (g)
Study number: TOX209B

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FEEDING STUDY/PALATABILITY

Animal Group		Day of Phase			
		16	17	18	19
Male Animals					
66	1	245.22	251.18	251.44	255.69
67		239.32	246.53	245.42	252.32
68		240.98	241.78	247.17	252.35
69		279.51	285.34	280.89	287.50
70		287.82	291.63	295.88	300.86
	(n)	5	5	5	5
	Means	258.57	263.29	264.16	269.74
	Sdevs	23.20	23.34	22.85	22.84
71	2	249.24	253.34	256.76	255.01
72		296.91	301.51	295.74	299.82
73		264.56	267.36	264.26	267.15
74		229.66	233.99	232.82	239.44
75		236.26	245.15	245.97	247.62
	(n)	5	5	5	5
	Means	255.33	260.27	259.11	261.81
	Sdevs	26.80	26.07	23.65	23.57
76	3	207.21	202.78	209.18	206.02
77		210.04	208.98	213.22	212.18
78		234.87	241.54	238.66	242.22
79		229.07	234.39	234.20	236.02
80		243.52	248.88	250.29	243.62
	(n)	5	5	5	5
	Means	224.94	227.31	229.11	228.01
	Sdevs	15.79	20.34	17.43	17.63
81	4	204.40	204.72	202.52	201.11
82		219.78	212.16	218.54	217.78
83		137.85	136.21	131.28	134.97
84		167.20	164.61	163.26	161.30
85		187.20	185.73	182.26	185.57
	(n)	5	5	5	5
	Means	183.29	180.69	179.57	180.15
	Sdevs	32.08	30.94	34.10	32.72

Note: Data for Exposure phase

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RAT/WISTAR HANOVER

Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209A
Quarantine/Acclimation (Day 6)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

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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	186.24	184.18	186.37	184.50	187.07	184.62	186.93	187.43	180.20	185.33	185.38	185.00	182.46
Standard deviation	21.38	20.65	21.44	21.29	19.27	25.49	20.50	23.08	21.08	22.58	22.17	17.98	20.80
Group diff.@ P=.05		38.94	38.94	38.94	38.94	38.94	38.94	38.94	38.94	38.94	38.94	38.94	38.94
Group diff.@ P=.01		47.00	47.00	47.00	47.00	47.00	47.00	47.00	47.00	47.00	47.00	47.00	47.00

Analysis of variance: F ratio = 0.04 Df = 12/ 52 F probability = 1.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209A
Exposure phase (Day 1)
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FEEDING STUDY/PALATABILITY

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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	189.78	190.52	193.20	190.84	192.79	190.01	193.89	194.84	186.50	188.56	188.47	189.93	186.69
Standard deviation	18.19	20.15	21.09	20.23	18.10	24.85	17.40	24.36	19.22	18.81	19.02	12.92	21.25
Group diff.@ P=.05		36.11	36.11	36.11	36.11	36.11	36.11	36.11	36.11	36.11	36.11	36.11	36.11
Group diff.@ P=.01		43.58	43.58	43.58	43.58	43.58	43.58	43.58	43.58	43.58	43.58	43.58	43.58

Analysis of variance: F ratio = 0.09 Df = 12/ 52 F probability = 1.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209A
Exposure phase (Day 2)
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FEEDING STUDY/PALATABILITY

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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	194.17	193.42	195.17	196.20	193.43	191.61	193.38	195.87	191.08	190.09	195.63	191.38	187.77
Standard deviation	16.60	18.78	20.22	20.42	21.43	24.26	18.14	24.33	18.53	16.16	19.94	12.64	18.31
Group diff.@ P=.05		35.35	35.35	35.35	35.35	35.35	35.35	35.35	35.35	35.35	35.35	35.35	35.35
Group diff.@ P=.01		42.66	42.66	42.66	42.66	42.66	42.66	42.66	42.66	42.66	42.66	42.66	42.66

Analysis of variance: F ratio = 0.08 Df = 12/ 52 F probability = 1.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209A
Exposure phase (Day 6)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

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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	206.24	217.75	220.55	219.36	223.00	212.69	221.11	224.32	218.80	215.35	217.77	218.32	212.94
Standard deviation	17.42	19.44	29.18	18.07	17.57	24.41	24.50	24.83	25.03	17.33	23.07	14.32	20.45
Group diff.@ P=.05		39.24	39.24	39.24	39.24	39.24	39.24	39.24	39.24	39.24	39.24	39.24	39.24
Group diff.@ P=.01		47.35	47.35	47.35	47.35	47.35	47.35	47.35	47.35	47.35	47.35	47.35	47.35

Analysis of variance: F ratio = 0.25 Df = 12/ 52 F probability = 0.994
Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209A
Exposure phase (Day 7)
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FEEDING STUDY/PALATABILITY

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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	211.60	220.78	225.57	217.20	217.85	209.59	214.04	230.70	214.95	215.99	215.30	215.28	208.03
Standard deviation	15.19	17.16	25.45	22.23	18.38	23.38	18.48	28.85	16.54	14.14	20.21	10.76	17.76
Group diff.@ P=.05		35.77	35.77	35.77	35.77	35.77	35.77	35.77	35.77	35.77	35.77	35.77	35.77
Group diff.@ P=.01		43.17	43.17	43.17	43.17	43.17	43.17	43.17	43.17	43.17	43.17	43.17	43.17

Analysis of variance: F ratio = 0.49 Df = 12/ 52 F probability = 0.909

Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209A
Exposure phase (Day 8)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

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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	217.31	222.53	229.54	218.82	220.04	206.76	208.73	232.01	219.66	220.33	220.15	216.67	207.84
Standard deviation	20.30	17.65	27.49	24.21	18.18	20.47	17.23	25.87	17.66	10.91	15.74	12.55	18.88
Group diff.@ P=.05		35.54	35.54	35.54	35.54	35.54	35.54	35.54	35.54	35.54	35.54	35.54	35.54
Group diff.@ P=.01		42.89	42.89	42.89	42.89	42.89	42.89	42.89	42.89	42.89	42.89	42.89	42.89

Analysis of variance: F ratio = 0.74 Df = 12/ 52 F probability = 0.704
Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
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Exposure phase (Day 9)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

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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	226.62	226.01	235.65	224.41	220.75	209.40	203.54	236.79	227.25	224.16	225.65	218.10	211.34
Standard deviation	16.94	17.44	27.79	27.92	22.23	24.67	16.34	29.12	21.18	14.55	16.38	12.42	17.65
Group diff.@ P=.05		38.23	38.23	38.23	38.23	38.23	38.23	38.23	38.23	38.23	38.23	38.23	38.23
Group diff.@ P=.01		46.14	46.14	46.14	46.14	46.14	46.14	46.14	46.14	46.14	46.14	46.14	46.14

Analysis of variance: F ratio = 1.06 Df = 12/ 52 F probability = 0.414
Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209A
Exposure phase (Day 10)
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FEEDING STUDY/PALATABILITY

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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	231.79	228.48	241.11	227.80	220.86	208.16	200.01	241.04	232.48	228.32	231.37	222.62	210.24
Standard deviation	15.84	17.66	22.80	24.70	25.02	23.40	16.78	28.39	19.80	11.43	12.34	10.84	14.48
Group diff.@ P=.05		35.47	35.47	35.47	35.47	35.47	35.47	35.47	35.47	35.47	35.47	35.47	35.47
Group diff.@ P=.01		42.81	42.81	42.81	42.81	42.81	42.81	42.81	42.81	42.81	42.81	42.81	42.81

Analysis of variance: F ratio = 2.01 Df = 12/ 52 F probability = 0.042
Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209A
Exposure phase (Day 11)
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FEEDING STUDY/PALATABILITY

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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	237.92	226.89	245.85	231.21	225.74	209.50	199.63	242.38	236.98	232.40	233.85	225.96	213.67
Standard deviation	16.36	17.58	22.79	24.97	24.85	22.31	17.88	30.86	19.19	12.00	10.05	10.95	16.57
Group diff.@ P=.05		36.04	36.04	36.04	36.04	36.04	36.04*	36.04	36.04	36.04	36.04	36.04	36.04
Group diff.@ P=.01		43.49	43.49	43.49	43.49	43.49	43.49	43.49	43.49	43.49	43.49	43.49	43.49

Analysis of variance: F ratio = 2.26 Df = 12/ 52 F probability = 0.022
Note: A * indicates group mean is significantly different from control at level of significance shown.

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Building 630/2
RAT/WISTAR HANOVER

Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209A
Exposure phase (Day 12)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

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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	243.19	232.15	250.72	236.89	229.03	212.52	199.91	248.89	242.59	235.95	239.54	229.02	211.21
Standard deviation	17.15	21.07	20.48	24.04	26.34	23.31	18.06	30.53	20.59	12.05	14.39	10.37	13.73
Group diff.@ P=.05		36.70	36.70	36.70	36.70	36.70	36.70*	36.70	36.70	36.70	36.70	36.70	36.70
Group diff.@ P=.01		44.29	44.29	44.29	44.29	44.29	44.29	44.29	44.29	44.29	44.29	44.29	44.29

Analysis of variance: F ratio = 2.89 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 Animal Body Weights in (g)
Study number: TOX209A
Exposure phase (Day 13)
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FEEDING STUDY/PALATABILITY

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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	249.43	246.03	258.68	245.84	232.39	216.75	201.89	251.72	250.86	243.57	244.13	234.97	219.14
Standard deviation	15.79	21.89	22.77	21.44	27.19	20.99	17.80	28.92	19.03	11.14	13.96	9.96	15.97
Group diff.@ P=.05		35.90	35.90	35.90	35.90	35.90	35.90*	35.90	35.90	35.90	35.90	35.90	35.90
Group diff.@ P=.01		43.32	43.32	43.32	43.32	43.32	43.32*	43.32	43.32	43.32	43.32	43.32	43.32

Analysis of variance: F ratio = 3.48 Df = 12/ 52 F probability = 0.001
Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209A
Exposure phase (Day 14)
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FEEDING STUDY/PALATABILITY

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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	255.53	253.48	263.35	251.23	240.46	213.38	199.06	258.71	253.74	248.01	248.81	237.24	212.02
Standard deviation	14.40	18.99	22.84	20.49	20.96	17.12	17.86	30.96	20.64	11.02	13.79	12.33	12.41
Group diff.@ P=.05		34.04	34.04	34.04	34.04	34.04*	34.04*	34.04	34.04	34.04	34.04	34.04	34.04*
Group diff.@ P=.01		41.08	41.08	41.08	41.08	41.08*	41.08*	41.08	41.08	41.08	41.08	41.08	41.08*

Analysis of variance: F ratio = 5.86 Df = 12/ 52 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209A
Exposure phase (Day 15)
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FEEDING STUDY/PALATABILITY

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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	261.14	260.01	266.84	254.98	243.32	215.91	197.68	262.66	258.38	250.24	251.12	239.22	208.72
Standard deviation	16.76	17.45	22.46	20.75	22.47	19.02	17.04	31.38	19.43	11.46	14.67	12.94	9.71
Group diff.@ P=.05		34.35	34.35	34.35	34.35	34.35*	34.35*	34.35	34.35	34.35	34.35	34.35	34.35*
Group diff.@ P=.01		41.46	41.46	41.46	41.46	41.46*	41.46*	41.46	41.46	41.46	41.46	41.46	41.46*

Analysis of variance: F ratio = 7.04 Df = 12/ 52 F probability = 0.000

Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209A
Exposure phase (Day 16)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

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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	264.08	262.78	269.75	256.50	246.15	218.84	196.94	264.78	259.50	254.28	254.00	239.76	207.44
Standard deviation	15.05	17.34	23.46	21.40	21.66	23.71	17.12	32.93	18.98	12.38	12.94	13.92	11.03
Group diff.@ P=.05		35.42	35.42	35.42	35.42	35.42*	35.42*	35.42	35.42	35.42	35.42	35.42	35.42*
Group diff.@ P=.01		42.74	42.74	42.74	42.74	42.74*	42.74*	42.74	42.74	42.74	42.74	42.74	42.74*

Analysis of variance: F ratio = 7.26 Df = 12/ 52 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209A
Exposure phase (Day 17)
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FEEDING STUDY/PALATABILITY

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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	268.34	269.41	273.29	262.51	250.04	222.43	199.52	271.18	265.21	256.84	259.91	243.48	206.46
Standard deviation	16.85	16.97	22.86	19.43	22.65	21.28	16.20	32.97	17.95	12.49	15.30	15.50	12.67
Group diff.@ P=.05		35.28	35.28	35.28	35.28	35.28*	35.28*	35.28	35.28	35.28	35.28	35.28	35.28*
Group diff.@ P=.01		42.57	42.57	42.57	42.57	42.57*	42.57*	42.57	42.57	42.57	42.57	42.57	42.57*

Analysis of variance: F ratio = 8.27 Df = 12/ 52 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209A
Exposure phase (Day 18)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

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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	270.97	273.29	275.04	262.34	250.34	225.35	200.07	271.11	266.21	252.22	259.08	242.31	204.68
Standard deviation	14.38	15.83	22.95	20.67	20.64	24.26	15.44	33.05	18.59	17.30	14.34	14.44	12.37
Group diff.@ P=.05		35.52	35.52	35.52	35.52	35.52*	35.52*	35.52	35.52	35.52	35.52	35.52	35.52*
Group diff.@ P=.01		42.87	42.87	42.87	42.87	42.87*	42.87*	42.87	42.87	42.87	42.87	42.87	42.87*

