RJReynolds

Research and Development Formal Report

AUTHORS: Paul H. Ayres, Chandra D. Williams, Suzana Theophilus, Charles D. Garner, and Christopher J. Cook
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2-Week Repeat Investigational Study of the Palatability of Smokeless Tobacco Blend and Extract Formulated in NTP-2000 Diets for Mice at Higher Doses

EXECUTIVE SUMMARY

Summary:
The objective of this study was to evaluate the palatability of diets formulated in NTP-2000 rodent feed with a smokeless tobacco blend, an aqueous tobacco extract of the tobacco blend or nicotine hydrogen tartrate as positive control when fed to male, CFW Swiss Webster mice at doses higher than evaluated in a previous study (TOX210). Dosing was based upon the nicotine content of the blend, extract and tartrate salt and all treatment groups received equivalent doses of nicotine. Mice provided feed formulated with either the tobacco blend, tobacco extract or nicotine tartrate demonstrated a dose dependent decrease in percent cumulative body weight gain and body weight during the 14-day study compared to the control group, which was feed NTP-2000 feed with no additions. The data indicate that a dose of 400 mg nicotine/kg body weight/day would be excessive while a dose of approximately 160 mg nicotine/kg body weight/day could be considered as the high dose for a longer term repeated dosing toxicology study. Additional doses that could be considered for a longer term study range between 2-80 mg nicotine/kg body weight/day or slightly higher. The data also indicate that the male, CFW Swiss Webster mouse is considerably less sensitive to the effects measured in this study than is the male, Wistar Hannover rat investigated in a similar study (TOX209).

Keywords:
TOX213 PALATABILITY TOBACCO BLEND EXTRACT NICOTINE RODENT MOUSE MICE FEED FEEDING WEIGHT NTP-2000

R. J. Reynolds Tobacco Company

Research and Development

Study Number: TOX213

Final Report

2- Week Repeat Investigational Study of the Palatability of Smokeless Tobacco Blend and Extract Formulated in NTP-2000 Diets for Mice at Higher Doses

Paul H. Ayres — 3/20/2009

Paul H. Ayres, Ph.D., D.A.B.T., Principal Scientist, In Vivo Toxicology Support, Product Integrity, Study Director — *Date*

Chandra D. Williams — 3/10/09

Chandra D. Williams, D.V.M. — *Date*

In Vivo Toxicology Support, Product Integrity, Attending Veterinarian

Theophilus — 3/10/09

Suzana Theophilus, Ph.D., D.A.B.T. — *Date*

Product Integrity
Senior Staff Toxicologist

Charles D. Garner — 3/20/09

Charles D. Garner, Ph.D., D.A.B.T., — *Date*

Senior Director
Product Integrity

Christopher J. Cook — 3/20/09

Christopher J. Cook, Ph.D. — *Date*

Vice-President, Product Integrity

A tobacco blend and an aqueous extract of the tobacco blend will be tested in a planned upcoming series of toxicology studies. Also, a positive control, nicotine hydrogen tartrate will be used in some of these studies. 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 male, CFW Swiss Webster mice at doses higher than evaluated in a previous study (TOX210).

Based upon the data from the previous mouse study (TOX210), it was necessary to repeat the study using significantly higher doses of the test articles and positive control to assess the palatability of the dosed feed to male, Swiss Webster mice. The study reported here used a protocol similar to the previous study except the doses of the test articles and positive control mixed into the rodent feed were significantly increased. The test articles and positive control were fed to mice at concentrations that resulted in intended doses of 40, 80, 160, 240 and 400 mg nicotine/kg body weight (bw)/day. The doses used for the current study were believed to encompass a dose range suitable to define the palatability of rodent feed formulated with the test articles and positive control in male, Swiss Webster mice.

Palatability was assessed by comparing the cumulative percent body weight gain and body weight of mice fed NTP-2000 diets formulated to contain increasing concentrations of the tobacco blend, the tobacco extract and the positive control to the cumulative percent body weight gain and body weight of the negative control mice fed the standard NTP-2000 feed with no additions. The duration of the feeding and data collection period was 14 days. Feed intake and body weight was 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.

The negative control mice demonstrated normal body weights and cumulative body weight gains for male, Swiss Webster mice of the age used in the study.

The data from this study demonstrate parallel trends in cumulative percent body weight gain and body weight between the test articles, tobacco blend and tobacco extract, and the positive control nicotine hydrogen tartrate when formulated into NTP-2000 rodent feed at equivalent nicotine doses. Because the positive control contained no tobacco components other than nicotine yet followed the trends seen in the data for the two test articles, this may indicate that the trends seen in this study are more dependent upon nicotine than any of the other tobacco components.

At a nicotine dose of 40 mg/kg bw/day, mice fed dosed feed containing either the tobacco blend or the tobacco extract demonstrated similar trends in percent body weight gain. There was an initial drop in body weight gain after the first day of the study, indicating the mice could detect the presence of the test articles in the feed at this dose in the feed and responded with a decrease in body weight gain. This was followed by an increase in percent body weight gain that resulted in no statistical difference in body weights at study

termination. This indicates the mice were able to acclimate to the feed containing nicotine at a dose of 40 mg/kg bw/day.

When the feed was formulated with the tobacco blend, tobacco extract or nicotine hydrogen tartrate to yield a nicotine dose of 80 mg/kg bw/day, there was a larger initial decrease in percent body weight gain than seen at 40 mg nicotine/kg bw/day for each test article. Again, after the initial drop in the percent body weight gain there was a gradual increase in percent body weight gain that paralleled the trends seen in the untreated control group. At the termination of the study, there were no statistically significant differences in body weights in any of the treatment groups when compared to the untreated control group. This demonstrates that the mice were able to acclimate to the presence of the test articles and nicotine hydrogen tartrate in their feed at this dose.

As the nicotine concentration in the feed was increased to a dose of 160 mg/kg bw/day by increased addition of the tobacco blend, tobacco extract or nicotine hydrogen tartrate to the feed, there was a dose related decrease in percent body weight gain after the first day of dosing. However, unlike the rebound seen at the lower doses during the first week of the study, there was not an increase in percent body weight gain after the initial decrease. The body weight gain continued to decrease slightly each day during the first week of the study, then slightly increased during the second week. The decrease in body weight gain seen at the 160 mg nicotine dose stabilized at approximately -15% compared to initial body weights with the tobacco blend and nicotine tartrate and about -10% for the tobacco extract. These data indicate that the mice could not acclimate to the presence of nicotine in their diet at this dose. At the study termination, the body weights of these mice were statistically significantly decreased compared to the untreated controls. These data indicate that a dose of 160 mg nicotine/kg bw/day is close or slightly higher than a Maximum Tolerated Dose (MTD) for male, Swiss Webster mice as defined by a 10% decrease in body weight. There is little evidence that these mice would acclimate to this dose in a longer term study.

At a dose of 240 mg nicotine/kg bw/day, the mice fed either of the test articles or the positive control demonstrated a severe reduction in percent body weight gain. There was a dose dependent decrease in body weight gain throughout the feeding period that exceeded that seen at 160 mg nicotine. This reduction in body weight was severe enough that feeding the formulated feed was discontinued on day six and the mice were returned to the control diet. A dose of 240 mg nicotine/kg body/day would likely be excessive in longer term studies.

When the dose was increased to 400 mg nicotine/kg bw/day there was a severe reduction in percent body weight gain that was again dose dependent in the groups fed either of the test articles or the positive control. The reduction in body weight gain was excessive enough to require discontinuation of the dosed feed on day four of the study and the mice were returned to the control diet.

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

Overall, this study demonstrated that doses between 40 and 160 mg nicotine/kg bw/day are palatable and tolerable to male, Swiss Webster mice and could be used for long term studies. However, caution needs to be observed at doses higher than 160 mg nicotine/kg bw/day in long term studies if the intent is to maintain the mice at or near an MTD, although doses higher than 160 mg nicotine/kg bw/day may be warranted to detect toxicological changes in intermediate term studies.

R. J. Reynolds Tobacco Company

Research and Development

Study Number: TOX213

Final Report

2- Week Repeat Investigational Study of the Palatability of Smokeless Tobacco Blend and Extract Formulated in NTP-2000 Diets for Mice at Higher Doses

3/20/2009

Paul H. Ayres, Ph.D., D.A.B.T., Principal Scientist,
In Vivo Toxicology Support, Product Integrity,
Study Director — *Date*

3/10/09

Chandra D. Williams, D.V.M.
In Vivo Toxicology Support, Product Integrity,
Attending Veterinarian — *Date*

3/10/09

Suzana Theophilus, Ph.D., D.A.B.T.
Product Integrity
Senior Staff Toxicologist — *Date*

3/20/09

Charles D. Garner, Ph.D., D.A.B.T.,
Senior Director
Product Integrity — *Date*

3/20/09

Christopher J. Cook, Ph.D.
Vice-President, Product Integrity — *Date*

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
Product Integrity
In Vivo Toxicology Support
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

Study Director: Principal Scientist Product Integrity	Paul H. Ayres, Ph.D., D.A.B.T.
Attending Veterinarian: Product Integrity	Chandra D. Williams, DVM
Program Manager: Research Resources of NC, Inc:	Jessica Baker, BS, LAT

5. Study Schedule

Quarantine Start:	May 21, 2008
End of Quarantine:	May 26, 2008
First Day of Dosing:	May 27, 2008
Last Day of Dosing	June 9, 2008
Study Termination:	June 11, 200

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V. LIST OF ABBREVIATIONS USED IN REPORT

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

QUALITY ASSURANCE STATEMENT

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

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

Quality Assurance Investigator

28-Apr-09
Date

FINAL REPORT TOX213

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

VI. EXECUTIVE SUMMARY

A tobacco blend and an aqueous extract of the tobacco blend will be tested in a planned upcoming series of toxicology studies. Also, a positive control, nicotine hydrogen tartrate will be used in some of these studies. 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 male, CFW Swiss Webster mice at doses higher than evaluated in a previous study (TOX210).

Based upon the data from the previous mouse study (TOX210), it was necessary to repeat the study using significantly higher doses of the test articles and positive control to assess the palatability of the dosed feed to male, Swiss Webster mice. The study reported here used a protocol similar to the previous study except the doses of the test articles and positive control mixed into the rodent feed were significantly increased. The test articles and positive control were fed to mice at concentrations that resulted in intended doses of 40, 80, 160, 240 and 400 mg nicotine/kg body weight (bw)/day. The doses used for the current study were believed to encompass a dose range suitable to define the palatability of rodent feed formulated with the test articles and positive control in male, Swiss Webster mice.

Palatability was assessed by comparing the cumulative percent body weight gain and body weight of mice fed NTP-2000 diets formulated to contain increasing concentrations of the tobacco blend, the tobacco extract and the positive control to the cumulative percent body weight gain and body weight of the negative control mice fed the standard NTP-2000 feed with no additions. The duration of the feeding and data collection period was 14 days. Feed intake and body weight were to be 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.

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At a nicotine dose of 40 mg/kg bw/day, mice fed dosed feed containing either the tobacco blend or the tobacco extract demonstrated similar trends in percent body weight gain. There was an

initial drop in body weight gain after the first day of the study, indicating the mice could detect the presence of the test articles in the feed at this dose and responded with a decrease in body weight gain. This was followed by an increase in percent body weight gain that resulted in no statistical difference in body weights at study termination. This indicates the mice were able to acclimate to the feed containing nicotine at a dose of 40 mg/kg bw/day.

When the feed was formulated with the tobacco blend, tobacco extract or nicotine hydrogen tartrate to yield a nicotine dose of 80 mg/kg bw/day, there was a larger initial decrease in percent body weight gain than seen at 40 mg nicotine/kg bw/day for each test article. Again, after the initial drop in the percent body weight gain there was a gradual increase in percent body weight gain that paralleled the trends seen in the untreated control group. At the termination of the study, there were no statistically significant differences in body weights in any of the treatment groups when compared to the untreated control group. This demonstrates that the mice were able to acclimate to the presence of the test articles and nicotine hydrogen tartrate in their feed at this dose.

As the nicotine concentration in the feed was increased to a dose of 160 mg/kg bw/day by increased addition of the tobacco blend, tobacco extract or nicotine hydrogen tartrate to the feed, there was a dose related decrease in percent body weight gain after the first day of dosing. However, unlike the rebound seen at the lower doses during the first week of the study, there was not an increase in percent body weight gain after the initial decrease. The body weight gain continued to decrease slightly each day during the first week of the study, then slightly increased during the second week. The decrease in body weight gain seen at the 160 mg nicotine dose stabilized at approximately -15% compared to initial body weights with the tobacco blend and nicotine tartrate and about -10% for the tobacco extract. These data indicate that the mice could not acclimate to the presence of nicotine in their diet at this dose. At the study termination, the body weights of these mice were statistically significantly decreased compared to the untreated controls. These data indicate that a dose of 160 mg nicotine/kg bw/day is close or slightly higher than a Maximum Tolerated Dose (MTD) for male, Swiss Webster mice as defined by a 10% decrease in body weight. There is little evidence that these mice would acclimate to this dose in a longer term study.

At a dose of 240 mg nicotine/kg bw/day, the mice fed either of the test articles or the positive control demonstrated a severe reduction in percent body weight gain. There was a dose dependent decrease in body weight gain throughout the feeding period that exceeded that seen at 160 mg nicotine. This reduction in body weight was severe enough that feeding the formulated feed was discontinued on day six and the mice were returned to the control diet. A dose of 240 mg nicotine/kg body/day would likely be excessive in longer term studies.

When the dose was increased to 400 mg nicotine/kg bw/day there was a severe reduction in percent body weight gain that was again dose dependent in the groups fed either of the test articles or the positive control. The reduction in body weight gain was excessive enough to require discontinuation of the dosed feed on day four of the study and the mice were returned to the control diet.

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

Overall, this study demonstrated that doses between 40 and 160 mg nicotine/kg bw/day are palatable and tolerable to male, Swiss Webster mice and could be used for long term studies. However, caution needs to be observed at doses higher than 160 mg nicotine/kg bw/day in long term studies if the intent is to maintain the mice at or near an MTD, although doses higher than 160 mg nicotine/kg bw may be warranted to detect toxicological changes in intermediate term studies.

VII. INTRODUCTION

The objective of this study was to further evaluate the palatability of diets formulated in NTP-2000 rodent feed with a tobacco blend, an aqueous extract of the tobacco blend or nicotine hydrogen tartrate as positive control when fed to Swiss Webster mice. A tobacco blend and an extract of the blend and a positive control (nicotine hydrogen tartrate) will be investigated in planned toxicology studies using this strain of mouse. Therefore, it is important to determine if feed containing these materials is palatable to mice.

A number of variables had to be considered in the design this 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 chemical complexity of the test article. Tobacco is a natural plant product that consists of large numbers 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 readily available in a number of laboratories. The toxicity of nicotine has been investigated in a number of animal species, including rats and mice (HSDB, 2008). These factors support the use of nicotine as the basis for dosing the mice and the formulation of the rodent feed for this study. Therefore, the dosing of the mice and the formulation of the dosed feed was based upon mg nicotine/kg body weight/day. This basis for dosing and feed formulation requires knowledge of the nicotine content of the tobacco, tobacco extract and the nicotine salt positive control used in the study. It also allows the determination of nicotine in the dosed feed to confirm the animals received their proper doses.

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

positive control than were rats. For example, at a dose of 40 mg nicotine/kg bw/day, rats demonstrated a severe depression of body weight gain and loss of body weight while mice exposed to this dose demonstrated minimal changes in these variables (TOX209, TOX210). Therefore, the previous mouse study was repeated in this study using nicotine doses of 0, 40, 80, 160, 240 and 400 mg nicotine/kg bw/day.

A third variable was whether to use both genders of mice or to use a single gender. To limit the size of these short term investigational studies to approximately 100 animals each, 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 there could be gender differences in the neurophysiological response to nicotine.

A fourth variable was the strain of mouse to be used in these studies. It had been decided that the most appropriate mouse strain for the planned toxicology studies was the outbred Swiss Webster strain. Supporting this decision was the choice of this mouse strain for longer term toxicology studies by the National Toxicology Program because of their robust nature.

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

With these considerations in mind and based upon the data from a previous mouse study (TOX210), it was necessary to conduct an additional study using significantly higher doses of the test articles and positive control to assess the palatability of the dosed feed to Swiss Webster mice. The study reported here used a protocol similar to the previous study except the doses for the test articles and positive control were significantly increased. The test articles and positive control were fed to mice at concentrations that resulted in doses of 40, 80, 160, 240 and 400 mg nicotine/kg bw/day. The doses used for the current study were believed to encompass a dose range suitable to define the palatability of rodent feed formulated with the test articles and positive control in Swiss Webster mice.

VII. MATERIALS AND METHODS

A. TEST ARTICLES

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

1. Test Article 1 Tobacco Blend

Test Article 1 was identified as Tobacco Blend Lot#0T162AF and consisted of a blend of natural tobaccos ground to a powder, which contained no preservatives or other additives. It was reported to contain 2.63% nicotine by weight 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 for Test Article 1 is on file with the Sponsor. Because tobacco is a complex mixture of natural components, its purity can not be ascertained. Upon arrival at the testing facility, the test article was stored at 4 ^{o}C. The Test Article was mixed to ensure uniformity before aliquots were removed for feed formulation. After the removal of the aliquots for this study, the test article was stored at 4 ^{o}C for potential additional use.

2. Test Article 2 Tobacco Extract

Test Article 2 was identified as Tobacco Extract Lot#0T162AE and consisted of an aqueous extract of Test Article 1. Its water content was adjusted to result in 1 ml of Test Article 2 being equivalent to 1 g of Test Article 1. It contained no components not contained in the tobacco and the water used for extraction. The water used for extraction of the tobacco was analyzed for a series of components and the results are on file with the sponsor. Because the aqueous extract is a complex mixture of materials extracted from the tobacco, its purity can not be ascertained. 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 ^{0}C. Before use for feed formulation, the Tobacco Extract was thawed at room temperature, shaken to ensure complete mixing and appropriate quantities of extract removed for dosed feed formulation and then refrozen. 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.

3. Positive Control Nicotine Hydrogen Tartrate

The positive control used in the study was nicotine hydrogen tartrate (Lot#077K1810) obtained from Sigma-Aldrich Co., St. Louis, MO. The Certificate of Analysis 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, except it was stored desiccated after opening.

4. Safety

Safety procedures were employed for personal protection, due to 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 with controlled entry.

During feed formulation and mixing, two people were present in case of any direct exposures to the technical staff 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 May 19, 2008, before arrival of the animals into the facility. Ninety five male, Swiss Webster mice (5-7 weeks of age) from Charles River Laboratories (Portage, Mich.) were received into the facility on May 21, 2008 along with 10 sentinel mice. Sentinel mice were retired breeders and maintained under identical conditions as the study animals, except they were fed Lab Diet, Certified Rodent Diet #5002 feed (PMI Nutrition International), provided as pellets throughout the study.

b) Animal Identification

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

Table 1: **Treatment Groups, Doses and Concentration of Test Article or Positive Control in Feed**[1]

Group Number	Treatment Group and Nicotine Dose (mg nicotine/kg body weight/day)		Concentration of Test Article, Positive Control in Feed[2] (mg/g feed)	Number of Mice	Mouse ID Numbers
	Control				
1	NTP-2000 feed (0)		0.00	10	1-10
	Tobacco Blend				
2	Dose 1 Tobacco in NTP-2000 feed	(40)	7.03	5	11-15
3	Dose 2 Tobacco in NTP-2000 feed	(80)	14.05	5	16-20
4	Dose 3 Tobacco in NTP-2000 feed	(160)	28.10	5	21-25
5	Dose 4 Tobacco in NTP-2000 feed	(240)	42.16	5	26-30
6	Dose 5 Tobacco in NTP-2000 feed	(400)	70.26	5	31-35
	Tobacco Extract				
7	Dose 1 Tobacco Extract in NTP-2000 feed	(40)	8.03	5	36-40
8	Dose 2 Tobacco Extract in NTP-2000 feed	(80)	16.05	5	41-45
9	Dose 3 Tobacco Extract in NTP-2000 feed	(160)	32.11	5	46-50
10	Dose 4 Tobacco Extract in NTP-2000 feed	(240)	48.16	5	51-55
11	Dose 5 Tobacco Extract in NTP-2000 feed	(400)	80.27	5	56-60
	Positive Control				
12	Dose 1 Nicotine Tartrate in NTP-2000 feed	(40)[3]	0.53[4]	5	61-65
13	Dose 2 Nicotine Tartrate in NTP-2000 feed	(80)	1.05	5	66-70
14	Dose 3 Nicotine Tartrate in NTP-2000 feed	(160)	2.10	5	71-75
15	Dose 4 Nicotine Tartrate in NTP-2000 feed	(240)	3.16	5	76-80
16	Dose 5 Nicotine Tartrate in NTP-2000 feed	(400)	5.26	5	81-85
	Sentinels				
	Sentinels (no treatment)			10	86-95

[1] Doses in parentheses represent the nicotine dose in mg nicotine/kg body weight/day.
[2] Concentration is expressed as the amount of test article added/g feed. For instance, 7.03 mg of the tobacco blend added to one gram of feed and 8.03 mg of tobacco extract added to one gram of feed.
[3] Actual dose was chemically confirmed as ~4 mg nicotine/kg body weight/day.
[4] The intended concentration was 0.53 mg nicotine/g of feed. The actual concentration was ~ 0.05 mg/g feed.

Data associated with the use of mice on this 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 TOX213A and TOX213B.

Because of the limitations in the Path/Tox system, two protocols were created to accommodate all 16 dosed groups. TOX213A contains Study Groups 1-11. TOX213B contains the five Nicotine Tartrate Positive Control Groups (i.e. Xybion protocol TOX213B Group 1 is study Group 12; Group 2 is Group 13; Group 3 is Group 14; Group 4 is Group 15 and Group 5 is Group 16).

The Xybion data collection protocols TOX213A and TOX213B were used for body weights, feed consumption and clinical observations for mice used on this study. Data were input into the Xybion Path/Tox collection protocols under the "A" module ("AINPUT").

c) Animal Housing

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

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

Mice were quarantined and acclimated to the facility for a minimum of six days prior to initiation of the study. The Attending Veterinarian performed a health examination of all mice within two days after delivery. Commencement of dosing the mice was dependent upon a favorable review of the health examination, as well as a written statement from the Attending Veterinarian releasing the mice from quarantine. Mice were approved for release from quarantine on May 24, 2008 but continued under quarantine conditions until May 26.

d) Feed and Water

All mice, with the exception of the sentinel mice, were fed *ad libitum* NTP-2000 powered feed (Zeigler Bros., Inc., Gardners, PA) throughout the study, including the quarantine period. The sentinel mice were fed *ad libitum* Certified Rodent Diet #5002 (PMI Nutrition International) pellets throughout the study. After initiation of the dosing period, NTP-2000 feed was provided as a powdered diet formulated with the appropriate doses of test articles, positive control or as a control diet with no test article. Clean feeders were provided weekly. Graphic or tabular representation of raw data for mice that spilled or contaminated their feed could be censored for days when excessive spillage was reported or when the data were unreasonable for the specific animal based upon group means and previous and subsequent feed intake for that specific animal. For instance, if an animal's feed intake more than doubled or was reduced by more than half, the data for that animal on that day could be censored.

Feed was provided to the mice in glass feed cups with stainless steel lids that minimized spillage but provided the mice access to the feed. The volume of the feed cups was adequate for several days feed; however, feed consumption was determined daily and fresh feed placed in the feed cups. This would have resulted in a large waste of feed each day. To minimize loss of feed

resulting from determination of daily feed consumption, a Delrin spacer was added to the feed cups to displace a portion of the feed. This minimized feed waste yet provided the mice adequate quantities of feed to insure *ad libitum* feeding. Since the spacer was below the surface of the feed, the mice did not have access to the spacer and there was no evidence of gnawing or biting on the spacers.

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

Mice were assigned to dose groups according to body weight using the "A" module of the PATH/TOX software (version 4.2.2; Xybion Medical Systems; Cedar Knolls, NJ) on May 27, 2008. Body weights and detailed clinical signs were recorded before allocation. At the discretion of the Study Director, mice exhibiting positive clinical signs, demonstrating body weight loss, or representing low or high extremes of body weight could be excluded from the allocation process. To ensure groups of similar mean body weight, all groups within the PATH/TOX protocol were compared by analysis of variance (ANOVA) and least significant difference criteria, and demonstrated not to be significantly different at a 5 percent, two-sided risk level. Following allocation into groups, mice were uniquely identified with their permanent identification number by tail marking on May 27, 2008 with their unique animal number using indelible ink and assigned to cages with permanent cage cards attached that provided 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 mice.

b) Dose Regimen

A total of 16 groups were used along with a sentinel group (Table 1). Each treatment group contained 5 male mice with the exception of the untreated control group (Group 1), which contained 10 mice. Mice in Group 1 were fed NTP-2000 feed without the addition of the test articles or positive control. Groups 2-6 were fed NTP-2000 feed with additions of the tobacco blend to yield the following mg nicotine/kg bw/day: 40, 80, 160, 240, and 400, respectively. Nicotine dosing was based upon data from TOX210 and chosen to encompass a dose range that

would allow determination of the palatability of the formulated feed for mice but below that which would produce acute toxicity. Groups 7-11 were fed NTP-2000 feed with additions of the tobacco extract to yield the mg nicotine/kg bw/day equivalent to those mice in Groups 2-6. The positive control (Groups 12-16), were fed NTP-2000 feed that contained nicotine hydrogen tartrate to yield nicotine doses equivalent to those of the test articles. Formulation of the feed to yield the required doses of nicotine for the duration of the study is dependent on two factors. First the mean body weight range of the mice for the duration of the feeding period must be assumed. An assumption of 30 g (0.03 kg) was used. Second, the mean feed intake range of the mice for the duration of the feeding period must also be assumed and is related to the mean body weight. An assumption of 6.5 g feed consumed/day was used. For this study, the data obtained from TOX210 was used as the basis for calculations related to formulation of the diets with the test articles and positive control for each dose. The calculations for the amount of both test articles and the positive control to yield the required nicotine concentrations at each dose are provided in Appendix III.

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

c) Dosed Feed Formulation

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

Formulated feed was prepared once for the study based upon the results of stability determinations conducted during the TOX209 and TOX210 studies, which indicated the test articles and positive control were stable in the feed for at least 30 days at room temperature.

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 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 required quantity positive control (nicotine hydrogen tartrate) was weighed and added to a clean porcelain mortar containing approximately five grams of NTP-2000 feed and ground lightly with the pestle to break up any lumps of nicotine hydrogen tartrate 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 premix. The premix was then subjected to mechanical mixing with the KitchenAid mixer 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. All mixing bowls and other apparatus used in feed formulation were cleaned before moving to the next higher dose formulation to minimize any carry over from

the previous formulation. Feed formulations were conducted during the week before initiation of feeding the formulated diets. 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.

Samples from the top, middle and bottom portions 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. The data for each sample portion for the homogeneity determination at the low and high dose was averaged for dose confirmation. Samples of each test article and positive control at the intermediate doses were removed for analysis of nicotine to confirm the proper dose formulation.

d) Analysis of Formulated Feed

The nicotine concentrations in the formulated feed were determined by a method developed at RJRT for analysis of nicotine in NTP-2000 powdered rodent feed. This method did not undergo complete validation but appears adequate to demonstrate the homogeneity and that dose formulation was conducted in a manner adequate for the purpose of this investigational study.

3. Biological Observations

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

a) Serology/Health Screens

Sentinel mice were handled identically to the study animals and placed in Room 40 with the study animals. Because of the short term nature of the study, prestudy sentinel mice were not employed. At study termination on June 11, 2008, the ten sentinel mice for health screening were anesthetized with 70% carbon dioxide (CO_2) in air and blood was drawn from either the vena cava or heart. While still under anesthesia, the animals were then euthanized by exsanguination. The health screen mice provided sera appropriate for measurement of the following antibodies to disease using the Charles River Laboratory Mouse Assessment Plus profile that consisted of the following: pneumonia virus of mice (PVM), mouse hepatitis virus (MHV), Minute virus of mice (MVM), Sendai virus, murine encephalomyelitis virus (GDVII), REO-3, *Mycoplasma pulmonis*, lymphocytic choriomeningitis virus (LCMV), Ectromelia (mousepox), K virus, polyoma virus, mouse adenovirus (MAV) 1 & 2, epizootic diarrhea of infant mice virus (EDIM), mouse cytomegalovirus (MCMV), Hantaan virus (HANT), *Encephalitozoon cuniculi* (ECUN), ciliated associated respiratory bacillus (CARB), mouse parvovirus (MPV) 1 & 2, mouse thymic virus (MTLV) and murine norovirus (MNV). Serology was performed by Charles River Research Animal Diagnostic Services, Wilmington, MA. In addition, during necropsy the lungs were removed and sent to Seventh Wave, Burlington, NC for histopathological examination for evidence of disease.

Commencement of animal dosing was dependent upon a favorable review of the health status of the animals and a written statement from the Attending Veterinarian releasing the animals from quarantine. The mice were released to the study from quarantine on May 24 and continued under quarantine conditions until dosing commenced on May 27, 2008.

b) Moribundity/Mortality Checks

Twice daily observations of all animals during weekdays, once in the morning and once in the afternoon (at least 6-hours apart), were performed to identify dead or moribund mice. For weekends and holidays, only one observation per day was performed. Mice whose conditions made it unlikely that they would survive to the next observation period or seemed to be in pain were to be euthanized at the discretion of the Attending Veterinarian and/or the Study Director. Clinical observations were to be recorded shortly before euthanasia. Any mice, including sentinels, euthanized in a moribund condition were to have serum collected for serology at the discretion of the Attending Veterinarian or Study Director.

c) Clinical Observations

The mice were subjected to observations for clinical signs twice each week. All findings were recorded using the "AINPUT" module of the PATH/TOX computer software. Negative findings (normal/no significant findings) were also recorded.

d) Body Weights

Individual non-fasted body weights were determined two days after delivery, again prior to study group allocation (i.e., prior to the initial dosing). Upon initiation of feeding the formulated feeds, body weights were recorded daily until the 15^{th} day of the study. This resulted in a lack of body weight data for the last day of the study. Determination of body weights was conducted generally between 7:00 and 11:00 AM. The "A" module of the Xybion PATH/TOX system was used for acquisition of body weight data. Individual body weights were used to calculate the group's mean body weight and body weight gain for each experimental group. Percent body weight gain was calculated from the group mean body weight data. Mouse weights were acquired using Mettler PM2000 balances (Mettler Instrument Corporation, Highstown, NJ).

Groups of mice that experienced a 20% or more cumulative group mean body weight loss for two consecutive days relative to the group mean body weight on the day prior to the onset of administration of dosed feed would be taken off the study and provided the control NTP-2000 feed.

e) Feed Consumption

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

f) Terminal Body Weights

The non-fasted, terminal body weights for the mice in each study group were determined either on the 14th day of the feeding period or upon removal of the dosed feed and return to the control feed based upon the decline in group mean body weight, as previously noted.

4. Statistics

Body Weights

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

5. Records Maintained

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

IX. RESULTS AND DISCUSSION

A. Feed Formulation Analysis

Calculations of feed requirements (average daily feed consumption and body weight) for this study were based upon extrapolation of the data from TOX210. Dosed feed preparation was conducted once before initiation of the feeding of the formulated feeds based upon previous stability data that indicated the nicotine in the formulated feed was stable for at least 30 days at room temperature. The formulated feed 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.

The two major sources of error in these data are the diet formulation and the analytical chemistry. As noted earlier, a new unvalidated analytical technique was developed to determine the nicotine concentrations in the NTP-2000 rodent feed. It is not possible, based upon these data, to determine the major source of the discrepancy.

Homogeneity data are presented in Table 2. These data are from the low dose formulation (40 mg nicotine/kg bw/day) and the high dose formulation (400 mg nicotine/kg bw/day) based upon the assumption that if the low and high doses demonstrated adequate homogeneity, then the intermediate doses can be assumed to have adequate homogeneity because all formulations were conducted using identical techniques. Samples were obtained from the top of the formulated feed mixture as well as the middle and bottom of the mixture.

Table 2: **Feed Formulations Homogeneity Data[1] and Dose Confirmation[1]**

Target Concentration (mg nic/g feed)	Sample Location **Top** (mg nic/g feed)	**Middle** (mg nic/g feed)	**Bottom** (mg nic/g feed)	Average Concentration (mg nic/g feed)
		Dose 40 mg nic/kg bw/day		
		Tobacco Blend		
0.18	0.17 (5.6%)	0.15 (17%)	0.13 (28%)	0.15 + 0.02[2] (16.9% ± 11.2%)[2]
		Tobacco Extract		
0.18	0.20 (11%)	0.16 (11%)	0.18 (0%)	0.18 ± 0.02 (7.3% ± 6.4%)
		Nicotine Tartrate		
0.18	0.017 (90.6%)	0.017 (90.6%)	0.016 (91.1%)	0.017 ± 0.01 (90.8% ± 0.3%)
		Dose 400 mg nic/kg bw/day		
		Tobacco Blend		
1.85	1.60 (14%)	1.69 (9%)	1.64 (11%)	1.64 ± 0.05 (11.3% ± 2.7%)
		Tobacco Extract		
1.85	1.59 (14%)	1.54 (17%)	1.67 (10%)	1.60 ± 0.07 (14% ± 3.5)
		Nicotine Tartrate		
1.85	1.52 (18%)	1.49 (19%)	1.51 (18%)	1.51 ± 0.03 (18% ± 0.5%)

[1] Analytical method uncertainty for nicotine analysis = ± 5.2%; data represent the mean of duplicate analytical runs. Data in parentheses represent the percent difference from the target concentration.
[2] Data represent mean ± standard deviation where appropriate.

At the low dose, the tobacco blend demonstrated adequate homogeneity for the objectives of this study. Samples taken form the bottom of the blender bowl demonstrated the greatest discrepancy (-28%) when compared to the expected nicotine content, while the overall discrepancy was -16.9%. At the high dose, the tobacco blend from the top of the bowl demonstrated the highest discrepancy (-14%) when compared to the expected values. Comparing the observed differences seen at the low and high dose implies a lack of systematic error in the diet formulations because the locations of greatest discrepancy, bottom of bowl in one case and top of bowl in another, are different. Overall, these data indicate that the homogeneity of the formulations were adequate for the purpose of this investigational study.

Feed formulated with the tobacco extract showed excellent homogeneity in respect to nicotine content at the low and high doses. This indicates that the difficulties encountered in obtaining complete homogeneity with this test article in earlier studies (TOX209 and TOX210) had been overcome. In respect to confirmation of the expected nicotine concentrations in feed, at the low dose, there was excellent agreement between the expected concentration and the analytically

determined concentration while at the high dose the analytically determined values were slightly lower than expected, although the agreement was adequate for the purpose of this study.

Homogeneity and dose confirmation data for the nicotine hydrogen tartrate containing NTP-2000 rodent feed followed the trends seen with the tobacco extract, with the exception of the low dose. At the low dose, the homogeneity of nicotine in the diet was excellent and at the high dose it was more than adequate. However, in respect to dose confirmation, the agreement between the expected nicotine concentration and the analytically determined nicotine was not adequate at the low dose. The anticipated concentration was 0.18 mg nicotine/g feed while the analytically determined concentration was 0.017 mg nicotine/g feed, an order of magnitude difference. Reanalysis of the formulated feeds for nicotine concentration again found a nicotine concentration of 0.017 mg nicotine/g feed, confirming the initial analytics. Therefore the body weight and body weight gain data obtained from Group 12, nicotine hydrogen tartrate at a dose of 40 mg/kg bw/day are not appropriate for use in this study because the dose of nicotine was approximately 4 mg nicotine/kg bw/day instead of the intended dose. All graphical representations of the low dose of nicotine hydrogen tartrate are noted as ~4 mg nicotine/kg bw/day. Indeed, there were no differences between the percent body weight gain from this group and the control. This indicates that at this inclusion of nicotine (approximately 4 mg nicotine/kg bw) this dose was within the No Observable Adverse Effect Level (NOAEL) for nicotine. The nicotine concentration at the high nicotine dose for the positive control was slightly below the anticipated concentration, but was adequate for the purposes of this investigational study.

Overall, both the homogeneity and dose confirmation data from the high and low dose concentrations indicate that the feed was adequately formulated with the test articles and positive control to meet the objectives of this investigational study, with the exception of the low dose nicotine hydrogen tartrate. Analytical data for the homogeneity analysis are provided in Appendix III.

Dose Confirmation Data: Dose confirmation data from two independent analyses for the feed formulations used in this study are presented in Tables 3–5. The original analytical methodology was not sensitive enough to determine the nicotine concentration at the lower doses of formulated feed and an improved analytical methodology was developed; however, this methodology 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. These data in combination with the dose responses seen in the study indicate that the proper formulated feeds were fed to the mice with the exception of the low dose nicotine hydrogen tartrate positive control data (Study Group 12 Nicotine Tartrate in NTP-2000 feed, 40 mg nicotine/kg bw/day). Data for this Group are included in this report because the actual dose (approximately 4 mg nicotine/kg bw/day) is known from the dose confirmation data and the discrepancy is noted in the data presentation.

Table 3: **Dose Confirmation Data Tobacco Blend**[1]

Target Dose (mg of nicotine/kg of bw/day)	**Target Concentration** (mg of nicotine/g of feed)	**Determined Feed Nicotine Concentration** (mg of nicotine/g of feed)
40	0.18	0.15
80	0.37	0.31
160	0.74	0.61
240	1.11	0.95
400	1.85	1.64

[1] Data for target doses of 80, 160 and 240 mg nicotine/kg bw/day are the means of duplicate determinations and data for the 40 and 400 doses are from Table 2.

Although the analytically determined nicotine concentration in feed formulated with the tobacco blend are slightly lower than expectations, in the 40-160 mg nicotine/kg of bw/day range these data double as the dose is doubled as would be expected. The percent difference between expected concentration at the 160-240 and 240-400 mg nicotine/kg bw/day compared to the analytically determined difference in nicotine concentration are almost identical. This reinforces the conclusion that the formulated feed containing the tobacco blend was adequately prepared.

Table 4: **Dose Confirmation Data Tobacco Extract**[1]

Target Dose (mg of nicotine/kg of bw/day)	**Target Concentration** (mg of nicotine/g of feed)	**Determined Feed Nicotine Concentration** (mg of nicotine/g of feed)
40	0.18	0.18
80	0.37	0.34
160	0.74	0.60
240	1.11	0.90
400	1.85	1.60

[1] Data for target doses of 80, 160 and 240 mg nicotine/kg bw/day are the means of duplicate determinations and data for 40 and 400 doses are from Table 2.

Data for the tobacco extract follow trends similar to those seen for the tobacco blend and indicate that the feed formulations were properly prepared.

Table 5: **Dose Confirmation Data Nicotine Tartrate**[1]

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)
40	0.18	0.017[2]
80	0.37	0.27
160	0.74	0.57
240	1.11	0.82
400	1.85	1.51

[1] Data for target doses of 80, 160 and 240 mg nicotine/kg bw are the means of duplicate determinations and data for 40 and 400 doses are from Table 2.

[2] These data indicate that feed formulated with nicotine tartrate at this dose was inadequate for the purpose of the study and actually represent a dose of ~ 4 mg nicotine/kg bw/day.

Data for the nicotine hydrogen tartrate concentrations in the NTP-2000 rodent feed indicate increasing nicotine concentrations in line with the increasing target concentrations and appear adequate for the objectives of this investigational study, with the exception of the low dose discussed previously. Analytical data for the dose confirmations are provided in Appendix III.

B. Biological Evaluations

1. Study Animals

A total of 95 male CFW Swiss Webster mice, age 5-7 weeks, along with 10 retired breeders for use as sentinel mice were received on May 21, 2008 from Charles River Laboratories, Portage, MI. The mice were placed in Room 40 and individually housed in stainless steel, wire bottomed cages on stainless steel racks. Mice were quarantined for 4 days and released to the study by the Attending Veterinarian on May 24, 2008. They were maintained under quarantine conditions until the initiation of feeding the formulated feed. Throughout the study, the environmental controls of the animal room maintained the following mean daily conditions: temperature range 21.6 $\pm$ 0.0 ºC (mean $\pm$ standard deviation) and relative humidity 57.7 $\pm$ 2.2 %. Filtered (HEPA and charcoal) air was provided with a mean of 124.7 $\pm$ 0.2 partial air changes per hour (> 12 room air changes per hour). The light cycle was maintained at 12 hours light/dark. All these variables were within the protocol specified ranges. Environmental conditions for the animal room housing the mice are maintained as part of the study file.

On May 27, 2008, 85 mice were assigned to dose groups, by body weight, using the "A" module of the PATH/TOX software. At the discretion of the Study Director, mice exhibiting positive clinical signs, demonstrating body weight loss (since the initial weighing), or representing low or high extremes of body weight were excluded from the allocation process. After allocation, all group mean body weights were compared by ANOVA and least significant difference criteria and demonstrated to be not significantly different at a $p \leq 0.05$ two-sided significance level. Study Day 1 was defined as the first day of dosing, May 27, 2008 and the last dosing day was June 9, 2008 and study termination was June 11, 2008. All study mice were transferred to TOX208 (a protocol used for procedural training) on June 10, 2008 with the exception of the retired breeders that served as sentinels, which were euthanized on June 11, 2008 for the health screen analysis.

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

During the quarantine period prior to initiation of dosing, mice were fed *ad libitum* NTP-2000 powdered feed. At the initiation of dosing, the mice were provided *ad libitum* access to NTP-2000 powdered feed containing the appropriate dose of Test Articles, positive control or non-dosed feed for the control group. After initiation of dosing, feeding bowls were weighted then the bowls refilled with feed on a daily basis. Clean feeding bowls were provided weekly.

Water was provided *ad libitum* by an automatic system. Samples of animal drinking water were obtained on March 19, 2008 and provided to Microbac Laboratories, Inc., Fayetteville, NC for analysis. The results of the analysis indicated there were no analytes detected that were outside the U.S. Environmental Protection Agency compliance range. The data for the water analysis are maintained as part of the study file.

2. Serology/Health Screens

On June 11, 2008 the 10 sentinel mice were euthanized for serology and necropsy to detect any signs of disease. There was no evidence of significant lesions, pathogenic microorganisms, nor antibodies to disease. Microscopic examination was performed on each of the five lung lobes from the 10 sentinel mice (retired breeders). Findings included congestion, hemorrhage, perivascular lymphocytic infiltrations, nonpigmented macrophages, chronic inflammation and a malignant lymphoma. The pathologist considered the occurrences of these changes random and nonspecific and not indicative of the presence of contagious disease. The congestion and hemorrhage reflect the mode of anesthesia/euthanasia while the nonpigmented macrophages and lymphocytic infiltrations were considered background changes typically seen in mice of this age and strain. Other than the findings indicating no evidence of contagious disease, the lung histopathology is not relevant to the study animals because the sentinel mice were retried breeders that were older than the study animals. Appendix IV provides the data from the serology and histopathology screening.

3. Survival

Survival was 100% during the study. This indicates, as anticipated, that the doses chosen were below those that could have produced acute toxicity and that the procedure of removing animals from treatment with test articles or the positive control after they exceeded $\geq$ 20% bw loss was effective. Survival data are presented in Appendix V.

4. Clinical Observations of Animals

Clinical observations reported throughout the study are provided in Table 6. There were no clinical observations indicating altered behavior or any other evidence of nicotine toxicity during the study. This indicates that the doses used in the study were below those that may elicit nicotinic effects in the animals detectable by routine clinical observations. Clinical observation data are provided in Appendix VI.

Table 6 **Group Incidences and Durations of Clinical Observations**[1]

Group	Treatment Group (Doses based on nicotine) (mg nicotine/kg body weight/day)	Observation Normal, No visible abnormalities	Thin/Emaciated
	Control		
1	NTP-2000 Feed	10/10 [100%, 14][2]	0/10 [100%, 14]
	Tobacco Blend		
2	Dose 1 Tobacco in NTP-2000 Feed (40)	5/5 [100%, 14]	0/5 [100%, 14]
3	Dose 2 Tobacco in NTP-2000 Feed (80)	5/5 [100%, 14]	0/5 [100%, 14]
4	Dose 3 Tobacco in NTP-2000 Feed (160)	5/5 [100%, 14]	0/5 [100%, 14]
5	Dose 4 Tobacco in NTP-2000 Feed (240)	5/5 [100%, 14]	0/5 [100%, 14]
6	Dose 5 Tobacco in NTP-2000 Feed (400)	4/5 [80%, 14]	1/5 [20%, 12]
	Tobacco Extract		
7	Dose 1 Tobacco Extract in NTP-2000 Feed (40)	5/5 [100%, 14]	0/5 [100%, 14]
8	Dose 2 Tobacco Extract in NTP-2000 Feed (80)	5/5 [100%, 14]	0/5 [100%, 14]
9	Dose 3 Tobacco Extract in NTP-2000 Feed (160)	5/5 [100%, 14]	0/5 [100%, 14]
10	Dose 4 Tobacco Extract in NTP-2000 Feed (240)	5/5 [100%, 14]	0/5 [100%, 14]
11	Dose 5 Tobacco Extract in NTP-2000 Feed (400)	4/5 [80%, 14]	1/5 [20%, 4]
	Positive Control		
12	Dose 1 Nicotine Tartrate in NTP-2000 Feed (40)	5/5 [100%, 14]	0/5 [100%, 14]
13	Dose 2 Nicotine Tartrate in NTP-2000 Feed (80)	5/5 [100%, 14]	0/5 [100%, 14]
14	Dose 3 Nicotine Tartrate in NTP-2000 Feed (160)	5/5 [100%, 14]	0/5 [100%, 14]
15	Dose 4 Nicotine Tartrate in NTP-2000 Feed (240)	5/5 [100%, 14]	0/5 [100%, 14]
16	Dose 4 Nicotine Tartrate in NTP-2000 Feed (400)	5/5 [100%, 14]	0/5 [100%, 14]

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

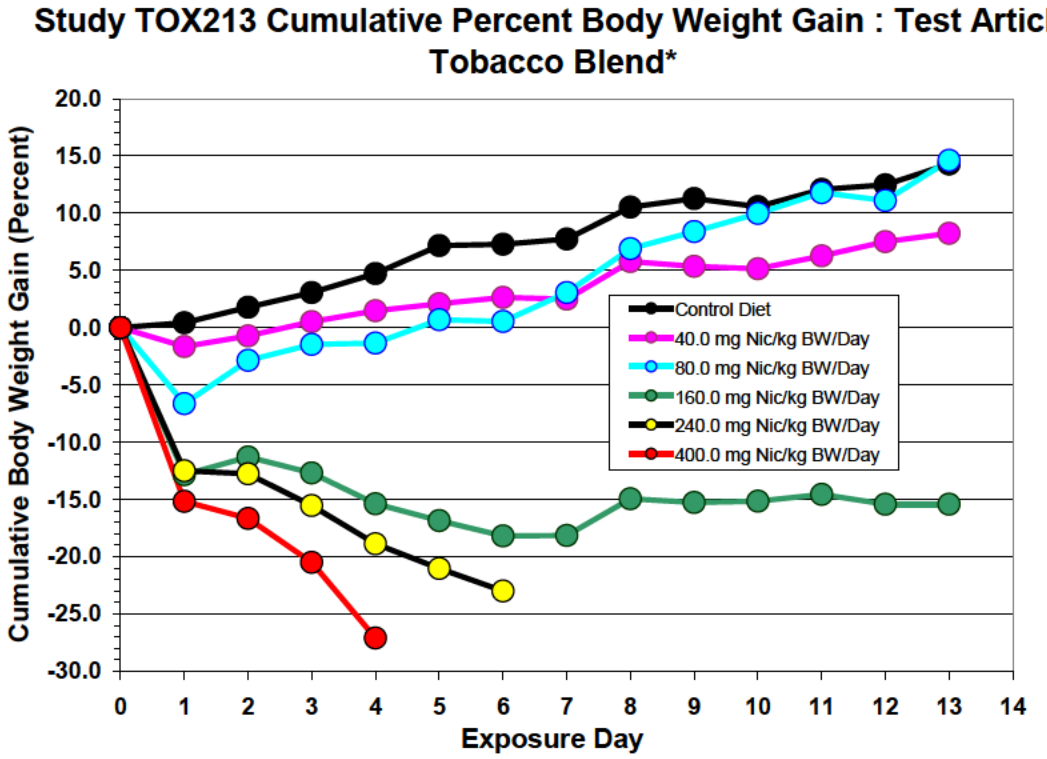
[2] One mouse in Group 1 and Group 16 was reported to have a portion of its tail necrotic. This is believed to not be associated with treatment and is not included in the data.

5. Body Weights

Group mean body weights and body weight gain were recorded daily throughout the 14 day study as Individual and Group Mean Animal Body Weights for each weighing period and are presented in Appendix VII.

Percent body weight gain data for mice fed the tobacco blend at different doses of nicotine are provided in Figure 1. Body weights in grams are presented in Figure 2. Data used for preparation of body weight and body weight gains figures are presented in Appendix VIII.

Figure 1



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

Figure 1 provides the body weight data for mice provided feed formulated with the tobacco blend normalized to cumulative percent body weight gain relative to the body weight on the day prior to the onset of treatment with dosed feed. This normalization removes any influence of differences in body weight between groups at the initiation of the study and thus provides the clearest picture of the effects of the different feed formulations on changes in body weight. The control group fed the NTP-2000 feed with no additions demonstrated normal body weight gains for male mice of this age. The addition of the tobacco blend to yield a nicotine concentration of 40 mg/kg bw/day resulted in a slight depression in body weight gain during the first day of the study, indicating the mice could detect the presence of the blend in their feed and reduced their feed intake. For the remainder of the study there were increases in percent body weight gain at this dose but the body weight gain was always less than that of the control group. This indicates that the mice acclimated to the diet but never reached the cumulative percent body weight gains of the control group. However, when examining the absolute body weight data, this trend does not apply (see later discussion).

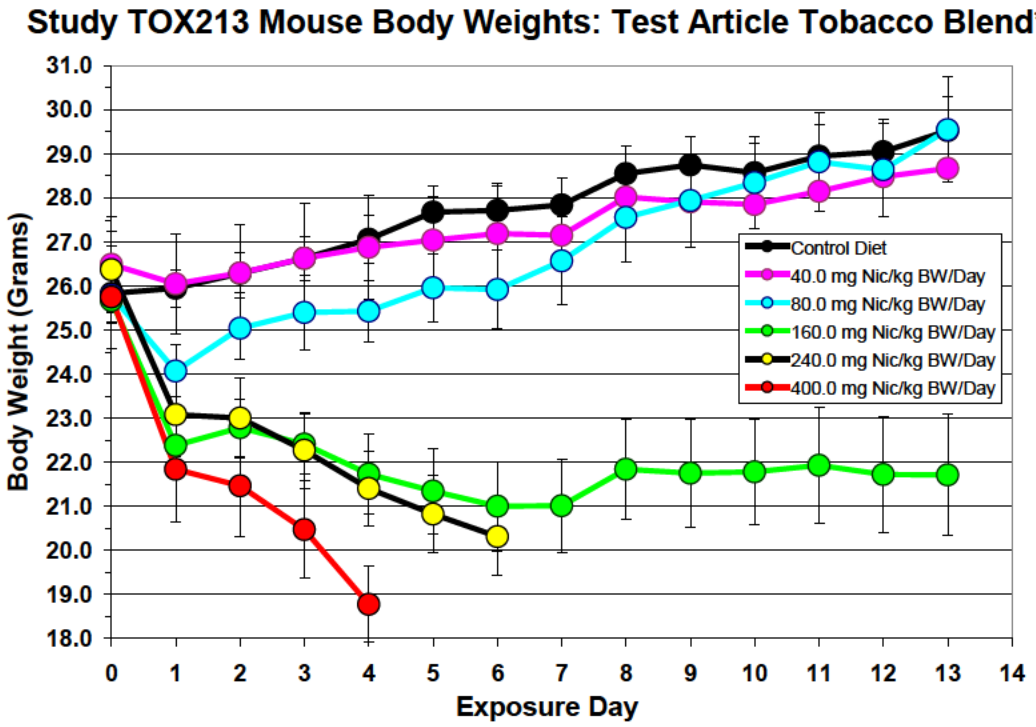
Addition of the tobacco blend to yield a nicotine concentration of 80 mg/kg bw/day resulted in a larger decrease in body weight gain during the first day of the study than seen at 40 mg/kg bw/day. However, by the second day on the formulated feed body weight gain had reached close to that of the 40 mg/kg bw/day group. This trend continued during the first week of the study at which time the body weight gain surpassed that of the 40 mg/kg bw/day group and was equivalent to that of the control group during the last four days of the study. This indicates that at the two lower doses of nicotine the mice acclimated to the feed containing the tobacco blend.

At the addition of the tobacco blend to yield a nicotine concentration of 160 mg/kg bw/day, the body weight gain decreased over 10% during the first day of the study. While there was a slight increase in percent body weight gain during the second day of the study, body weight gain of the mice at this dose did not increase throughout the remainder of the study. This indicates that the mice did not acclimate to feed containing the tobacco blend at this nicotine concentration. Lack of acclimation to the diet could be based upon either an organoleptic effect or a neurophysiological effect that could not be overcome by the mice.

When the quantity of tobacco blend in the feed was increased to yield a nicotine dose of 240 mg/kg bw/day, body weight gain decreased in a manner similar to the 160 mg nicotine dose and continued to decrease. On the sixth day of the study, the mice were taken off the formulated feed and returned to the unformulated NTP-2000 feed. This was based upon the protocol specified limit of a 20% loss of body weight that continued for two consecutive days. At this dose, the mice could not acclimate to feed containing the tobacco blend. The data for the mice provided feed containing the tobacco blend to yield a nicotine dose 400 mg/kg bw/day are similar to the data seen with the group provided feed at a nicotine dose of 240 mg/kg bw/day with the exception that for the 400 mg/kg bw/day reductions of body weight were greater and the protocol specified limit was reached by day four of the study instead of day six.

Figure 2 provides the data for the tobacco blend in terms of group mean body weight and the associated standard deviations. The trends in the data follow closely those seen when the data are plotted as percent body weight gain. However, there are a few notable exceptions. For instance, comparing the 40 mg nicotine/kg bw/day data to the control group appears to indicate that at the low dose there was little difference in body weight while the cumulative percent body weight gain data show a clear difference. This results from the higher initial body weight of the 40 mg nicotine/kg bw/day dose group compared to the control. The initial decrease in body weight gain in the 40 mg nicotine/kg bw/day dose group results in a body weight equivalent to that of the control group that continues for the first week of the study. This phenomenon can also be seen with the 240 mg nicotine/kg bw/day group, which follows a trend essentially equivalent to the 160 mg nicotine/kg bw/day group and does not indicate a dose response. Again, this results from the higher initial body weight of the 240 mg nicotine/kg bw/day dose group compared to the 160 mg nicotine/kg bw/day dose group.

Figure 2



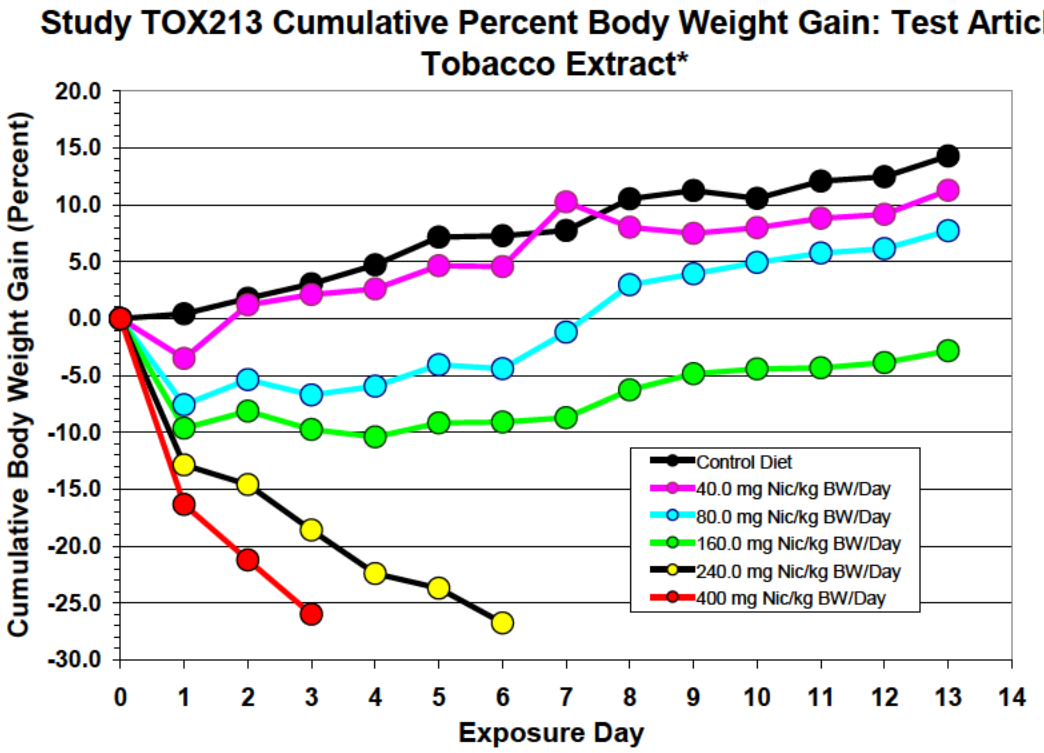
* Data represent the group mean daily body weight ± standard deviation. Exposure day zero represents the body weights before exposure to the dosed feed. Exposure day one represents data acquired after one day of exposure to the untreated control feed or feed formulated with the tobacco blend. Body weights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

At least two possibilities should be considered in respect to explaining these data. First, at an organoleptic level, the mice may consider diets containing the tobacco 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 effects in the peripheral or central nervous system 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 body weight gain.

Regardless of the mechanisms producing the decreases in body weight gain and body weight, it is obvious that doses of 240 and 400 mg nicotine/kg bw/day are excessive because of the large decreases in body weight gain. It may be possible that in a longer term study the mice may acclimate to the 240 mg nicotine/kg bw/day dose and recover some of the body weight. A decrease in body weight of 10% or less in longer term toxicology studies is generally acceptable and believed to not jeopardize the utility of the data.

Body weight data for male mice fed feed formulated to contain different concentrations of tobacco extract that resulted in nicotine doses equivalent to those used for the tobacco blend are provided in Figures 3 and 4.

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 weights of the mice before being exposed to the dosed feed. Exposure day one represents data acquired after one day of exposure to the untreated control feed or feed formulated with the tobacco extract. Body weights were not determined on day 15 after the 14th day of exposure, resulting in a lack of data for day 14.

Figure 3 presents the body weight data for mice provided feed formulated with the tobacco extract normalized to cumulative percent body weight gain relative to the body weight on the day prior to the provision of dosed feed to the mice. After an initial one day drop in body weight gain, there was little to no difference between mice fed the tobacco extract at a concentration in the feed that resulted in a nicotine dose of 40 mg/kg body weight/day. This indicates that even though the mice detected the presence of the extract in the diet, they rapidly acclimated to the formulated diet and regained a normal body weight gain pattern.

At a dose of 80 mg nicotine/kg bw/day, the mice demonstrated a larger drop in body weight gain during the first day of the study than did the mice receiving the 40 mg nicotine dose. However, unlike the 40 mg nicotine dosed mice, the 80 mg nicotine dosed mice did not demonstrate the rapid recovery in body weight gain. Body weight gain appears to stabilize to around -5% for the

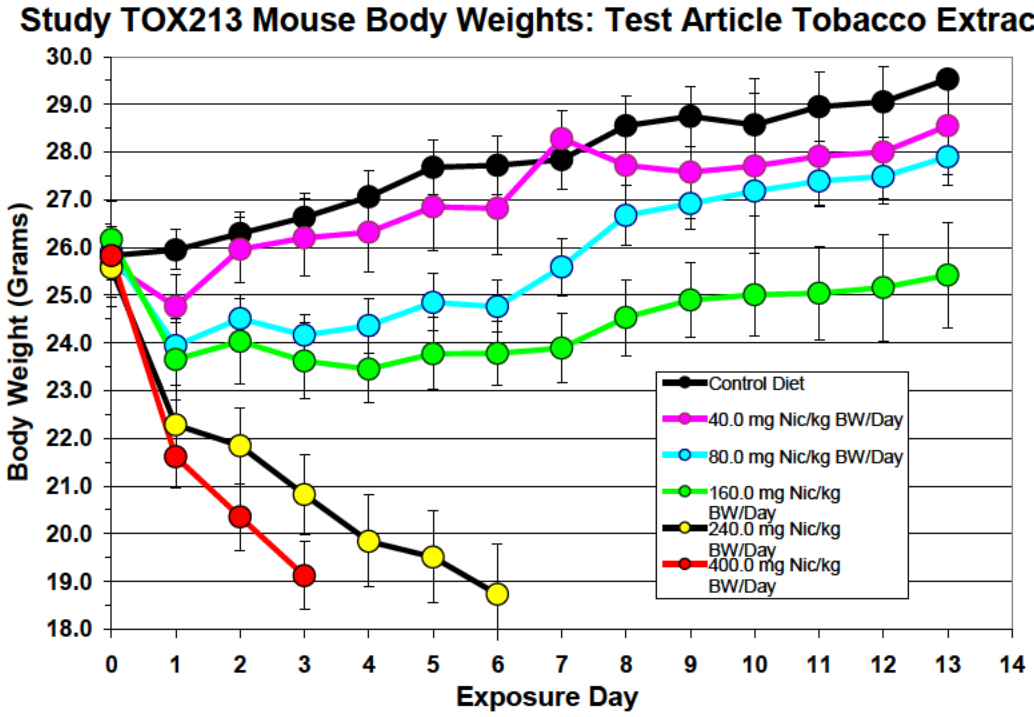
next six days after which there is an increase that resulted in a 7% increase by the end of the study. This indicates that the mice do acclimate to the feed containing the tobacco extract at 80 mg nicotine/kg bw/day but at a slower rate than at 40 mg nicotine/kg bw/day. In a longer term study, mice at this nicotine dose may demonstrate comparable body weight gains to the control group.

A trend similar to that seen at 80 mg nicotine/kg bw/day was seen at 160 mg nicotine/kg bw/day, with the exception that acclimation to the formulated feed was incomplete and the mice never reached the body weight gain seen in the control group. Body weight gain was reduced to about -10% during the first day of the study and was maintained near this for the next six days followed by a slow but steady increase to about -3% by the end of the study. Extrapolating these data to a longer term study implies that mice receiving the tobacco extract at concentrations that provide a nicotine dose of 160 mg/kg bw/day may slightly increase their body weight gain but may not reach that of the control group.

Similar to the data seen with the tobacco blend, doses of 240 and 400 mg nicotine/kg bw/day resulted in excessive body weight gain losses. There was a definitive dose response with mice receiving the 400 mg nicotine/kg bw/day dose demonstrating a greater and more rapid loss of percent body weight gain than those receiving the 240 mg/kg bw/day nicotine dose. Based upon the protocol requirements, mice receiving the 240 mg/kg bw/day nicotine dose were removed from the formulated feed on day six and provided NTP-2000 feed while those on the 400 mg nicotine/kg bw/day dose were removed from the formulated feed on day three. These doses were beyond the ability of the mice to acclimate to the feed.