Analysis of variance: F ratio = 8.47 Df = 12/ 52 F probability = 0.000

Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209A
Exposure phase (Day 19)
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FEEDING STUDY/PALATABILITY

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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	272.28	272.36	277.40	269.27	253.95	225.27	199.19	273.63	269.73	255.42	261.59	245.47	204.82
Standard deviation	15.86	17.81	24.15	20.98	19.70	22.73	13.13	32.67	18.79	17.33	14.39	15.20	11.23
Group diff.@ P=.05		35.47	35.47	35.47	35.47	35.47*	35.47*	35.47	35.47	35.47	35.47	35.47	35.47*
Group diff.@ P=.01		42.81	42.81	42.81	42.81	42.81*	42.81*	42.81	42.81	42.81	42.81	42.81	42.81*

Analysis of variance: F ratio = 9.22 Df = 12/ 52 F probability = 0.000

Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209B
Quarantine/Acclimation (Day 6)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

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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	189.47	187.96	186.01	191.75	
Standard deviation	21.31	20.26	12.30	14.01	
Group diff.@ P=.05		28.54	28.54	28.54	
Group diff.@ P=.01		37.32	37.32	37.32	

Analysis of variance: F ratio = 0.10 Df = 3/ 16 F probability = 0.956
Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209B
Exposure phase (Day 1)
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FEEDING STUDY/PALATABILITY

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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	195.75	195.33	192.53	186.38	
Standard deviation	21.41	26.12	13.23	24.45	
Group diff.@ P=.05		35.86	35.86	35.86	
Group diff.@ P=.01		46.90	46.90	46.90	

Analysis of variance: F ratio = 0.20 Df = 3/ 16 F probability = 0.896
Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209B
Exposure phase (Day 2)
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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	4	4	5	5	
Mean	193.51	195.31	190.43	187.96	
Standard deviation	17.93	27.36	14.76	25.57	
Group diff.@ P=.05		40.69	38.60	38.60	
Group diff.@ P=.01		53.67	50.92	50.92	

Analysis of variance: F ratio = 0.10 Df = 3/ 14 F probability = 0.954
Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209B
Exposure phase (Day 6)
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FEEDING STUDY/PALATABILITY

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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	211.02	222.59	215.49	214.99	
Standard deviation	29.67	29.22	15.83	29.60	
Group diff.@ P=.05		43.84	43.84	43.84	
Group diff.@ P=.01		57.34	57.34	57.34	

Analysis of variance: F ratio = 0.16 Df = 3/ 16 F probability = 0.917

Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209B
Exposure phase (Day 7)
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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	4	5	5	5	
Mean	225.24	224.00	213.69	204.78	
Standard deviation	25.29	27.47	14.69	27.78	
Group diff.@ P=.05		42.62	42.62	42.62	
Group diff.@ P=.01		55.96	55.96	55.96	

Analysis of variance: F ratio = 0.74 Df = 3/ 15 F probability = 0.548

Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209B
Exposure phase (Day 8)
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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	4	5	5	5	
Mean	230.50	223.16	213.17	199.88	
Standard deviation	22.68	29.85	15.37	28.36	
Group diff.@ P=.05		43.52	43.52	43.52	
Group diff.@ P=.01		57.14	57.14	57.14	

Analysis of variance: F ratio = 1.31 Df = 3/ 15 F probability = 0.306
Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209B
Exposure phase (Day 9)
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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	4	5	5	5	
Mean	234.10	230.74	213.19	196.25	
Standard deviation	23.73	26.97	14.82	27.03	
Group diff.@ P=.05		41.43	41.43	41.43	
Group diff.@ P=.01		54.40	54.40	54.40	

Analysis of variance: F ratio = 2.58 Df = 3/ 15 F probability = 0.092

Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209B
Exposure phase (Day 10)
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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	231.12	233.89	214.52	195.18	
Standard deviation	28.64	27.08	13.41	28.22	
Group diff.@ P=.05		41.23	41.23	41.23	
Group diff.@ P=.01		53.92	53.92	53.92	

Analysis of variance: F ratio = 2.52 Df = 3/ 16 F probability = 0.094

Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209B
Exposure phase (Day 11)
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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	234.43	238.03	213.75	189.56	
Standard deviation	24.67	25.76	14.14	29.26	
Group diff.@ P=.05		39.55	39.55	39.55*	
Group diff.@ P=.01		51.73	51.73	51.73	

Analysis of variance: F ratio = 4.28 Df = 3/ 16 F probability = 0.021
Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
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Exposure phase (Day 12)
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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	238.92	239.72	216.48	189.97	
Standard deviation	24.70	24.35	12.89	31.23	
Group diff.@ P=.05		39.69	39.69	39.69*	
Group diff.@ P=.01		51.91	51.91	51.91	

Analysis of variance: F ratio = 4.70 Df = 3/ 16 F probability = 0.015

Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
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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	246.07	246.26	219.89	188.73	
Standard deviation	22.38	25.02	14.86	30.12	
Group diff.@ P=.05		38.92	38.92	38.92*	
Group diff.@ P=.01		50.90	50.90	50.90*	

Analysis of variance: F ratio = 6.62 Df = 3/ 16 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 Animal Body Weights in (g)
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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	251.35	248.24	220.09	186.48	
Standard deviation	24.46	27.00	15.01	29.68	
Group diff.@ P=.05		40.44	40.44	40.44*	
Group diff.@ P=.01		52.88	52.88	52.88*	

Analysis of variance: F ratio = 7.49 Df = 3/ 16 F probability = 0.002

Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209B
Exposure phase (Day 15)
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FEEDING STUDY/PALATABILITY

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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	256.79	251.19	224.49	185.76	
Standard deviation	22.44	27.23	19.23	31.30	
Group diff.@ P=.05		41.75	41.75	41.75*	
Group diff.@ P=.01		54.61	54.61	54.61*	

Analysis of variance: F ratio = 8.10 Df = 3/ 16 F probability = 0.002

Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209B
Exposure phase (Day 16)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

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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	258.57	255.33	224.94	183.29	
Standard deviation	23.20	26.80	15.79	32.08	
Group diff.@ P=.05		41.27	41.27	41.27*	
Group diff.@ P=.01		53.97	53.97	53.97*	

Analysis of variance: F ratio = 9.64 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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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209B
Exposure phase (Day 17)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

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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	263.29	260.27	227.31	180.69	
Standard deviation	23.34	26.07	20.34	30.94	
Group diff.@ P=.05		41.77	41.77	41.77*	
Group diff.@ P=.01		54.62	54.62	54.62*	

Analysis of variance: F ratio = 11.38 Df = 3/ 16 F probability = 0.000

Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209B
Exposure phase (Day 18)
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	264.16	259.11	229.11	179.57	
Standard deviation	22.85	23.65	17.43	34.10	
Group diff.@ P=.05		41.38	41.38	41.38*	
Group diff.@ P=.01		54.11	54.11	54.11*	

Analysis of variance: F ratio = 11.83 Df = 3/ 16 F probability = 0.000

Note: A * indicates group mean is significantly different from control at level of significance shown.

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Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX209B
Exposure phase (Day 19)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

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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	269.74	261.81	228.01	180.15	
Standard deviation	22.84	23.57	17.63	32.72	
Group diff.@ P=.05		40.64	40.64*	40.64*	
Group diff.@ P=.01		53.15	53.15	53.15*	

Analysis of variance: F ratio = 13.51 Df = 3/ 16 F probability = 0.000

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: TOX209A
Exposure phase (Day 6) (Reference Day 2)
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	12.07	24.33	25.37	23.16	29.57	21.08	27.73	28.45	27.72	25.26	22.14	26.94	25.17
Standard deviation	11.93	7.62	11.08	7.05	5.35	15.18	7.57	2.16	9.88	7.17	6.72	9.61	9.79
Group diff.@ P=.05		16.53	16.53	16.53	16.53*	16.53	16.53	16.53	16.53	16.53	16.53	16.53	16.53
Group diff.@ P=.01		19.95	19.95	19.95	19.95	19.95	19.95	19.95	19.95	19.95	19.95	19.95	19.95

Analysis of variance: F ratio = 1.23 Df = 12/ 52 F probability = 0.291
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: TOX209A
Exposure phase (Day 7) (Reference Day 2)
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	17.43	27.37	30.39	21.00	24.42	17.97	20.66	34.83	23.87	25.90	19.66	23.90	20.26
Standard deviation	14.39	12.16	6.54	7.44	6.23	11.42	2.29	5.67	8.19	6.58	5.65	8.52	6.81
Group diff.@ P=.05		15.29	15.29	15.29	15.29	15.29	15.29	15.29*	15.29	15.29	15.29	15.29	15.29
Group diff.@ P=.01		18.46	18.46	18.46	18.46	18.46	18.46	18.46	18.46	18.46	18.46	18.46	18.46

Analysis of variance: F ratio = 1.79 Df = 12/ 52 F probability = 0.075
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: TOX209A
Exposure phase (Day 8) (Reference Day 2)
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	23.14	29.11	34.37	22.61	26.61	15.15	15.35	36.13	28.58	30.24	24.51	25.29	20.07
Standard deviation	17.06	15.99	10.08	8.09	6.75	11.96	4.26	2.02	11.58	7.27	8.42	9.78	7.57
Group diff.@ P=.05		18.41	18.41	18.41	18.41	18.41	18.41	18.41	18.41	18.41	18.41	18.41	18.41
Group diff.@ P=.01		22.22	22.22	22.22	22.22	22.22	22.22	22.22	22.22	22.22	22.22	22.22	22.22

Analysis of variance: F ratio = 2.00 Df = 12/ 52 F probability = 0.043
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: TOX209A
Exposure phase (Day 9) (Reference Day 2)
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	32.44	32.59	40.48	28.21	27.33	17.79	10.16	40.91	36.17	34.08	30.02	26.72	23.57
Standard deviation	11.43	17.94	10.92	10.74	7.69	14.40	3.55	6.99	10.82	9.25	5.75	10.51	5.04
Group diff.@ P=.05		18.76	18.76	18.76	18.76	18.76	18.76*	18.76	18.76	18.76	18.76	18.76	18.76
Group diff.@ P=.01		22.64	22.64	22.64	22.64	22.64	22.64	22.64	22.64	22.64	22.64	22.64	22.64

Analysis of variance: F ratio = 3.50 Df = 12/ 52 F probability = 0.001
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: TOX209A
Exposure phase (Day 10) (Reference Day 2)
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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	37.62	35.07	45.93	31.59	27.43	16.55	6.63	45.17	41.40	38.23	35.74	31.24	22.47
Standard deviation	10.23	19.37	6.05	9.92	10.03	12.88	4.88	5.86	10.00	8.78	8.21	11.41	6.10
Group diff.@ P=.05		18.52	18.52	18.52	18.52	18.52*	18.52*	18.52	18.52	18.52	18.52	18.52	18.52
Group diff.@ P=.01		22.36	22.36	22.36	22.36	22.36	22.36*	22.36	22.36	22.36	22.36	22.36	22.36

Analysis of variance: F ratio = 6.19 Df = 12/ 52 F probability = 0.000

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: TOX209A
Exposure phase (Day 11) (Reference Day 2)
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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	43.75	33.47	50.67	35.01	32.32	17.89	6.25	46.51	45.90	42.31	38.22	34.58	25.90
Standard deviation	9.42	19.32	5.84	11.51	10.64	13.48	3.52	9.11	9.65	8.68	10.19	11.58	4.05
Group diff.@ P=.05		19.13	19.13	19.13	19.13	19.13*	19.13*	19.13	19.13	19.13	19.13	19.13	19.13
Group diff.@ P=.01		23.09	23.09	23.09	23.09	23.09*	23.09*	23.09	23.09	23.09	23.09	23.09	23.09

Analysis of variance: F ratio = 6.95 Df = 12/ 52 F probability = 0.000
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: TOX209A
Exposure phase (Day 12) (Reference Day 2)
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	49.02	38.73	55.55	40.68	35.60	20.91	6.53	53.02	51.51	45.86	43.91	37.64	23.44
Standard deviation	8.07	18.51	3.75	6.93	11.96	15.10	5.05	7.32	9.20	8.42	6.94	10.44	7.68
Group diff.@ P=.05		18.11	18.11	18.11	18.11	18.11*	18.11*	18.11	18.11	18.11	18.11	18.11	18.11*
Group diff.@ P=.01		21.86	21.86	21.86	21.86	21.86*	21.86*	21.86	21.86	21.86	21.86	21.86	21.86*

Analysis of variance: F ratio = 10.22 Df = 12/ 52 F probability = 0.000

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: TOX209A
Exposure phase (Day 13) (Reference Day 2)
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	55.26	52.61	63.51	49.63	38.97	25.14	8.51	55.84	59.78	53.48	48.50	43.59	31.37
Standard deviation	7.83	19.72	6.38	5.50	13.32	15.33	5.83	7.11	9.27	12.14	6.44	12.03	3.91
Group diff.@ P=.05		19.21	19.21	19.21	19.21	19.21*	19.21*	19.21	19.21	19.21	19.21	19.21	19.21*
Group diff.@ P=.01		23.18	23.18	23.18	23.18	23.18*	23.18*	23.18	23.18	23.18	23.18	23.18	23.18*