Figure 4 provides the mean body weights and their standard deviations for the mice fed feed formulated with different concentrations of the tobacco extract. The trends in the body weight data are equivalent to those seen with cumulative percent body weight gain, as would be expected, and demonstrate a strong dose response. Slight differences from the percent body weight gain data result from differences in body weight at the initiation of feeding the feed formulated with tobacco extract.

Figure 4

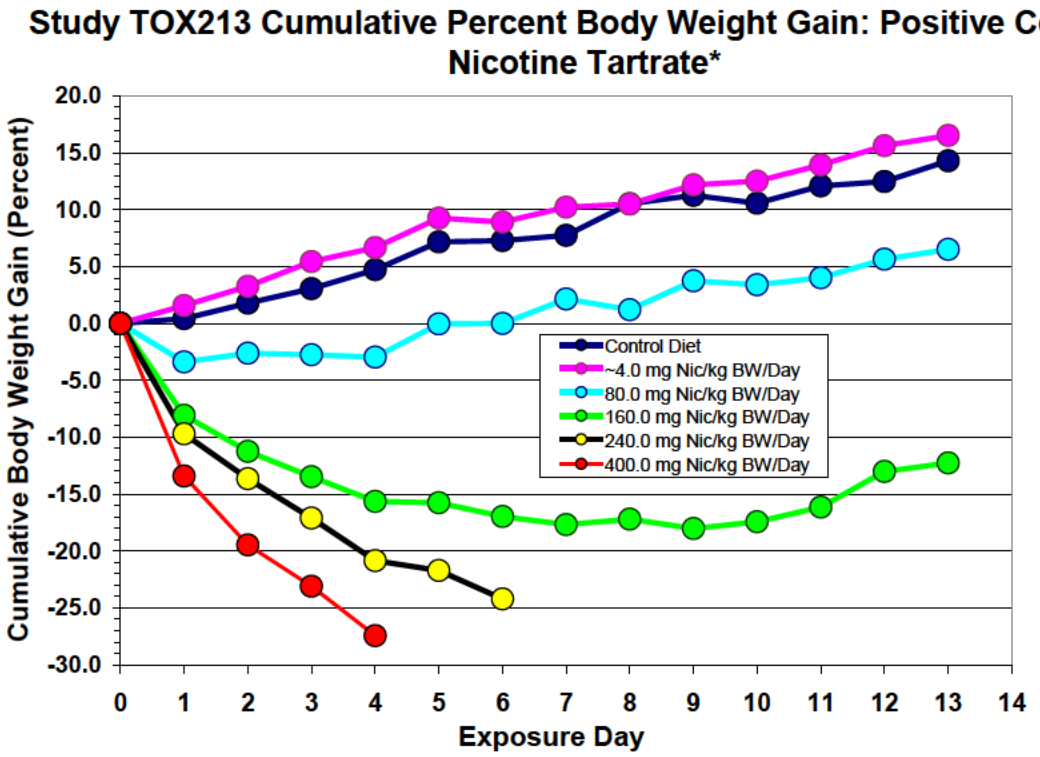


* Data represent the group mean daily body weight ± standard deviation. Exposure day zero represents the body weights before exposure to the dosed feed. Exposure day one represents data acquired after one day of exposure to the untreated control feed or feed formulated with the tobacco extract. Body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

As seen with the tobacco blend, doses of 240 and 400 mg nicotine/kg bw/day are excessive for long term toxicological studies because of the decreases in body weight gain. Again, the use of a dose as high as 160 mg nicotine/kg bw/day for male mice in long term studies may be questionable because of the decrease in body gain that continued throughout this 14-day study, although this dose may be appropriate for toxicological studies of 28 day duration. Again, in a longer term study, the mice may acclimate to this dose and recover some of the body weight loss. As noted earlier, a decrease in body weight of 10% or less in longer term toxicology studies is generally acceptable and believed to not jeopardize the utility of the data.

Data for mice fed feed formulated to contain nicotine hydrogen tartrate (positive control) are provided in Figures 5 and 6.

Figure 5



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

Figure 5 provides the cumulative percent body weight gain data for mice fed diets containing the positive control, nicotine hydrogen tartrate. There is remarkable similarity between the data for the tobacco blend, tobacco extract and positive control considering the differences between these materials. As seen with the two test articles, there is a definitive dose response in the data for the positive control.

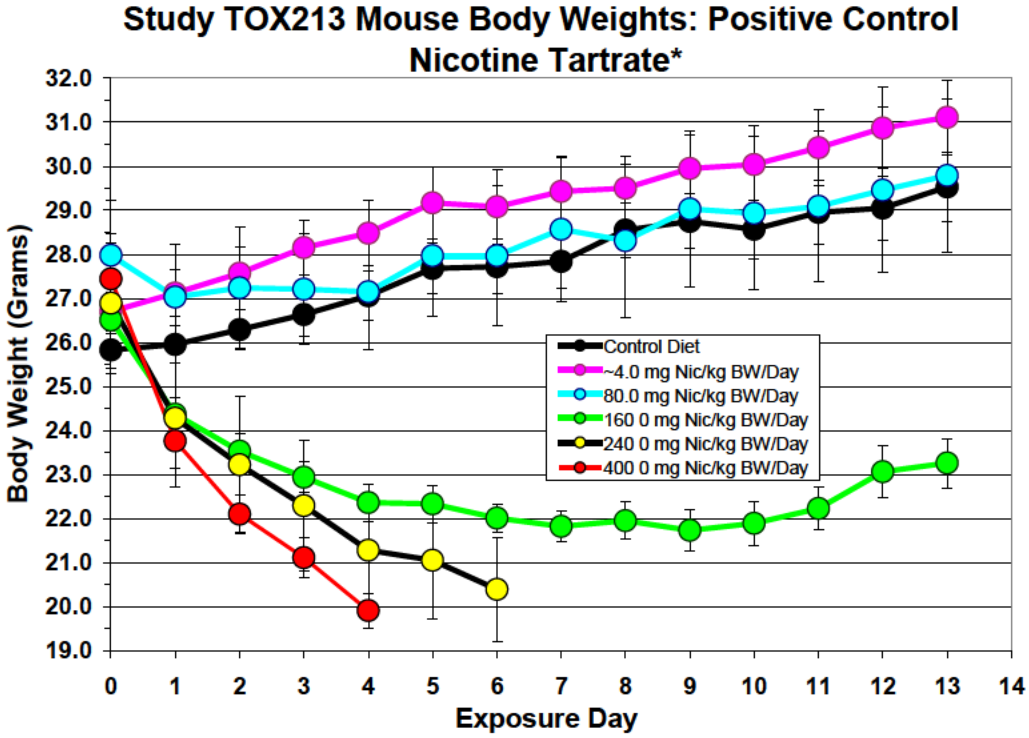
As noted earlier, chemical analysis of the formulated feeds to confirm the nicotine doses indicated that the 40 mg nicotine/kg bw/day did not contain an appropriate amount of nicotine hydrogen tartrate. This resulted in an approximate order of magnitude difference between the anticipated nicotine content in the feed and analytically determined nicotine. This resulted in the 40 mg nicotine/kg bw/day dose being closer to 4 mg nicotine/kg bw/day. As would be expected, based upon this low nicotine content, there were no differences between the percent body weight gains for the mice fed the control feed compared to those fed feed formulated with nicotine hydrogen tartrate at this dose.

At 80 mg nicotine/kg bw, there was a reduction in body weight gain during the first day of feeding which stabilized for the next four days of the study and then increased for the remainder of the study but did not reach values seen in the control. Again, this indicates that the mice began to acclimatize to the formulated feed, although acclimation was not adequate to return the body weight gain to that of the control group during the period of this study. At a dose of 160 mg nicotine/kg bw/day, there was a sharp drop in percent body weight gain that began to stabilize during the next seven days, similar to the data seen at this dose with the tobacco blend and the tobacco extract. However, there appears to be a slight increase in percent body weight gain during the last three days of the study but it was never greater than a -10% reduction in body weight gain. Whether this increase would continue in a longer term study or if the increase was spurious can not be ascertained from the data developed in this study.

Doses of 240 and 400 mg nicotine/kg bw/day produced rapid decreases in body weight gain. As seen with the tobacco blend and the tobacco extract, there was a definitive dose response with mice receiving the 400 mg nicotine/kg bw/day dose demonstrating a greater and more rapid loss of percent body weight gain than those receiving the 240 mg nicotine/kg bw/day dose. Based upon the protocol requirements mice receiving the 240 mg nicotine/kg bw/day dose were removed from the formulated feed on day six and provided NTP-2000 feed while those on the 400 mg nicotine /kg bw/day dose were removed from the formulated feed on day four. These doses were beyond the ability of the mice to acclimate to the feed.

The body weight data with associated standard deviations are shown in Figure 6. These data mimic those based upon percent body weight gain, but are modulated by the differing initial mean body weights of the dose groups; again, demonstrating how initial body weight can influence body weight data from short term studies and the utility of normalizing the data.

Figure 6



* Data represent the group mean daily body weight ± standard deviation. Exposure day zero represents the body weights before exposure to the dosed feed. Exposure day one represents data acquired after one day of exposure to the untreated control feed or feed formulated with the nicotine hydrogen tartrate positive control. Body weights were not determined on day 15.

Feed containing nicotine hydrogen tartrate to yield a nicotine concentration of ~4 mg/kg bw/day did not differ from the control in respect to body weight. Increasing the dose to 80 mg nicotine/kg resulted in decreases in body weight gain on the first day of the study followed by a gradual increase throughout remainder of the study as the mice acclimated to the diet, as seen with the two test articles. Although the body weight gain of this group did not reach that of the control group this dose would be adequate for future toxicology studies. At 160 mg nicotine/kg bw/day, there was a steady decline that resulted in an almost -20% decline in body weight followed by an increase to slightly below a -10% decline. Whether or not this increase would continue is unknown. Based upon these data, it is questionable whether doses higher than 160 mg nicotine/kg bw/day would be useful in long term studies with male, Swiss Webster mice but may be appropriate for intermediate term toxicology studies (e.g., 28-day studies).

Because the nicotine tartrate dosed group contained no tobacco but was comparable to those groups receiving the tobacco blend and tobacco extract, these data may indicate that the decreased percent body weight gains seen in the various treatment groups that comprised this

study may be associated, in part, with their nicotine content more so than with the presence of other tobacco components.

6. Terminal Body Weights

Group mean terminal body weights either at study termination or at termination of feeding the formulated feed are provided in Table 7. Individual animal terminal body weights are presented in Appendix IX.

Table 7: **Terminal Body Weights**

Group	Treatment	Terminal Body Weight (g) ± SD
1	NTP-2000 Feed (0)	29.53 ± 2.46
	Smokeless Tobacco Blend	
2	Dose 1 Tobacco Blend in NTP-2000 Feed (40)[1]	28.67 ± 2.99
3	Dose 2 Tobacco Blend in NTP-2000 Feed (80)	29.55 ± 2.69
4	Dose 3 Tobacco Blend in NTP-2000 Feed (160)	21.71 ± 3.09*
5	Dose 4 Tobacco Blend in NTP-2000 Feed (240)	20.82 ± 1.95* [27.68 ± 1.84][&]
6	Dose 5 Tobacco Blend in NTP-2000 Feed (400)	20.47 ± 2.47* [26.63 ± 1.58][@]
	Tobacco Extract	
7	Dose 1 Tobacco Extract in NTP-2000 Feed (40)	28.55 ± 2.29
8	Dose 2 Tobacco Extract in NTP-2000 Feed (80)	27.90 ± 1.34
9	Dose 3 Tobacco Extract in NTP-2000 Feed (160)	25.42 ± 2.47*
10	Dose 4 Tobacco Extract in NTP-2000 Feed (240)	19.51 ± 2.17* [27.68 ± 1.84][&]
11	Dose 5 Tobacco Extract in NTP-2000 Feed (400)	20.35 ± 1.57* [26.29 ± 1.43][#]
	Positive Control	
12	Dose 1 Nicotine Tartrate in NTP-2000 Feed (~4)	31.11 ± 1.88
13	Dose 2 Nicotine Tartrate in NTP-2000 Feed (80)	29.79 ± 3.91
14	Dose 3 Nicotine Tartrate in NTP-2000 Feed (160)	23.26 ± 1.25*
15	Dose 4 Nicotine Tartrate in NTP-2000 Feed (240)	21.05 ± 2.95* [27.68 ± 1.84][&]
16	Dose 5 Nicotine Tartrate in NTP-2000 Feed (400)	21.11 ± 1.02* [26.63 ± 1.58][@]

[1] Target nicotine doses in mg nicotine/kg bw/day are provided in parentheses. * Statistically significant from Group 1 NTP-2000 Feed ($p \leq 0.05$). [#] Taken off study on day 3; for comparison. data in brackets are Group 1 data for day 3. [@] Taken off study on day 4, data in brackets are Group 1 data for day 4. [&] Taken off study on day 6, data in brackets are Group 1 data for day 6.

As would be expected, terminal body weights follow the trends seen in the daily percent body weight gain and body weight plots. The two highest doses (240 and 400 mg nicotine/kg bw/day) were removed from the study in each treatment group because of severe body weight loss. When the terminal body weights of these groups were statistically compared to the control group on their last day of treatment, each of these doses from each treatment group was statistically significantly lower than the control group provided NTP-2000 feed.

At the low dose (40 mg nicotine/kg bw/day) (~4 mg nicotine/kg bw/day for the positive control) there were no statistical differences when compared to the control group in any of the treatment groups. This indicates that the low dose was a No Observable Adverse Effect Level (NOAEL) in respect to body weight.

Feeding diets containing 80 mg nicotine/kg bw/day for the duration of the study resulted in recovery of the early reductions in body weight gain and body weight resulting in no statistical

differences compared to the control in the mice fed diets containing the tobacco blend, tobacco extract and nicotine hydrogen tartrate. These data support the conclusion that the mice acclimated to the inclusion of these materials in their feed at this dose.

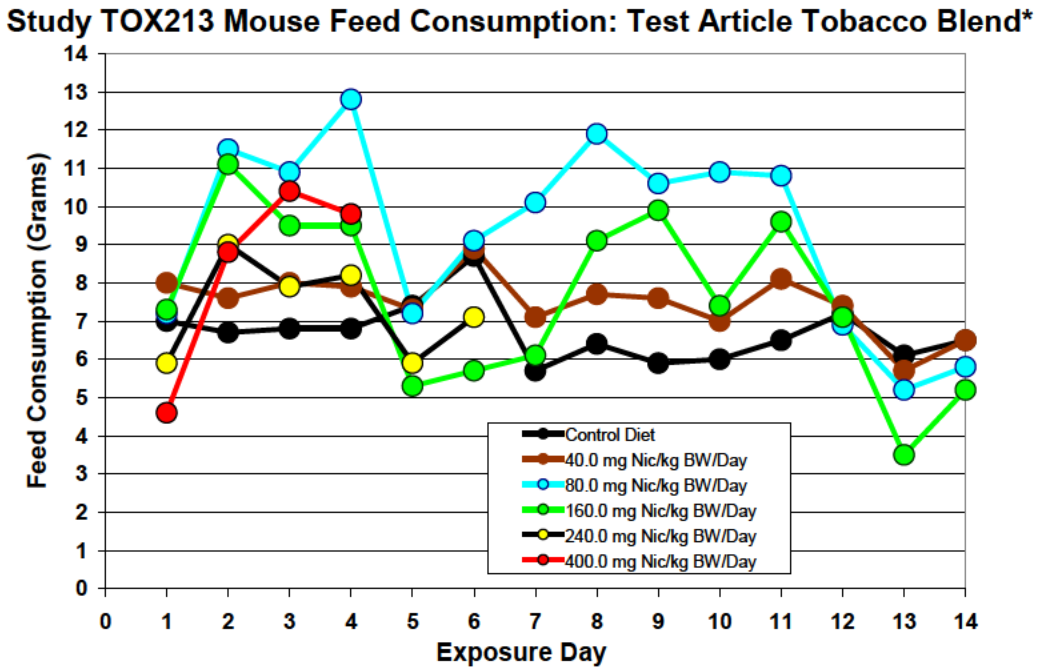
At the 160 mg nicotine/kg bw/day dose, body weights were statistically significantly lower than mean body weight of the control group for both test articles and the positive control. This indicates that the mice were not able to acclimate to inclusion of these materials in their feeds at this dose of nicotine.

7. Feed Consumption

Determination of the feed consumption of rodents fed powdered feed is notoriously difficult, especially for mice and young rats. These animals have a tendency to spill significant quantities of feed through playful exploratory activities and while feeding. Even though attempts were made to minimize spillage in this study, the feed consumption data can only be considered estimates. Daily feed consumption data are provided in Appendix X.

Feed consumption data for mice fed feed containing the tobacco blend, tobacco extract or nicotine hydrogen tartrate are shown in Figures 7-12.

Figure 7



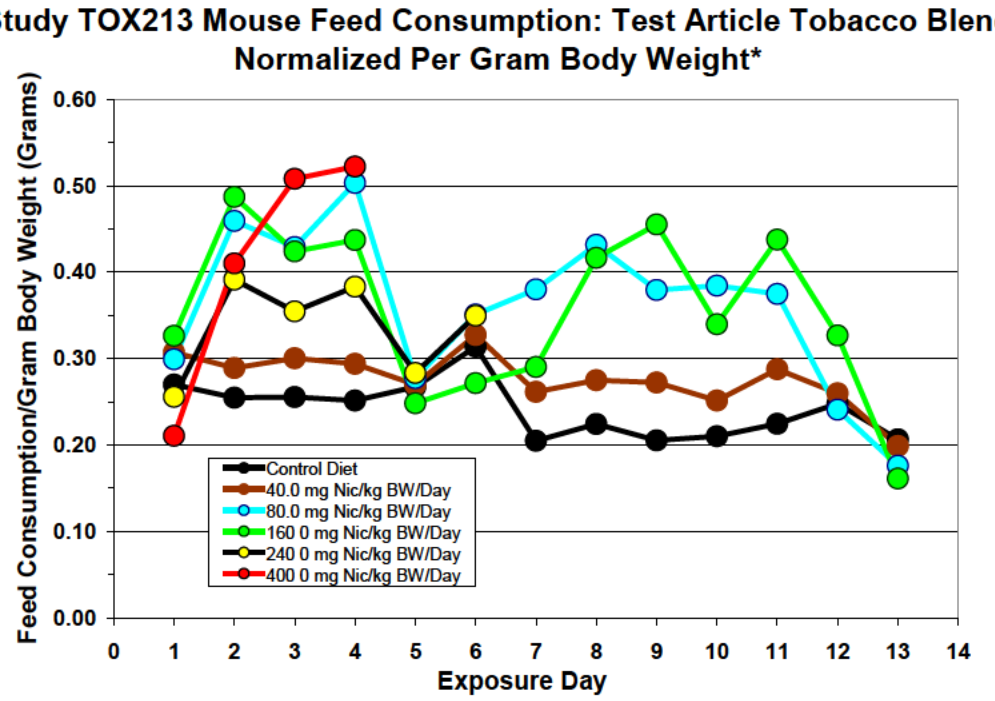
* Data represent the group mean feed consumption for each nicotine dose for mice fed feed formulated with the tobacco blend.

Data for feed consumption for the mice in this study appear erratic. Attempts to normalize the data by expressing in terms of feed consumption/g of body weight, among other methods, did not

greatly improve the data set, as seen in Figure 8. No dose related trends can be ascertained with certainty: however there are instances where apparent trends may be evident.

Figure 8 represents the feed consumption normalized to gram of body weight. This normalization accounts for the body weights of the mice unlike feed consumption simply measured in grams. Since food consumption is related to the weight of an animal, this normalization often reveals trends not seen with expression of the data on other bases. However, it does not assist in interpretation of the data in this case. This is probably, in most part, related to the short term nature of this study.

Figure 8

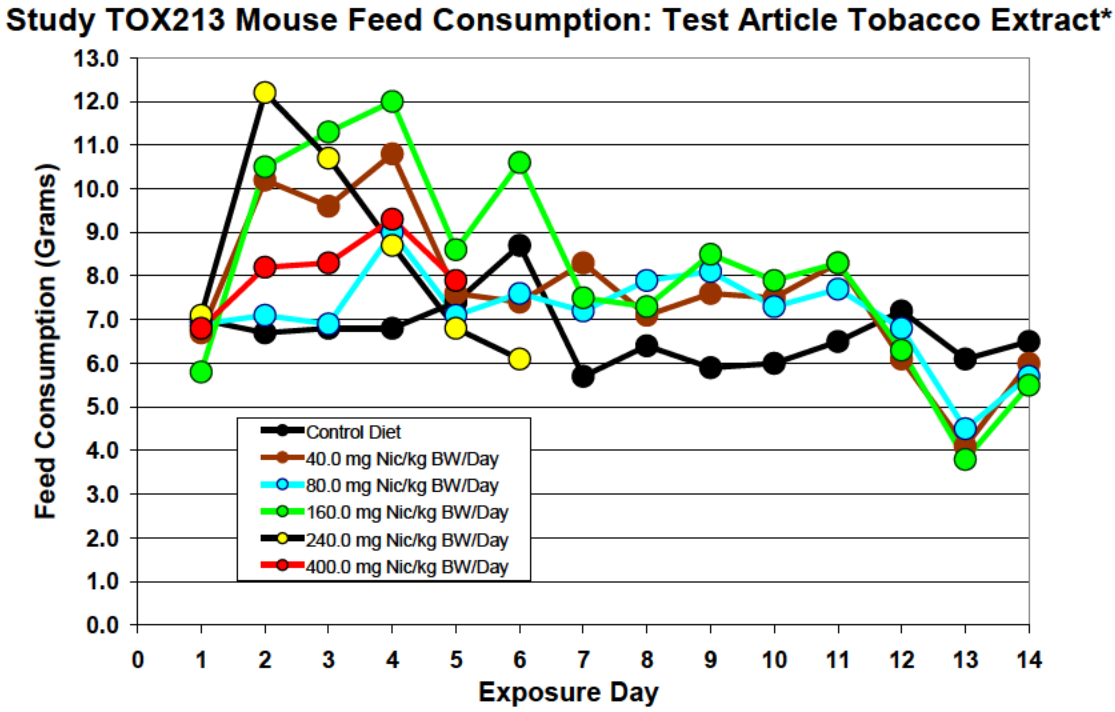


* Data represent the group mean feed consumption normalized to feed consumption/group mean gram of body weight for mice fed different doses of nicotine in feed formulated with the tobacco blend.

One aspect of these data is that the feed consumption of the control group appears relatively constant, as does the low nicotine dose groups. This is consistent with the body weight gain data seen with the tobacco blend. At the 80 mg nicotine/kg bw/day dose, feed consumption appears to increase over that of the control group as the mice compensate for the initial decrease in body weight gain. This trend also appears with the two highest nicotine doses but may be more related to higher feed spillage as the animals search for more palatable feed in their bowls. A factor that can influence the data when expressed as per gram of body weight is the body weights of the high dose groups are decreasing. This tends to emphasize the feed consumption in these groups compared to the lower dose groups and the control group where the body weights are increasing. Again, because of the erratic nature and difficulty obtaining adequate feed consumption data in this study, any observed trends have to be considered uncertain.

Feed consumption data for mice fed feed containing the tobacco extract are shown in Figure 9.

Figure 9

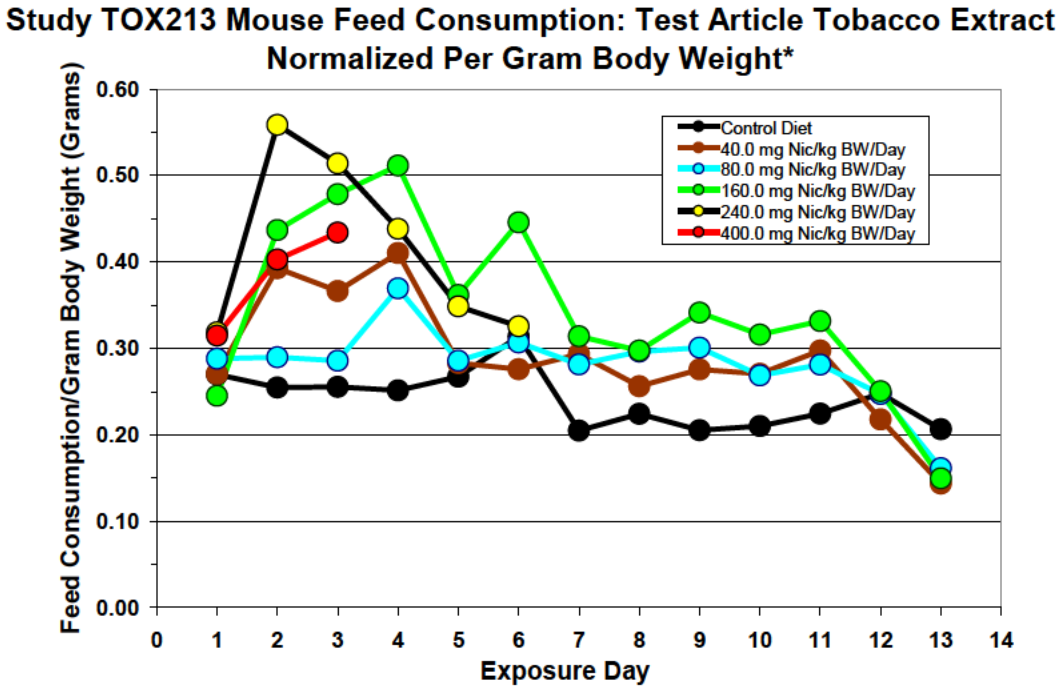


* Data represent the group mean feed consumption for each nicotine dose for mice fed feed formulated with the tobacco extract.

Again, the food consumption data are erratic but follow the trends seen with the tobacco blend. It does appear that the mice were trying to acclimate to dosed feed at the higher doses but were not successful.

Feed consumption data normalized to gram of body weight for the mice fed feed containing the tobacco extract are shown in Figure 10.

Figure 10

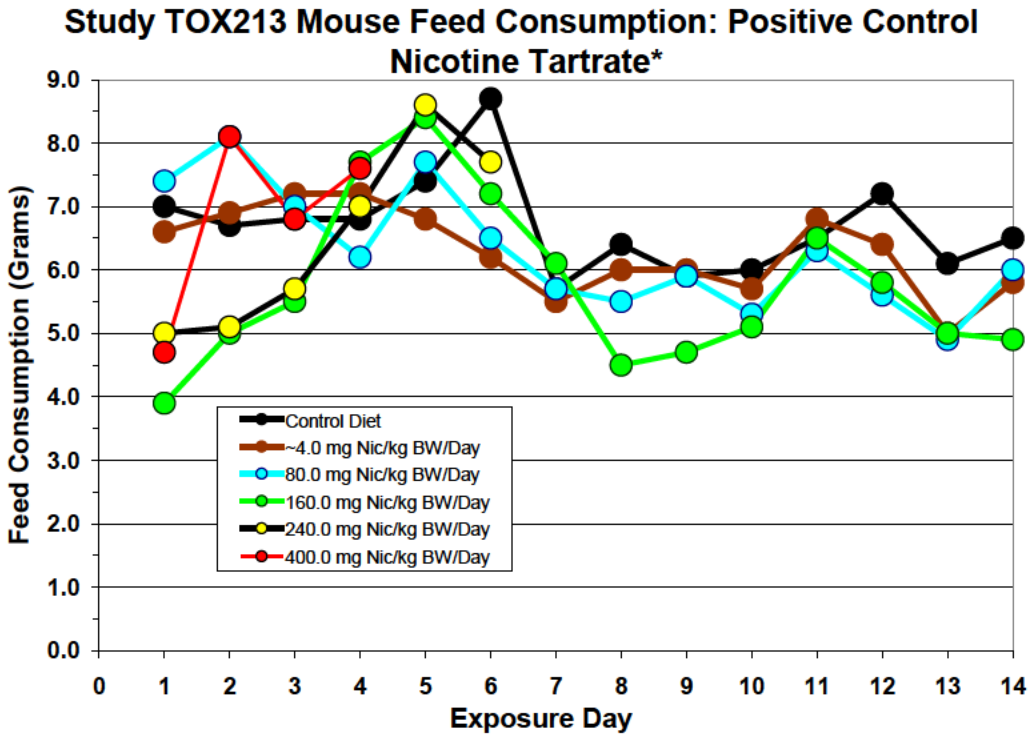


* Data represent the group mean feed consumption normalized to feed consumption/group mean gram of body weight for mice fed different doses of nicotine in feed formulated with the tobacco extract.

These data follow the general trends seen for the tobacco blend.

Data for feed consumption of mice fed feed dosed with the positive control are provided in Figure 11.

Figure 11

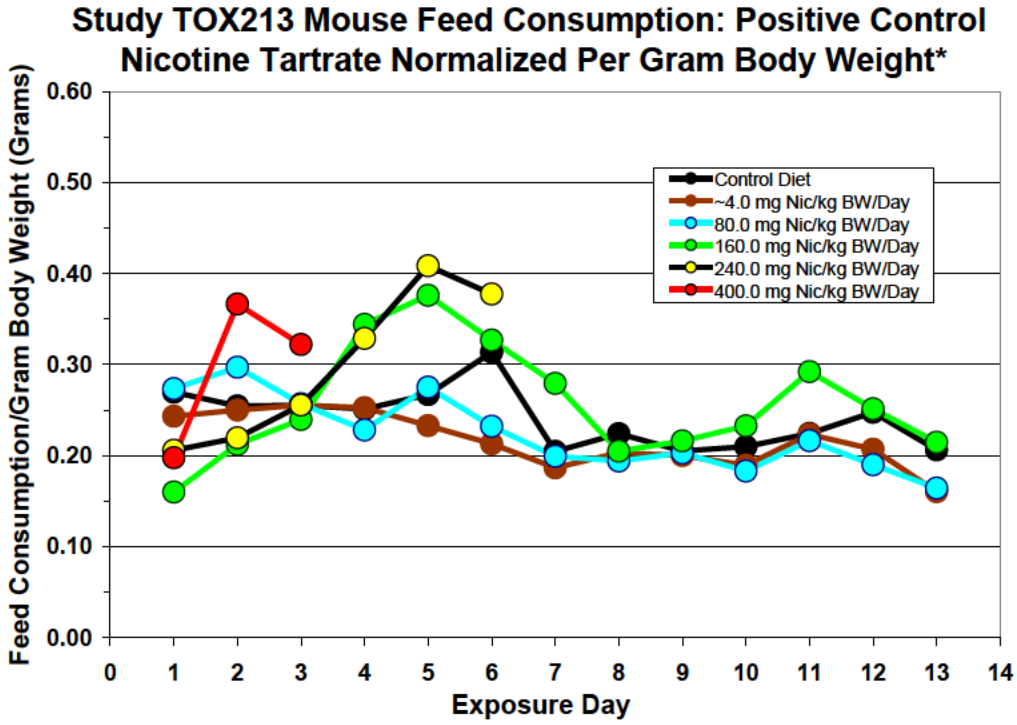


* Data represent the group mean feed consumption for each nicotine dose for mice fed feed formulated with the nicotine hydrogen tartrate positive control. Note that the low dose for the positive control actually was approximately 4.0 mg nicotine/kg bw/day.

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

Feed consumption data normalized to gram of body weight are provided in Table 12.

Figure 12



* Data represent the group mean feed consumption normalized to feed consumption/group mean gram of body weight for mice fed different doses of nicotine in feed formulated with the nicotine hydrogen tartrate positive control. Note that the low dose was approximately 4 mg nicotine/kg bw/day.

Expression of the data on the basis of gram of body weight appears to decrease the apparent erratic nature of the data for the positive control. These data do reinforce the idea that the increases seen in feed consumption in the high dose groups in this study are based upon the reductions in body weight seen at nicotine doses of 160, 240 and 400 mg/kg bw/day. These increases in apparent feed consumption parallel the decrease in body weight seen in these dose groups. This supports the hypothesis that the increases in food consumption are related to spillage associated with the mice digging through the feed in their bowls in an attempt to find more palatable feed.

Overall, the feed consumption data from this study do not indicate any biologically significant differences between the tobacco blend, tobacco extract or nicotine hydrogen tartrate.

8. Conclusions:

The data from this 14 day mouse study demonstrate parallel trends in cumulative percent bw gain and bw between the test articles, tobacco blend and tobacco extract, and the positive control nicotine hydrogen tartrate when formulated into NTP-2000 rodent feed at equivalent nicotine doses. Because the positive control contained no tobacco components other than nicotine, yet followed the trends seen in the data for the two test articles, this may indicate that the changes seen in this study are more dependent upon nicotine than any of the other tobacco components.

At a nicotine dose of 40 mg/kg bw/day, mice fed feed containing either the tobacco blend or the tobacco extract demonstrated similar trends in percent body weight gain. There was an initial drop in body weight gain after the first day of the study, indicating the mice could detect the presence of the test articles at this dose in the diet and responded with a drop in body weight. This was followed by an increase in percent body weight gain that resulted in no statistical difference in body weights at study termination. This indicates the mice were able to acclimate to the feed containing nicotine at a dose of 40 mg/kg body/day.

When the feed was formulated with the tobacco blend, tobacco extract and nicotine hydrogen tartrate to yield a nicotine dose of 80 mg/kg bw/day, there was a larger initial decrease in percent body weight gain than seen at 40 mg nicotine/kg bw/day for each test article. Again, after the initial drop in the percent body weight gain there was a gradual increase in body weight that paralleled the trends seen in the control group. At the termination of the study, there were no statistically significant differences in body weights in any of the treatment groups at this dose when compared to the control group. This demonstrates that the mice were able to acclimate to the presence of the test articles and nicotine hydrogen tartrate in their feed at this dose.

As the nicotine concentration in the feed was increased to a dose of 160 mg/kg bw/day by increased addition of the tobacco blend, tobacco extract or nicotine hydrogen tartrate to the feed, there was a dose related decrease in percent body weight gain after the first day of dosing. However, unlike the decrease seen at the lower doses, there was not in increase in percent body weight gain after the initial decrease. The body weight gain continued to decrease slightly each day during the first week of the study, then slightly increased during the second week. The decrease in body weight gain seen at the 160 mg nicotine dose stabilized at approximately -15% with the tobacco blend and nicotine tartrate and about -10% for the tobacco extract. These data indicate that the mice could not acclimate to the presence of nicotine in their diet at this dose. At the termination of this 14 day study, the body weights of the mice were statistically significantly decreased in all treatment groups at this dose. These data indicate that a in a study of this duration a dose of 160 mg nicotine/kg bw/day is close or slightly higher than a Maximum Tolerated Dose (MTD) for male, Swiss Webster mice as defined by a 10% decrease in body weight.

At a dose of 240 mg nicotine/kg bw/day, the mice in each treatment group demonstrated a severe reduction in percent body weight gain. There was a dose dependent decrease in body weight gain throughout the feeding period that exceeded that seen at 160 mg nicotine. This reduction in body weight was severe enough that feeding the formulated feed was stopped on day six and the mice were reverted to the control diet.

When the dose was increased to 400 mg nicotine/kg bw/day there was a severe reduction in percent body weight gain that was again dose dependent in all treatment groups. The reduction in body weight gain was excessive enough to require discontinuation of the dosed feed on day 3-4 of the study and the mice were returned to the control diet.

Overall, this study demonstrated that doses between 40 and 160 mg nicotine/kg bw/day are tolerable to male, Swiss Webster mice and could be used for longer term studies; however, caution needs to be observed at doses higher than 160 mg nicotine/kg bw/day if the intent is to maintain the mice at or near to an MTD. Doses higher than 160 mg nicotine/kg bw may be warranted to detect toxicological changes in shorter term studies.

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. Jason Hull and Andre Bryant and other members of 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. 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, who insured the health of the animals is acknowledged.

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Appendix I

Study Protocol, Amendments to Protocol, Protocol Deviations, Notes to Study File

RJReynolds

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

Protocol Identifier: TOX213

Repeat Investigational Study of the Palatability of Tobacco Test Articles Formulated in NTP-2000 Diets for Mice at Higher Doses

Scientist III, Preclinical Models of Disease In Vivo Toxicology: Study Director: Jenny L. Smith — Date: 5-20-08
Jenny L. Smith, B.S.

Director, Product Integrity Preclinical Models of Disease In Vivo Toxicology: Chairman, Institutional Animal Care and Use Committee: Paul H. Ayres — Date: 5-19-08
Paul H. Ayres, Ph.D., DABT

Attending Veterinarian: Preclinical Models of Disease In-Vivo Toxicology Chandra D. Williams — Date: 5-20-08
Chandra D. Williams, D.V.M.

Senior Director, Stewardship: Charles D. Garner — Date: 5/21/08
Charles D. Garner, Ph.D., DABT

Senior Staff Toxicologist, Stewardship: Theophilus — Date: 5/21/08
Suzana Theophilus, Ph.D., DABT.

Senior Director; Preclinical Models Of Disease: Natalie Takenaka — Date: 19 May 08
Natalie Takenaka, Ph.D.

Vice-President, Product Integrity: Christopher J. Cook — Date: 5/21/08
Christopher J. Cook, Ph.D.

Anticipated Mouse Delivery Date: May 21, 2008
Anticipated Final Report Date: November 5, 2008

Table of Contents

Facilities and Administration

Sponsor

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

Testing Facility

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

Contractors

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

Study Administration

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

Investigational Study of the Palatability of Tobacco Test Articles Formulated in NTP-2000 Diets for Mice at Observable Effect Doses

Executive Summary

A series of in vivo toxicology studies to investigate the potential toxicity of a blend of tobaccos and an aqueous extract of this tobacco blend along with a positive control (nicotine hydrogen tartrate) are planned to be sponsored by R. J. Reynolds Tobacco Co. and to be conducted in a Contract Research Organization. The route of exposure to the mice is oral through a formulation of the test articles into NTP-2000 feed. A previous study (TOX210) did not adequately define Observable Effect Doses (OED). Addition of the test articles to the feed at higher doses may make it unpalatable to the mice. An unpalatable diet would severely compromise the results from the planned studies. The current study is designed to determine the palatability of formulated diets each of which contains one of the test articles or the positive control by comparison to the control diet at anticipated OED. This will be accomplished by feeding the formulated diets and the control diet to Swiss Webster mice (the strain to be used in the toxicology studies) that are closely matched to the age of the mice to be used in the toxicology studies. Feed consumption and body weight will be determined during the 14-day study to assess what effect, if any, addition of the test articles at anticipated OED levels to the feed has on its palatability and acceptance by the mice. Conducting the study in our laboratory will significantly decrease any potential delay in initiating the planned studies and the chance of obtaining data from these studies that are not useful.

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 tobacco blend, an aqueous tobacco extract of the tobacco blend and nicotine tartrate as positive control when fed to Swiss Webster mice at anticipated Observable Effect Doses (OED).

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 feed of mice (non-certified, NTP-2000 manufactured by Zeiglar Brothers, Inc., Gardners, PA). There is the possibility that incorporation of the test articles and positive control in the feed may alter its palatability to the mice. If the feed is less palatable than the control diet, the mice may consume less feed with a resulting decrease in body weight gain. This would also result in lower than anticipated doses during the planned toxicology studies. Therefore, it is necessary to ascertain the palatability of the dosed feed to mice at OED. The time frame for the studies in the CRO is short to produce the required data at an appropriate time. This preliminary investigational study of the palatability of the diets will be conducted in RJRT facilities to expedite the CRO studies.

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

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

Group Number	*Treatment Group (Doses based on Nicotine) (mg/kg body weight/day)*	*Number of Mice*	*Mouse ID Numbers*
	Control		
1	NTP-2000 feed	10	1-10
	Smokeless Tobacco Blend		
2	Dose 1 Tobacco in NTP-2000 feed (40)	5	11-15
3	Dose 2 Tobacco in NTP-2000 feed (80)	5	16-20
4	Dose 3 Tobacco in NTP-2000 feed (160)	5	21-25
5	Dose 4 Tobacco in NTP-2000 feed (240)	5	26-30
6	Dose 5 Tobacco in NTP-2000 feed (400)	5	31-35
	Tobacco Extract		
7	Dose 1 Tobacco Extract in NTP-2000 feed (40)	5	36-40
8	Dose 2 Tobacco Extract in NTP-2000 feed (80)	5	41-45
9	Dose 3 Tobacco Extract in NTP-2000 feed (160)	5	46-50
10	Dose 4 Tobacco Extract in NTP-2000 feed (240)	5	51-55
11	Dose 5 Tobacco Extract in NTP-2000 feed (400)	5	56-60
	Positive Control		
12	Dose 1 Nicotine Tartrate in NTP-2000 feed (40)	5	61-65
13	Dose 2 Nicotine Tartrate in NTP-2000 feed (80)	5	66-70
14	Dose 3 Nicotine Tartrate in NTP-2000 feed (160)	5	71-75
15	Dose 4 Nicotine Tartrate in NTP-2000 feed (240)	5	76-80
16	Dose 5 Nicotine Tartrate in NTP-2000 feed (400)	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 (TOX210) that did not adequately identify OED. The doses to be used in this study are expected to provide data required to set doses for future toxicology studies in the laboratory of the CRO.

Experimental Use of Mice

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

Duplication of the Study

To determine if this proposed study duplicates any previous studies, a literature search was conducted (Appendix 2). The literature revealed one published study where snus tobacco was incorporated into the feed of mice (Stenstrom, et al., 2007). Although the data were not adequately reported, the authors noted that a short-term pilot study had indicated when snus tobacco was incorporated into the feed of the mice, body weight was decreased (data not provided). The relevance of this study to the present study cannot be ascertained because the feed was not the same, the tobacco was not the same, dosing was based on grams of

tobacco instead of nicotine and no tobacco extract was used in the study nor was a positive control employed. Therefore, the current study is not a duplication of this study. A similar study (TOX210) has been conducted at RJRT and the current study is based upon the results from that study. In TOX210, it was discovered that mice respond to the presence of the test articles in the feed in a very different manner when compared to rats. Whereas, both feed intake and body weight gain were decreased in a dose dependent manner in rats this was not true for mice. Mice appear to tolerate the test articles as opposed to rats. Since it is important to provide data useful for the design of a short term repeated study with mice, this study is designed with higher doses than TOX210 to further define the mouse's tolerance to the test articles.

Rationale for the Use of Animals

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

Animal Selection and Justification for Test System

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

Selection of the Swiss Webster Mouse

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

Justification for Areas of Investigation

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

of these formulated diets in a dose dependent manner compared to control feed and will provide important information for the design of the upcoming toxicology studies.

Animal Requirements

The number of mice to be used is the minimum associated with meaningful statistical analyses of the data. A minimum of 90 male, Swiss Webster, juvenile mice (5-7 weeks of age) will be received from Charles River Laboratories (Raleigh, NC) for conduct of this study. Assigned to the TOX213 Xybion protocol will be 85 male mice for the experimental groups, as well as 10 male mice (retired breeders) to be used for health screening and sentinels [***SOP TOX061***]; assessments of the sentinel population will occur at the conclusion of the feeding study. The design for the current study uses five male mice/study group and uses five dose groups for each of the tobacco test articles and 10 mice in the control group fed diet without the addition of test article. The positive control group uses five dose groups consisting of five male mice each. Extra mice (five male) will be utilized during the allocation and randomization process, i.e., to ensure that an adequate number of healthy animals are available for placement onto the study in the event that any of the mice demonstrate abnormal clinical signs, or die unexpectedly (e.g., are euthanized for humane reasons) during the quarantine/acclimation period. Any additional animals shipped by the vendor in excess of the number ordered will be used for studies approved by the Institutional Animal Care and Use Committee (IACUC) or euthanized using 70% carbon dioxide (CO_2) in air [***SOP TOX057***]. The final fate of each animal will be documented.

Quarantine/Acclimation and Serological Evaluation

Mice received into the facility (***SOP TOX015***) will be quarantined for a minimum of 3 days under conditions simulating those of the study (***SOP TOX012***). All mice will be assigned a pre-allocation identification number, and that number will be indicated on the corresponding cage card.

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

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

The Attending Veterinarian will perform a health examination of all mice within four days after delivery. Commencement of mouse dosing is dependent upon a favorable review of

the health examination, as well as a written statement from the Attending Veterinarian releasing the mice from quarantine.

Allocation of Animals to Study Groups

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

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

Animal Husbandry

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

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

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

Mice will have *ad libitum* access to NTP-2000 feed, with the exception of the sentinel mice, which will be fed Lab Diet, Certified Rodent Diet #5002 feed (PMI Nutrition International), presented as pellets (***SOP TOX017***). Feed will be presented as a powdered diet formulated with the test articles, positive control or as a control diet with no test articles. Water will be provided to mice on an *ad libitum* basis through an automatic system (***SOP EQP048***). The water source originates from the municipal supply of the City of

Winston-Salem, and is subsequently filtered through activated carbon and 5-micron particulate filters prior to mouse delivery. This water is analyzed semi-annually. There are no known contaminants expected to be present in the feed or water that would be anticipated to interfere with the outcome of the study.

Invasive Techniques

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

Survival Surgery

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

Pain/Distress

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

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

Euthanasia

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

Hazardous Materials and Safety

Hazardous Materials

The test articles will be stored in a refrigerator/freezer (Freezer 10) in Lab #95 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. Only non-formulated diet will be stored in the Isotemp refrigerator in Lab #95 (***SOP EQP076***).

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 with nicotine hydrogen tartrate, will take place under a certified exhaust hood (***SOP EQP056***). The tobacco extract may present a hazard through skin exposure because nicotine can be absorbed through the skin. Therefore, gloves and safety glasses will be required along with appropriate attire to minimize the possibility of skin contact when working with the tobacco extract and positive control. Only the smallest quantities (of hazardous materials) needed for a particular procedure will be used. Excess material will be disposed of as described below.

Safety Procedures

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

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

Disposal of Contaminated Wastes

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

Test Articles

Smokeless Tobacco Blend

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

Aqueous Tobacco Extract

The aqueous tobacco extract test article consists of a water extraction of the tobacco test article. It will contain no components not contained in the tobacco and the water used for extraction. Information concerning the identity, processing and other characteristics of the water extract will be on file. Because the tobacco extract is a complex mixture of natural components, its purity cannot be ascertained. The tobacco extract will be assayed for nicotine. The aqueous tobacco extract will be identified by Manufacture Date. A Material Safety Data Sheet for the tobacco extract test article will be provided. It will be stored frozen ($\leq 0^{o}$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 re-frozen. An archival sample (~ 5 ml) of the extract used to prepare each diet formulation shall be maintained frozen ($\leq 0^{o}$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). Mouse dosing will be based upon nicotine and not the bulk salt. A Certificate of Analysis and a Material Safety Data Sheet will be obtained from the supplier and maintained in the study file. The nicotine tartrate will be stored under conditions recommended by the supplier and should be stored desiccated. An archival sample (~ 0.1 g) of the nicotine tartrate shall be maintained under the storage conditions recommended by the supplier.

Dosed Diet Formulation

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

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

Test and Control Article Exposure

Dosing Regimen

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

Biological Effect Evaluation During In-Life Phase

Evaluation of Dead or Moribund Animals

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

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

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

Body Weights

Individual non-fasted body weights will be determined prior to study group allocation (i.e., prior to the initial dosing). Body weights will be recorded daily for the duration of the 14-day study (***SOPs EQP034, TOX038***). Weighing will take place at approximately the same time each day. Individual body weights will be used to calculate the mean body weight for each experimental group. The "A" module of the PATH/TOX system will be used for acquisition of body weight data. Unscheduled body weight determinations may be made at any time if deemed necessary by the Attending Veterinarian or Study Director. All mouse weights will be acquired using Mettler PM2000 balances (Mettler Instrument Corporation, Highstown, NJ) (***SOP EQP034).*** Groups of mice that experience a twenty percent or more cumulative group mean body weight loss for two consecutive days relative to the group mean body weight on the day prior to the onset of the administration of dosed feed will be returned to the control NTP-2000 diet.

A non-fasted, terminal body weight will be obtained from mice euthanized at study completion. In addition, terminal weights will be taken for mice that are euthanized due to moribundity or for humane reasons. Data will be entered into the "A" module of the

PATH/TOX computer software. No terminal body weight will be obtained for mice found dead.

Feed Consumption

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

Clinical Observations

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

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

Biological Effect Evaluation at Termination of In-Life Phase

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

Statistical Analyses

The following statistical tests will be used unless other tests are recommended.

Body Weights

Statistical evaluations of group mean body weights and terminal body weights can 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 can be performed; if the data are non-homogeneous, then Cochran and Cox's modified t-test can be used.

Feed Consumption

Statistical evaluations of group mean feed consumption can 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 can be performed; if the data are non-homogeneous, then Cochran and Cox's modified t-test will be used.

Significance

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

Records to be Maintained

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

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

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

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

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

Reporting

Final Report

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

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

Statement By The Study Director

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

The Study Director assures that this study does not represent any unnecessary duplication of experimental studies using animal resources. The Study Director assures that this study will

follow practices set forth in the *Guide for the Care and Use of Laboratory Animals* and IACUC policies.

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

R**eference**s

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

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

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

Appendix I: Proposed Study Schedule

Test Article Receipt:	
Smokeless tobacco blend:	March 7-March 15, 2008
Tobacco Extract:	March 7-March 15, 2008
Nicotine hydrogen tartrate	March 7-March 20, 2008
Animal Quarantine/Acclimation Start:	May 21, 2008
Animal Randomization:	May 25, 2008
Animal Identification:	May 25, 2008
Initiation of Feeding Formulated Diets	May 26, 2008
Study Termination:	June 8, 2008
Report Dates:	
Data Report	June 20, 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 data databases were again searched or added to the search strategy on May 11, 2008

PubMed

alttox.org (alternatives to animal research website)

go3r.org (Website that conducts database searches using filters that highlight the 3 R's, replacement, reduction and refinement. Appears excellent for this purpose.

Examples of search terms: palatability, snus, snus and nicotine, nicotine taste etc.

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

	May 11, 2008 results
Oral nicotine rat: 186 titles and abstracts retrieved	No new titles
Oral nicotine mouse: 92 titles and abstracts retrieved	One new title (not relevant)
Diet nicotine mouse: 13 titles and abstracts retrieved	No new titles
Diet tobacco mouse: 83 titles and abstracts retrieved	No new titles
Diet tobacco rat: 79 titles and abstracts retrieved	No new titles

Feed tobacco: 78 titles and abstracts retrieved	One new title (not relevant)
Oral tobacco rat: 66 titles and abstracts retrieved	No new titles
Oral tobacco mouse: 51 titles and abstracts retrieved	No new titles
Snuff diet: 4 titles and abstracts retrieved	No new titles
Snuff diet rat: 76 titles and abstracts retrieved	No new titles
Snuff diet mouse: 4 titles and abstracts retrieved	No new titles
Palatability tobacco: 2 titles and abstracts retrieved	No new titles
Palatability snuff: 0 titles and abstracts retrieved	No new titles
Snus animal: 1 title and abstract retrieved	No new titles
Snus mice: 2 titles and abstracts retrieved	No new titles
Snus rats: 0 title and abstract retrieved	No new titles
Snus: 78 titles and abstracts retrieved	One new titles (not relevant)
Palatability nicotine: 6 titles and abstracts retrieved	No new titles
Palatability nicotine refinement alternative:	
0 titles and abstracts retrieved	No new titles
Palatability study refinement alternative:	
0 titles and abstracts retrieved	No new titles
Feed palatability alternative:	
6 titles and abstracts retrieved	No new titles
Feeding study palatability refinement:	
0 titles and abstracts retrieved	No new titles
Feeding study palatability replacement alternative:	
0 titles and abstracts retrieved	No new titles
Nicotine palatability replacement alternative:	
0 titles and abstracts retrieved	No new titles
Nicotine palatability refinement alternative:	
0 titles and abstracts retrieved	No new titles
Palatability study nicotine:	
2 titles and abstracts retrieved	No new titles
Palatability study tobacco: 1 title and abstract retrieved	No new titles
Palatability study snus: 0 titles and abstracts retrieved	No new titles
Palatability study: 588 titles and abstracts retrieved	No new titles

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: TOX213
Protocol Title: Repeat Investigational Study of the Palatability of Smokeless Tobacco Test Articles Formulated in NTP-2000 Diets for Mice at Higher Doses
Study Director: Jenny L. Smith
Euthanasia: Change this section to read.

All mice with identities of 1-85 from TOX213 will be transferred to TOX208 (Training for Performing Procedures in Rodents) instead of Euthanasia by 70% CO_2 in air.

Reason for change: Optimal scientific use since the animals were only used for dose feeding, no additional tissues are required and the reduction of animal numbers used for training.

Approval

Jenny L. Smith, Study Director

Jenny L. Smith

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

Protocol Amendment

Amendment # 2

Protocol Identifier: TOX213

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

Study Director: Jenny L. Smith

Protocol Amendment:

The protocol for Study TOX213 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

12-11-2008
Date

NOTICE OF DEVIATION

Section 1 (To Be Completed By The Person Discovering The Deviation)

Study Number: TOX213

Source Document(s): ☒ Protocol ☐ SOP (List SOP Number(s)):

Describe the Requirement (give section numbers for SOPs): There was no requirement for histopathological examination of the lungs of the sentinel mice in the protocol.

Describe the Deviation: Date of Occurrence: June 11,2008

Lungs were removed from each sentinel mouse, preserved and provided to Seventh Wave for histopathological examination. Seventh Wave conducted the examination and provided a report to RJRT.

Documented By: Paul Ayres Date: Nov 3, 2008

Section 2 (To Be Completed By The Study Director)

Describe the Corrective Action(s) To Be Taken In Response To The Deviation:

No corrective action is possible. It should be noted that the data obtained from the histopathological examination can not be directly related to the health status of the study animals. The sentinel mice were retired breeders and therefore older than the mice used in this study.

Assessment Of The Deviation's Impact Upon The Outcome Of The Study:

This deviation had no effect on the outcome of the study. Again, the data from the histological examination of lungs of the sentinel mice are not directly comparable to the health status of the mice used on the study because of significant differences in the ages of the sentinel mice and the ages of the study mice.

Study Director's Signature: Paul N Ayres Date: 12-1-2008

NOTICE OF DEVIATION

Section 1 (To Be Completed By The Person Discovering The Deviation)

Study Number: TOX213

Source Document(s): ☒ Protocol ☐ SOP (List SOP Number(s)):

Describe the Requirement (give section numbers for SOPs):

"A preliminary test batch of diet formulated with each test article will be made to refine the techniques required and will not be provided to the mice."

Describe the Deviation: Date of Occurrence: May, 25-26, 2008

A preliminary test batch of diet was not formulated. No preliminary test batch of diet was needed because this had been done in previous studies (TOX209 and TOX210). Experience was also gained in formulating the diets during these previous studies. Analytical confirmation of feed homogeneity and dose confirmations indicated the techniques used to prepare the dosed feed were adequate for the purposes of these studies. Therefore the decision was made to not prepare a test batch of feed.

Documented By: Paul Ayres Date: Nov 3, 08

Section 2 (To Be Completed By The Study Director)

Describe the Corrective Action(s) To Be Taken In Response To The Deviation:

No corrective action was necessary.

Assessment Of The Deviation's Impact Upon The Outcome Of The Study:

This deviation had no impact upon the results of the study because the process had been completed during previous studies and the techniques for formulation of the feed with the test article and positive control had been deemed adequate for the purposes of this study.

Study Director's Signature: Paul N Ayres Date: 12-11-2008

NOTICE OF DEVIATION

Section 1 (To Be Completed By The Person Discovering The Deviation)

Study Number: TOX213

Source Document(s): ☒ Protocol ☐ SOP (List SOP Number(s)):

Describe the Requirement (give section numbers for SOPs):

"The formulated feeds will be stored at room temperature during their one week use."

Describe the Deviation: Date of Occurrence: ~ May 23, 2008

The formulated feeds were stored a room temperature during their two weeks of use. Based upon the availability of data indicating that nicotine was stable in the formulated feeds for at least 30-days at room temperature, it was decided to prepare only one feed formulation. Therefore, the formulated feeds were stored at room temperature during the duration of this 14-day study.

Documented By: Paul Ayres Date: Nov. 3, 08

Section 2 (To Be Completed By The Study Director)

Describe the Corrective Action(s) To Be Taken In Response To The Deviation:

No corrective action was necessary

Assessment Of The Deviation's Impact Upon The Outcome Of The Study:

There was no impact upon the outcome of the study based upon the decision to prepare a single formulation of the dosed feed and the storage of these feeds for two weeks at room temperature instead of one week.

Study Director's Signature: Paul N Ayres Date: 12/1-2008

NOTICE OF DEVIATION

Section 1 (To Be Completed By The Person Discovering The Deviation)

Study Number: TOX213

Source Document(s): ☒ Protocol ☐ SOP (List SOP Number(s)):

Describe the Requirement (give section numbers for SOPs):

"A non-fasted, terminal body weight will be obtained from mice euthanized at study completion."

Describe the Deviation: Date of Occurrence:

While a non-fasted, terminal body weight was obtained from mice at the study completion, the mice were not euthanized. Mice were transferred to another protocol (TOX208).

Documented By: Paul Ayres Date: Nov. 3, 08

Section 2 (To Be Completed By The Study Director)

Describe the Corrective Action(s) To Be Taken In Response To The Deviation:

No corrective action was necessary.

Assessment Of The Deviation's Impact Upon The Outcome Of The Study:

Transfer of the mice to TOX208 had no impact upon the outcome of this study because no other procedures, including necropsy, were stipulated for the mice at the completion of the study.

Study Director's Signature: Paul N Ayres Date: 12-11-2008

NOTICE OF DEVIATION

Section 1 (To Be Completed By The Person Discovering The Deviation)

Study Number: TOX213

Source Document(s): ☒ Protocol ☐ SOP (List SOP Number(s)):

Describe the Requirement (give section numbers for SOPs):

"A non-fasted, terminal body weight will be obtained from mice euthanized at study completion."

Describe the Deviation: Date of Occurrence: ~ May 23, 2008

The last day of the study was actually day 15 not day 14. Technical staff were confused about taking the last body weight on day 15 not day 14.

Documented By: Paul Ayres Date: Nov. 18, 08

Section 2 (To Be Completed By The Study Director)

Describe the Corrective Action(s) To Be Taken In Response To The Deviation:

No corrective action is possible.

Assessment Of The Deviation's Impact Upon The Outcome Of The Study:

Body weights were not obtained 24 hours after the last feeding period (day 14). However, this had little impact upon the outcome of the study because body weights had stabilized during the last few days of the study for each study group that remained on dosed feed.

Study Director's Signature: Paul W Ayres Date: 12-1-2008

Note to the TOX213 Study File

Formulated feed from each test article (tobacco blend and tobacco extract) and the positive control (nicotine hydrogen tartrate) at each dose was submitted for chemical analysis of its nicotine concentration to confirm the doses were within an appropriate and expected range. Each sample was analyzed in duplicate and the individual analysis and the mean of the duplicates were reported by the analytical chemists.

When the results were reported by the analytical chemist the Excel spread sheet was annotated for the formulation for the tobacco extract at 80 mg nicotine/kg body weight/day and the tobacco extract at 160 mg nicotine/kg body weight/day to indicate a possible mix up between these two samples, as seen below.

	mg/g	Average		
gn77202			gn77202	
AD1	0.512			
AD2	0.571	0.5414	TOB EXT 80	*These two samples look like they are mixed up.
AE1	0.308			We are going to reprep to verify our results. I will
AE2	0.335	0.3218	TOB EXT 160	let you know on Tuesday what we find out.

A second analysis of the formulated feeds repeated the data from the first analysis and confirmed that the nicotine concentrations in the feed samples for Group 8 and Group 9 were opposite from their intended concentrations.

Review of the data from the study indicates that these two doses were originally mislabeled. Therefore, the feed formulated for the 80 mg nicotine/kg body weight/day was mislabeled during preparation as the 160 mg nicotine/kg body weight/day and the feed formulated as the 160 mg nicotine/kg body weight/day was mislabeled as 80 mg nicotine/kg body weight/day during preparation.

The mislabeling would have resulted in those mice in Group 8 that were to be fed the 80 mg nicotine/kg body weight/day formulated feed actually receiving 160 mg nicotine/kg body weight/day while the Group 9 mice received 80 mg nicotine instead of their intended 160 mg nicotine/kg body weight/day.

A number of factors support the conclusion that these two diets were mislabeled.

1) The analytical chemistry data, as noted above, indicate that nicotine analysis of the formulated feed produced results opposite to what was expected.

2) During analysis of the data the original Study Director switched the body weight data and body weight gain data originally obtained from Xybion for Groups 8 and 9. In other words, the Xybion data for Group 8 were plotted as Group 9 and the Group 9 data were plotted as Group 8. This indicates the Study Director thought the data should have been reversed and the body weight and body weight gain data in Xybion was opposite to the Xybion group dose designation. This could have occurred through the mice being fed from the mislabeled container every day of the study [see 3) below] or the groups were switched every day of the study when they were weighed [see 4) below].

3) Discussions with the technical staff responsible for feeding the mice indicate that there were no problems in respect to feeding the mice that would have resulted in the wrong formulated feed being provided to the mice. In other words, the feed in the container labeled for Group 8, mice numbers 41-45, 80 mg nicotine/kg body weight/day was fed to Group 8 while the feed in the container labeled Group 9, mice numbers 46-50, 160 mg nicotine/kg body weight/day was feed to Group 9. If the labels on the containers were switched the mice actually would have been fed the opposite dosed feeds. It is possible that the wrong container could have been used during a single day of the study but highly unlikely that this mistake could have been made every day during the study.

4) Discussions with the technical staff responsible for weighing the mice indicated that there were no problems in weighing and capture of the data in Xybion. Therefore Group 8 mice were weighed as Group 8 mice and Group 9 mice were weighed as Group 9 mice. This indicates that Group 8 mice were not weighted as Group 9 mice and vice versa. While it is possible that, on occasion, a group could be switched when weighed during a study it is highly unlikely that this would happen every day during the study.

5) Scientific and biological plausibility indicate that Group 8 and Group 9 were fed the opposite formulated feeds compared to their protocol specified requirements. Group 8 and Group 9 show steady incremental changes in body weight and body weight gain, as would be expected and in concordance with the other test article and positive control. In terms of dose response, when the Xybion data for these two groups are reversed the dose response curves follow expected trends of decreased body weight and body weight gains and are in complete concordance with the other test article and positive control. If the Xybion data are not reversed, then the dose responses do not followed expected trends and are completely out of concordance with the data for the other test article and positive control when equivalent nicotine doses are compared.

Based upon these facts, especially 1) and 5), it appears that during formulation of the diets the formulated feed prepared for Group 8 (80 mg nicotine/kg body weight) was mislabeled as 160 mg nicotine and the Group 9 feed (160 mg nicotine/kg body weight) was mislabeled as 80 mg nicotine/kg body weight/day. These findings justify exchanging the body weight and body weight gain data for Group 8 and 9 as recorded in Xybion, as was done by the original Study Director. Therefore, this change has no impact on the interpretation of the data from this study.