Analysis of variance: F ratio = 10.80 Df = 12/ 52 F probability = 0.000

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: TOX209A
Exposure phase (Day 14) (Reference Day 2)
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	61.36	60.06	68.18	55.02	47.03	21.76	5.68	62.83	62.66	57.92	53.18	45.86	24.25
Standard deviation	8.49	13.58	5.55	6.54	11.78	11.24	7.57	8.68	10.87	11.00	6.47	15.04	7.05
Group diff.@ P=.05		18.06	18.06	18.06	18.06	18.06*	18.06*	18.06	18.06	18.06	18.06	18.06	18.06*
Group diff.@ P=.01		21.80	21.80	21.80	21.80	21.80*	21.80*	21.80	21.80	21.80	21.80	21.80	21.80*

Analysis of variance: F ratio = 18.50 Df = 12/ 52 F probability = 0.000

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: TOX209A
Exposure phase (Day 15) (Reference Day 2)
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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	66.97	66.59	71.67	58.78	49.89	24.29	4.30	66.79	67.30	60.15	55.49	47.84	20.95
Standard deviation	10.11	12.98	5.61	6.36	11.99	12.66	7.62	8.20	10.74	12.76	6.78	16.18	9.19
Group diff.@ P=.05		19.14	19.14	19.14	19.14	19.14*	19.14*	19.14	19.14	19.14	19.14	19.14	19.14*
Group diff.@ P=.01		23.10	23.10	23.10	23.10	23.10*	23.10*	23.10	23.10	23.10	23.10	23.10	23.10*

Analysis of variance: F ratio = 20.33 Df = 12/ 52 F probability = 0.000

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: TOX209A
Exposure phase (Day 16) (Reference Day 2)
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	69.90	69.36	74.58	60.30	52.73	27.23	3.56	68.90	68.42	64.19	58.36	48.38	19.67
Standard deviation	10.48	9.22	5.58	5.37	12.45	12.42	9.80	11.69	10.50	12.64	8.67	16.39	8.39
Group diff.@ P=.05		19.39	19.39	19.39	19.39	19.39*	19.39*	19.39	19.39	19.39	19.39	19.39*	19.39*
Group diff.@ P=.01		23.40	23.40	23.40	23.40	23.40*	23.40*	23.40	23.40	23.40	23.40	23.40	23.40*

Analysis of variance: F ratio = 21.79 Df = 12/ 52 F probability = 0.000

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: TOX209A
Exposure phase (Day 17) (Reference Day 2)
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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	74.16	75.99	78.12	66.30	56.61	30.81	6.14	75.31	74.13	66.75	64.28	52.10	18.69
Standard deviation	10.93	9.70	6.46	5.55	13.44	11.09	7.99	11.62	9.07	10.78	8.28	18.70	9.25
Group diff.@ P=.05		19.46	19.46	19.46	19.46	19.46*	19.46*	19.46	19.46	19.46	19.46	19.46*	19.46*
Group diff.@ P=.01		23.48	23.48	23.48	23.48	23.48*	23.48*	23.48	23.48	23.48	23.48	23.48	23.48*

Analysis of variance: F ratio = 24.45 Df = 12/ 52 F probability = 0.000

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: TOX209A
Exposure phase (Day 18) (Reference Day 2)
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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	76.79	79.88	79.86	66.14	56.91	33.74	6.69	75.24	75.13	62.14	63.44	50.93	16.91
Standard deviation	6.53	7.39	4.42	6.23	15.26	12.89	8.86	11.46	8.63	13.44	9.07	16.82	8.38
Group diff.@ P=.05		19.22	19.22	19.22	19.22*	19.22*	19.22*	19.22	19.22	19.22	19.22	19.22*	19.22*
Group diff.@ P=.01		23.19	23.19	23.19	23.19	23.19*	23.19*	23.19	23.19	23.19	23.19	23.19*	23.19*

Analysis of variance: F ratio = 25.94 Df = 12/ 52 F probability = 0.000

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: TOX209A
Exposure phase (Day 19) (Reference Day 2)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

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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	78.10	78.94	82.23	73.07	60.52	33.66	5.81	77.75	78.65	65.33	65.95	54.09	17.05
Standard deviation	8.02	11.19	5.42	6.58	13.99	13.35	10.82	11.30	7.33	13.17	8.24	17.21	8.44
Group diff.@ P=.05		19.80	19.80	19.80	19.80	19.80*	19.80*	19.80	19.80	19.80	19.80	19.80*	19.80*
Group diff.@ P=.01		23.89	23.89	23.89	23.89	23.89*	23.89*	23.89	23.89	23.89	23.89	23.89*	23.89*

Analysis of variance: F ratio = 26.61 Df = 12/ 52 F probability = 0.000

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: TOX209B
Exposure phase (Day 6) (Reference Day 2)
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	4	4	5	5	
Mean	26.42	30.65	25.06	27.03	
Standard deviation	9.60	7.08	6.19	5.77	
Group diff.@ P=.05		13.28	12.60	12.60	
Group diff.@ P=.01		17.52	16.62	16.62	

Analysis of variance: F ratio = 0.48 Df = 3/ 14 F probability = 0.706
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: TOX209B
Exposure phase (Day 7) (Reference Day 2)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

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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	4	4	5	5	
Mean	31.72	28.53	23.26	16.82	
Standard deviation	8.32	7.75	3.06	6.12	
Group diff.@ P=.05		11.92	11.31	11.31*	
Group diff.@ P=.01		15.73	14.92	14.92	

Analysis of variance: F ratio = 4.67 Df = 3/ 14 F probability = 0.018

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: TOX209B
Exposure phase (Day 8) (Reference Day 2)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

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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	4	4	5	5	
Mean	36.99	30.06	22.74	11.92	
Standard deviation	5.83	9.18	5.74	6.94	
Group diff.@ P=.05		12.95	12.29*	12.29*	
Group diff.@ P=.01		17.09	16.21	16.21*	

Analysis of variance: F ratio = 10.68 Df = 3/ 14 F probability = 0.001
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: TOX209B
Exposure phase (Day 9) (Reference Day 2)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

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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	4	4	5	5	
Mean	40.59	36.62	22.76	8.30	
Standard deviation	6.70	7.70	6.14	6.48	
Group diff.@ P=.05		12.49	11.85*	11.85*	
Group diff.@ P=.01		16.48	15.63*	15.63*	

Analysis of variance: F ratio = 21.59 Df = 3/ 14 F probability = 0.000

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: TOX209B
Exposure phase (Day 10) (Reference Day 2)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

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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	4	4	5	5	
Mean	47.06	41.47	24.09	7.22	
Standard deviation	5.45	9.24	5.85	5.97	
Group diff.@ P=.05		12.42	11.79*	11.79*	
Group diff.@ P=.01		16.39	15.55*	15.55*	

Analysis of variance: F ratio = 32.96 Df = 3/ 14 F probability = 0.000

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: TOX209B
Exposure phase (Day 11) (Reference Day 2)
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	4	4	5	5	
Mean	48.30	44.28	23.32	1.60	
Standard deviation	4.71	9.52	6.39	5.52	
Group diff.@ P=.05		12.41	11.78*	11.78*	
Group diff.@ P=.01		16.38	15.54*	15.54*	

Analysis of variance: F ratio = 47.12 Df = 3/ 14 F probability = 0.000

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: TOX209B
Exposure phase (Day 12) (Reference Day 2)
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	4	4	5	5	
Mean	52.16	45.62	26.05	2.01	
Standard deviation	6.82	7.61	5.87	7.08	
Group diff.@ P=.05		12.69	12.04*	12.04*	
Group diff.@ P=.01		16.74	15.88*	15.88*	

Analysis of variance: F ratio = 49.65 Df = 3/ 14 F probability = 0.000

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: TOX209B
Exposure phase (Day 13) (Reference Day 2)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

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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	4	4	5	5	
Mean	58.66	52.05	29.46	0.77	
Standard deviation	4.40	8.45	7.32	6.44	
Group diff.@ P=.05		12.69	12.04*	12.04*	
Group diff.@ P=.01		16.74	15.88*	15.88*	

Analysis of variance: F ratio = 66.88 Df = 3/ 14 F probability = 0.000

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: TOX209B
Exposure phase (Day 14) (Reference Day 2)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

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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	4	4	5	5	
Mean	64.39	55.72	29.66	-1.48	
Standard deviation	6.65	9.52	6.85	7.27	
Group diff.@ P=.05		14.09	13.37*	13.37*	
Group diff.@ P=.01		18.59	17.63*	17.63*	

Analysis of variance: F ratio = 69.50 Df = 3/ 14 F probability = 0.000

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: TOX209B
Exposure phase (Day 15) (Reference Day 2)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

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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	4	4	5	5	
Mean	69.36	59.69	34.06	-2.20	
Standard deviation	5.55	9.47	10.37	9.46	
Group diff.@ P=.05		16.85	15.99*	15.99*	
Group diff.@ P=.01		22.23	21.09*	21.09*	

Analysis of variance: F ratio = 56.72 Df = 3/ 14 F probability = 0.000

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: TOX209B
Exposure phase (Day 16) (Reference Day 2)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

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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	4	4	5	5	
Mean	69.87	61.54	34.51	-4.67	
Standard deviation	8.11	8.87	9.32	9.85	
Group diff.@ P=.05		16.99	16.12*	16.12*	
Group diff.@ P=.01		22.42	21.27*	21.27*	

Analysis of variance: F ratio = 61.65 Df = 3/ 14 F probability = 0.000

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: TOX209B
Exposure phase (Day 17) (Reference Day 2)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

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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	4	4	5	5	
Mean	73.97	66.69	36.88	-7.27	
Standard deviation	9.43	8.31	12.98	10.18	
Group diff.@ P=.05		19.66	18.65*	18.65*	
Group diff.@ P=.01		25.93	24.60*	24.60*	

Analysis of variance: F ratio = 55.77 Df = 3/ 14 F probability = 0.000

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: TOX209B
Exposure phase (Day 18) (Reference Day 2)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

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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	4	4	5	5	
Mean	75.33	64.39	38.68	-8.38	
Standard deviation	9.44	7.26	9.69	11.87	
Group diff.@ P=.05		18.36	17.42*	17.42*	
Group diff.@ P=.01		24.23	22.98*	22.98*	

Analysis of variance: F ratio = 65.25 Df = 3/ 14 F probability = 0.000

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: TOX209B
Exposure phase (Day 19) (Reference Day 2)
Dosing start date: 16-Apr-08

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FEEDING STUDY/PALATABILITY

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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	4	4	5	5	
Mean	80.59	68.20	37.58	-7.81	
Standard deviation	9.14	8.67	12.93	9.73	
Group diff.@ P=.05		19.41	18.41*	18.41*	
Group diff.@ P=.01		25.60	24.29*	24.29*	

Analysis of variance: F ratio = 64.89 Df = 3/ 14 F probability = 0.000

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: TOX209A

PRINTED: 11-Nov-08
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Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Animal Group		D a y o f P h a s e													
		6!	1"	2	6	7	8	9	10	11	12	13	14	15	
		M a l e A n i m a l s													
1	1	5.550	0.560	4.030	6.710	6.560	4.530	4.680	6.820	4.160	3.480	7.480	8.990	5.620	
2		6.083	5.095	2.400	1.412	3.630	1.420	12.290	8.380	6.410	7.990	6.430	6.180	4.640	
3		6.017	-3.745	1.400	4.768	-5.300	15.420	6.930	3.800	7.080	6.390	3.730	4.050	9.770	
4		5.483	5.315	5.830	-1.018	34.980	6.980	4.740	1.150	6.060	2.460	6.440	4.840	6.060	
5		5.097	1.620	8.330	3.215	-13.090	0.220	17.880	5.730	6.940	6.020	7.130	6.440	1.960	
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5	
	Means	5.646	1.769	4.398	3.017	5.356	5.714	9.304	5.176	6.130	5.268	6.242	6.100	5.610	
	Sdevs	0.408	3.726	2.767	2.983	18.274	6.038	5.708	2.801	1.175	2.254	1.475	1.888	2.818	
6	2	3.843	2.625	4.100	2.862	-1.110	-3.710	2.830	1.860	-0.740	2.510	7.480	18.360	7.650	
7		6.797	1.985	0.530	6.827	-5.790	-1.960	-1.380	-0.470	-0.930	17.060	15.460	9.640	4.330	
8		5.257	3.990	2.350	5.940	4.120	4.540	5.650	3.760	2.530	4.940	7.620	7.720	4.160	
9		6.430	2.735	4.260	7.098	7.210	6.990	5.710	3.810	4.660	5.700	8.580	5.370	4.880	
10		5.430	4.495	3.270	7.682	10.760	2.880	4.570	3.420	-13.500	-3.920	30.290	-3.840	11.610	
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5	
	Means	5.551	3.166	2.902	6.082	3.038	1.748	3.476	2.476	-1.596	5.258	13.886	7.450	6.526	
	Sdevs	1.156	1.039	1.529	1.906	6.583	4.475	2.954	1.829	7.054	7.605	9.747	7.995	3.172	
11	3	6.090	3.005	-1.880	8.665	-2.830	8.190	3.160	4.180	-0.280	8.760	7.420	3.030	5.730	
12		5.693	4.005	7.270	7.490	2.360	6.210	6.880	2.060	7.530	2.920	10.160	5.410	1.370	
13		5.527	2.990	-1.730	3.098	19.230	4.660	10.370	5.420	5.380	2.700	9.910	3.200	4.180	
14		5.200	3.080	-0.970	8.825	1.550	3.580	5.630	0.920	3.850	2.350	9.360	5.230	3.640	
15		6.153	4.000	7.180	3.640	4.780	-2.770	4.510	14.700	7.230	7.650	2.940	6.490	2.520	
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5	
	Means	5.733	3.416	1.974	6.344	5.018	3.974	6.110	5.456	4.742	4.876	7.958	4.672	3.488	
	Sdevs	0.397	0.536	4.806	2.771	8.407	4.150	2.749	5.459	3.178	3.071	3.004	1.502	1.655	
16	4	6.210	2.900	5.400	5.110	-1.350	2.360	1.160	-1.580	0.680	12.110	8.010	8.610	1.380	
17		5.447	2.380	7.380	3.618	1.200	3.950	12.110	-0.030	2.540	8.260	7.390	-0.180	5.720	
18		3.920	3.490	1.520	8.340	-0.070	1.980	4.770	3.920	6.290	-0.710	7.780	8.460	1.550	
19		4.420	4.110	5.270	6.522	-11.180	-1.790	-0.520	10.060	4.020	6.800	15.420	3.990	3.070	
20		5.943	2.970	7.260	5.355	0.610	1.580	10.460	4.540	3.550	1.910	6.160	6.070	7.050	
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5	
	Means	5.188	3.170	5.366	5.789	-2.158	1.616	5.596	3.382	3.416	5.674	8.952	5.390	3.754	
	Sdevs	0.985	0.656	2.369	1.761	5.132	2.106	5.565	4.541	2.055	5.106	3.685	3.648	2.534	
21	5	6.327	2.160	5.970	5.860	-7.100	1.660	3.400	0.150	4.890	1.430	3.290	0.360	4.060	
22		6.007	3.445	-1.260	8.550	-10.050	1.240	-3.170	-3.240	-1.280	8.160	1.360	20.920	0.230	
23		6.807	2.360	2.450	6.598	-1.940	1.770	6.040	5.660	2.740	9.900	3.440	4.410	4.790	
24		5.100	2.865	-2.720	9.017	-2.230	3.100	1.580	0.290	5.110	2.690	6.340	5.070	2.810	

Note: ! = Quarantine/Acclimation; " = Exposure phase

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Animal weight gains per day in (g)
Study number: TOX209A

PRINTED: 11-Nov-08
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Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Animal	Group	D a y o f P h a s e													
		6!	1"	2	6	7	8	9	10	11	12	13	14	15	

25	5		4.393	3.465	-1.240	6.938	-4.410	3.160	-4.270	-2.330	12.970	-5.770	2.400	9.570	2.410
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	5.727	2.859	0.640	7.393	-5.146	2.186	0.716	0.106	4.886	3.282	3.366	8.066	2.860
		Sdevs	0.972	0.602	3.540	1.338	3.435	0.884	4.366	3.465	5.197	6.191	1.858	7.893	1.753
26	6		5.147	2.070	1.930	3.713	-1.440	-5.150	5.820	2.050	-2.370	-0.880	0.930	2.210	1.660
			4.010	2.580	3.010	6.070	-2.950	1.980	0.740	-0.320	4.060	1.580	7.320	0.050	1.420
			6.997	2.370	-0.620	8.575	-7.630	-1.990	-0.460	0.580	-1.010	3.240	4.780	-4.400	2.140
			4.313	4.850	6.480	-0.498	2.330	-3.030	-2.660	-0.290	1.740	2.600	5.520	2.830	-1.240
			6.640	1.595	-2.780	8.485	-5.820	-5.940	9.750	-8.200	4.270	8.570	2.580	-17.550	8.670
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	5.421	2.693	1.604	5.269	-3.102	-2.826	2.638	-1.236	1.338	3.022	4.226	-3.372	2.530
		Sdevs	1.348	1.261	3.536	3.795	3.879	3.120	5.049	4.010	2.976	3.475	2.506	8.417	3.676
31	7		7.563	2.460	2.550	8.965	-15.100	-8.150	-4.820	-0.970	1.100	0.960	-1.730	4.080	-1.530
			5.940	2.905	0.600	6.950	-8.660	-2.740	-2.230	-0.500	-2.680	-0.130	4.030	-3.430	-2.440
			5.743	1.145	1.000	8.588	-12.320	-5.550	-7.530	-5.180	0.790	0.630	3.790	-8.230	-2.670
			5.790	5.115	-7.400	4.530	-0.320	-7.750	-3.790	-5.560	0.910	-3.290	1.200	-4.790	-0.590
			4.610	5.795	0.680	5.633	1.060	-2.370	-7.610	-5.400	-2.060	3.270	2.600	-1.770	0.310
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	5.929	3.484	-0.514	6.933	-7.068	-5.312	-5.196	-3.522	-0.388	0.288	1.978	-2.828	-1.384
		Sdevs	1.056	1.927	3.930	1.893	7.180	2.708	2.355	2.553	1.826	2.368	2.358	4.534	1.254
36	8		5.267	3.390	3.580	7.327	3.830	1.770	-4.780	7.440	-4.940	11.780	3.860	4.190	4.600
			4.377	4.665	-4.660	6.943	16.950	-8.390	10.500	0.900	8.990	2.590	3.830	8.050	3.820
			5.543	2.625	-2.860	6.502	5.230	2.390	4.080	2.660	2.930	2.370	5.370	4.540	3.320
			5.487	3.265	6.910	6.867	6.530	4.960	5.240	5.020	-0.050	8.370	-4.430	10.620	7.240
			5.627	4.575	2.200	7.923	-0.660	5.810	8.860	5.260	-0.250	7.450	5.500	7.550	0.800
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	5.260	3.704	1.034	7.112	6.376	1.308	4.780	4.256	1.336	6.512	2.826	6.990	3.956
		Sdevs	0.511	0.886	4.742	0.540	6.503	5.680	5.947	2.527	5.122	4.019	4.134	2.667	2.323
41	9		3.833	4.275	5.900	3.620	9.020	2.280	1.710	9.050	6.100	3.110	11.390	1.360	6.830
			7.497	2.465	2.850	7.982	-17.390	-1.830	15.560	8.210	5.300	8.660	7.830	5.170	2.360
			7.030	3.630	-0.890	10.260	-4.550	7.930	9.240	4.840	3.850	4.070	7.500	5.190	5.480
			5.940	4.165	6.920	6.988	-2.600	7.970	5.900	2.590	3.610	6.990	8.880	1.850	2.800
			5.220	1.235	8.100	5.805	-3.760	7.210	5.550	1.440	3.640	5.220	5.760	0.820	5.760
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	5.904	3.154	4.576	6.931	-3.856	4.712	7.592	5.226	4.500	5.610	8.272	2.878	4.646
		Sdevs	1.463	1.291	3.624	2.471	9.368	4.357	5.192	3.353	1.135	2.235	2.073	2.133	1.958

Note: ! = Quarantine/Acclimation; " = Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Animal weight gains per day in (g)
Study number: TOX209A

PRINTED: 11-Nov-08
Page: 3

Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Animal	Group	D a y o f P h a s e												
		6!	1"	2	6	7	8	9	10	11	12	13	14	15

		M a l e						A n i m a l s						
46	10	5.373	2.985	0.810	8.530	-0.520	2.220	8.360	-0.050	5.530	1.690	9.800	2.580	3.890
47		5.947	1.185	2.780	6.747	-2.500	1.110	11.720	0.850	4.630	4.900	5.030	4.090	2.920
48		4.213	-18.095	2.510	3.788	5.020	12.020	-3.410	13.320	1.040	5.400	12.350	5.490	2.660
49		5.863	2.110	4.800	7.085	3.570	5.580	1.760	4.400	4.540	2.490	9.290	3.440	2.730
50		6.090	19.885	-3.250	5.425	-2.370	0.790	0.730	2.240	4.670	3.270	1.630	6.620	-1.050
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	5.497	1.614	1.530	6.315	0.640	4.344	3.832	4.152	4.082	3.550	7.620	4.444	2.230
	Sdevs	0.767	13.459	3.024	1.793	3.465	4.692	6.105	5.393	1.747	1.574	4.257	1.615	1.899
51	11	7.597	-1.675	8.170	7.295	-6.670	0.260	6.360	-3.420	0.970	14.600	2.080	4.250	5.120
52		5.943	3.235	6.180	3.442	1.220	11.620	5.930	8.650	6.760	2.970	3.610	5.170	0.910
53		5.333	2.045	7.730	4.083	-4.080	-1.420	9.620	8.190	-0.770	5.590	6.830	5.370	-0.040
54		5.653	2.605	5.670	6.685	-2.120	6.330	4.010	9.860	1.290	2.950	5.240	4.990	3.450
55		5.213	1.510	8.050	6.165	-0.710	7.460	1.600	5.320	4.160	2.350	5.180	3.630	2.100
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	5.948	1.544	7.160	5.534	-2.472	4.850	5.504	5.720	2.482	5.692	4.588	4.682	2.308
	Sdevs	0.965	1.910	1.153	1.681	3.044	5.367	2.972	5.374	2.975	5.135	1.806	0.725	2.045
56	12	4.313	-1.830	1.380	3.555	-4.800	0.060	-0.530	1.950	3.170	4.960	3.110	-3.020	-0.110
57		5.500	3.875	2.240	4.873	3.690	-0.710	3.730	9.100	4.750	0.900	7.220	6.180	2.050
58		6.067	5.500	1.720	7.700	-2.330	1.110	0.890	5.150	2.240	4.560	8.050	0.480	2.840
59		6.447	2.980	-3.960	8.422	-3.220	0.610	1.250	5.420	4.360	0.410	5.430	2.790	2.730
60		5.887	1.790	5.870	9.125	-8.550	5.870	1.840	0.980	2.170	4.460	5.970	4.880	2.430
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	5.643	2.463	1.450	6.735	-3.042	1.388	1.436	4.520	3.338	3.058	5.956	2.262	1.988
	Sdevs	0.818	2.755	3.518	2.401	4.452	2.595	1.551	3.214	1.187	2.208	1.895	3.659	1.212
61	13	5.723	2.475	1.290	2.405	0.760	-1.810	9.280	-2.180	4.750	-3.780	10.160	-7.100	2.980
62		5.220	0.820	9.280	6.373	-7.550	-0.990	2.880	4.090	2.980	-4.060	9.510	-7.150	-2.860
63		5.957	3.080	-5.320	5.935	0.750	-0.740	3.580	-7.160	3.830	-4.970	13.530	-10.550	-10.250
64		4.673	1.765	-0.750	8.522	-13.900	2.750	-0.010	-2.390	6.230	-4.480	6.110	-10.030	-5.110
65		4.307	2.440	0.890	8.228	-4.610	-0.140	1.750	2.120	-0.620	5.010	0.330	-0.760	-1.280
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	5.176	2.116	1.078	6.293	-4.910	-0.186	3.496	-1.104	3.434	-2.456	7.928	-7.118	-3.304
	Sdevs	0.693	0.861	5.283	2.448	6.166	1.747	3.506	4.383	2.566	4.198	4.998	3.895	4.881

Note: ! = Quarantine/Acclimation; " = Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Animal weight gains per day in (g)
Study number: TOX209A

PRINTED: 11-Nov-08
Page: 4

Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Animal Group		Day of Phase			
		16	17	18	19
Male Animals					
1	1	5.950	1.270	0.270	2.570
2		3.900	-0.580	6.480	3.930
3		-0.340	8.690	-1.430	2.600
4		1.410	8.250	-1.970	-0.130
5		3.750	3.660	9.800	-2.410
	(n)	5	5	5	5
	Means	2.934	4.258	2.630	1.312
	Sdevs	2.436	4.131	5.231	2.551
6	2	8.550	6.360	8.040	-8.310
7		4.750	6.530	2.100	1.960
8		2.920	5.920	1.850	0.610
9		-0.720	7.980	-0.430	3.900
10		-1.650	6.370	7.860	-2.840
	(n)	5	5	5	5
	Means	2.770	6.632	3.884	-0.936
	Sdevs	4.155	0.787	3.841	4.800
11	3	2.160	2.940	-0.410	5.120
12		6.180	3.490	1.690	3.600
13		-0.210	6.700	-2.480	0.200
14		3.140	1.700	3.840	3.170
15		3.280	2.860	6.090	-0.250
	(n)	5	5	5	5
	Means	2.910	3.538	1.746	2.368
	Sdevs	2.301	1.884	3.383	2.307
16	4	1.570	7.400	-3.310	7.250
17		4.310	2.060	3.060	7.300
18		-1.060	8.470	0.210	7.880
19		1.190	9.750	0.050	7.760
20		1.600	2.330	-0.820	4.450
	(n)	5	5	5	5
	Means	1.522	6.002	-0.162	6.928
	Sdevs	1.908	3.575	2.286	1.413
21	5	0.770	5.430	-4.820	4.210
22		7.640	7.880	5.890	-0.410
23		4.050	3.080	-0.260	1.560
24		-0.780	4.480	-1.270	7.130