1.0 STUDY TITLE

Repeat Investigational Study of the Palatability of Tobacco Test Articles Formulated in NTP-2000 diets for Mice at Higher Doses

1.1 Purpose of Study

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 at anticipated Observable Effect Doses.

1.2 Sponsor

R. J. Reynolds Tobacco Company (RJRT)
Research and Development
Product Integrity
Bowman Gray Technical Center
Winston-Salem, NC. 27012

1.3 Test Facility

R.J. Reynolds Tobacco Company
Toxicology Research Division
Building 630/2
Winston-Salem, North Carolina 27102

2.0 STUDY PERSONNEL

2.1 Study Director_____ Approval date: Tue. 20-May-08
JENNY SMITH

2.2 Reviewer _____ Approval date: Tue. 20-May-08
DANIEL R. MECKLEY

2.3 Study Pathologist	JOHN SAGARTZ, DVM, ACVP
Study Technician:	VICKI HOCKER
Study Technician:	PAMELA SMOOT
Study Technician:	KIM STANLEY, BS, LAT
Research Assistant:	KEITH SHREVE, BS.
Study Technician:	WALDEN HEARN, JR.
Study Technician:	CHANDRA D. WILLIAMS, DVM
Study Technician:	Abraham Doby
Study Technician:	JESSICA BAKER, BS, LAT
Study Technician:	MONICA L. PAITSEL
Study Technician:	PATRICIA BATCHELOR
Study Technician:	TABATHA GALLIMORE
Study Technician:	JAYSON HULL
Study Technician:	JOHNNIE R. HAYES

Study Technician: LIZ CHIASSON
Study Technician: DEBORA TRAIL
Study Technician: ANDRE BRYANT
Prinicipal Scientist PAUL AYRES, PH.D., DABT

3.0 PROPOSED DATES

Dosing initiation date - - - - - - - - - - - - Tue. 27-May-08
Study completion date - - - - - - - - - - - - - Mon. 09-Jun-08

4.0 STUDY TYPE AND SPECIES SPECIFICATIONS

4.1

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

4.2

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

4.3 Animal Supplier

CHARLES RIVER BREEDING LABS,INC.; RALEIGH, NC

5.0 NUMBER OF ANIMALS ON STUDY

Pretest: 63 # Males: 63 # Females:
Study: 60 # Males: 60 # Females:

5.1 Number of Animals Per Group

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

6.0 Test Article Descriptions

6.1 Test Article: TOB BLEND

Test article identification - - TOB BLEND

6.2 Test Article: TOB EXTRACT

Test article identification - - TOB EXTRACT

7.0 Control Article Descriptions

7.1 CONTROL ARTICLE NTP-2000

10.0 Study Phases, Laboratory Determinations, and Schedules

Quarantine/Acclimation	21-May-08 (Receipt date)	M 1 F 1 MFS NDZ
Exposure phase	27-May-08 (Start of dosing)	M 1 F 1 MFS NDZ
	09-Jun-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: 2 6

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

10.1.2 CLINICAL SIGNS 2 /day

Scheduled Days: 2 6

Abv	Parameter
CS	CLINICAL SIGNS

10.2 ANIMAL ROOM FUNCTIONS (Exposure phase)

10.2.1 BODY WEIGHT FUNCTIONS

Starting on day 1 every day through day 14

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

10.2.2 FULL FEEDER WEIGHT FUNCTIONS

Starting on day 1 every day through day 14

Abv	Parameter	Units	Parameter range limits Male Low	Male High	Female Low	Female High	# Dec Pts
FF	FULL FEEDER WEIGHTS	GRAMS	380.00	420.00	380.00	420.00	0

10.2.3 EMPTY FEEDER WEIGHT FUNCTIONS

Starting on day 2 every day through day 15

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

10.2.4 CLINICAL SIGNS 2 /day

Scheduled Days: 1 4 7 11 14

Abv	Parameter
CS	CLINICAL SIGNS

10.3 DOSING (Exposure phase)

10.3.1 DOSED FEED

Starting on day 1 every day through day 14

Abv	Parameter Name	Parameter Type	# Dec Pts
FD	DOSED FEEDING	Solid Dose Units - mg/kg	1

10.4 Necropsy Procedures (F0 - Exposure phase)

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

10.5 Tissues to process

10.5.1 Organs to Weigh

Organ		Sex	Expected % of Body Weight # Low Range	High Range	Dec	Paired	Subset
BN*	BRAIN	-	0.4700	0.8700	3	-	Y
HT	HEART	-	0.2600	0.4300	3	-	Y
KD	KIDNEYS	-	0.5500	0.9500	3	-	Y
LI	LIVER	-	2.0000	3.4000	2	-	Y
LU	LUNGS	-	0.4000	0.7400	3	Y	Y
SP	SPLEEN	-	0.0600	0.2800	3	-	Y
TE	TESTES	M	0.3800	0.6200	2	Y	Y
TH	THYROID GLANDS	-	0.0050	0.0100	3	Y	Y
PG	PITUITARY GLAND	-	0.0040	0.0090	4	-	Y
OV	OVARIES	F	0.0330	0.0650	3	Y	Y
AD	ADRENAL GLANDS	-	0.0160	0.0420	4	Y	Y
AO	AORTA	-	0.0000	0.0000	3	-	Y

10.5.2 Tissues to Collect None

10.5.3 Tissues for Microscopic Examination None

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	10	M	------	------	------
2	5	M	40.0	------	------
3	5	M	80.0	------	------
4	5	M	160.0	------	------
5	5	M	240.0	------	------
6	5	M	400.0	------	------
7	5	M	------	40.0	------
8	5	M	------	80.0	------
9	5	M	------	160.0	------
10	5	M	------	240.0	------
11	5	M	------	400.0	------

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

11.0 Protocol amendments:

11.1 Date: 20-May-08 Approved by: JENNY SMITH

Time: 14:28

Reason: Did not account for extra animals ordered

o NUMBER OF ANIMALS ON STUDY

Pretest:	63	# Males:	63	# Females:	
Study:	60	# Males:	60	# Females:	

o Number of Animals Per Group

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

o Starting Animal Number Per Group

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

11.2 Date: 22-May-08 Approved by: KEITH SHREVE, BS.

Time: 09:12

added andre bryant as technician wks 5-22-08

11.3 Date: 22-May-08 Approved by: JENNY SMITH

Time: 13:34

Reason: Delayed exposure phase start

o PROPOSED DATES

Dosing initiation date - - - - - - - - - - - - Tue. 27-May-08
Study completion date - - - - - - - - - - - - - Mon. 09-Jun-08

11.4 Date: 22-May-08 Approved by: JENNY SMITH

Time: 13:36

Reason: Delayed exposure phase start date

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o NUMBER OF ANIMALS ON STUDY

Pretest: 63 # Males: 63 # Females:
Study: 60 # Males: 60 # Females:

o Number of Animals Per Group

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

o Starting Animal Number Per Group

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

11.5 Date: 22-May-08 Approved by: JENNY SMITH

Time: 13:40

Reason: Delayed start of exposure phase

Edited phase description
F0 - Quarantine/Acclimation 21-May-08 (Receipt date) M 1 F 1 MFS NDZ
27-May-08 (Start of dosing)

11.6 Date: 22-May-08 Approved by: JENNY SMITH

Time: 13:42

Reason: did not need day 5 clinicals

o ANIMAL ROOM FUNCTIONS (Quarantine/Acclimation)

o BODY WEIGHT FUNCTIONS

Scheduled Days: 2

11.7 Date: 22-May-08 Approved by: JENNY SMITH

Time: 13:43

Reason: did not need 5 day clinical

o ANIMAL ROOM FUNCTIONS (Quarantine/Acclimation)

o CLINICAL SIGNS 2 /day

Scheduled Days: 2

11.8 Date: 22-May-08 Approved by: JENNY SMITH

Time: 13:46

Reason: delayed exposure phase start day

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o CLINICAL SIGNS 2 /day

Scheduled Days: 1 4 7 11

11.9 Date: 22-May-08 Approved by: JENNY SMITH

Time: 13:48

Reason: delayed exposure start date

o Necropsy Procedures

o Tissues to process

o Organs to Weigh

Organ		Sex	Expected % of Body Weight # Low Range	High Range	Dec	Paired	Subset
BN*	BRAIN	-	0.4700	0.8700	3	-	Y
HT	HEART	-	0.2600	0.4300	3	-	Y
KD	KIDNEYS	-	0.5500	0.9500	3	-	Y
LI	LIVER	-	2.0000	3.4000	2	-	Y
LU	LUNGS	-	0.4000	0.7400	3	Y	Y
SP	SPLEEN	-	0.0600	0.2800	3	-	Y
TE	TESTES	M	0.3800	0.6200	2	Y	Y
TH	THYROID GLANDS	-	0.0050	0.0100	3	Y	Y
PG	PITUITARY GLAND	-	0.0040	0.0090	4	-	Y
OV	OVARIES	F	0.0330	0.0650	3	Y	Y
AD	ADRENAL GLANDS	-	0.0160	0.0420	4	Y	Y
AO	AORTA	-	0.0000	0.0000	3	-	Y

o Tissues to Collect None

11.10 Date: 27-May-08 Approved by: JENNY SMITH

Time: 08:47

Reason: Last day of Quarantine and Exposure Dates

---

o ANIMAL ROOM FUNCTIONS (Quarantine/Acclimation)

o BODY WEIGHT FUNCTIONS

Scheduled Days: 2 6

11.11 Date: 27-May-08 Approved by: JENNY SMITH

Time: 08:48

Reason: Add date for quarantine

o ANIMAL ROOM FUNCTIONS (Quarantine/Acclimation)

o CLINICAL SIGNS 2 /day

Scheduled Days: 2 6

11.12 Date: 09-Jun-08 Approved by: JENNY SMITH

Time: 09:14

corrected

11.13 Date: 01-Oct-08 Approved by: DANIEL R. MECKLEY

Time: 13:33

Reason: Added Dr. Ayres.

o STUDY PERSONNEL

o Study Director_____ Approval date: Tue. 20-May-08
JENNY SMITH

o Reviewer _____ Approval date: Tue. 20-May-08
DANIEL R. MECKLEY

o Study Pathologist	JOHN SAGARTZ, DVM, ACVP
Study Technician:	VICKI HOCKER
Study Technician:	PAMELA SMOOT
Study Technician:	KIM STANLEY, BS, LAT
Research Assistant:	KEITH SHREVE, BS.
Study Technician:	WALDEN HEARN, JR.
Study Technician:	CHANDRA D. WILLIAMS, DVM
Study Technician:	Abraham Doby
Study Technician:	JESSICA BAKER, BS, LAT
Study Technician:	MONICA L. PAITSEL
Study Technician:	PATRICIA BATCHELOR
Study Technician:	TABATHA GALLIMORE
Study Technician:	JAYSON HULL
Study Technician:	JOHNNIE R. HAYES

Study Technician: LIZ CHIASSON

Study Technician:	DEBORA TRAIL
Study Technician:	ANDRE BRYANT
Prinicipal Scientist	PAUL AYRES, PH.D., DABT

1.0 STUDY TITLE

Repeat Investigational Study of the Palatability of Tobacco Arti
les Formulated in NTP-2000 Diets for Mice at Higher Doses

1.1 Purpose of Study

To determine the palatability of formulated diets with Swiss
Webster mice, each of which
contains one of the test articles or the positive control
by comparison
to the control diet at anticipated Observable Effect Doses (OED)

1.2 Sponsor

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

1.3 Test Facility

R.J.R. Tobacco
Toxicology Research Division
Building 630/2
Winston-Salem, North Carolina 27102

2.0 STUDY PERSONNEL

2.1 Study Director_____ Approval date: Tue. 20-May-08
JENNY SMITH

2.2 Reviewer _____ Approval date: Tue. 20-May-08
DANIEL R. MECKLEY

2.3

Study Pathologist	JOHN SAGARTZ, DVM, ACVP
Study Technician:	VICKI HOCKER
Study Technician:	PAMELA SMOOT
Study Technician:	KIM STANLEY, BS, LAT
Study Technician:	KEITH SHREVE, BS.
Study Technician:	WALDEN HEARN, JR.
Study Technician:	Abraham Doby
Study Technician:	ANDRE BRYANT
Study Technician:	MONICA L. PAITSEL
Study Technician:	PATRICIA BATCHELOR

Study Technician:	CHANDRA D. WILLIAMS, DVM
Study Technician:	TABATHA GALLIMORE
Study Technician:	JAYSON HULL
Study Technician:	JAYSON HULL
Study Technician:	JOHNNIE R. HAYES
Study Technician:	LIZ CHIASSON
Study Technician:	DEBORA TRAIL
Study Technician:	PAUL AYRES, PH.D., DABT
Study Technician:	JESSICA BAKER, BS, LAT
Study Technician:	WALDEN HEARN, JR.
Study Technician:	Abraham Doby
Study Technician:	ANDRE BRYANT

3.0 PROPOSED DATES

Dosing initiation date - - - - - - - - - - - - - Mon. 26-May-08
Study completion date - - - - - - - - - - - - - Sun. 08-Jun-08

4.0 STUDY TYPE AND SPECIES SPECIFICATIONS

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

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

4.3 Animal Supplier

CHARLES RIVER BREEDING LABS,INC.; RALEIGH, NC

5.0 NUMBER OF ANIMALS ON STUDY

Pretest: 37 # Males: 37 # Females:
Study: 35 # Males: 35 # Females:

5.1 Number of Animals Per Group

Group	1	2	3	4	5	6
Males	5	5	5	5	5	10

5.2 Starting Animal Number Per Group

Group	1	2	3	4	5	6
Males	61	66	71	76	81	86

6.0 Test Article Descriptions

6.1 Test Article: NIC TAR

Test article identification - - NIC TAR

7.0 Control Article Descriptions

10.0 Study Phases, Laboratory Determinations, and Schedules

Quarantine/Acclimation	21-May-08 (Receipt date)	M 1 F 1 MFS NDZ
Exposure phase	27-May-08 (Start of dosing)	M 1 F 1 MFS NDZ
	09-Jun-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: 2 6

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

10.1.2 CLINICAL SIGNS 2 /day

Scheduled Days: 2 6

Abv	Parameter
CS	CLINICAL SIGNS

10.2 ANIMAL ROOM FUNCTIONS (Exposure phase)

10.2.1 BODY WEIGHT FUNCTIONS

Starting on day 1 every day through day 14

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

10.2.2 FULL FEEDER WEIGHT FUNCTIONS

Starting on day 1 every day through day 14

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

10.2.4 CLINICAL SIGNS 2 /day

Scheduled Days: 1 4 7 11 14

Abv	Parameter
CS	CLINICAL SIGNS

10.3 DOSING (Exposure phase)

10.3.1 DOSED FEED

Starting on day 1 every day through day 14

Abv	Parameter Name	Parameter Type	# Dec Pts
FD	DOSED FEEDING	Solid Dose Units - mg/kg	1

10.4 Necropsy Procedures (F0 - Exposure phase)

Method of sacrifice - - - - - - - - - - - - - Carbon dioxide inhalation
Anesthetic - - - - - - - - - - - - - - - - - - CO 2
Randomization algorithm for sacrifices - - - No
Skip unscheduled dead during selection - - - No
Select animals from top of groups - - - - - Yes
At final, sacrifice all remaining animals - Yes
Final phase sacrifice on day - - - - - - - - 15
Grace days: - - - - - - - - - - - - - - - - [0, +2]

11.0 Treatment Groups and Dosages

11.1 Doses: Exposure phase

Group No./ No.	Group	Sex	Dosage in mg/kg * Articles A
1	5	M	40.0
2	5	M	80.0
3	5	M	160.0
4	5	M	240.0
5	5	M	400.0
6	10	M	------

* Article codes: A=NIC TAR

11.0 Protocol amendments:

11.1 Date: 27-May-08 Approved by: JENNY SMITH

Time: 09:05

Reason: Changed bwts date

o ANIMAL ROOM FUNCTIONS (Quarantine/Acclimation)

o BODY WEIGHT FUNCTIONS

Scheduled Days: 2 6

11.2 Date: 27-May-08 Approved by: JENNY SMITH

Time: 09:05

Reason: changed clinical dates

o ANIMAL ROOM FUNCTIONS (Quarantine/Acclimation)

o CLINICAL SIGNS 2 /day

Scheduled Days: 2 6

11.3 Date: 27-May-08 Approved by: JENNY SMITH

Time: 11:36

Reason: Corrected start of exposure phase date

Edited phase description
F0 - Exposure phase 27-May-08 (Start of dosing) M 1 F 1 MFS NDZ
09-Jun-08 (Final sacrifice day)

11.4 Date: 30-May-08 Approved by: JENNY SMITH

Time: 10:44

Reason: updated clinicals

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o CLINICAL SIGNS 2 /day

Scheduled Days: 1 4 7 11

11.5 Date: 09-Jun-08 Approved by: JENNY SMITH

Time: 09:16

Reason: corrected

---

o ANIMAL ROOM FUNCTIONS (Exposure phase)

o CLINICAL SIGNS 2 /day

Scheduled Days: 1 4 7 11 14

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	g/ml
density 2:	1.2028	g/ml
density 3:	1.2036	g/ml
Average:	1.2032	g/ml
Standard Deviation:	0.4	

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

TOX316.001.062907

Appendix III

Calculations for the Formulation of Dosed Feed and
Analytical Chemistry Data for Homogeneity and Dose Confirmation

Calculations for Formulation of Dosed Feed

TEST ARTICLE PREPARATION FOR UPCOMING PALATABILITY STUDY FOR **MOUSE: Potential Dose Levels**

Group Number	Tobacco Blend (mg nic/kg bw/day)	Assumed Average BW kg	Needed mg of NIC	Needed g of tob	Assumed g of feed consumed per mouse per day	Amount of tob (g) in g feed	Total g Feed Mix	Total g Tobacco in Diet Prep	Total g of feed Mix	Grams tobacco blend to add to correct blend diet mix	Revised total tobacco in mix
Group	40	0.03	1.2	0.04567	6.500	0.007026	1000	7.0259	1007.0259	0.0500	7.0759
Group	80.0	0.03	2.4	0.09134	6.500	0.014052	1000	14.0517	1014.0517	0.2010	14.2527
Group	160.0	0.03	4.8	0.18267	6.500	0.028103	1000	28.1035	1028.1035	0.8120	28.9155
Group	240.0	0.03	7.2	0.27401	6.500	0.042155	1000	42.1552	1042.1552	1.8550	44.0102
Group	400.0	0.03	12	0.45668	6.500	0.070259	1000	70.2587	1070.2587	5.3100	75.5687

	Tobacco Extract (mg nic/kg bw/day)	Assumed Average BW kg	Needed mg of Nic	Needed g of tob	Assumed g of feed consumed per mouse per day	Amount of tob (g) in g feed	Total g Feed Mix	Total g Tobacco Ext in Diet Prep	Total g of feed in Diet Prep	Percent Tobacco in Feed	mg of nic/g of feed
Group	40	0.03	1.2	0.05217	6.500	0.00803	1000	8.0268	991.9732	0.8027	0.1846
Group	80.0	0.03	2.4	0.10435	6.500	0.01605	1000	16.0535	983.9465	1.6054	0.3692
Group	160.0	0.03	4.8	0.20870	6.500	0.03211	1000	32.1070	967.8930	3.2107	0.7385
Group	240.0	0.03	7.2	0.31304	6.500	0.04816	1000	48.1605	951.8395	4.8161	1.1077
Group	400.0	0.03	12.0	0.52174	6.500	0.08027	1000	80.2676	919.7324	8.0268	1.8462

	Nicotine Hydrogen Tartr (mg nic/kg bw/day)	Assumed Average BW kg	Needed mg of Nic	Needed mg of nic tartrate	Assumed g of feed consumed per mouse per day	mg amount of nic tar per g feed	Total g Feed Mix	Total g of nic tartrate in Diet Prep	Total g of feed in Diet Prep	Total g of nic tartrate in Diet Prep	mg of nic/g of feed
Group	40.0	0.03	1.2	3.419	6.500	0.5260	1000	0.526	999.474	0.52597	0.1846
Group	80.0	0.03	2.4	6.838	6.500	1.0519	1000	1.052	998.948	1.05194	0.3692
Group	160.0	0.03	4.8	13.675	6.500	2.1039	1000	2.104	997.896	2.10388	0.7385
Group	240.0	0.03	7.2	20.513	6.500	3.1558	1000	3.156	996.844	3.15582	1.1077
Group	400.0	0.03	12	34.188	6.500	5.2597	1000	5.260	994.740	5.25970	1.8462

Note: 1000 grams of dosed feed were prepared for 5 mice for 7 days with sufficient excess for chemistry samples.

The actual dose levels to be used can be inserted into column B.
Column F is the assumed amount of feed consumed per mouse per day. It can be adjusted.

Revised Total mass of feed mix	Percent tobacco in blend	Percent Tobacco in Feed	mg of nic/g of feed
1007.0759	0.7026	0.7026	0.1846
1014.2527	1.4052	1.4052	0.3692
1028.9155	2.8103	2.8103	0.7385
1044.0102	4.2155	4.2155	1.1077
1075.5687	7.0259	7.0259	1.8462

26.3 mg of nic per g of tobacco blend

23.0 mg of nic per g of tobacco extract

1 g of NIC in 2.85 g of NICOTINE HYDROGEN TARTRATE

0.351 otine in Nicotine Hydrogen Tartrate

Analytical Chemistry Data

TOX213 Summary Analytics

			Initial Formulated Feed Analytics			Repeat Formulated Feed Analytics		
Group #	Test Article	Dose mg nic/g bw	GN #	Mean [ ] mg nic/g feed	Anticipated mg nic/g feed	GN #	Mean [ ] mg nic/g feed	Anaytical Mean [ ] mg nic/g feed
1	Control	0	Not tested					
2	Tob. Blend	40	77172	0.150**	0.18	77522AA	0.155 #	0.153
3	Tob. Blend	80	77172AD	0.303	0.37	77522AC	0.307	0.305
4	Tob. Blend	160	77172AE	0.613	0.74	77522AD	0.612	0.613
5	Tob. Blend	240	77172AF	0.898	1.11	77522AE	0.992	0.945
6	Tob. Blend	400	77172	1.646**	1.85	77522AB	1.448 #	1.547
7	Tob. Blend	40	77172AD	0.180**	0.18	77522AF	0.152 #	0.166
8	Tob. Extract	80*	77202AE	0.322	0.37	77522AI	0.351	0.337
9	Tob. Extract	160*	77202AD	0.541	0.74	77522AH	0.665	0.603
10	Tob. Extract	240	77202AF	0.940	1.11	77522AJ	0.866	0.903
11	Tob. Extract	400	77202	1.600**	1.85	77522AG	1.563 #	1.582
12	Nic. Tartrate	4***	77202	0.017**	0.18	77522AK	0.017 #	0.017
13	Nic. Tartrate	80	77202AM	0.279	0.37	77522AM	0.260	0.270
14	Nic. Tartrate	160	77202AN	0.594	0.74	77522AN	0.540	0.567
15	Nic. Tartrate	240	77202AO	0.859	1.11	77522AO	0.784	0.822
16	Nic. Tartrate	400	77202	1.502**	1.85	77522AL	1.359 #	1.431

*Original feed formulations mislabelled. 80 mg nic/kg BW/day was labelled 160 mg nig/kg/day and vice versa. Table has been corrected.
**Data represent the mean of the three feed homogeneity determinations at this dose.
***Intentended dose was 40 mg nic/g bw, actual dose was ~4 mg nic/g bw.
Data from an independent analytical run not from homogeneity analysis

TOX213 Summary Analytics

Feed Homogeneity Analytics

Group #	Test Article	Dose mg nic/g bw	Sample Location	GN#	Mean [ ] mg nic/g feed	Anticipated mg nic/g feed
2	Tob. Blend	40	Top	77172AA	0.170	0.18
			Middle	77172AB	0.148	0.18
			Bottom	77172AC	0.133	0.18
			Mean		**0.150**	0.18
6		400	Top	77172AG	1.599	1.85
			Middle	77172AH	1.693	1.85
			Bottom	77172AI	1.644	1.85
			Mean		1.645	1.85
7	Tob. Extract	40	Top	77202AA	0.196	0.18
			Middle	77202AB	0.159	0.18
			Bottom	77202AC	0.184	0.18
			Mean		**0.180**	0.18
11		400	Top	77202AG	1.591	1.85
			Middle	77202AH	1.544	1.85
			Bottom	77202AI	1.665	1.85
			Mean		**1.600**	1.85
	Nic. Tartrate	4*	Top	77202AJ	0.017	0.018
			Middle	77202AK	0.017	0.018
			Bottom	77202AL	0.016	0.018
			Mean		0.017	0.018
16		400	Top	77202AP	1.517	1.85
			Middle	77202AQ	1.485	1.85
			Bottom	77202AR	1.505	1.85
			Mean		**1.502**	1.85

*Intended dose was 40 mg/kg bw/day

SAMPLE SUBMISSION RECORD

Study Number: TOX213

The following sample(s) are being submitted for analysis:
Submitted by: G Smith Submitted on: 5-21-08

Sample Identification (GN number)	Analysis	Sample Identification (GN number)	Analysis
GN77202AA	46	GN77202AL	46
GN77202AB	46	GN77202AM	46
GN77202AC	46	GN77202AN	46
GN77202AD	46	GN77202AO	46
GN77202AE	46	GN77202AP	46
GN77202AF	46	GN77202AQ	46
GN77202AG	46	GN77202AR	46
GN77202AH	46		
GN77202AI	46		
GN77202AJ	46		
GN77202AK	46		

Comments:

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

Sample(s) Received By: [signature] Received On: 5-22-08

Testno: GN77202 Prog no: 900 Protocol: GENERAL Needed: //
Requester: SMITH, JENNY L Phone: 741-0125
Description: TOB EXT/NIC TAR DIET(5-21-08)
PDR Number: TOX213
Lab Instructions:
Please run duplicates

Part: GN77202AA	Points: 1	Butt Len: 0	Part Name:	TOB EXT 40.0 TOP
Date: 20080521	Shift:	Comments:		
Part: GN77202AB	Points: 1	Butt Len: 0	Part Name:	TOB EXT 40.0 MID
Date: 20080521	Shift:	Comments:		
Part: GN77202AC	Points: 1	Butt Len: 0	Part Name:	TOB EXT 40.0 BOT
Date: 20080521	Shift:	Comments:		
Part: GN77202AD	Points: 1	Butt Len: 0	Part Name:	TOB EXT 80.0 MG/KG
Date: 20080521	Shift:	Comments:		
Part: GN77202AE	Points: 1	Butt Len: 0	Part Name:	TOB EXT 160.0 MG/KG
Date: 20080521	Shift:	Comments:		
Part: GN77202AF	Points: 1	Butt Len: 0	Part Name:	TOB EXT 240.0 MG/KG
Date: 20080521	Shift:	Comments:		
Part: GN77202AG	Points: 1	Butt Len: 0	Part Name:	TOB EXT 400 TOPMG/KG
Date: 20080521	Shift:	Comments:		
Part: GN77202AH	Points: 1	Butt Len: 0	Part Name:	TOB EXT 400 MID
Date: 20080521	Shift:	Comments:		
Part: GN77202AI	Points: 1	Butt Len: 0	Part Name:	TOB EXT 400 BOT
Date: 20080521	Shift:	Comments:		
Part: GN77202AJ	Points: 1	Butt Len: 0	Part Name:	NIC TAR 40.0 TOP
Date: 20080521	Shift:	Comments:		
Part: GN77202AK	Points: 1	Butt Len: 0	Part Name:	NIC TAR 40.0 MID
Date: 20080521	Shift:	Comments:		
Part: GN77202AL	Points: 1	Butt Len: 0	Part Name:	NIC TAR 40.0 BOT
Date: 20080521	Shift:	Comments:		

Part: GN77202AM Points: 1 Butt Len: 0 Part Name: NIC TAR 80.0
Date: 20080521 Shift: Comments:

Part: GN77202AN Points: 1 Butt Len: 0 Part Name: NIC TAR 160.0
Date: 20080521 Shift: Comments:

Part: GN77202AO Points: 1 Butt Len: 0 Part Name: NIC TAR 240.0
Date: 20080521 Shift: Comments:

Part: GN77202AP Points: 1 Butt Len: 0 Part Name: NIC TAR 400.0 TOP
Date: 20080521 Shift: Comments:

Part: GN77202AQ Points: 1 Butt Len: 0 Part Name: NIC TAR 400.0 MID
Date: 20080521 Shift: Comments:

Part: GN77202AR Points: 1 Butt Len: 0 Part Name: NIC TAR 400.0 BOT
Date: 20080521 Shift: Comments:

SAMPLE SUBMISSION RECORD

Study Number: TOX213A

The following sample(s) are being submitted for analysis:
Submitted by: Gp Smith Submitted on: 5-20-08

Sample Identification (GN number)	Analysis	Sample Identification (GN number)	Analysis
GN77172AA	46		
GN77172AB	46		
GN77172AC	46		
GN77172AD	46		
GN77172AE	46		
GN77172AF	46		
GN77172AG	46		
GN77172AH	46		
GN77172AI	46		

Comments:

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

Sample(s) Received By: Lue Flynt Received On: 5-21-08

TOX215.004 041808

From EXCEL Spreadsheet Titled: TOX 213 Feed Study Series 1 Diet

	mg/mL	Weight (g)	vol solution	mg/g	Average	
gn77202						
AA1	0.049594	1.0205	5	0.243		
AA2	0.029899	1.0093	5	0.148	0.1956	TOB EXT 40 TOP
AB1	0.029939	1.0146	5	0.148		
AB2	0.035079	1.0243	5	0.171	0.1594	TOB EXT 40 MID
AC1	0.035160	1.0325	5	0.170		
AC2	0.040342	1.0184	5	0.198	0.1842	TOB EXT 40 BOTTOM
AD1	0.102954	1.0055	5	0.512		
AD2	0.118826	1.0407	5	0.571	0.5414	TOB EXT 80
AE1	0.062703	1.0166	5	0.308		
AE2	0.067291	1.0035	5	0.335	0.3218	TOB EXT 160
AF1	0.182087	1.0034	5	0.907		
AF2	0.203445	1.0465	5	0.972	0.9397	TOB EXT 240
AG1	0.321826	1.0103	5	1.593		
AG2	0.329134	1.0359	5	1.589	1.5907	TOB EXT 400 TOP
AH1	0.310266	1.0407	5	1.491		
AH2	0.326093	1.0213	5	1.596	1.5436	TOB EXT 400 MID
AI1	0.360656	1.0363	5	1.740		
AI2	0.319771	1.0052	5	1.591	1.6653	TOB EXT 400 BOTTOM
Aj1	0.003398	1.0097	5	0.017		
Aj2	0.003391	1.0509	5	0.016	0.0165	NIC TAR 40 TOP
Ak1	0.003429	1.0262	5	0.017		
Ak2	0.003404	1.0314	5	0.017	0.0166	NIC TAR 40 MID
Al1	0.003471	1.0355	5	0.017		
Al2	0.003116	1.0035	5	0.016	0.0161	NIC TAR 40 BOTTOM
AM1	0.055637	0.9992	5	0.278		
AM2	0.056026	0.9998	5	0.280	0.2793	NIC TAR 80
AN1	0.120239	1.021	5	0.589		
AN2	0.124802	1.0426	5	0.599	0.5937	NIC TAR 160
AO1	0.169854	0.998	5	0.851		
AO2	0.180741	1.0415	5	0.868	0.8593	NIC TAR 240
AP1	0.299080	0.9988	5	1.497		
AP2	0.322030	1.0481	5	1.536	1.5167	NIC TAR 400 TOP
AQ3	0.306078	1.0318	5	1.483		
AQ2	0.299617	1.0077	5	1.487	1.4849	NIC TAR 400 MID
AR1	0.313688	1.0316	5	1.520		
AR2	0.303836	1.0195	5	1.490	1.5053	NIC TAR 400 BOTTOM

	mg/mL	Weight (g)	vol solution	mg/g	Average	
gn77172						
AA1	0.034764	1.045	5	0.166		TOB BLEND 40 TOP
AA2	0.034755	1.0044	5	0.173	0.1697	
AB1	0.028557	1.0192	5	0.140		TOB BLEND 40 MID
AB2	0.031585	1.0108	5	0.156	0.1482	
AC1	0.025023	1.0365	5	0.121		TOB BLEND 40 BOTTOM
AC2	0.029308	1.0073	5	0.145	0.1331	
AD1	0.056118	1.0353	5	0.271		TOP BLEND 80
AD2	0.069403	1.0393	5	0.334	0.3025	
AE1	0.126837	1.0102	5	0.628		TOP BLEND 160
AE2	0.120484	1.0086	5	0.597	0.6125	
AF1	0.198685	1.0202	5	0.974		TOP BLEND 240
AF2	0.168088	1.0231	5	0.821	0.8976	
AG1	0.300267	0.999	5	1.503		TOP BLEND 400 TOP
AG2	0.342028	1.0091	5	1.695	1.5988	
AH1	0.352509	1.0174	5	1.732		TOP BLEND 400 MID
AH2	0.335790	1.015	5	1.654	1.6933	
AI1	0.343125	1.0221	5	1.679		TOP BLEND 400 BOTTOM
AI2	0.326535	1.0142	5	1.610	1.6442	

*These two samples look like they are mixed up. We are going to reprep to verify our results. I will let you know on Tuesday what we find out.

	mg/mL	Weight (g)	vol solutior	mg/g	Average	
gn77202						
AA1	0.049594	1.0205	5	0.243		
AA2	0.029899	1.0093	5	0.148	0.1956	TOB EXT 40 TOP
AB1	0.029939	1.0146	5	0.148		
AB2	0.035079	1.0243	5	0.171	0.1594	TOB EXT 40 MID
AC1	0.035160	1.0325	5	0.170		
AC2	0.040342	1.0184	5	0.198	0.1842	TOB EXT 40 BOTTOM
AD1	0.102954	1.0055	5	0.512		
AD2	0.118826	1.0407	5	0.571	0.5414	TOB EXT 80
AE1	0.062703	1.0166	5	0.308		
AE2	0.067291	1.0035	5	0.335	0.3218	TOB EXT 160
AF1	0.182087	1.0034	5	0.907		
AF2	0.203445	1.0465	5	0.972	0.9397	TOB EXT 240
AG1	0.321826	1.0103	5	1.593		
AG2	0.329134	1.0359	5	1.589	1.5907	TOB EXT 400 TOP
AH1	0.310266	1.0407	5	1.491		
AH2	0.326093	1.0213	5	1.596	1.5436	TOB EXT 400 MID
AI1	0.360656	1.0363	5	1.740		
AI2	0.319771	1.0052	5	1.591	1.6653	TOB EXT 400 BOTTOM
Aj1	0.003398	1.0097	5	0.017		
Aj2	0.003391	1.0509	5	0.016	0.0165	NIC TAR 40 TOP
Ak1	0.003429	1.0262	5	0.017		
Ak2	0.003404	1.0314	5	0.017	0.0166	NIC TAR 40 MID
AI1	0.003471	1.0355	5	0.017		
AI2	0.003116	1.0035	5	0.016	0.0161	NIC TAR 40 BOTTOM
AM1	0.055637	0.9992	5	0.278		
AM2	0.056026	0.9998	5	0.280	0.2793	NIC TAR 80
AN1	0.120239	1.021	5	0.589		
AN2	0.124802	1.0426	5	0.599	0.5937	NIC TAR 160
AO1	0.169854	0.998	5	0.851		
AO2	0.180741	1.0415	5	0.868	0.8593	NIC TAR 240
AP1	0.299080	0.9988	5	1.497		
AP2	0.322030	1.0481	5	1.536	1.5167	NIC TAR 400 TOP
AQ3	0.306078	1.0318	5	1.483		
AQ2	0.299617	1.0077	5	1.487	1.4849	NIC TAR 400 MID
AR1	0.313688	1.0316	5	1.520		
AR2	0.303836	1.0195	5	1.490	1.5053	NIC TAR 400 BOTTOM

*These two samples look like they are mixed up. We are going to reprep to verify our results. I will let you know on Tuesday what we find out.

	mg/mL	Weight (g)	vol solutior	mg/g	Average	
gn77172						
AA1	0.034764	1.045	5	0.166		TOB BLEND 40 TOP
AA2	0.034755	1.0044	5	0.173	0.1697	
AB1	0.028557	1.0192	5	0.140		TOB BLEND 40 MID
AB2	0.031585	1.0108	5	0.156	0.1482	
AC1	0.025023	1.0365	5	0.121		TOB BLEND 40 BOTTOM
AC2	0.029308	1.0073	5	0.145	0.1331	
AD1	0.056118	1.0353	5	0.271		TOP BLEND 80
AD2	0.069403	1.0393	5	0.334	0.3025	
AE1	0.126837	1.0102	5	0.628		TOP BLEND 160
AE2	0.120484	1.0086	5	0.597	0.6125	
AF1	0.198685	1.0202	5	0.974		TOP BLEND 240
AF2	0.168088	1.0231	5	0.821	0.8976	
AG1	0.300267	0.999	5	1.503		TOP BLEND 400 TOP
AG2	0.342028	1.0091	5	1.695	1.5988	
AH1	0.352509	1.0174	5	1.732		TOP BLEND 400 MID
AH2	0.335790	1.015	5	1.654	1.6933	
AI1	0.343125	1.0221	5	1.679		TOP BLEND 400 BOTTOM
AI2	0.326535	1.0142	5	1.610	1.6442	

SAMPLE SUBMISSION RECORD

Study Number: TOX213

The following sample(s) are being submitted for analysis:
Submitted by: GSmith Submitted on: 6-9-08

Sample Identification (GN number)	Analysis	Sample Identification (GN number)	Analysis
GN77522AA	46	GN77522AL	46
GN77522AB	46	GN77522AM	46
GN77522AC	46	GN77522AN	46
GN77522AD	46	GN77522AO	46
GN77522AE	46		46 ①
GN77522AF	46		
GN77522AG	46		
GN77522AH	46		
GN77522AI	46		
GN77522AJ	46		
GN77522AK	46		

Comments: ① Entry Error GS 6-9-08

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

Sample(s) Received By: [signature] Received On: 6-9-2008

TOX215.004 041808

Testno: GN77522 Prog no: 900 Protocol: GENERAL Needed: //
Requester: SMITH, JENNY L Phone: 741-0125
Description: FEED STUDY
PDR Number: TOX213
Lab Instructions:
Please run duplicates

Part: GN77522AA	Points:	1	Butt Len:	0	Part Name: TOB BLEND 40.0
Date: 20080609	Shift:		Comments:		
Part: GN77522AB	Points:	1	Butt Len:	0	Part Name: TOB BLEND 400.0
Date: 20080609	Shift:		Comments:		
Part: GN77522AC	Points:	1	Butt Len:	0	Part Name: TOB BLEND 80.0
Date: 20080609	Shift:		Comments:		
Part: GN77522AD	Points:	1	Butt Len:	0	Part Name: TOB BLEND 160.0
Date: 20080609	Shift:		Comments:		
Part: GN77522AE	Points:	1	Butt Len:	0	Part Name: TOB BLEND 240.0
Date: 20080609	Shift:		Comments:		
Part: GN77522AF	Points:	1	Butt Len:	0	Part Name: TOB EXTRACT 40.0
Date: 20080609	Shift:		Comments:		
Part: GN77522AG	Points:	1	Butt Len:	0	Part Name: TOB EXTRACT 400.0
Date: 20080609	Shift:		Comments:		
Part: GN77522AH	Points:	1	Butt Len:	0	Part Name: TOB EXTRACT 80.0
Date: 20080609	Shift:		Comments:		
Part: GN77522AI	Points:	1	Butt Len:	0	Part Name: TOB EXTRACT 160.0
Date: 20080609	Shift:		Comments:		
Part: GN77522AJ	Points:	1	Butt Len:	0	Part Name: TOB EXTRACT 240.0
Date: 20080609	Shift:		Comments:		
Part: GN77522AK	Points:	1	Butt Len:	0	Part Name: NIC TAR 40.0
Date: 20080609	Shift:		Comments:		
Part: GN77522AL	Points:	1	Butt Len:	0	Part Name: NIC TAR 400.0
Date: 20080609	Shift:		Comments:		

Part: GN77522AM Points: 1 Butt Len: 0 Part Name: NIC TAR 80.0
Date: 20080609 Shift: Comments:

Part: GN77522AN Points: 1 Butt Len: 0 Part Name: NIC TAR 160.0
Date: 20080609 Shift: Comments:

Part: GN77522AO Points: 1 Butt Len: 0 Part Name: NIC TAR 240.0
Date: 20080609 Shift: Comments:

PAD TEST MEMORANDUM

AUTHOR: Karen B. Kilby
Timothy A. Ellisor

DATE: August 5, 2008

DEPARTMENT: Product Quality

DIVISION: Product Assessment

CLIENTS: Jenny Smith
Suzanne Theophilus

PREVIOUS REPORTS: PAD-MKBK 2008, 217

PROJECT CHARTER: Smokeless Tobacco Stewardship Animal Feed Palatability Project

MANHOURS: 40

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

OBJECTIVE:

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

SUMMARY:

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

STATUS:

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

KEYWORDS:

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

Karen B. Kilby, Scientist III

Timothy A. Ellisor, Technician III

Reviewed by:

S. Mark DeBusk, Lead Manager Product Quality

INTRODUCTION:

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

EXPERIMENTAL:

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

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

RESULTS AND DISCUSSION:

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

Table 1: Nicotine Results GN77624, GN77620, and GN77622

GN77624	mg/g	Average	GN77620	mg/g	Average	GN77662	mg/g	Average
AA1	<0.001		AA1	0.001		AA1	0.002	
AA2	<0.001	<0.001	AA2	<0.001	0.001	AA2	0.001	0.002
AB1	0.008		AB1	0.009		AB1	0.012	
AB2	0.013	0.011	AB2	0.008	0.009	AB2	0.013	0.012
AC1	0.021		AC1	0.015		AC1	0.015	
AC2	0.015	0.018	AC2	0.017	0.016	AC2	0.013	0.014
AD1	0.034		AD1	0.077		AD1	0.029	
AD2	0.030	0.032	AD2	0.078	0.077	AD2	0.029	0.029
AE1	0.082		AE1	0.153		AE1	0.092	
AE2	0.071	0.077	AE2	0.157	0.155	AE2	0.078	0.085
AF1	0.171		AF1	0.337		AF1	0.125	
AF2	0.151	0.161	AF2	0.339	0.338	AF2	0.144	0.135
AG1	0.001		AG1	0.001		AG1	0.001	
AG2	0.000	0.001	AG2	0.001	0.001	AG2	0.001	0.001
AH1	0.003		AH1	0.011		AH1	0.006	
AH2	0.004	0.003	AH2	0.016	0.014	AH2	0.005	0.006
AI1	0.064		AI1	0.018		AI1	0.011	
AI2	0.072	0.068	AI2	0.024	0.021	AI2	0.017	0.014
AJ1	0.019		AJ1	0.058		AJ1	0.044	
AJ2	0.017	0.018	AJ2	0.048	0.053	AJ2	0.047	0.046
AK1	0.048		AK1	0.108		AK1	0.011	
AK2	0.034	0.041	AK2	0.176	0.142	AK2	0.096	0.054
AL1	0.118		AL1	0.354		AL1	0.288	
AL2	0.062	0.090	AL2	0.393	0.374	AL2	0.280	0.284
AM1	0.007		AM1	0.017		AM1	0.011	
AM2	0.007	0.007	AM2	0.017	0.017	AM2	0.010	0.011
AN1	0.027		AN1	0.066		AN1	0.030	
AN2	0.027	0.027	AN2	0.065	0.065	AN2	0.030	0.030
AO1	0.068		AO1	0.156		AO1	0.001	
AO2	0.064	0.066	AO2	0.151	0.154	AO2	0.001	0.001
AP1	0.137		AP1	0.303		AP1	0.209	
AP2	0.123	0.130	AP2	0.310	0.306	AP2	0.203	0.206
			AQ1	0.045				
			AQ2	0.038	0.042			

control ?

AL

Table 2:Nicotine Results GN77615, GN77522, And GN77172

GN77615	mg/g	Average
AA1	<0.001	
AA2	<0.001	<0.001
AB1	0.002	
AB2	0.003	0.002
AC1	0.015	
AC2	0.012	0.014
AD1	0.028	
AD2	0.042	0.035
AE1	0.050	
AE2	0.075	0.062
AF1	0.122	
AF2	0.153	0.138
AG1	0.307	
AG2	0.298	0.302
AH1	0.002	
AH2	0.003	0.002
AI1	0.068	
AI2	0.032	0.050
AJ1	0.054	
AJ2	0.052	0.053
AK1	0.052	
AK2	0.067	0.060
AL1	0.191	
AL2	0.164	0.178
AM1	0.291	
AM2	0.239	0.265
AN1	0.017	
AN2	0.018	0.018
AO1	0.061	
AO2	0.064	0.063
AP1	0.152	
AP2	0.155	0.153
AQ1	0.287	
AQ2	0.306	0.296

GN77522	mg/g	Average
AA1	0.176	
AA2	0.134	0.155
AB1	1.415	
AB2	1.481	1.448
AC1	0.304	
AC2	0.311	0.307
AD1	0.648	
AD2	0.577	0.612
AE1	0.875	
AE2	0.969	0.922
AF1	0.159	
AF2	0.156	0.158
AG1	1.581	*
AG2	1.546	1.563
AH1*	0.647	
AH2*	0.683	0.665
AI1*	0.380	
AI2*	0.322	0.351
AJ1	0.853	
AJ2	0.879	0.866
AK1	0.018	
AK2	0.016	0.017
AL1	1.379	
AL2	1.340	1.359
AM1	0.261	
AM2	0.259	0.260
AN1	0.542	
AN2	0.539	0.540
AO1	0.779	
AO2	0.789	0.784

GN77172	mg/g	Average
AA1	0.166	
AA2	0.173	0.170
AB1	0.140	
AB2	0.156	0.148
AC1	0.121	
AC2	0.145	0.133
AD1	0.271	
AD2	0.334	0.302
AE1	0.628	
AE2	0.597	0.613
AF1	0.974	
AF2	0.821	0.898
AG1	1.503	
AG2	1.695	1.599
AH1	1.732	
AH2	1.654	1.693
AI1	1.679	
AI2	1.610	1.644

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

Table 3: Nicotine Results GN77202, GN76746, and GN76747

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

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

Table 4: Nicotine Results GN76749 and GN76750

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

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

Decode 1 Jest

PAD TEST MEMORANDUM

AUTHOR: Karen B. Kilby
Timothy A. Ellisor

DATE: August 5, 2008

DEPARTMENT: Product Quality

DIVISION: Product Assessment

CLIENTS: Jenny Smith
Suzanne Theophilus

PREVIOUS REPORTS: PAD-MKBK 2008, 217

PROJECT CHARTER: Smokeless Tobacco Stewardship Animal Feed Palatability Project

MANHOURS: 40

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

OBJECTIVE:

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

SUMMARY:

GN 76746

listed on report as GN76746, which corrected with submission sheet.

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

STATUS:

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

KEYWORDS:

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

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

Karen B. Kilby, 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.

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Table 1: Nicotine Results GN77624, GN77620, and GN77622

	GN77624	mg/g	Dose (mg) Average		GN77620	mg/g	Dose Average		GN77622	mg/g	Dose Average
control TB	AA1	<0.001	0.2	control	AA1	0.001	0	TB	AA1	0.002	0.2
	AA2	<0.001	<0.001		AA2	<0.001	0.001		AA2	0.001	0.002
TB	AB1	0.008	2.0	TB	AB1	0.009	0.2	TB	AB1	0.012	2.0
	AB2	0.013	0.011		AB2	0.008	0.009		AB2	0.013	0.012
TB	AC1	0.021	4.0	TB	AC1	0.015	2.0	TB	AC1	0.015	4.0
	AC2	0.015	0.018		AC2	0.017	0.016		AC2	0.013	0.014
TB	AD1	0.034	8.0	TB	AD1	0.077	8.0	TB	AD1	0.029	8.0
	AD2	0.030	0.032		AD2	0.078	0.077		AD2	0.029	0.029
TB	AE1	0.082	20.0	TB	AE1	0.153	20.0	TB	AE1	0.092	20.0
	AE2	0.071	0.077		AE2	0.157	0.155		AE2	0.078	0.085
TB	AF1	0.171	40.0	TB	AF1	0.337	40.0	TB	AF1	0.125	40.0
	AF2	0.151	0.161		AF2	0.339	0.338		AF2	0.144	0.135
TE	AG1	0.001	0.2	TE	AG1	0.001	0.2	TE	AG1	0.001	0.2
	AG2	0.000	0.001		AG2	0.001	0.001		AG2	0.001	0.001
TE	AH1	0.003	2.0	TE	AH1	0.011	2.0	TE	AH1	0.006	2.0
	AH2	0.004	0.003		AH2	0.016	0.014		AH2	0.005	0.006
TE	AI1	0.064	4.0	TE	AI1	0.018	4.0	TE	AI1	0.011	4.0
	AI2	0.072	0.068		AI2	0.024	0.021		AI2	0.017	0.014
TE	AJ1	0.019	8.0	TE	AJ1	0.058	8.0	TE	AJ1	0.044	8.0
	AJ2	0.017	0.018		AJ2	0.048	0.053		AJ2	0.047	0.046
TE	AK1	0.048	20.0	TE	AK1	0.108	20.0	TE	AK1	0.011	20.0
	AK2	0.034	0.041		AK2	0.176	0.142		AK2	0.096	0.054
TE	AL1	0.118	40.0	TE	AL1	0.354	40.0	TE	AL1	0.288	40.0
	AL2	0.062	0.090		AL2	0.393	0.374		AL2	0.280	0.284
NT	AM1	0.007	2.0	NT	AM1	0.017	2.0	NT	AM1	0.011	2.0
NT	AM2	0.007	0.007		AM2	0.017	0.017		AM2	0.010	0.011
L	AN1	0.027	8.0	NT	AN1	0.066	8.0	NT	AN1	0.030	8.0
	AN2	0.027	0.027		AN2	0.065	0.065		AN2	0.030	0.030
NT	AO1	0.068	20.0	NT	AO1	0.156	20.0	NT	AO1	0.001	20.0 *
	AO2	0.064	0.066		AO2	0.151	0.154		AO2	0.001	0.001
NT	AP1	0.137	40.0	NT	AP1	0.303	40.0	NT	AP1	0.209	40.0
	AP2	0.123	0.130		AP2	0.310	0.306		AP2	0.203	0.206
	• TOX210 Series 2 Feed Formulation Data			TB	AQ1	0.045	4.0		• TOX 210 Series 1 Feed Formulation Data		
					AQ2	0.038	0.042				

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

AL

- Data from PAD report

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

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

* Sample problem - data not used

Table 2:Nicotine Results GN77615, GN77522, And GN77172

	GN77615	mg/g	Dose Average
cont'd TB	AA1	<0.001	0.0
	AA2	<0.001	<0.001
TB	AB1	0.002	0.2
	AB2	0.003	0.002
TB	AC1	0.015	2.0
	AC2	0.012	0.014
TB	AD1	0.028	4.0
	AD2	0.042	0.035
TB	AE1	0.050	8.0
	AE2	0.075	0.062
TB	AF1	0.122	20.0
	AF2	0.153	0.138
TB	AG1	0.307	40.0
	AG2	0.298	0.302
TE	AH1	0.002	0.2
	AH2	0.003	0.002
TE	AI1	0.068	2.0
	AI2	0.032	0.050
TE	AJ1	0.054	4.0
	AJ2	0.052	0.053
TE	AK1	0.052	8.0
	AK2	0.067	0.060
TE	AL1	0.191	20.0
	AL2	0.164	0.178
TE	AM1	0.291	40.0
	AM2	0.239	0.265
NT	AN1	0.017	2.0
	AN2	0.018	0.018
NT	AO1	0.061	8.0
	AO2	0.064	0.063
NT	AP1	0.152	20.0
	AP2	0.155	0.153
NT	AQ1	0.287	40.0
	AQ2	0.306	0.296

	GN77522	mg/g	Dose Average
TB	AA1	0.176	40
	AA2	0.134	0.155
TB	AB1	1.415	400
	AB2	1.481	1.448
TB	AC1	0.304	80
	AC2	0.311	0.307
TB	AD1	0.648	160
	AD2	0.577	0.612
TB	AE1	0.875	240
	AE2	0.969	0.922
TB	AF1	0.159	40
	AF2	0.156	0.158
TB	AG1	1.581	400
	AG2	1.546	1.563
TE	AH1*	0.647	160
	AH2*	0.683	0.665
TE	AI1*	0.380	80
	AI2*	0.322	0.351
TE	AJ1	0.853	240
	AJ2	0.879	0.866
NT	AK1	0.018	40
	AK2	0.016	0.017
	AL1	1.379	400
	AL2	1.340	1.359
	AM1	0.261	80
	AM2	0.259	0.260
	AN1	0.542	160
	AN2	0.539	0.540
	AO1	0.779	240
	AO2	0.789	0.784

GN77172	mg/g	Average
AA1	0.166	
AA2	0.173	0.170
AB1	0.140	
AB2	0.156	0.148
AC1	0.121	
AC2	0.145	0.133
AD1	0.271	
AD2	0.334	0.302
AE1	0.628	
AE2	0.597	0.613
AF1	0.974	
AF2	0.821	0.898
AG1	1.503	
AG2	1.695	1.599
AH1	1.732	
AH2	1.654	1.693
AI1	1.679	
AI2	1.610	1.644

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

TOX209 series 1
- Feed Formulation Data
- Dose Confirmation
- Decoded from sample cups by Duane

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- Data from TOX 213
- Feed Formulation
- Dose Confirmation
- Decoded from KK email
- Only one diet formulated

~~listed as GN76747~~ or not listed

Table 3: Nicotine Results GN77202, GN76746, and GN76747

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

TOX209 series 2 Feed Formulation Data
- Summission 5/02/08
- Feed formulated 4/24-25

- TOX210 series 2 Feed Formulation Data
- Submission May 2
- Confirmed by sample cups

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

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Table 4: Nicotine Results GN76749 and GN76750

	GN76749	mg/g	Dose (mg) Average		GN76750	mg/g	Dose (mg) Average
TB	AA1	0.466	40	TB	AA1	0.416	40
	AA2	0.411	0.438		AA2	0.402	0.409
TB	AB1	0.002	0.2	TB	AB1	0.002	0.2
	AB2	0.002	0.002		AB2	0.001	0.002
TE	AC1	0.448	40	TE	AC1	0.408	40
	AC2	0.424	0.436		AC2	0.325	0.367
TE	AD1	0.002	0.2	TE	AD1	0.001	0.2
	AD2	0.002	0.002		AD2	0.001	0.001
NT	AE1	0.415	40	NT	AE1	0.360	40
	AE2	0.407	0.411		AE2	0.348	0.354
NT	AF1	0.019	2.0	NT	AF1	0.019	2.0
	AF2	0.021	0.020		AF2	0.019	0.019

- TOX209 Stability Data (1 month)
- TOX209 ~~One-week~~ Stability Data (10-day stability)
- LIMS Submission Sheet dated 05/02/08

CONCLUSION:

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

- 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

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

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PAD TEST MEMORANDUM

AUTHOR: Karen B. Kilby
Timothy A. Ellisor

DATE: August 4, 2008

DEPARTMENT: Product Quality

DIVISION: Product Assessment

CLIENTS: Jenny Smith
Suzanne Theophilus

PREVIOUS REPORTS: none

PROJECT CHARTER: Smokeless Tobacco Stewardship Animal Feed Palatability Project

MANHOURS: 40

Validation of a New Method for the Determination of the amount of Nicotine Applied to Rat/Mouse Feed

OBJECTIVE:

The purpose of this study was to develop a method to determine the amount of nicotine applied to rat/mouse feed samples to support the Smokeless Tobacco Stewardship Animal Feed Palatability Project.

SUMMARY:

A method was developed to determine the amount of nicotine applied to rat/mouse feed to support the Smokeless Tobacco Stewardship Animal Feed Palatability Project. The proposed method is detailed in Appendix A. The method was validated based on several factors including: linearity, accuracy, instrument precision, and method reproducibility.

The linearity of the end determination was determined by analyzing standard solutions with various concentrations of nicotine. Statistical analysis showed excellent linearity with an r^2 greater than 0.999. The accuracy of the method was determined by two standard addition experiments. These experiments showed percent recoveries of 92 to 106%. Statistical analysis of the results measured in the standard addition experiment showed excellent linearity of the method (including sample preparation, extraction, and end determination), with an r^2 greater than 0.995 for both experiments. The instrument precision was determined by calculating the variation from 6 replicate injections of the same sample vial. The result showed the instrument precision to be 1.1%RSD. The method reproducibility was determined by calculating the variation of 6 replicate preparations of the same sample. The method reproducibility was calculated to be 1.6%RSD.

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

STATUS:
The validation of a method for the determination of the amount of nicotine applied to rat/mouse feed is complete.

KEYWORDS:
GC/MS, nicotine, smokeless tobacco, SNUS, tobacco extract, tobacco blend, nicotine tartrate

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 µL of the standard solution (0.0145 mg nicotine), the second level contained 500 µL of the standard solution (0.1445 mg nicotine), and the third level contained 1000 µL of the standard solution (0.2890 mg nicotine). The samples were extracted and analyzed according to the procedures described in the proposed method (Appendix A).

RESULTS AND DISCUSSION:

This method was validated based on several factors including: linearity, accuracy, instrument precision, and method reproducibility,. These factors are discussed individually below.

Linearity

The linearity of the end determination was evaluated by analyzing standard solutions with various concentrations (0.000099 mg/mL-0.198000 mg/mL) of nicotine. The quantitation limit of the method was determined to be equal to the lowest standard. Statistical analysis of the results from the standard solutions was performed using least squares regression of the concentration versus the peak area ratio of nicotine to quinoline-d_7. The regression coefficient, r^2 is a measure of random error associated with the calibration and a measure of the linearity of the responses. The results measured were directly proportional to the analyte concentration. Excellent linearity was observed for nicotine, with an r^2 greater than 0.999, which is within the typical range observed for other methods.

Accuracy

The accuracy of the method was determined by two standard addition experiments (1). For this experiment, a standard solution was prepared by adding a known amount of nicotine to MTBE

(concentration 0.289 mg/mL). Two sets of samples were prepared using the blank rat/mouse feed samples. Six replicates were prepared for each level. The amount of nicotine measured in the blank feed sample was below the quantitation limit for this method (0.000099 mg/mL or 0.00099 mg). One set of standard addition samples were prepared by adding known amounts of the nicotine solution to the samples at the beginning of the sample extraction procedure; while a second set of samples were prepared by adding known amounts of the nicotine solution to the samples at the end of the extraction procedure. Recovery was calculated as follows:

$$\%\text{Recovery} = \frac{\text{Feed Plus Standard (mg)} - \text{Blank Feed (mg)}}{\text{Amount Standard Added (mg)}} \times 100\%$$

Table 1 shows the calculated percent Recovery for each level to be 92 to 106% for both sets of samples.

Table 1: Accuracy- Standard Addition Experiment (n=6)

Level	Amount Added (mg)	Average Amount Standard Measured (mg)	Average %Recovery
Standard Added Before Extraction			
Level 0	0	<0.00099	
Level 1	0.0145	0.01532	106.0
Level 2	0.1445	0.14561	100.8
Level 3	0.2890	0.26675	92.3
		% Recovery	**99.7**
Standard Added After Extraction			
Level 0	0	<0.00099	
Level 1	0.0145	0.01501	103.9
Level 2	0.1445	0.13927	96.4
Level 3	0.2890	0.27408	94.8
		% Recovery	**98.4**

Further evaluation of the data collected during the standard addition experiment shows the linearity of the method (including sample preparation, extraction and the end determination). The results measured are directly proportional to the concentration of nicotine added to each sample. Statistical analysis of the results from the standard addition experiments were performed using least squares regression of concentration versus the peak area ratio of nicotine to quinoline-d_7 (Figures 1 and 2). A regression coefficient of 0.9974 and 1.0000, (r^2) indicates excellent linearity of the method. A near zero intercept (0.0060 and 0.0019) is an indication that a constant systematic error between the

amount of standard added and the amount of standard measured is not present and that a co-elution is highly unlikely.