Note: Data for Exposure phase

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Building 630/2
RAT/WISTAR HANOVER

Animal weight gains per day in (g)
Study number: TOX209A

PRINTED: 11-Nov-08
Page: 5

Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Animal Group		Day of Phase			
		16	17	18	19
		Male Animals			
25	5	2.500	-1.450	1.960	5.550
	(n)	5	5	5	5
	Means	2.836	3.884	0.300	3.608
	Sdevs	3.241	3.457	3.969	3.036
26	6	3.210	4.800	-3.220	1.870
27		-1.850	4.600	-0.770	2.140
28		-5.170	8.970	-3.060	5.310
29		5.890	2.530	8.670	-6.390
30		12.610	-2.990	13.010	-3.320
	(n)	5	5	5	5
	Means	2.938	3.582	2.926	-0.078
	Sdevs	6.910	4.355	7.449	4.693
31	7	2.290	-1.230	-4.570	-4.970
32		0.740	-1.800	6.290	-1.760
33		-4.470	5.580	2.280	-2.820
34		-1.990	4.700	-1.740	0.210
35		-0.260	5.630	0.510	4.910
	(n)	5	5	5	5
	Means	-0.738	2.576	0.554	-0.886
	Sdevs	2.602	3.758	4.105	3.741
36	8	-1.960	5.750	-0.100	3.560
37		-1.500	7.990	-1.350	0.710
38		-0.750	7.110	0.000	2.190
39		4.750	5.300	3.340	3.020
40		10.030	5.870	-2.250	3.110
	(n)	5	5	5	5
	Means	2.114	6.404	-0.072	2.518
	Sdevs	5.183	1.112	2.123	1.125
41	9	-0.710	8.940	-0.220	2.800
42		-2.980	8.440	2.460	4.870
43		0.410	3.440	-0.160	-0.090
44		5.760	2.340	2.060	5.920
45		3.090	5.420	0.860	4.100
	(n)	5	5	5	5
	Means	1.114	5.716	1.000	3.520
	Sdevs	3.394	2.936	1.236	2.317

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Animal weight gains per day in (g)
Study number: TOX209A

PRINTED: 11-Nov-08
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Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Animal Group		Day of Phase			
		16	17	18	19
Male Animals					
46	10	1.140	5.940	-0.060	4.990
47		7.940	3.080	-0.880	1.480
48		5.000	-7.830	-18.460	4.340
49		2.300	5.250	-2.620	1.950
50		3.800	6.360	-1.050	3.220
	(n)	5	5	5	5
	Means	4.036	2.560	-4.614	3.196
	Sdevs	2.628	5.944	7.795	1.502
51	11	1.180	11.050	-2.040	1.250
52		5.080	5.740	0.250	0.440
53		0.130	5.470	-2.170	4.750
54		3.710	4.810	-0.510	4.210
55		4.270	2.510	0.290	1.900
	(n)	5	5	5	5
	Means	2.874	5.916	-0.836	2.510
	Sdevs	2.116	3.139	1.202	1.881
56	12	0.470	-0.360	2.410	2.740
57		1.910	4.120	-0.100	3.790
58		-2.680	5.930	-4.460	2.260
59		3.790	0.320	1.870	1.620
60		-0.790	8.590	-5.580	5.400
	(n)	5	5	5	5
	Means	0.540	3.720	-1.172	3.162
	Sdevs	2.479	3.774	3.656	1.481
61	13	-1.210	0.600	-5.210	0.950
62		-4.590	-5.610	-0.120	1.980
63		1.020	-1.530	0.730	0.970
64		-0.140	1.470	-2.330	-2.930
65		-1.470	0.150	-1.950	-0.290
	(n)	5	5	5	5
	Means	-1.278	-0.984	-1.776	0.136
	Sdevs	2.097	2.807	2.300	1.893

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Animal weight gains per day in (g)
Study number: TOX209B

PRINTED: 11-Nov-08
Page: 1

Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Animal Group		6!	1"	2	6	D a y		o f		P h a s e		11	12	13	14	15
						7	8	9	10							
		M a l e A n i m a l s														
66	1	5.137	3.400	7.590	5.857	4.080	4.590	5.640	6.640	3.260	5.030	4.820	1.390	9.300		
67		7.447	2.400							11.610	6.980	9.730	3.530	7.260		
68		4.927	2.650	3.130	4.255	6.090	10.780	-0.660	8.840	1.120	0.510	11.780	7.040	3.840		
69		6.650	2.710	3.370	6.360	8.590	2.660	5.820	4.690	0.540	4.150	4.650	5.550	3.320		
70		7.200	4.560	-4.960	9.942	2.470	3.020	3.620	5.720	0.010	5.780	4.750	8.920	3.440		
	(n)	5	5	4	4	4	4	4	4	5	5	5	5	5		
	Means	6.272	3.144	2.283	6.604	5.308	5.263	3.605	6.473	3.308	4.490	7.146	5.286	5.432		
	Sdevs	1.171	0.874	5.245	2.400	2.642	3.773	3.013	1.768	4.802	2.455	3.374	2.942	2.705		
71	2	6.733	3.565		1.074	15.510	-10.300	11.650	-3.670	9.460	3.080	7.010	-4.750	-1.140		
72		5.863	11.350	-4.800	10.313	-1.750	3.270	4.120	5.670	2.000	-1.620	7.810	6.160	3.280		
73		5.910	1.860	8.180	6.627	-5.210	1.970	6.740	1.310	2.040	2.470	6.560	3.130	4.370		
74		5.353	2.560	3.420	6.893	-0.370	-3.810	14.700	6.750	4.580	0.680	7.140	2.550	5.140		
75		7.540	-0.910	1.550	6.815	-1.130	4.680	0.670	5.680	2.610	3.840	4.190	2.840	3.100		
	(n)	5	5	4	5	5	5	5	5	5	5	5	5	5		
	Means	6.280	3.685	2.088	6.344	1.410	-0.838	7.576	3.148	4.138	1.690	6.542	1.986	2.950		
	Sdevs	0.861	4.596	5.373	3.321	8.097	6.196	5.650	4.348	3.155	2.187	1.389	4.036	2.433		
76	3	5.287	2.290	-0.170	7.633	-4.890	0.090	-0.350	1.540	0.020	-1.220	1.020	-0.260	1.110		
77		5.567	3.665	-14.630	4.042	4.850	-5.580	-0.750	2.010	-2.760	7.320	1.040	0.790	-2.260		
78		6.620	2.610	4.570	5.397	-2.270	-0.090	1.940	4.630	2.570	3.390	6.960	-0.330	7.190		
79		4.830	4.100	-4.090	7.638	-3.960	3.700	1.070	-0.040	-3.380	4.230	3.500	-0.140	7.480		
80		6.090	3.650	3.810	6.620	-2.760	-0.700	-1.800	-1.520	-0.280	-0.060	4.520	0.910	8.500		
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5		
	Means	5.679	3.263	-2.102	6.266	-1.806	-0.516	0.022	1.324	-0.766	2.732	3.408	0.194	4.404		
	Sdevs	0.697	0.772	7.812	1.547	3.859	3.317	1.486	2.313	2.387	3.433	2.509	0.604	4.724		
81	4	7.383	4.050	-2.370	8.785	-8.180	-2.880	-4.290	-2.470	-7.480	1.500	-1.610	0.430	2.010		
82		7.010	5.195	5.330	6.920	-14.570	-2.560	-5.160	1.670	-2.520	1.530	-2.920	-6.110	3.590		
83		6.223	-27.315	3.120	5.498	-8.470	-4.580	-2.120	-4.510	-6.040	-3.680	-0.470	-2.080	-2.170		
84		5.027	2.300	-2.940	5.250	-8.700	-5.590	-2.260	1.650	-7.150	0.660	0.630	-2.980	-0.090		
85		6.040	2.325	4.760	7.337	-11.130	-8.900	-4.290	-1.700	-4.910	2.040	-1.850	-0.500	-6.950		
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5		
	Means	6.337	-2.689	1.580	6.758	-10.210	-4.902	-3.624	-1.072	-5.620	0.410	-1.244	-2.248	-0.722		
	Sdevs	0.917	13.821	3.955	1.443	2.706	2.556	1.357	2.697	2.006	2.339	1.362	2.535	4.103		

Note: ! = Quarantine/Acclimation; " = Exposure phase

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RAT/WISTAR HANOVER

Animal weight gains per day in (g)
Study number: TOX209B

PRINTED: 11-Nov-08
Page: 2

Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Animal Group		D a y o f P h a s e			
		16	17	18	19
		M a l e A n i m a l s			
66	1	1.320	5.960	0.260	4.250
67		6.890	7.210	-1.110	6.900
68		-5.870	0.800	5.390	5.180
69		3.780	5.830	-4.450	6.610
70		2.800	3.810	4.250	4.980
	(n)	5	5	5	5
	Means	1.784	4.722	0.868	5.584
	Sdevs	4.740	2.508	4.014	1.128
71	2	13.260	4.100	3.420	-1.750
72		1.630	4.600	-5.770	4.080
73		5.090	2.800	-3.100	2.890
74		1.080	4.330	-1.170	6.620
75		-0.400	8.890	0.820	1.650
	(n)	5	5	5	5
	Means	4.132	4.944	-1.160	2.698
	Sdevs	5.485	2.312	3.532	3.091
76	3	2.210	-4.430	6.400	-3.160
77		4.820	-1.060	4.240	-1.040
78		-0.660	6.670	-2.880	3.560
79		1.250	5.320	-0.190	1.820
80		-5.360	5.360	1.410	-6.670
	(n)	5	5	5	5
	Means	0.452	2.372	1.796	-1.098
	Sdevs	3.802	4.851	3.644	4.047
81	4	-3.220	0.320	-2.200	-1.410
82		-0.280	-7.620	6.380	-0.760
83		-3.550	-1.640	-4.930	3.690
84		-1.990	-2.590	-1.350	-1.960
85		-3.310	-1.470	-3.470	3.310
	(n)	5	5	5	5
	Means	-2.470	-2.600	-1.114	0.574
	Sdevs	1.366	2.997	4.402	2.708

Note: Data for Exposure phase

Appendix VIII

Data Used for Preparation of Body Weight Figures

TOX209 A & B Rat Data

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	206.24	17.42	217.75	19.44	220.55	29.18	219.36	18.07	223.00	17.57	212.69	24.41	221.50	24.50
1	211.60	15.19	220.78	17.16	225.57	25.45	217.20	22.23	217.85	18.38	209.59	23.38	214.04	18.48
2	217.31	20.30	222.53	17.65	229.54	27.49	218.82	24.21	220.04	18.18	206.76	20.47	208.73	17.23
3	226.62	16.94	226.01	17.44	235.65	27.79	224.41	27.92	220.75	22.23	209.40	24.67	203.54	16.34
4	231.79	15.84	228.48	17.66	241.11	22.80	227.80	24.70	220.86	25.02	208.16	23.40	200.01	16.78
5	237.92	16.36	226.89	17.58	245.85	22.79	231.21	24.97	225.74	24.85	209.50	22.31	199.63	17.88
6	243.19	17.15	232.15	21.07	250.72	20.48	236.89	24.04	229.03	26.34	212.52	23.31	199.91	18.06
7	249.43	15.79	246.03	21.89	258.68	22.77	245.84	21.44	232.39	27.19	216.75	20.99	201.89	17.80
8	255.53	14.40	253.48	18.99	263.35	22.84	251.23	20.49	240.46	20.96	213.38	17.12	199.06	17.86
9	261.14	16.76	260.01	17.45	266.84	22.46	254.98	20.75	243.32	22.47	215.91	19.02	197.68	17.04
10	264.08	15.05	262.78	17.34	269.75	23.46	256.50	21.40	246.15	21.66	218.84	23.71	196.94	17.12
11	268.34	16.85	269.41	16.97	273.29	22.86	262.51	19.43	250.04	22.65	222.43	21.28	199.52	16.20
12	270.97	14.38	273.29	15.83	275.04	22.95	262.34	20.67	250.34	20.64	225.35	24.26	200.07	15.44
13	272.28	15.86	272.36	17.81	277.40	24.15	269.27	20.98	253.95	19.70	225.27	22.73	199.19	13.13

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	206.24	17.42	224.32	24.83	218.80	25.03	215.35	17.33	217.77	23.07	218.32	14.32	212.94	20.45
1	211.60	15.19	230.70	28.85	214.95	16.54	215.99	14.14	215.30	20.21	215.28	10.76	208.03	17.76
2	217.31	20.30	232.01	25.87	219.66	17.66	220.33	10.91	220.15	15.74	216.67	12.55	207.84	18.88
3	226.62	16.94	236.79	29.12	227.25	21.18	224.16	14.55	225.65	16.38	218.10	12.42	211.34	17.65
4	231.79	15.84	241.04	28.39	232.48	19.80	228.32	11.43	231.37	12.34	222.62	10.84	210.24	14.48
5	237.92	16.36	242.38	30.86	236.98	19.19	232.4	12.0	233.85	10.05	225.96	10.95	213.67	16.57
6	243.19	17.15	248.89	30.53	242.59	20.59	235.95	12.05	239.54	14.39	229.02	10.37	211.21	13.73
7	249.43	15.79	251.72	28.92	250.86	19.03	243.57	11.14	244.13	13.96	234.97	9.96	219.14	15.97
8	255.53	14.40	258.71	30.96	253.74	20.64	248.01	11.02	248.81	13.79	237.24	12.33	212.02	12.41
9	261.14	16.76	262.66	31.38	258.38	19.43	250.24	11.46	251.12	14.67	239.22	12.94	208.72	9.71
10	264.08	15.05	264.78	32.93	259.50	18.98	254.28	12.38	254.00	12.94	239.76	13.92	207.44	11.03
11	268.34	16.85	271.18	32.97	265.21	17.95	256.84	12.49	259.91	15.3	243.48	15.5	206.46	12.67
12	270.97	14.38	271.11	33.05	266.21	18.59	252.22	17.30	259.08	14.34	242.31	14.44	204.68	12.37
13	272.28	15.86	273.63	32.67	269.73	18.79	255.42	17.33	261.59	14.39	245.47	15.2	204.82	11.23