Figure 1: Method Linearity Standard Addition Test 1

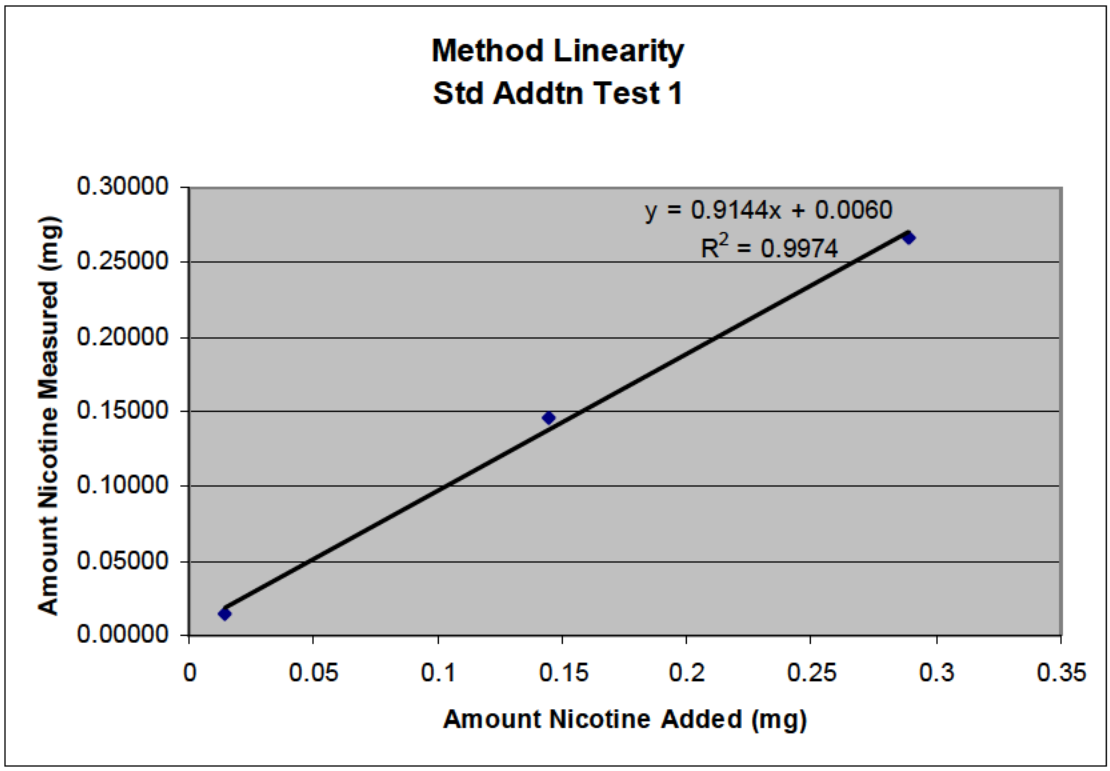
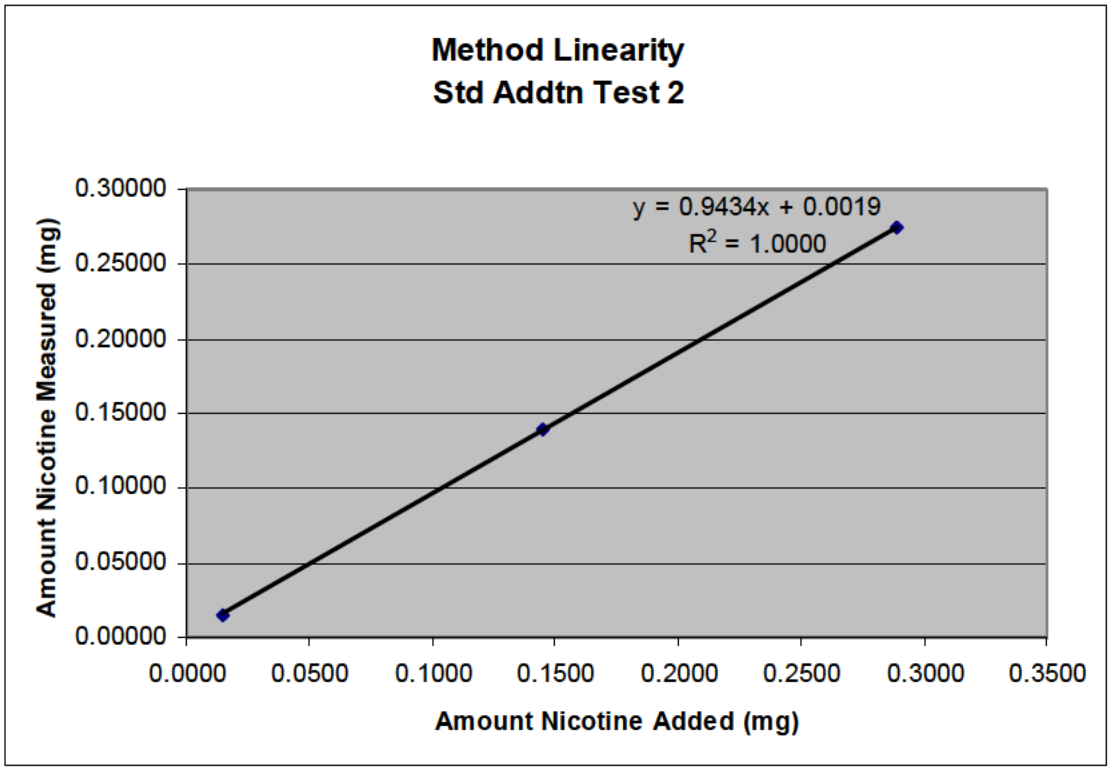


Figure 2: Method Linearity -Standard Addition Test 2



Instrument Precision

The instrument precision was determined by calculating the variation from 6 replicate injections of the same sample vial. The results showed the instrument precision to be 1.1%RSD.

Table 2: Instrument Precision

Sample	Nicotine (mg/g)
1	0.043
2	0.044
3	0.043
4	0.043
5	0.043
6	0.043
Average	0.043
Std Dev	0.0005
%RSD	1.1

Method Reproducibility

The method reproducibility was determined by calculating the variation of 6 replicate preparations of the same sample. The method reproducibility was calculated to be 1.6%RSD.

Table 3: Method Reproducibility

Sample	
	Nicotine (mg/g)
1	0.059
2	0.058
3	0.059
4	0.057
5	0.058
6	0.057
Avg.	0.058
Std dev.	0.0009
% RSD	**1.6**

CONCLUSION:

The validation of a new method for the determination of nicotine applied to rat/mouse feed samples is complete. The proposed method showed good linearity (r^2>0.999), accuracy (92-106%), instrument precision (1.1%RSD), and method reproducibility (1.6%RSD). The proposed method shall be used for the determination of nicotine in rat/mouse feed to support the Smokeless Tobacco Stewardship Animal Feed Palatability Project.

REFERENCES:

1. Meier, P. C. and Zünd, R. E., “Statistical Methods in Analytical Chemistry”, John Wiley and Sons, New York, 1993, pp. 109-110.

Appendix A
Proposed Method

1. Scope

This method specifies procedures for the determination of the amount of nicotine in rat/mouse feed samples by gas chromatography/mass spectrometry (GC/MS)

2. Principle

Rat/mouse feed samples with known amounts of applied nicotine are treated with aqueous sodium hydroxide and the nicotine is extracted into tert-butyl methyl ether (MTBE). The amount of nicotine is then quantitated by gas chromatography/mass spectrometry. Results are reported in mg nicotine/g feed units.

3. Equipment/Apparatus

3.1 Equipment

3.1.1 Agilent Technologies 6890/5973 gas chromatograph/mass spectrometer with an Agilent Technologies 7683 automatic sampler, or equivalent.

3.1.2 Mettler AE 163 analytical balance, or equivalent.

3.1.3 Burrell wrist action shaker, model 75, or equivalent.

3.1.4 Thermolyne Maxi-Mix II, or equivalent.

3.1.5 Rainin Micropipettes- various dispense capabilities, or equivalent

3.2 Apparatus

3.2.1 Class A volumetric pipets –1 mL, 5 mL, 10 mL, 25 mL.

3.2.2 Class A volumetric flasks – 50 mL, 100 mL, 1 L.

3.2.3 Bottletop Dispensors- 5.0 mL capability, or equivalent.

3.2.4 Glass tubes 25 X 200 with screw caps, Kimax catalog # 45066-25200, or equivalent.

3.2.5 GC vials with crimp-top caps.

3.2.6 Fisherbrand 9” Pasteur Pipets flint glass (catalog no. 13-678-6B), or equivalent.

3.2.7 Liner, straight with glass wool in the middle (Agilent Technologies part no.19251-60540), or equivalent.

3.2.8 Column – J & W Scientific Co., DB-WAX, 30 m x 0.25 mm id, 0.5 micron film thickness (catalog no. 122-7033), or equivalent.

4. Reagents/Safety

4.1 Reagents

4.1.1 L-nicotine, minimum 99% purity, Acros, catalog # AC 18142-0250.

4.1.5 Quinoline-d_7 (Internal Standard) CDN Isotopes, catalog no. D-1450.

4.1.6 Tert-butyl methyl ether, MTBE-Aldrich catalog no. 29-321-0.

4.1.7 NaOH – Pellets, Fisher # S318-500.

4.2 Safety

The chemicals used in this method are possible carcinogens, mutagens, toxins, etc. The analysts shall refer to section 6.1 of this document and the Material Safety Data Sheets for each chemical for appropriate handling instructions.

5. Set Up GC:

5.1 Suitable chromatographic conditions for an Agilent Technologies 6890/5973 gas chromatograph/mass spectrometer with an Agilent Technologies 7683 automatic sampler and a J & W Scientific Co., DB-WAX, 30 m x 0.25 mm id, 0.5 micron film thickness, include:

Table 1: Oven Program

	ºC/min	ºC	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 ºC
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 ºC
Split Ratio	25:1
Split Flow	50 mL/min
Column	
Constant flow	2.0 mL/min
Detector	MSD
MSD Transfer line	150 ºC
SIM Parameters	**m/z ***
Quinoline-d_7 (IS)	136
Nicotine	84

*The components and their selected ions listed above were identified as the optimal ions of interest for the quantification of nicotine in rat/mouse feed. See Figures 1-3 for example chromatograms of the calibration standards and product extracts. All ions are monitored concurrently for the entire run.

6. Preparation of Extraction Solution, Standards, and Checks:

6.1 Preparation of Solutions:

Safety Alert!

Nicotine is extremely toxic and readily absorbed through the skin, as well as a possible teratogen. Always wear nitrile gloves when handling and use appropriate glassware for pipetting.

6.1.1 Extraction Solution: Add approximately 0.0500 g of Quinoline-d_7 (Internal standard) in 4 liters of MTBE and mix well.

6.1.2 2N NaOH solution: Weigh 80 g of NaOH pellets into a 1 L volumetric flask. Dilute to volume with distilled water. Add a stir bar and stir to dissolve pellets. Mix well and transfer a portion of the solution to a container equipped with a bottle top dispenser to dispense 5 mL.

6.2 Prepare Standard Solutions:

6.2.1 Prepare Primary Standard Stock Solution
Weigh 0.4000 g (to the nearest 0.1 mg) nicotine into a 100 mL volumetric flask and dilute to volume with extraction solution.

Example calculation:

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

6.2.2 Prepare Diluted Standard Stock Solution
Pipette 5 mL of the Primary Standard Stock Solution into a 100 mL volumetric flask and dilute to volume with extraction solution. This solution is also used as the highest standard.

Example calculation:

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

6.2.3 Prepare Standard Solutions
Pipette (using a micro-pipette or Class A volumetric pipette) the following amounts of Standard Stock Solution to the appropriate 50 mL volumetric flask. Dilute to volume with extraction solution and mix well. Determine the concentration of nicotine for each standard as shown in the example below.

Table 3: Standard Preparation

Level	Amount of Standard Stock Solution	Nicotine Concentration mg/mL
L1 (Std 1)	25 µL	0.000099
L2 (Std 2)	50 µL	0.000198
L3 (Std 3)	100 µL	0.000396
L4 (Std 4)	500 µL	0.001980
L5 (Std 5)	1 mL	0.003960
L6 (Std 6)	5 mL	0.019800
L7 (Std 7)	10 mL	0.039600
L8 (Std 8)	25 mL	0.099000
L9 (Diluted stock)	Diluted Stock Solution	0.198000

Notes: All solutions shall be stored in a freezer, when not in use. New standards shall be prepared when extraction solution is made.

The calibration range concentrations may be expanded or changed to encompass varying levels, if necessary.

7. Process Standards:

7.1 Calibration is normally performed at the beginning of each week prior to sample analysis or when new extraction solution is prepared.

7.2 Using a Pasteur pipette, transfer an aliquot of each standard solution to GC vials and cap.

7.3 Prime the GC System.

7.4 Inject the standards in duplicate.

7.5 ChemStation performs a “quadratic regression” fit. Obtain a printout of the calibration report.

7.6 If the calibration curve is acceptable ($r^2 \geq 0.999$), continue with sample analysis. If it is not acceptable, take the necessary corrective action before continuing.

8. Prepare Test Portion(s):

8.1 Label glass tubes (25 X 200mm) to correspond to the samples to be analyzed.

8.2 Add 5 mL of 2N NaOH to each glass tube.

8.3 Accurately weigh approximately 1.0000 g (to the nearest 0.1 mg) of sample into the corresponding glass tube.

8.4 Shake each tube on the Maxi-Mix II (mini vortexer) to ensure saturation of the sample with the NaOH solution.

8.5 Allow the sample solutions to sit for 30 minutes.

8.6 Add 5.0 mL of extraction solution to each tube and cap tightly.

8.7 Shake each tube on the Maxi-Mix II shaker to ensure complete mixing.

8.8 Shake tubes for 2 hours on a wrist action shaker at full speed. (Make sure the extraction solution is completely mixing with the sample.)

8.9 After shaking, allow layers to separate (approximately 15 minutes).

8.10 Transfer a portion of the top layer into corresponding GC vials using a new disposable Pasteur pipette for each sample.

8.11 Use a crimper to cap the GC vials to ensure a good seal is formed.

9. Analyze Extracts:

9.1 Transfer the GC vials to the appropriate GC/MS system.

9.3 Results are expressed in mg nicotine per gram feed units and may be calculated manually according to the following equations:

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

where: C= Concentration of nicotine
a = quadratic term

b = linear term

c = constant term

x = component peak area/internal standard peak area

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

10. Sample Disposal

Extracted samples are disposed of in accordance with the CHP. Sample Disposal shall be performed as follows:

10.1 Test tube caps are removed and placed in a container to be washed and re-used. The MTBE waste is poured into an appropriate chemical waste container labeled MTBE waste. When the container is full, it is transferred to the proper location. Test tubes are rinsed and transferred to the washroom to be washed and re-used.

10.2 Used GC vials are placed into plastic buckets obtained from the stockroom. When the containers are full, the buckets are transferred to the appropriate disposal location.

Figure 1: Selected Ion 136 Internal Standard Peak (Quinolined_7)**:**

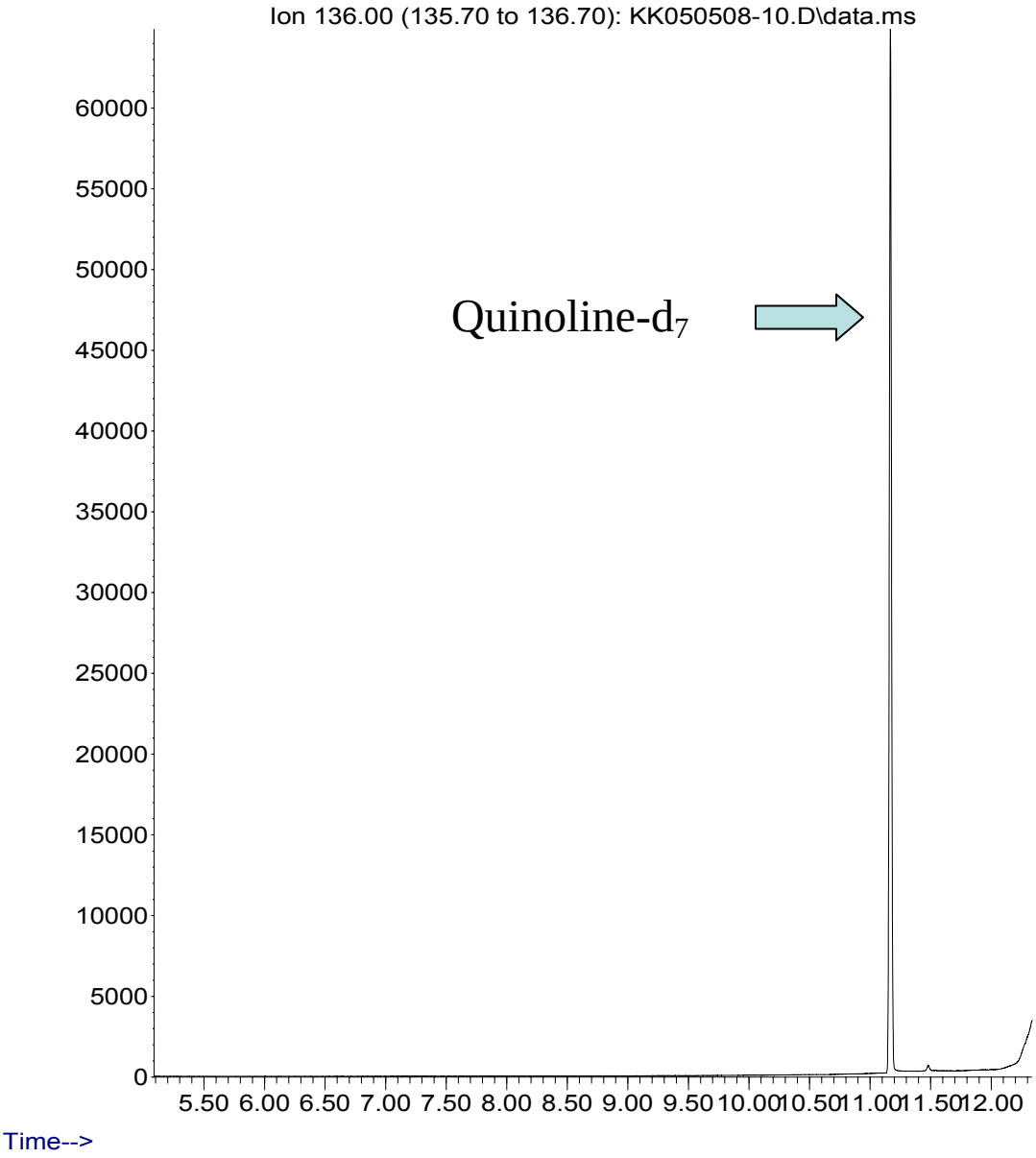


Figure 2: TIC Chromatogram of Calibration Standard (Std 5):

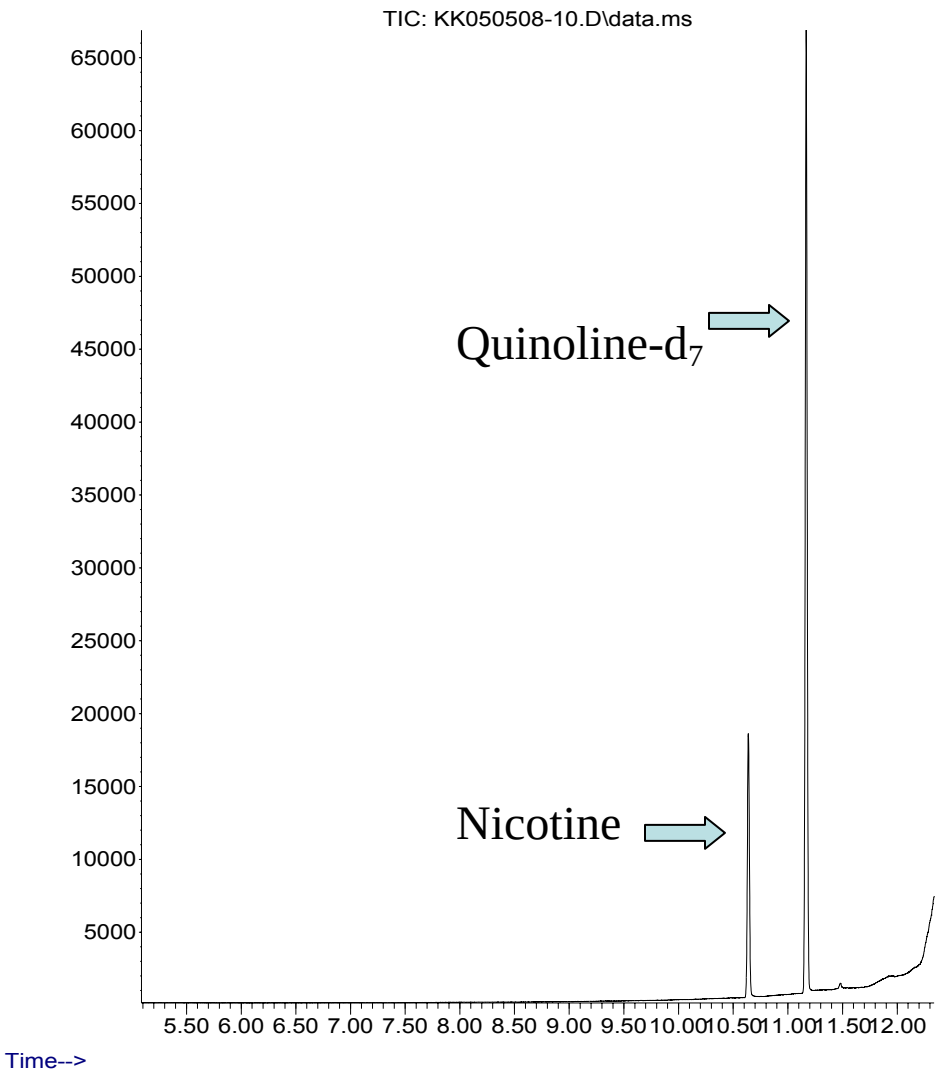
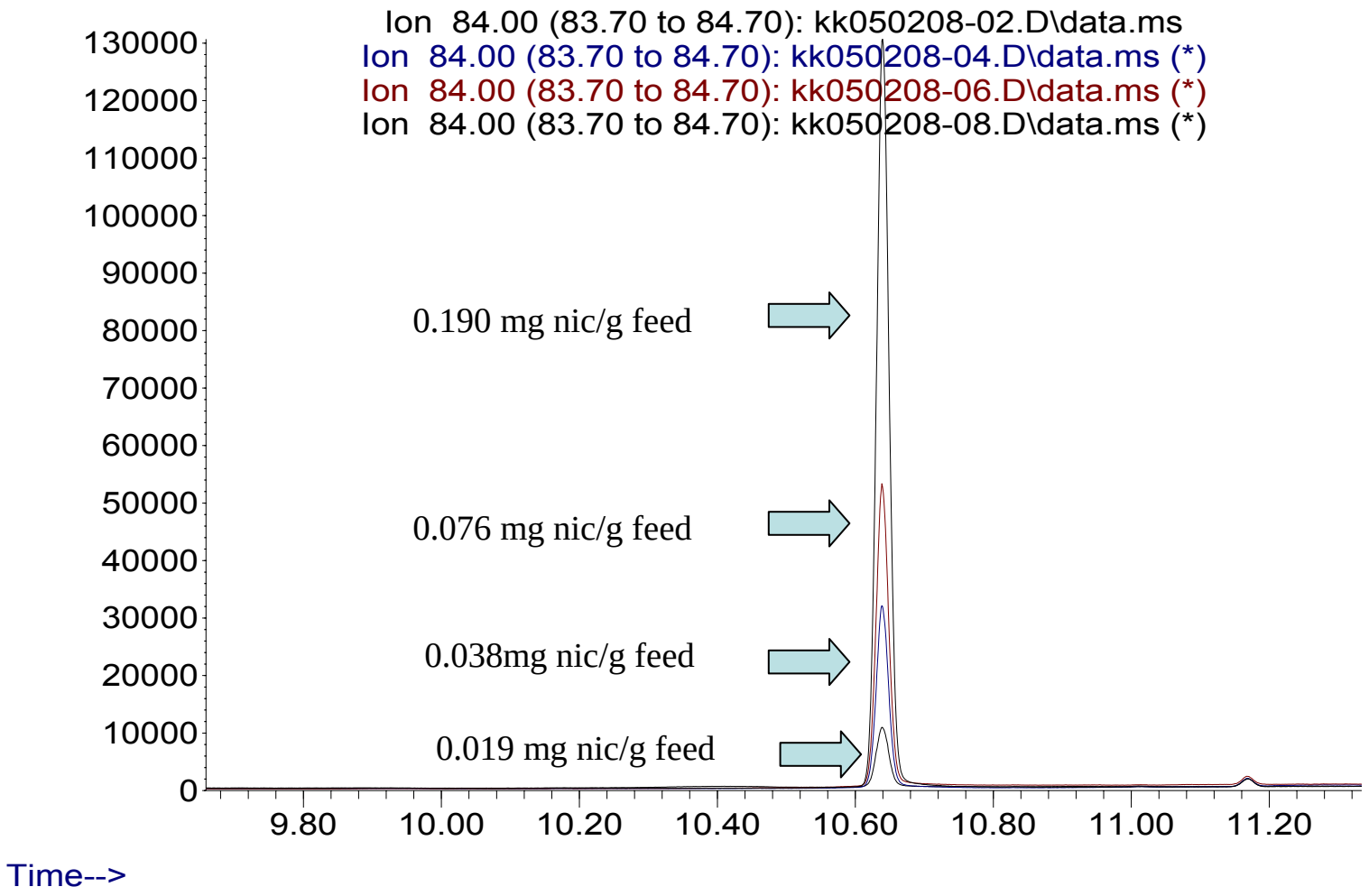


Figure 3: Overlaid Chromatograms (Selected Ion 84) of four Test Extracts Ranging from 0.019 to 0.19 mg nicotine per gram rat feed:



Appendix IV

Serology and Histopathology of the Lung

Subject: Serology Results for TOX213 **Date:** June 19, 2008

To: Jenny Smith **From:** Chandra Williams, DVM

Attached are the serology results of serum samples taken from animals on the TOX213 study. The samples were collected from 10 male (animal numbers 28-37) mice. Mice were euthanized for whole blood sample collection. Serum was removed from the whole blood samples and submitted on 10 animals (28-37). The samples were collected on June 11, 2008.

The serum was submitted to Charles River Laboratory (CRL) and was analyzed for the presence of antibodies to the mouse pathogens (CRL Mouse Assessment Plus profile) listed below.

Sendai Virus	Mouse Adenovirus (MAV) 1 & 2
Pneumonia Virus of Mice (PVM)	Epizootic Diarrhea of Infant Mice Virus (EDIM)
Mouse Hepatitis Virus (MHV)	Mouse Cytomegalovirus (MCMV)
Minute Virus of Mice (MVM)	Hantaviruses
GDVII (Murine Encephalomyelitis Virus)	*Encephalitozoon cuniculi*
REO-3	Cilia Associated Respiratory Bacillus
Mycoplasma pulmonis	Mouse Parvovirus (MPV)* 1 & 2
Lymphocytic Choriomeningitis Virus	Mouse Thymic Virus (MTLV)
Ectromelia (Mousepox)	Murine Norovirus (MNV)
K virus	Polyoma Virus
Sendai Virus	

*MPV testing also includes testing for the non-structural protein of MPV, denoted as *MFIA 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

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 #: 2008-029876**

Diagnostic Summary Report

PO Box 1236
Winston-Salem, NC 27102 USA

Attn: Dr.Chandra Williams

Tel: 336-741-0121

Received: 12 Jun 2008
Approved: 18 Jun 2008, 08:05
Bill Method: PO# 4534386478
Test Specimen: Mouse

Sample Set	Service (# Tested)	Profile	Assay	Tested	+	+/-	?
#1	Serology (10)	All Results Negative					

+ = Positive, +/- = Equivocal, ? = Indeterminate

Service Approvals

Service	Approved By*	Date
Serology	Rosanilis Tejada	18 Jun 2008, 08:05

**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.*

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 #: 2008-029876**

Product: Not Indicated **Test Specimen:** Mouse **Received:** 12 Jun 2008

Serology Results Report

Department Review: Approved by Rosanilis Tejada, 18 Jun 2008, 08:05*

Sample #: / Code :	1 TOX 213M28	2 TOX 213M29	3 TOX 213M30	4 TOX 213M31	5 TOX 213M32	6 TOX 213M33	7 TOX 213M34	8 TOX 213M35	9 TOX 213M36	10 TOX 213M37
MFIA SEND	-	-	-	-	-	-	-	-	-	-
MFIA PVM	-	-	-	-	-	-	-	-	-	-
MFIA MHV	-	-	-	-	-	-	-	-	-	-
MFIA MVM	-	-	-	-	-	-	-	-	-	-
MFIA MPV-1	-	-	-	-	-	-	-	-	-	-
MFIA MPV-2	-	-	-	-	-	-	-	-	-	-
MFIA NS-1	-	-	-	-	-	-	-	-	-	-
MFIA MNV	-	-	-	-	-	-	-	-	-	-
MFIA GDVII	-	-	-	-	-	-	-	-	-	-
MFIA REO	-	-	-	-	-	-	-	-	-	-
MFIA EDIM	-	-	-	-	-	-	-	-	-	-
MFIA LCMV	-	-	-	-	-	-	-	-	-	-
MFIA ECTRO	-	-	-	-	-	-	-	-	-	-
MFIA MAV 1 & 2	-	-	-	-	-	-	-	-	-	-
MFIA MCMV	-	-	-	-	-	-	-	-	-	-
MFIA K	-	-	-	-	-	-	-	-	-	-
MFIA POLY	-	-	-	-	-	-	-	-	-	-
MFIA HANT	-	-	-	-	-	-	-	-	-	-
MFIA MPUL	-	-	-	-	-	-	-	-	-	-
MFIA ECUN	-	-	-	-	-	-	-	-	-	-
MFIA CARB	-	-	-	-	-	-	-	-	-	-
IFA MTLV	-	-	-	-	-	-	-	-	-	-
MFIA Anti-Ig	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**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.*

SEVENTH WAVE

where chemistry meets biology

June 22, 2008

Dr. Chandra Williams
R.J. Reynolds Tobacco Company
Toxicology Division
P. O. Box 1236
Winston Salem, NC 27102

REFERENCE: TOX-213 (Seventh Wave Study No. SW08-0175)

SUBJECT: Scheduled Health Screen, Histopathology, Final Necropsy

Dear Doctor Williams:

Formalin-preserved samples of infused lungs from ten male Sencar mice were processed at Seventh Wave, beginning on June 12, 2008. Microscopic examination was performed on each of the five lung lobes from every mouse. As shown in the attached STARPATH Overall Incidence Table and Single Tabulated Animal Report, histopathologic changes in the lungs included congestion, hemorrhage, peribronchiolar/perivascular lymphocytic infiltrations, nonpigmented macrophages, and chronic inflammation. The chronic inflammation was of minimal intensity and noted in one lobe of each of two mice (Nos. 32 and 36). The occurrences of these changes are regarded as random and nonspecific and do not indicate contagious disease.

The congestion and hemorrhage probably reflect the mode of anesthesia/euthanasia via carbon dioxide/exsanguination. The nonpigmented macrophages and lymphocytic infiltrations are anticipated background changes typically seen in mice of this age and strain.

CONCLUSION

Histopathologic examination revealed no evidence of intercurrent infectious disease in any of the mice examined.

John W. Sagartz, D.V.M.
Diplomate, A.C.V.P.

JWS: cjh

Seventh Wave Document No.: 313

cc: Jenny Smith
Paul Ayres
Jessica Baker
Sheri Bowman

QUALITY CONTOL STATEMENT

This study meets the Sponsor's requirements for quality control.

This study was performed without deviation from SOPs.

All SOPs used in this study were properly authorized.

The final report has been reviewed. The results accurately reflect the raw data of the study.

Any discrepancies are of an inconsequential nature or have been properly explained and documented.

The following phases of this study were inspected by Seventh Wave Laboratories Quality Control Unit. The dates of the inspections performed are as indicated below.

June 12. 2008	Part 1 of 9 - Project Sheet Review
June 16. 2008	Part 2 of 9 - Master Individual/Multiple Animal Worksheet
June 12. 2008	Part 3 of 9 - Histology Setup
June 16. 2008	Part 4 of 9 - Histology Completion
June 16. 2008	Part 5 of 9 - Slide/Block Match (100%)
June 16. 2008	Part 6 of 9 - Slide/Label Check (100%)
June 16. 2008	Part 7 of 9 - Wet Tissue Check (100%)
June 22. 2008	Part 8 of 9 - Rough Draft Report
June 22. 2008	Part 9 of 9 - Final Report

Vickie R. Hocker 6/22/08

Vickie R. Hocker (Date)

The Starpath Project Documentation File

Project Title: Scheduled Health Screen, Histopathology, Final Necropsy

Institution: R. J. Reynolds Tobacco Company

Project Number: TOX-213 (SW08-0175) Species: Swiss Webster Mice Deaths will be reported in days.

This report was printed: 06-22-2008 This file was edited 06-22-2008 Reports will be paginated.

The Dosage Group Names

1	Health Screen	11	21
2		12	22
3		13	23
4		14	24
5		15	25
6		16	26
7		17	27
8		18	28
9		19	29
10		20	30

The Currently Defined Sacrifice Definitions

1	HS F Health Screen	11	21
2		12	22
3		13	23
4		14	24
5		15	25
6		16	26
7		17	27
8		18	28
9		19	29
10		20	30

The Project Organ File (No.=Organ Number S=Sex where M=male, F=female & B=both sexes)

No.	S	Name	No.	S	Name	No.	S	Name
186	B	LUNG, LEFT LOBE, H&E						
187	B	LUNG, INTERMEDIATE LOBE, H&E						
188	B	LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E						
189	B	LUNG, RIGHT CARDIAC LOBE, H&E						
190	B	LUNG, RIGHT APICAL LOBE, H&E						

Single Tabulated Animal Report
Individual Macroscopic and Microscopic Observations
R. J. Reynolds Tobacco Company
Scheduled Health Screen, Histopathology, Final Necropsy

ANIMAL NUMBER: 28 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 06-22-2008.