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	206.24	17.42	211.02	29.67	222.59	29.22	215.49	15.83	214.99	29.60
1	211.60	15.19	213.53	34.13	224.00	27.47	213.69	14.69	204.78	27.78
2	217.31	20.30	216.38	37.17	223.16	29.85	213.17	15.37	199.88	28.36
3	226.62	16.94	217.87	41.71	230.74	26.97	213.19	14.82	196.25	27.03
4	231.79	15.84	231.12	28.64	233.89	27.08	214.52	13.41	195.18	28.22
5	237.92	16.36	234.43	24.67	238.03	25.76	213.75	14.14	189.56	29.26
6	243.19	17.15	238.92	24.70	239.72	24.35	216.48	12.89	189.97	31.23
7	249.43	15.79	246.07	22.38	246.26	25.02	219.89	14.86	188.73	30.12
8	255.53	14.40	251.35	24.46	248.24	27.00	220.09	15.01	186.48	29.68
9	261.14	16.76	256.79	22.44	251.19	27.23	224.49	19.23	185.76	31.30
10	264.08	15.05	258.57	23.20	255.33	26.80	224.94	15.79	183.29	32.08
11	268.34	16.85	263.29	23.34	260.27	26.07	227.31	20.34	180.69	30.94
12	270.97	14.38	264.16	22.85	259.11	23.65	229.11	17.43	179.57	34.10
13	272.28	15.86	269.74	22.84	261.81	23.57	228.01	17.63	180.15	32.72

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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	5.36	18.27	3.04	6.58	5.02	8.41	-2.16	5.13	-5.15	3.44	-3.10	3.88	-7.07	7.18
2	11.07	19.78	4.79	10.39	8.99	8.65	-0.54	7.12	-2.96	4.05	-5.93	5.00	-12.38	8.52
3	20.37	15.58	8.26	13.03	15.10	11.21	5.05	11.85	-2.24	6.95	-3.29	3.98	-17.58	8.58
4	25.55	13.67	10.74	14.77	20.56	11.36	8.44	9.50	-2.14	10.18	-4.53	5.18	-21.10	8.08
5	31.68	13.50	9.14	15.73	25.30	12.86	11.85	10.07	2.75	11.56	-3.19	5.29	-21.49	6.77
6	36.95	12.40	14.40	14.49	30.18	12.06	17.53	8.04	6.03	12.28	-0.17	4.74	-21.20	6.75
7	43.19	12.30	28.29	13.00	38.13	12.17	26.48	5.72	9.40	13.39	4.06	6.01	-19.22	7.81
8	49.29	11.75	35.74	8.49	42.81	11.90	31.87	3.53	17.46	9.62	0.69	10.44	-22.05	8.21
9	54.90	12.46	42.26	7.27	46.29	11.62	35.62	5.52	20.32	10.49	3.22	7.84	-23.43	9.02
10	57.83	11.95	45.03	4.99	49.20	10.55	37.14	6.70	23.16	10.83	6.16	9.45	-24.17	10.70
11	62.09	13.26	51.66	5.51	52.74	12.28	43.15	3.21	27.04	11.41	9.74	7.40	-21.59	10.30
12	64.72	10.15	55.55	3.97	54.49	11.16	42.98	5.02	27.34	12.63	12.66	11.21	-21.04	11.16
13	66.03	10.45	54.61	5.61	56.86	9.30	49.91	4.85	30.95	10.99	12.59	7.26	-21.93	14.37

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	5.36	18.27	6.38	6.50	-3.86	9.37	0.64	3.47	-2.47	3.04	-3.04	4.45	-4.91	6.17
2	11.07	19.78	7.68	2.55	0.86	11.68	4.98	8.02	2.38	8.23	-1.65	2.88	-5.10	4.77
3	20.37	15.58	12.46	7.08	8.45	6.95	8.82	5.59	7.88	7.00	-0.22	4.34	-1.60	7.64
4	25.55	13.67	16.72	5.46	13.67	6.68	12.97	9.31	13.60	11.23	4.30	7.21	-2.70	7.06
5	31.68	13.50	18.06	9.36	18.17	7.00	17.05	7.94	16.08	13.30	7.64	8.02	0.73	6.55
6	36.95	12.40	24.57	7.00	23.78	5.17	20.60	8.73	21.78	9.41	10.70	6.52	-1.73	7.33
7	43.19	12.30	27.39	6.41	32.06	7.01	28.22	12.49	26.36	9.54	16.65	7.92	6.20	8.29
8	49.29	11.75	34.38	8.08	34.93	6.82	32.66	12.20	31.05	9.88	18.92	10.69	-0.92	9.45
9	54.90	12.46	38.34	7.93	39.58	7.76	34.89	13.35	33.35	8.76	20.90	11.43	-4.22	12.96
10	57.83	11.95	40.45	10.85	40.69	7.76	38.93	13.85	36.23	10.46	21.44	11.98	-5.50	12.34
11	62.09	13.26	46.86	10.86	46.41	7.92	41.49	9.10	42.14	9.46	25.16	13.57	-6.48	12.84
12	64.72	10.15	46.79	10.92	47.41	7.15	36.88	7.55	41.31	10.29	23.99	12.69	-8.26	11.29
13	66.03	10.45	49.3	10.65	50.93	6.92	40.07	7.16	43.82	9.53	27.15	13.06	-8.12	12.27

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	5.36	18.27	5.31	2.64	1.41	8.10	-1.81	3.86	-10.21	2.71
2	11.07	19.78	10.57	4.82	0.57	4.13	-2.32	1.89	-15.11	3.52
3	20.37	15.58	14.18	3.57	8.15	5.59	-2.30	2.77	-18.74	4.27
4	25.55	13.67	20.11	3.89	11.30	4.56	-0.98	4.27	-19.81	4.10
5	31.68	13.50	23.41	5.49	15.43	6.71	-1.74	5.13	-25.43	3.43
6	36.95	12.40	27.90	5.76	17.12	6.97	0.99	7.09	-25.02	3.89
7	43.19	12.30	35.05	7.87	23.67	7.18	4.40	8.39	-26.26	4.09
8	49.29	11.75	40.34	6.89	25.65	4.95	4.59	8.20	-28.51	3.84
9	54.90	12.46	45.77	7.73	28.60	5.28	9.00	10.04	-29.23	6.66
10	57.83	11.95	47.55	9.37	32.73	6.21	9.45	9.73	-31.70	7.34
11	62.09	13.26	52.27	11.08	37.68	5.55	11.82	13.3	-34.30	7.18
12	64.72	10.15	53.14	9.79	36.52	8.22	13.62	9.91	-35.42	9.11
13	66.03	10.45	58.73	10.33	39.22	7.55	12.52	13.47	-34.84	6.52

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
1	17.75	4.71	20.52	7.70	22.68	5.33	17.63	4.65	16.06	3.16	10.90	0.18	9.29	2.52
2	23.28	6.35	23.15	7.38	24.28	4.66	19.57	6.06	17.79	3.99	11.71	1.38	10.08	4.14
3	23.89	1.10	21.66	7.67	22.05	4.80	20.96	7.87	19.00	7.48	20.40	7.41	11.85	4.52
4	23.75	3.38	21.81	5.91	24.39	4.60	23.50	5.95	20.28	6.93	14.82	2.95	11.05	2.49
5	23.18	1.26	21.34	8.27	24.42	2.52	21.98	5.10	22.59	6.17	16.46	3.94	13.86	4.78
6	23.65	0.92	24.01	7.58	27.84	5.85	23.74	4.31	18.97	3.88	15.12	3.69	16.99	7.24
7	24.74	2.50	27.56	6.14	28.12	5.40	27.28	5.33	21.42	7.62	17.74	3.03	14.65	2.43
8	21.50	3.44	24.33	3.00	23.94	2.28	22.06	4.15	27.31	3.37	15.21	5.08	16.29	9.48
9	22.62	3.65	27.95	3.03	24.47	2.98	24.42	2.58	24.29	1.86	16.37	1.52	16.67	9.31
10	23.69	3.28	25.56	2.26	23.81	4.24	23.72	2.89	25.03	5.01	18.24	3.41	21.48	11.22
11	22.49	3.71	27.71	2.55	23.87	0.98	22.61	2.02	24.70	7.86	19.43	1.99	18.55	11.02
12	25.31	2.41	27.36	1.55	22.60	1.93	22.61	4.34	24.35	5.47	22.12	3.99	20.51	11.94
13	24.28	4.75	22.29	3.63	22.54	3.32	25.92	2.72	25.85	6.88	15.10	3.72	16.33	6.64
14	24.01	6.87	25.81	9.35	22.26	2.43	22.62	4.27	23.31	4.61	15.29	1.95	20.23	8.42

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
1	17.75	4.71	25.36	4.37	20.09	5.31	17.77	2.00	18.13	3.05	13.93	0.85	11.01	2.28
2	23.28	6.35	22.90	3.54	20.81	5.74	21.73	4.26	20.54	3.25	18.40	4.79	13.78	3.00
3	23.89	1.10	23.12	7.09	24.85	5.43	21.78	4.92	22.96	2.61	18.73	4.21	18.08	3.13
4	23.75	3.38	25.39	3.93	27.10	3.34	25.43	4.27	24.09	2.23	22.81	5.34	16.90	5.06
5	23.18	1.26	22.55	6.00	25.61	4.14	24.35	3.74	22.92	2.63	26.22	8.20	21.35	1.82
6	23.65	0.92	25.22	1.70	28.00	4.02	24.26	3.59	22.07	4.09	29.00	9.08	13.22	2.94
7	24.74	2.50	19.69	5.90	26.30	4.89	26.56	1.94	23.49	2.91	25.15	6.24	20.90	3.94
8	21.50	3.44	23.47	4.37	21.55	4.99	24.86	2.85	21.64	3.07	26.83	11.17	15.31	7.53
9	22.62	3.65	24.62	4.32	24.12	6.32	24.12	4.29	22.33	3.03	30.01	13.17	16.96	7.40
10	23.69	3.28	23.21	3.19	26.26	5.02	24.11	4.29	24.64	6.89	24.93	10.45	21.51	10.85
11	22.49	3.71	25.50	3.56	24.19	3.79	26.89	2.83	24.60	4.53	23.89	12.93	16.86	10.19
12	25.31	2.41	23.64	4.3	24.49	3.33	22.81	5.57	22.15	2.28	29.90	14.96	18.63	9.28
13	24.28	4.75	22.00	4.2	21.78	5.84	21.75	3.07	20.65	1.86	26.27	11.94	20.87	10.22
14	24.01	6.87	20.83	3.3	20.39	6.05	28.04	4.81	24.99	12.48	28.01	9.52	23.06	10.93

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
1	17.75	4.71	20.75	2.71	18.07	4.04	11.52	3.41	4.99	1.54
2	23.28	6.35	23.34	3.05	18.23	5.30	12.47	1.56	6.67	2.27
3	23.89	1.10	23.55	4.52	25.02	2.35	14.53	1.29	7.60	1.35
4	23.75	3.38	27.03	3.94	21.17	4.40	16.35	4.02	10.28	5.72
5	23.18	1.26	24.87	4.96	25.64	5.17	17.42	5.59	13.19	5.95
6	23.65	0.92	24.17	3.03	18.87	4.13	19.51	0.56	10.83	6.75
7	24.74	2.50	25.26	3.21	21.18	2.34	15.90	3.79	9.58	5.70
8	21.50	3.44	27.68	4.20	22.42	5.54	21.69	6.23	17.82	5.49
9	22.62	3.65	23.32	2.17	21.10	3.03	18.20	5.69	10.60	7.49
10	23.69	3.28	23.33	5.62	24.05	3.92	19.71	4.42	12.53	4.39
11	22.49	3.71	23.71	3.76	22.33	5.38	14.45	4.02	9.86	3.66
12	25.31	2.41	26.34	2.41	23.27	8.23	18.71	2.40	11.56	7.29
13	24.28	4.75	24.83	4.99	19.70	3.89	14.49	4.33	8.75	2.91
14	24.01	6.87	24.21	3.40	20.31	5.85	16.82	4.07	8.40	2.20

Tobacco Blend
Percent Body Weight Gain (g)
Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	0.2 (2A)	2.0 (3A)	4.0 (4A)	8.0 (5A)	20.0 (6A)	40.0 (7A)
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	2.60	1.40	2.28	-0.98	-2.31	-1.46	-3.19
2	5.37	2.20	4.08	-0.25	-1.33	-2.79	-5.59
3	9.88	3.79	6.85	2.30	-1.00	-1.55	-7.94
4	12.39	4.93	9.32	3.85	-0.96	-2.13	-9.53
5	15.36	4.20	11.47	5.40	1.23	-1.50	-9.70
6	17.92	6.61	13.68	7.99	2.70	-0.08	-9.57
7	20.94	12.99	17.29	12.07	4.22	1.91	-8.68
8	23.90	16.41	19.41	14.53	7.83	0.32	-9.95
9	26.62	19.41	20.99	16.24	9.11	1.51	-10.58
10	28.04	20.68	22.31	16.93	10.39	2.90	-10.91
11	30.11	23.72	23.91	19.67	12.13	4.58	-9.75
12	31.38	25.51	24.71	19.59	12.26	5.95	-9.50
13	32.02	25.08	25.78	22.75	13.88	5.92	-9.90

Tobacco Extract
Percent Body Weight Gain (g)
Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	0.2 (8A)	2.0 (9A)	4.0 (10A)	8.0 (11A)	20.0 (12A)	40.0 (13A)
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	2.60	2.84	-1.76	0.30	-1.13	-1.39	-2.31
2	5.37	3.42	0.39	2.31	1.09	-0.76	-2.40
3	9.88	5.55	3.86	4.10	3.62	-0.10	-0.75
4	12.39	7.45	6.25	6.02	6.25	1.97	-1.27
5	15.36	8.05	8.30	7.92	7.38	3.50	0.34
6	17.92	10.95	10.87	9.57	10.00	4.90	-0.81
7	20.94	12.21	14.65	13.10	12.10	7.63	2.91
8	23.90	15.33	15.96	15.17	14.26	8.67	-0.43
9	26.62	17.09	18.09	16.20	15.31	9.57	-1.98
10	28.04	18.03	18.60	18.08	16.64	9.82	-2.58
11	30.11	20.89	21.21	19.27	19.35	11.52	-3.04
12	31.38	20.86	21.67	17.13	18.97	10.99	-3.88
13	32.02	21.98	23.28	18.61	20.12	12.44	-3.81

Nicotine Hydrogen Tartrate
Percent Body Weight Gain (g)
 Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	2.0 (1B)	8.0 (2B)	20.0 (3B)	40.0 (4B)
0	0	0	0	0	0
1	2.60	2.52	0.63	-0.84	-4.75
2	5.37	5.01	0.26	-1.08	-7.03
3	9.88	6.72	3.70	-1.07	-8.72
4	12.39	9.53	5.08	-0.45	-9.21
5	15.36	11.09	6.93	-0.81	-11.83
6	17.92	13.22	7.69	0.46	-11.64
7	20.94	16.61	10.63	2.04	-12.21
8	23.90	19.12	11.52	2.13	-13.26
9	26.62	21.69	12.85	4.18	-13.60
10	28.04	22.53	14.70	4.39	-14.74
11	30.11	24.77	16.93	5.49	-15.95
12	31.38	25.18	16.41	6.32	-16.48
13	32.02	27.83	17.62	5.81	-16.21

Appendix IX

Terminal Body Weights

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Mean Animal Body Weights in (g)
Study number: TOX209A

PRINTED: 26-Mar-09
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Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Group(s)		D a y o f P h a s e	
		19	
		M a l e	A n i m a l s
1	(N) Means Sdevs		5 272.28 15.86
2	(N) Means Sdevs		5 272.36 17.81
3	(N) Means Sdevs		5 277.40 24.15
4	(N) Means Sdevs		5 269.27 20.98
5	(N) Means Sdevs		5 253.95 19.70
6	(N) Means Sdevs		5 225.27+ 22.73
7	(N) Means Sdevs		5 199.19+ 13.13
8	(N) Means Sdevs		5 273.63 32.67
9	(N) Means Sdevs		5 269.73 18.79
10	(N) Means Sdevs		5 255.42 17.33
11	(N) Means Sdevs		5 261.59 14.39

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Mean Animal Body Weights in (g)
Study number: TOX209A

PRINTED: 26-Mar-09
Page: 2

Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

		D a y o f P h a s e	
Group(s)		19	
12	(N)	5	
	Means	245.47	
	Sdevs	15.20	
13	(N)	5	
	Means	204.82+	
	Sdevs	11.23	

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
RAT/WISTAR HANOVER

Mean Animal Body Weights in (g)
Study number: TOX209A

PRINTED: 26-Mar-09
Page: 1

Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Group(s)		D a y o f P h a s e	
		19	
		M a l e	A n i m a l s
1	(N) Means Sdevs		5 272.28 15.86
2	(N) Means Sdevs		5 272.36 17.81
3	(N) Means Sdevs		5 277.40 24.15
4	(N) Means Sdevs		5 269.27 20.98
5	(N) Means Sdevs		5 253.95 19.70
6	(N) Means Sdevs		5 225.27+ 22.73
7	(N) Means Sdevs		5 199.19+ 13.13
8	(N) Means Sdevs		5 273.63 32.67
9	(N) Means Sdevs		5 269.73 18.79
10	(N) Means Sdevs		5 255.42 17.33
11	(N) Means Sdevs		5 261.59 14.39

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Mean Animal Body Weights in (g)
Study number: TOX209A

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Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

		D a y o f P h a s e	
Group(s)		19	
12	(N)	5	
	Means	245.47	
	Sdevs	15.20	
13	(N)	5	
	Means	204.82+	
	Sdevs	11.23	

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
RAT/WISTAR HANOVER

Mean Animal Body Weights in (g)
Study number: TOX209B

PRINTED: 26-Mar-09
Page: 1

Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Group(s)		D a y o f P h a s e	
		19	
		M a l e	A n i m a l s
1	(N) Means		5 269.74
2	(N) Means		5 261.81
3	(N) Means		5 228.01*
4	(N) Means		5 180.15+
5	(N) Means		

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
RAT/WISTAR HANOVER

Individual Animal Feed Consumed/day (g)
Study number: TOX209A

PRINTED: 11-Nov-08
Page: 1

Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Animal Group		D a y o f P h a s e												
		7	8	9	10	11	12	13	14	15	16	17	18	19
		M a l e A n i m a l s												
1	1	21.77	25.08	24.03	28.85	22.35	24.06	22.95	25.31	26.23	28.36	26.71	28.26	31.16
2		16.28	18.85	24.61	24.99	22.19	23.93	25.58	21.00	23.95	23.68	24.03	26.16	25.59
3		13.59	30.38	22.11	20.48	22.86	22.84	24.27	16.20	18.13	22.96	21.89	21.61	22.32
4		23.65	27.30	23.78	21.02	25.32	22.60	28.61	23.61	25.41	24.29	23.16	25.35	24.14
5		13.48	14.79	24.94	23.41	23.19	24.82	22.30	21.37	19.36	19.17	16.66	25.17	18.18
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	17.75	23.28	23.89	23.75	23.18	23.65	24.74	21.50	22.62	23.69	22.49	25.31	24.28
	Sdevs	4.71	6.35	1.10	3.38	1.26	0.92	2.50	3.44	3.65	3.28	3.71	2.41	4.75
6	2	11.57	12.66	14.22	17.09	14.42	12.83	16.90	21.95	23.46	26.20	25.44	28.91	20.25
7		13.20		14.33	14.67	15.10	29.09	31.53	26.12	29.85	27.76	28.81	27.31	24.48
8		22.98	26.81	24.96	25.60	23.95	25.84	28.06	26.69	26.92	26.67	29.29	27.92	25.49
9		28.43	29.46	32.38	28.98	31.89	28.28	31.59	26.62	31.39	25.31	30.41	27.86	24.34
10		26.40	23.66	22.41	22.72			29.73	20.29	28.12	21.84	24.58	24.78	16.88
	(n)	5	4	5	5	4	4	5	5	5	5	5	5	5
	Means	20.52	23.15	21.66	21.81	21.34	24.01	27.56	24.33	27.95	25.56	27.71	27.36	22.29
	Sdevs	7.70	7.38	7.67	5.91	8.27	7.58	6.14	3.00	3.03	2.26	2.55	1.55	3.63
11	3	18.35	24.03	21.42	21.41	21.83	23.86	26.44		25.49	23.86	22.79	19.89	21.25
12		22.76	23.77	28.61	21.32	23.30	22.59	25.26	24.81	24.17	27.74	24.63	24.59	25.99
13		31.67	30.85		31.47	24.19	37.32	37.68		19.45	16.78	22.96	21.34	17.53
14		21.48	25.00	21.10	21.08	24.21	29.08	26.51	25.66	26.59	24.22	23.98	23.40	24.82
15		19.12	17.73	17.06	26.66	28.59	26.34	24.70	21.35	26.65	26.43	24.99	23.80	23.10
	(n)	5	5	4	5	5	5	5	3	5	5	5	5	5
	Means	22.68	24.28	22.05	24.39	24.42	27.84	28.12	23.94	24.47	23.81	23.87	22.60	22.54
	Sdevs	5.33	4.66	4.80	4.60	2.52	5.85	5.40	2.28	2.98	4.24	0.98	1.93	3.32
16	4	15.61	17.14	15.45	16.04	15.72	21.62	22.38	20.18	20.41	20.51	20.17	15.82	22.25
17		21.09	23.07	28.90	22.51	26.05	29.27	24.22	17.73	24.67	25.52	22.93	27.30	27.02
18		22.21	26.94	24.18	32.03	28.29		35.20	27.78	27.18	27.24	25.47	25.24	29.52
19		10.66	10.94	10.15	21.01	19.79	19.31	30.24	19.68	23.84	21.06	23.22	22.52	24.56
20		18.60	19.78	26.14	25.93	20.06	24.75	24.34	24.92	25.99	24.27	21.28	22.18	26.24
	(n)	5	5	5	5	5	4	5	5	5	5	5	5	5
	Means	17.63	19.57	20.96	23.50	21.98	23.74	27.28	22.06	24.42	23.72	22.61	22.61	25.92
	Sdevs	4.65	6.06	7.87	5.95	5.10	4.31	5.33	4.15	2.58	2.89	2.02	4.34	2.72
21	5	16.30	19.32	23.19	22.66	27.41	21.11	23.24	28.96	22.85	25.95	28.82	21.91	20.34
22		10.54	10.77	10.25	11.52	12.02	17.71	12.02	25.62	25.89	21.72	23.09	24.06	19.86
23		17.98	20.79	20.53	26.29	25.07	23.03	23.69	27.45	22.52	27.99	21.85	23.48	22.71
24		17.45	19.07	28.34	26.53	25.93	20.07	31.86	31.71		31.04	35.35	33.43	34.89

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Individual Animal Feed Consumed/day (g)
Study number: TOX209A

PRINTED: 11-Nov-08
Page: 2

Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Animal Group		D a y o f P h a s e												
		7	8	9	10	11	12	13	14	15	16	17	18	19
		M a l e A n i m a l s												
25	5	18.03	19.00	12.68	14.39	22.52	12.94	16.28	22.79	25.90	18.43	14.39	18.85	31.45
	(n)	5	5	5	5	5	5	5	5	4	5	5	5	5
	Means	16.06	17.79	19.00	20.28	22.59	18.97	21.42	27.31	24.29	25.03	24.70	24.35	25.85
	Sdevs	3.16	3.99	7.48	6.93	6.17	3.88	7.62	3.37	1.86	5.01	7.86	5.47	6.88
26	6	11.10	10.80			14.65	12.30	14.44	15.23	16.52	17.00	17.72	17.57	
27		10.72	13.76	22.45	18.12	22.20	14.78	19.66	20.73	14.56	15.34	22.31	22.25	15.02
29		11.00	11.25	12.18	13.87	13.35	12.97	15.97	16.42	16.13	17.46	18.79	21.39	11.42
30		10.76	11.01	26.58	12.46	15.65	20.42	20.88	8.47	18.26	23.17	18.90	27.26	18.85
	(n)	4	4	3	3	4	4	4	4	4	4	4	4	3
	Means	10.90	11.71	20.40	14.82	16.46	15.12	17.74	15.21	16.37	18.24	19.43	22.12	15.10
	Sdevs	0.18	1.38	7.41	2.95	3.94	3.69	3.03	5.08	1.52	3.41	1.99	3.99	3.72
31	7	12.75		16.85	13.06	18.63	20.94	16.52	17.39	14.39	23.59	20.64		11.55
32		7.39	6.45	6.59	8.50	9.88	11.78	12.28	10.47	10.17	18.98	9.48	17.21	13.63
33		6.68	7.39	8.70	8.90	9.94	11.84	13.47	9.11	9.03	11.01	12.24	13.02	
34		10.90	15.58	16.13	14.12	19.45	27.93	17.92	32.28	32.16	39.64	36.84	38.20	26.16
35	7	8.72	10.88	10.98	10.68	11.41	12.47	13.08	12.18	17.61	14.16	13.57	13.59	13.96
	(n)	5	4	5	5	5	5	5	5	5	5	5	4	4
	Means	9.29	10.08	11.85	11.05	13.86	16.99	14.65	16.29	16.67	21.48	18.55	20.51	16.33
	Sdevs	2.52	4.14	4.52	2.49	4.78	7.24	2.43	9.48	9.31	11.22	11.02	11.94	6.64
36	8	20.35	19.57	10.81	21.31	11.99	25.31	21.87	20.11	24.05	17.65	19.55	18.78	16.85
37		29.05	21.28	28.37	24.79	26.56	24.01	22.68	26.52	24.55	24.05	27.73	24.02	22.72
38		25.33	23.78	23.76	24.58	24.28	23.97	17.88	23.01	20.21	24.44	28.34	27.16	23.72
39		30.33	28.65	26.97	31.95	25.94		10.38	29.10	31.73	25.81	26.91	28.45	27.66
40	8	21.75	21.29	25.67	24.34	23.97	27.60	25.66	18.59	22.55	24.11	24.99	19.77	19.04
	(n)	5	5	5	5	5	4	5	5	5	5	5	5	5
	Means	25.36	22.91	23.12	25.39	22.55	25.22	19.69	23.47	24.62	23.21	25.50	23.64	22.00
	Sdevs	4.37	3.54	7.09	3.93	6.00	1.70	5.90	4.37	4.32	3.19	3.56	4.31	4.21
41	9	22.46	19.54	16.74	22.80	18.98	21.39	18.87	15.52	19.11	21.55	20.01	19.89	17.94
42		10.82	11.56	25.38	25.16	25.08	27.26	27.89	23.86	19.15	21.37	25.18	22.34	14.22
43		20.88	26.45	31.80	31.06	30.18	29.17	32.44	28.67	33.95	33.04	29.73	28.19	27.88
44		22.09	22.52	26.46	26.79	27.04	30.79	25.73	20.66	21.62	26.15	24.69	26.31	27.01
45	9	24.19	23.98	23.85	29.68	26.75	31.37	26.57	19.04	26.79	29.20	21.33	25.72	21.87
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	20.09	20.81	24.85	27.10	25.61	28.00	26.30	21.55	24.12	26.26	24.19	24.49	21.78
	Sdevs	5.31	5.74	5.43	3.34	4.14	4.02	4.89	4.99	6.32	5.02	3.79	3.33	5.84

Note: Data for Exposure phase

R.J.R. TOBACCO
TOXICOLOGY DIVISION
Building 630/2
RAT/WISTAR HANOVER

Individual Animal Feed Consumed/day (g)
Study number: TOX209A

PRINTED: 11-Nov-08
Page: 3

Dosing start date: 16-Apr-08

FEEDING STUDY/PALATABILITY

Animal Group		D a y o f P h a s e												
		7	8	9	10	11	12	13	14	15	16	17	18	19
		M a l e A n i m a l s												
46	10	20.59	23.12	26.34	22.95	30.33	27.27	27.06	24.74	29.37	28.25	30.35	29.12	
47		15.46	18.21	27.74	23.96	24.63	28.05	25.18	25.52	20.25	27.53	27.89	25.59	22.73
48		18.49	28.56	16.96	31.63	22.01	24.71	29.57	23.44	25.85	24.73			19.72
49		16.30	19.90	18.18	20.87	20.54	19.78	24.60	21.45	21.02	21.69	23.92	19.66	25.64
50		18.00	18.86	19.66	27.75	24.23	21.47	26.37	29.16		18.34	25.38	16.85	18.92
	(n)	5	5	5	5	5	5	5	5	4	5	4	4	4
	Means	17.77	21.73	21.78	25.43	24.35	24.26	26.56	24.86	24.12	24.11	26.89	22.81	21.75
	Sdevs	2.00	4.26	4.92	4.27	3.74	3.59	1.94	2.85	4.29	4.13	2.83	5.57	3.07
51	11	19.85	17.48	27.34		22.05	26.45	20.91	20.96	23.10	36.31	31.65	25.72	
52		13.43	24.62	22.56	22.99	22.33	18.02	22.39	19.58	20.31	21.87	20.89	20.24	18.73
53		16.99	17.00	22.29	21.87	19.34	18.90	21.15	18.70	18.25	18.15	20.41	20.38	19.41
54		19.05	22.38	22.34	27.04	26.20	20.57	27.57	26.49	25.78	22.80	25.68	22.98	21.88
55		21.31	21.21	20.29	24.44	24.66	26.42	25.44	22.49	24.22	24.06	24.36	21.43	22.57
	(n)	5	5	5	4	5	5	5	5	5	5	5	5	4
	Means	18.13	20.54	22.96	24.09	22.92	22.07	23.49	21.64	22.33	24.64	24.60	22.15	20.65
	Sdevs	3.05	3.25	2.61	2.23	2.63	4.09	2.91	3.07	3.03	6.89	4.53	2.28	1.86
56	12	14.99	16.43	17.56	16.85	26.70	35.12	24.27	20.27	31.25	18.41	13.53	20.98	18.54
57		14.20	14.33	15.81	23.98	17.58	22.19	21.61	20.94	19.84	18.24	21.63	22.57	23.06
58		12.87	15.32	14.23	18.51	20.38	20.39	19.37	16.04	17.05	16.03	17.60	17.05	15.44
59		14.32	19.74	21.64	30.32	27.67	25.62	24.91	34.23	31.42	32.37	20.32	35.18	28.59
60		13.28	26.16	24.40	24.37	38.79	41.66	35.60	42.65	50.48	39.59	46.35	53.74	45.70
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	13.93	18.40	18.73	22.81	26.22	29.00	25.15	26.83	30.01	24.93	23.89	29.90	26.27
	Sdevs	0.85	4.79	4.21	5.34	8.20	9.08	6.24	11.17	13.17	10.45	12.93	14.96	11.94
61	13	7.93	9.38	17.43	12.41	20.89	14.36	20.76	10.45	11.73		17.76	15.21	16.86
62		11.49	14.41	14.17	18.31	19.26	13.50	18.20	9.26	9.56	9.93	9.37	12.27	12.14
63		11.74	12.27	18.79	11.86	20.13	13.27	26.02			21.42	11.46	16.30	18.86
64		9.84	16.50	17.23	17.62	23.11	16.50	16.15	15.72	22.19	18.64	11.51	14.36	
65		14.04	16.32	22.80	24.28	23.36	8.49	23.35	25.79	24.36	36.04	34.20	35.01	35.60
	(n)	5	5	5	5	5	5	5	4	4	4	5	5	4
	Means	11.01	13.78	18.08	16.90	21.35	13.22	20.90	15.31	16.96	21.51	16.86	18.63	20.87
	Sdevs	2.28	3.00	3.13	5.06	1.82	2.94	3.94	7.53	7.40	10.85	10.19	9.28	10.22

Note: Data for Exposure phase

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FEEDING STUDY/PALATABILITY

Animal Group		Day of Phase	
		20	
		Male Animals	
1	1		18.43
2			25.63
4			33.06
5			18.93
	(n)		4
	Means		24.01
	Sdevs		6.87
6	2		35.49
7			30.04
9			24.07
10			13.65
	(n)		4
	Means		25.81
	Sdevs		9.35
11	3		18.81
12			21.86
13			21.67
14			23.67
15			25.31
	(n)		5
	Means		22.26
	Sdevs		2.43
16	4		19.41
17			22.91
18			28.65
19			17.81
20			24.34
	(n)		5
	Means		22.62
	Sdevs		4.27
21	5		18.02
22			22.38
23			23.62
24			29.22
	(n)		4
	Means		23.31
	Sdevs		4.61

Note: Data for Exposure phase

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FEEDING STUDY/PALATABILITY

Animal Group		Day of Phase	
		20	
		Male	Animals
26	6		13.07
27			16.08
29			16.71
	(n)		3
	Means		15.29
	Sdevs		1.95
31	7		22.71
32			14.82
34			31.00
35			12.39
	(n)		4
	Means		20.23
	Sdevs		8.42
36	8		16.18
37			21.05
38			20.16
39			25.51
40			21.24
	(n)		5
	Means		20.83
	Sdevs		3.32
41	9		15.08
42			15.70
43			29.27
44			18.06
45			23.83
	(n)		5
	Means		20.39
	Sdevs		6.05
46	10		30.90
47			29.76
48			33.38
49			24.46
50			21.72
	(n)		5
	Means		28.04
	Sdevs		4.81

Note: Data for Exposure phase

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FEEDING STUDY/PALATABILITY

Animal Group		Day of Phase	
		20	
		Male Animals	
51	11		43.49
52			13.89
53			14.89
54			21.24
55			31.43
	(n)		5
	Means		24.99
	Sdevs		12.48
56	12		25.77
57			25.07
58			21.64
59			22.78
60			44.78
	(n)		5
	Means		28.01
	Sdevs		9.52
61	13		19.64
62			11.14
63			24.12
64			37.33
65			4
	(n)		4
	Means		23.06
	Sdevs		10.93

Note: Data for Exposure phase

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FEEDING STUDY/PALATABILITY

Animal	Group	D a y o f P h a s e												
		7	8	9	10	11	12	13	14	15	16	17	18	19
M a l e A n i m a l s														
66	1	17.40	19.28	20.91	22.37	21.63	23.01	21.04	20.60	21.40	24.71	23.55	22.79	22.68
67		18.79	22.07	30.49	31.65	33.58	29.29	27.78	29.18	20.87	25.25	21.90	29.40	32.47
68		22.33	27.05	18.47	27.03	22.12	21.26	26.89	28.79	25.12	13.40	21.50	27.41	26.69
69		24.17	22.73	23.53	23.98	24.11	23.42	22.61	28.08	23.46	26.30	21.36	26.22	19.39
70		21.07	25.58	24.34	30.13	22.92	23.89	27.97	31.76	25.74	26.99	30.25	25.90	22.92
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	20.75	23.34	23.55	27.03	24.87	24.17	25.26	27.68	23.32	23.33	23.71	26.34	24.83
	Sdevs	2.71	3.05	4.52	3.94	4.96	3.03	3.21	4.20	2.17	5.62	3.76	2.41	4.99
71	2	23.70	14.47	25.08	15.71	25.97	19.66	20.70	16.98	17.42	28.62	21.46	30.93	16.79
72		20.59	23.36	27.09	24.78	34.43	12.91	24.53	31.06	20.75	25.17	28.41	32.08	24.55
73		17.06	20.72	24.21	26.22	23.61	21.46	22.40	20.61	25.85	20.48	27.14	13.51	21.26
74		13.95	10.90	27.22	20.98	22.23	16.84	18.60	19.04	20.45	19.49	15.77	22.84	21.12
75		15.03	21.68	21.50	18.18	21.95	23.48	19.67	24.41	21.02	26.48	18.87	17.00	14.80
	(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	18.07	18.23	25.02	21.17	25.64	18.87	21.18	22.42	21.10	24.05	22.33	23.27	19.70
	Sdevs	4.04	5.30	2.35	4.40	5.17	4.13	2.34	5.54	3.03	3.92	5.38	8.23	3.89
76	3	9.34	13.67	14.32	15.38	13.57	19.13	13.01	19.45		17.93	10.61	20.26	8.96
77		13.35	11.72	13.20	14.74	13.67	18.81	12.67	13.40	13.97	16.81	13.63	15.89	12.99
78		14.96	14.34	14.72	23.15	27.11	19.85	21.72	22.95	24.67	25.81	17.11	21.95	19.83
79		6.68	12.20	16.59	12.50	16.23	20.21	14.52	30.63		22.77	10.96	17.45	12.88
80		13.26	10.42	13.80	15.98	16.52	19.55	17.60	22.03	15.97	15.24	19.93	18.00	17.80
	(n)	5	5	5	5	5	5	5	5	3	5	5	5	5
	Means	11.52	12.47	14.53	16.35	17.42	19.51	15.90	21.69	18.20	19.71	14.45	18.71	14.49
	Sdevs	3.41	1.56	1.29	4.02	5.59	0.56	3.79	6.23	5.69	4.42	4.02	2.40	4.33
81	4	7.02	8.93	8.86	11.54	12.42	18.39	12.30	16.25	11.65	13.96	11.55	11.63	10.94
82			7.26		2.81			9.29	23.44	2.23	9.66	8.66	12.77	10.22
83		4.91	6.24	7.10	7.80	7.66	8.69	8.33	9.25	8.33	9.44	6.34		10.69
84		4.72	7.90	8.52	18.47			1.24	18.82		9.96	7.35	2.05	7.87
85		3.29	3.00	5.93	10.80	19.49	5.40	16.74	21.32	20.18	19.64	15.39	19.77	4.01
	(n)	4	5	4	5	3	3	5	5	4	5	5	4	5
	Means	4.99	6.67	7.60	10.28	13.19	10.83	9.58	17.82	10.60	12.53	9.86	11.56	8.75
	Sdevs	1.54	2.27	1.35	5.72	5.95	6.75	5.70	5.49	7.49	4.39	3.66	7.29	2.91

Note: Data for Exposure phase

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FEEDING STUDY/PALATABILITY

Animal Group		Day of Phase	
		20	
		Male	Animals
66	1		21.06
67			25.77
68			27.27
69			20.06
70			26.89
	(n)		5
	Means		24.21
	Sdevs		3.40
71	2		17.53
72			26.78
73			25.12
74			19.81
75			12.31
	(n)		5
	Means		20.31
	Sdevs		5.85
76	3		12.14
77			13.25
78			18.28
79			18.40
80			22.01
	(n)		5
	Means		16.82
	Sdevs		4.07
81	4		11.76
82			8.79
83			6.42
84			6.40
85			8.62
	(n)		5
	Means		8.40
	Sdevs		2.20

Note: Data for Exposure phase