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	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD -INFLAMMATION, CHRONIC, FOCAL, MINIMAL
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL -CONGESTION, DIFFUSE, MINIMAL
LUNG, RIGHT CARDIAC LOBE, H&E	-CONGESTION, DIFFUSE, MINIMAL -INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MINIMAL -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT APICAL LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL

ANIMAL NUMBER: 29 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 06-22-2008.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

LUNG, LEFT LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -CONGESTION, DIFFUSE, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
LUNG, INTERMEDIATE LOBE, H&E	-CONGESTION, DIFFUSE, MILD -INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD -HEMORRHAGE, MULTIFOCAL, MINIMAL

SPECIES: Swiss Webster Mice
PROJECT NUMBER: TOX-213 (SW08-0175)

ANIMAL NUMBER: 29 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 06-22-2008.

MICROSCOPIC OBSERVATIONS (continued):

LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -CONGESTION, DIFFUSE, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD -HEMORRHAGE, MULTIFOCAL, MINIMAL
LUNG, RIGHT CARDIAC LOBE, H&E	-CONGESTION, DIFFUSE, MILD -INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MINIMAL -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT APICAL LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -CONGESTION, DIFFUSE, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL

ANIMAL NUMBER: 30 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 06-22-2008.

MACROSCOPIC OBSERVATIONS:

No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

LUNG, LEFT LOBE, H&E	-CONGESTION, DIFFUSE, MILD -INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD -HEMORRHAGE, MULTIFOCAL, MINIMAL
LUNG, INTERMEDIATE LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT CARDIAC LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -CONGESTION, DIFFUSE, MINIMAL -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT APICAL LOBE, H&E	-CONGESTION, DIFFUSE, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL

ANIMAL NUMBER: 31 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 06-22-2008.

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	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	-HEMORRHAGE, MULTIFOCAL, MINIMAL -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, MINIMAL
LUNG, RIGHT APICAL LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL

ANIMAL NUMBER: 32 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 06-22-2008.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

LUNG, LEFT LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -HEMORRHAGE, MULTIFOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD

ANIMAL NUMBER: 32 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 06-22-2008.

MICROSCOPIC OBSERVATIONS (continued):

LUNG, INTERMEDIATE LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MINIMAL -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT CARDIAC LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MINIMAL -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
LUNG, RIGHT APICAL LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -CONGESTION, DIFFUSE, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL

ANIMAL NUMBER: 33 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 06-22-2008.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

LUNG, LEFT LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MINIMAL -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, INTERMEDIATE LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
LUNG, RIGHT CARDIAC LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT APICAL LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL

ANIMAL NUMBER: 34 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 06-22-2008.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

LUNG, LEFT LOBE, H&E	-CONGESTION, DIFFUSE, MILD -INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
LUNG, INTERMEDIATE LOBE, H&E	-CONGESTION, DIFFUSE, MINIMAL -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	-CONGESTION, DIFFUSE, MILD -INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
LUNG, RIGHT CARDIAC LOBE, H&E	-HEMORRHAGE, FOCAL, MINIMAL -INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT APICAL LOBE, H&E	-CONGESTION, DIFFUSE, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD

ANIMAL NUMBER: 35 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 06-22-2008.

MACROSCOPIC OBSERVATIONS:
No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

LUNG, LEFT LOBE, H&E	-CONGESTION, DIFFUSE, MILD -INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD
LUNG, INTERMEDIATE LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD -CONGESTION, DIFFUSE, MILD
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	-CONGESTION, DIFFUSE, MILD -HEMORRHAGE, MULTIFOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL

ANIMAL NUMBER: 35 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 06-22-2008.

MICROSCOPIC OBSERVATIONS (continued):

LUNG, RIGHT CARDIAC LOBE, H&E	-CONGESTION, DIFFUSE, MINIMAL -INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MINIMAL -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT APICAL LOBE, H&E	-CONGESTION, DIFFUSE, MILD -INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MILD

ANIMAL NUMBER: 36 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 06-22-2008.

MACROSCOPIC OBSERVATIONS:

No macroscopic entries are on file.

MICROSCOPIC OBSERVATIONS:

LUNG, LEFT LOBE, H&E	-CONGESTION, DIFFUSE, MILD -INFLAMMATION, CHRONIC, FOCAL, MINIMAL -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, INTERMEDIATE LOBE, H&E	-HEMORRHAGE, FOCAL, MILD -INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	-CONGESTION, DIFFUSE, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT CARDIAC LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT APICAL LOBE, H&E	-CONGESTION, DIFFUSE, MINIMAL -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL

ANIMAL NUMBER: 37 SEX: Male GROUP: (1) Health Screen
Fate: Health Screen Printed on 06-22-2008.

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	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL -HEMORRHAGE, MULTIFOCAL, MINIMAL
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	-INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT CARDIAC LOBE, H&E	-HEMORRHAGE, MULTIFOCAL, MILD -INFILTRATION, LYMPHOCYTIC, PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD -MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL
LUNG, RIGHT APICAL LOBE, H&E	-MACROPHAGES, NONPIGMENTED, MULTIFOCAL, MINIMAL

Overall Incidence for Males
R. J. Reynolds Tobacco Company
Scheduled Health Screen, Histopathology, Final Necropsy

PROJECT NUMBER: TOX-213 (SW08-0175) SPECIES: Swiss Webster Mice
Printed on 06-22-2008.

Tissue/ Diagnosis/ Modifier(s)	Health Screen
LUNG, LEFT LOBE, H&E	(10)
CONGESTION	5
DIFFUSE, MILD	5
HEMORRHAGE	2
MULTIFOCAL, MINIMAL	1
MULTIFOCAL, MILD	1
INFILTRATION, LYMPHOCYTIC	9
PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD	1
PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MINIMAL	1
PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD	7
INFLAMMATION, CHRONIC	1
FOCAL, MINIMAL	1
MACROPHAGES, NONPIGMENTED	10
MULTIFOCAL, MINIMAL	5
MULTIFOCAL, MILD	5
LUNG, INTERMEDIATE LOBE, H&E	(10)
CONGESTION	3
DIFFUSE, MINIMAL	1
DIFFUSE, MILD	2
HEMORRHAGE	3
FOCAL, MILD	1
MULTIFOCAL, MINIMAL	2
INFILTRATION, LYMPHOCYTIC	7
PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MINIMAL	1
PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD	3
PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD	3
INFLAMMATION, CHRONIC	1
FOCAL, MINIMAL	1
MACROPHAGES, NONPIGMENTED	10
MULTIFOCAL, MINIMAL	7
MULTIFOCAL, MILD	3
LUNG, RIGHT DIAPHRAGMATIC LOBE, H&E	(10)
CONGESTION	5
DIFFUSE, MINIMAL	1
DIFFUSE, MILD	4
HEMORRHAGE	3
MULTIFOCAL, MINIMAL	2
MULTIFOCAL, MILD	1
INFILTRATION, LYMPHOCYTIC	4
PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD	2
PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD	2
MACROPHAGES, NONPIGMENTED	10
MULTIFOCAL, MINIMAL	6
MULTIFOCAL, MILD	4

() = Number Of Animals Examined For This Tissue

All modifiers are printed.

Overall Incidence for Males (continued)
R. J. Reynolds Tobacco Company
Scheduled Health Screen, Histopathology, Final Necropsy

PROJECT NUMBER: TOX-213 (SW08-0175) SPECIES: Swiss Webster Mice
Printed on 06-22-2008.

Tissue/ Diagnosis/ Modifier(s)	Health Screen
LUNG, RIGHT CARDIAC LOBE, H&E	(10)
CONGESTION	4
DIFFUSE, MINIMAL	3
DIFFUSE, MILD	1
HEMORRHAGE	2
FOCAL, MINIMAL	1
MULTIFOCAL, MILD	1
INFILTRATION, LYMPHOCYTIC	8
PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MINIMAL	2
PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MINIMAL	2
PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD	4
MACROPHAGES, NONPIGMENTED	10
MULTIFOCAL, MINIMAL	9
MULTIFOCAL, MILD	1
LUNG, RIGHT APICAL LOBE, H&E	(10)
CONGESTION	6
DIFFUSE, MINIMAL	1
DIFFUSE, MILD	5
INFILTRATION, LYMPHOCYTIC	5
PERIBRONCHIOLAR, PERIVASCULAR, FOCAL, MILD	3
PERIBRONCHIOLAR, PERIVASCULAR, MULTIFOCAL, MILD	2
MACROPHAGES, NONPIGMENTED	10
MULTIFOCAL, MINIMAL	8
MULTIFOCAL, MILD	2

() = Number Of Animals Examined For This Tissue

All modifiers are printed.

Appendix V

Survival

Group Survival and Mortality Table - Males
Study number: TOX213A
Deaths: All
Dosing start date: 27-May-08

		Day of Phase													
mg/kg		1	2	3	4	5	6	7	8	9	10	11	12	13	14
0.0	a	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
40.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0
80.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0
160.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0
240.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0
400.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.0	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	b	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

mg/kg		Day of Phase 1	2	3	4	5	6	7	8	9	10	11	12	13	14
	a	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
	c	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5
40.0	b	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
	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5
80.0	b	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
	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5
160.0	b	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
	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5
240.0	b	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
	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5
400.0	b	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
	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5
0.0	b	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
	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5
0.0	b	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
	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5
0.0	b	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
	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5
0.0	b	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
	a	5	5	5	5	5	5	5	5	5	5	5	5	5	5
0.0	b	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

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

Group Survival and Mortality Table - Males
Study number: TOX213B
Deaths: All
Dosing start date: 26-May-08

		Day of Phase												
mg/kg		1	2	3	4	5	6	7	8	9	10	11	12	13
	a	5	5	5	5	5	5	5	5	5	5	5	5	5
40.0	b	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
	a	5	5	5	5	5	5	5	5	5	5	5	5	5
80.0	b	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
	a	5	5	5	5	5	5	5	5	5	5	5	5	5
160.0	b	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
	a	5	5	5	5	5	5	5	5	5	5	5	5	5
240.0	b	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
	a	5	5	5	5	5	5	5	5	5	5	5	5	5
400.0	b	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
	a	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
	c	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

Group Survival and Mortality Table - Males
Study number: TOX213B
Deaths: All
Dosing start date: 26-May-08

		Day of Phase												
mg/kg		1	2	3	4	5	6	7	8	9	10	11	12	13
	a	5	5	5	5	5	5	5	5	5	5	5	5	5
40.0	b	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
	a	5	5	5	5	5	5	5	5	5	5	5	5	5
80.0	b	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
	a	5	5	5	5	5	5	5	5	5	5	5	5	5
160.0	b	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
	a	5	5	5	5	5	5	5	5	5	5	5	5	5
240.0	b	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
	a	5	5	5	5	5	5	5	5	5	5	5	5	5
400.0	b	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
	a	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
	c	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 that died from start of interval
Note: Data for Exposure phase

Appendix VI

Clinical Observations

Interval: 4 - 15 Days — Males

Group	1		2		3		4		5		6	
Observation	(10)		(5)		(5)		(5)		(5)		(5)	
	a	b	a	b	a	b	a	b	a	b	a	b
Normal												
Normal/no visible abnormalities	10	100.0	5	100.0	5	100.0	5	100.0	5	100.0	4	80.0
Body surface												
Thin/emaciated	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	20.0
Limbs/tail												
Tail/portion of tail necrotic	1	10.0	0	0.0	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

Interval: 4 - 15 Days — Males

Group	7		8		9		10		11	
Observation	(5)		(5)		(5)		(5)		(5)	
	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	5	100.0
Body surface										
Thin/emaciated	0	0.0	0	0.0	0	0.0	0	0.0	1	20.0
Limbs/tail										
Tail/portion of tail necrotic	0	0.0	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

Interval: 4 - 15 Days — Males

Group	1		2		3		4		5		6	
Observation	(5)		(5)		(5)		(5)		(5)		(10)	
	a	b	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	5	100.0	0	0.0
Limbs/tail												
Tail/portion of tail necrotic	0	0.0	0	0.0	0	0.0	0	0.0	1	20.0	0	0.0

Key: () = Number of animals alive at start of interval
a = Number animals affected
b = Percent of animals with observation during interval

Appendix VII

Body Weights

Animal	Group		Day of Phase 6!	2"	3	4	5	6	7	8	9	10	11	12	13
							Male Animals								
1	1		26.43	26.54	27.32	27.57	28.26	28.60	28.94	28.93	29.64	29.69	28.92	29.21	28.85
2			25.61	25.85	26.54	26.95	27.05	27.76	27.89	28.05	28.99	28.92	28.60	29.28	29.51
3			24.95	25.46	25.74	26.05	26.17	26.94	26.77	26.87	27.55	27.83	27.79	27.89	28.15
4			26.32	27.27	27.62	28.37	29.28	30.22	30.07	30.36	31.09	31.08	31.40	31.58	31.97
5			27.42	27.35	27.88	28.62	29.36	30.41	30.90	30.89	31.78	32.31	32.16	33.08	33.15
6			25.37	25.28	25.90	26.20	26.30	27.58	27.40	27.30	27.56	27.27	27.00	26.91	26.66
7			24.80	24.75	25.35	25.75	26.38	27.21	27.26	26.99	27.94	27.96	28.12	28.60	28.68
8			26.01	25.90	26.27	25.22	25.92	25.81	25.97	26.45	27.20	27.66	27.07	27.44	27.63
9			23.44	23.36	23.01	23.57	23.63	24.32	24.05	24.33	25.06	25.37	25.02	25.21	25.58
10			27.99	27.72	27.28	27.95	28.20	27.98	27.93	28.20	28.70	29.38	29.58	30.29	30.31
		(n)	10	10	10	10	10	10	10	10	10	10	10	10	10
		Means	25.83	25.95	26.29	26.63	27.06	27.68	27.72	27.84	28.55	28.75	28.57	28.95	29.05
		Sdevs	1.32	1.33	1.43	1.58	1.76	1.84	1.97	1.92	1.96	1.99	2.11	2.30	2.31
11	2		26.21	25.31	25.44	25.66	25.92	26.45	26.45	26.47	26.51	26.62	25.94	26.26	26.71
12			26.67	25.83	26.27	26.22	26.83	26.35	26.46	26.53	27.87	27.51	27.64	27.91	28.47
13			23.87	23.57	23.69	24.02	24.12	24.72	24.87	24.76	25.36	25.32	25.33	25.72	25.36
14			30.41	30.34	30.34	31.33	31.24	30.62	31.34	31.11	32.48	31.93	32.01	32.39	32.80
15			25.29	25.20	25.76	25.93	26.31	27.06	26.81	26.87	27.89	28.15	28.32	28.45	29.08
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	26.49	26.05	26.30	26.63	26.88	27.04	27.19	27.15	28.02	27.91	27.85	28.15	28.48
		Sdevs	2.44	2.54	2.46	2.76	2.64	2.18	2.44	2.36	2.71	2.49	2.63	2.63	2.82
16	3		25.99	24.29	25.51	25.05	25.63	25.84	25.77	26.42	26.95	27.04	27.42	27.82	27.49
17			26.80	25.02	26.10	26.08	26.04	26.16	26.09	26.63	27.60	28.20	28.68	30.34	29.69
18			25.60	23.96	24.28	24.59	24.77	25.51	25.59	26.28	27.89	27.97	28.58	29.05	28.51
19			23.62	21.88	22.71	23.11	23.26	23.73	23.27	23.59	24.49	24.98	25.36	25.14	25.61
20			26.89	25.21	26.62	28.19	27.44	28.56	28.88	29.93	30.89	31.53	31.73	31.73	31.92
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	25.78	24.07	25.04	25.40	25.43	25.96	25.92	26.57	27.56	27.94	28.35	28.82	28.64
		Sdevs	1.32	1.33	1.57	1.89	1.55	1.73	2.00	2.25	2.29	2.37	2.31	2.52	2.37
21	4		26.34	23.20	23.73	23.08	22.52	22.60	21.83	21.85	22.61	22.18	22.56	22.88	23.65
22			27.18	23.99	24.57	24.52	24.69	24.30	24.45	24.65	25.90	26.26	26.00	26.30	25.35
23			25.01	21.68	22.31	21.67	20.73	20.12	19.46	19.58	19.89	19.78	19.93	19.45	18.51
24			25.69	22.36	22.48	22.32	21.34	21.03	20.59	20.53	21.12	20.98	21.08	22.03	21.97
25			24.15	20.66	20.79	20.48	19.36	18.65	18.67	18.45	19.66	19.55	19.35	18.99	19.11
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	25.67	22.38	22.78	22.41	21.73	21.34	21.00	21.01	21.84	21.75	21.78	21.93	21.72
		Sdevs	1.17	1.30	1.45	1.52	2.01	2.19	2.27	2.39	2.56	2.73	2.66	2.95	2.92

Note: ! = Quarantine/Acclimation; " = Exposure phase

Animal	Group		Day of Phase 6!	2"	3	4	5	6	7	8	9	10	11	12	13
							Male Animals								
26	5		25.60	22.93	22.87	21.82	21.12	20.46	19.87	24.72	25.27	26.33	27.41	27.66	28.10
27			26.25	22.66	21.72	21.00	20.29	19.73	18.64	23.69	24.54	25.72	26.44	27.28	27.28
28			29.68	26.69	26.56	25.67	24.76	24.25	23.60	28.38	28.68	29.94	31.91	33.31	34.62
29			24.63	21.54	21.93	21.59	20.64	20.08	20.32	24.06	24.42	25.78	27.01	27.29	27.83
30			25.71	21.56	21.93	21.27	20.18	19.59	19.10	22.96	23.76	25.79	27.40	28.37	28.02
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	26.37	23.08	23.00	22.27	21.40	20.82	20.31	24.76	25.33	26.71	28.03	28.78	29.17
		Sdevs	1.94	2.12	2.04	1.93	1.91	1.95	1.95	2.12	1.95	1.82	2.20	2.57	3.06
31	6		29.40	25.24	24.32	23.01	21.05	25.86	27.58	29.14	30.76	32.06	33.17	34.34	34.17
32			24.91	20.75	20.30	19.25	18.00	22.60	22.61	24.55	26.81	27.86	28.85	27.97	27.42
33			25.87	21.70	21.89	20.94	19.27	24.89	24.44	26.72	28.09	29.00	29.19	29.44	29.62
34			26.41	23.34	23.08	22.28	19.62	24.96	25.69	27.14	27.62	28.62	28.77	28.71	29.18
35			22.15	18.17	17.70	16.88	15.89	20.38	20.68	21.61	22.67	23.54	24.58	24.83	25.16
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	25.75	21.84	21.46	20.47	18.77	23.74	24.20	25.83	27.19	28.22	28.91	29.06	29.11
		Sdevs	2.62	2.67	2.57	2.47	1.94	2.23	2.67	2.87	2.93	3.06	3.04	3.44	3.33
36	7		23.16	22.24	23.64	23.56	23.67	23.81	23.56	29.09	24.65	24.46	24.26	24.71	25.02
37			27.21	25.48	27.14	27.89	27.97	28.37	28.77	29.44	30.00	29.50	29.96	30.43	30.53
38			26.71	25.92	27.52	27.74	28.01	28.90	28.60	29.04	29.22	29.66	29.88	29.93	29.56
39			25.49	24.63	25.51	25.31	25.20	25.97	26.03	26.42	26.92	26.45	26.92	26.63	26.68
40			25.70	25.52	26.00	26.48	26.76	27.18	27.15	27.42	27.80	27.81	27.52	27.87	28.22
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	25.65	24.76	25.96	26.20	26.32	26.85	26.82	28.28	27.72	27.58	27.71	27.91	28.00
		Sdevs	1.56	1.48	1.53	1.81	1.87	2.04	2.14	1.30	2.09	2.18	2.36	2.36	2.21
41	8		25.06	22.46	23.18	22.97	23.13	23.19	23.31	23.40	23.61	24.13	24.03	23.64	23.21
42			24.34	21.38	21.37	21.18	20.94	21.14	21.36	21.27	21.82	22.16	22.10	21.95	21.82
43			26.82	24.51	24.98	24.41	24.13	24.75	24.81	25.00	26.05	26.29	26.87	27.15	27.48
44			28.98	26.33	26.74	25.96	25.01	25.48	24.85	24.87	25.39	26.00	26.08	25.77	26.20
45			25.64	23.56	23.90	23.58	24.04	24.28	24.59	24.92	25.78	25.93	25.98	26.67	27.07
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	26.17	23.65	24.03	23.62	23.45	23.77	23.78	23.89	24.53	24.90	25.01	25.04	25.16
		Sdevs	1.82	1.90	2.00	1.77	1.55	1.69	1.49	1.61	1.79	1.75	1.93	2.19	2.50
46	9		25.08	23.43	24.46	24.23	24.53	25.21	25.31	26.01	26.70	26.57	27.22	27.68	28.38
47			26.64	24.83	24.78	24.90	25.62	26.39	26.33	27.33	28.76	28.64	28.69	28.84	28.87
48			24.41	22.32	22.78	22.63	22.74	23.58	23.64	24.52	25.26	25.77	26.08	25.74	25.95
49			26.02	24.18	24.73	23.97	23.36	23.31	23.42	23.95	25.51	25.98	26.07	26.90	26.43

Note: ! = Quarantine/Acclimation; " = Exposure phase

Animal	Group		Day of Phase 6!	2"	3	4	5	6	7	8	9	10	11	12	13
							Male Animals								
50	9		27.35	24.96	25.81	25.06	25.53	25.77	25.09	26.12	27.14	27.63	27.82	27.80	27.82
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	25.90	23.94	24.51	24.16	24.36	24.85	24.76	25.59	26.67	26.92	27.18	27.39	27.49
		Sdevs	1.18	1.09	1.10	0.97	1.29	1.35	1.22	1.35	1.41	1.20	1.13	1.15	1.26
51	10		25.98	22.60	21.68	20.25	19.45	18.87	17.81	18.69	23.07	23.85	26.21	27.75	29.41
52			25.01	21.43	21.73	20.85	19.93	19.68	19.54	21.24	23.88	24.84	26.65	27.74	28.66
53			26.65	23.24	22.44	21.36	20.13	19.78	18.57	20.12	24.74	25.84	27.18	28.39	29.52
54			27.43	24.53	24.19	23.41	22.88	22.62	22.07	23.53	26.57	27.48	28.96	29.79	30.13
55			22.78	19.58	19.16	18.23	16.81	16.58	15.64	16.69	20.72	20.21	23.03	23.54	24.71
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	25.57	22.28	21.84	20.82	19.84	19.51	18.73	20.05	23.80	24.44	26.41	27.44	28.49
		Sdevs	1.80	1.88	1.81	1.87	2.16	2.17	2.36	2.58	2.16	2.72	2.16	2.34	2.17
56	11		27.51	23.77	22.50	21.14	25.17	25.74	27.02	28.29	29.49	29.65	30.54	30.68	31.32
57			26.36	22.23	20.36	18.89	23.67	24.23	26.24	28.03	29.34	29.48	29.21	29.48	29.71
58			25.00	20.04	18.18	16.91	20.75	21.95	23.41	24.79	26.39	27.05	27.26	28.04	27.91
59			25.65	21.50	20.88	20.03	23.42	24.53	25.33	27.01	27.86	27.90	27.88	28.08	29.21
60			24.63	20.51	19.85	18.62	21.30	22.33	24.21	25.98	26.71	27.65	28.33	28.53	28.70
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	25.83	21.61	20.35	19.12	22.86	23.76	25.24	26.82	27.96	28.35	28.64	28.96	29.37
		Sdevs	1.15	1.48	1.57	1.59	1.82	1.59	1.47	1.46	1.44	1.16	1.28	1.12	1.28

Note: ! = Quarantine/Acclimation; " = Exposure phase

Animal	Group		Day of Phase 14
		Male	Animals
1	1		29.22
2			29.67
3			28.08
4			32.73
5			34.10
6			27.17
7			29.16
8			28.65
9			25.84
10			30.63
		(n)	10
		Means	29.53
		Sdevs	2.46
11	2		26.70
12			28.15
13			25.65
14			33.35
15			29.48
		(n)	5
		Means	28.67
		Sdevs	2.99
16	3		28.18
17			31.26
18			29.87
19			25.77
20			32.68
		(n)	5
		Means	29.55
		Sdevs	2.69
21	4		23.19
22			26.08
23			18.39
24			21.65
25			19.26
		(n)	5
		Means	21.71
		Sdevs	3.09

Note: Data for Exposure phase

Animal	Group		Day of Phase 14
			Male Animals
26	5		28.47
27			27.43
28			34.74
29			28.01
30			28.89
		(n)	5
		Means	29.51
		Sdevs	2.97
31	6		34.19
32			27.87
33			29.42
34			29.06
35			26.24
		(n)	5
		Means	29.36
		Sdevs	2.97
36	7		25.54
37			30.77
38			30.48
39			26.85
40			29.09
		(n)	5
		Means	28.55
		Sdevs	2.29
41	8		24.11
42			21.82
43			27.93
44			26.17
45			27.09
		(n)	5
		Means	25.42
		Sdevs	2.47
46	9		28.64
47			29.63
48			26.70
49			26.44

Note: Data for Exposure phase

Animal	Group		Day of Phase 14
			Male Animals
50	9		28.08
		(n)	5
		Means	27.90
		Sdevs	1.34
51	10		29.94
52			29.20
53			29.14
54			30.41
55			25.13
		(n)	5
		Means	28.76
		Sdevs	2.10
56	11		31.62
57			29.78
58			28.48
59			28.93
60			29.26
		(n)	5
		Means	29.61
		Sdevs	1.22

Note: Data for Exposure phase

Animal	Group		Day of Phase 6!	2"	3	4	5	6	7	8	9	10	11	12	13
							Male Animals								
61	1		26.45	26.81	27.52	28.22	28.16	29.08	28.91	29.11	29.18	29.85	30.17	29.93	30.40
62			26.05	27.88	28.22	29.13	29.96	30.64	30.83	30.97	30.63	31.16	31.84	32.58	33.36
63			25.94	25.63	26.37	26.78	27.00	27.47	27.44	28.01	28.11	28.26	27.96	28.66	29.12
64			26.70	26.57	26.32	26.77	26.89	27.28	27.04	27.65	27.91	28.36	28.15	28.60	28.78
65			28.36	28.69	29.44	29.83	30.40	31.40	31.17	31.42	31.66	32.12	32.07	32.34	32.67
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	26.70	27.12	27.57	28.15	28.48	29.17	29.08	29.43	29.50	29.95	30.04	30.42	30.87
		Sdevs	0.98	1.19	1.32	1.38	1.64	1.84	1.89	1.70	1.62	1.70	1.95	1.94	2.07
66	2		25.17	23.86	23.83	24.38	24.46	25.12	25.06	25.27	25.56	25.88	26.08	26.32	26.56
67			28.24	26.01	25.76	25.45	25.22	26.06	25.78	26.67	26.25	27.06	27.18	28.13	28.18
68			26.48	27.13	27.80	27.63	27.60	28.01	27.88	28.16	28.20	28.48	28.26	27.83	27.88
69			32.51	31.28	32.07	31.67	31.98	33.08	33.97	34.83	35.09	35.88	35.74	35.81	36.95
70			27.48	26.85	26.75	26.92	26.49	27.57	27.18	27.94	26.47	27.85	27.39	27.38	28.22
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	27.98	27.03	27.24	27.21	27.15	27.97	27.97	28.57	28.31	29.03	28.93	29.09	29.56
		Sdevs	2.78	2.70	3.07	2.80	2.95	3.08	3.53	3.68	3.91	3.95	3.89	3.82	4.19
71	3		26.18	23.90	23.54	23.31	22.82	23.25	22.97	23.05	23.17	23.25	23.61	23.96	25.04
72			27.31	25.51	24.82	23.99	23.64	23.45	22.36	22.10	22.53	22.24	22.38	22.65	23.30
73			26.14	24.22	22.84	22.22	21.24	21.32	21.14	21.02	20.68	20.67	20.98	21.84	23.23
74			27.23	24.87	23.79	23.01	22.43	21.90	22.16	21.59	21.82	21.46	21.25	21.36	22.11
75			25.69	23.33	22.64	22.18	21.65	21.71	21.44	21.32	21.57	21.04	21.25	21.36	21.64
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	26.51	24.37	23.53	22.94	22.36	22.33	22.01	21.82	21.95	21.73	21.89	22.23	23.06
		Sdevs	0.72	0.85	0.87	0.76	0.95	0.96	0.73	0.80	0.95	1.03	1.10	1.10	1.32
76	4		22.62	20.57	19.46	18.70	18.16	18.37	18.22	18.78	21.95	22.86	23.73	24.52	25.72
77			31.25	28.85	27.87	26.65	25.09	24.89	23.98	24.00	28.02	28.57	30.56	31.85	34.07
78			27.54	24.32	23.48	22.38	21.21	20.61	19.56	19.82	24.68	25.27	26.46	27.09	25.11
79			24.01	21.26	20.12	19.52	18.78	18.21	17.91	18.54	26.44	27.54	23.07	24.91	25.23
80			29.05	26.42	25.19	24.22	23.17	23.19	22.26	23.12	21.74	22.76	28.42	30.25	30.79
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	26.89	24.28	23.22	22.29	21.28	21.05	20.39	20.85	24.57	25.40	26.45	27.72	28.18
		Sdevs	3.56	3.48	3.51	3.29	2.92	2.95	2.64	2.54	2.75	2.65	3.15	3.24	4.05
81	5		26.57	23.42	21.80	20.82	19.66	25.18	24.29	26.79	28.59	28.74	29.38	29.60	30.52
82			25.93	22.28	20.97	19.98	18.86	24.16	23.44	24.93	26.26	27.28	27.87	28.34	29.25
83			27.86	24.49	22.64	21.35	19.98	25.14	25.58	27.58	29.32	30.52	31.46	32.00	33.02
84			30.47	25.78	23.45	22.71	21.29	26.19	26.86	29.12	30.37	31.17	31.62	31.76	31.40

Note: ! = Quarantine/Acclimation; " = Exposure phase

Animal	Group		Day of Phase 6!	2"	3	4	5	6	7	8	9	10	11	12	13
			Male Animals												
85	5		26.39	22.84	21.64	20.67	19.78	24.50	24.52	26.05	27.66	28.15	28.63	28.88	29.20
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	27.44	23.76	22.10	21.11	19.91	25.03	24.94	26.89	28.44	29.17	29.79	30.12	30.68
		Sdevs	1.84	1.39	0.96	1.02	0.88	0.78	1.32	1.58	1.57	1.63	1.68	1.67	1.60

Note: ! = Quarantine/Acclimation; " = Exposure phase

Animal	Group		Day of Phase 14
			Male Animals
61	1		31.31
62			33.20
63			29.47
64			28.95
65			32.63
		(n)	5
		Means	31.11
		Sdevs	1.88
66	2		26.95
67			28.87
68			27.98
69			36.67
70			28.49
		(n)	5
		Means	29.79
		Sdevs	3.91
71	3		25.39
72			23.21
73			22.81
74			22.71
75			22.16
		(n)	5
		Means	23.26
		Sdevs	1.25
76	4		25.93
77			33.56
78			28.19
79			25.48
80			31.93
		(n)	5
		Means	29.02
		Sdevs	3.60
81	5		29.97
82			29.34
83			33.12
84			32.86

Note: Data for Exposure phase

Animal	Group		Day of Phase 14
		Male Animals	
85	5		29.39
		(n)	5
		Means	30.94
		Sdevs	1.89

Note: Data for Exposure phase

Mean Animal Body Weights in (g)
Study number: TOX213A
Dosing start date: 27-May-08

FEEDING STUDY/PALATABILITY

Group(s)		Day of Phase 6!	2"	3	4	5	6	7	8	9	10	11	12	13
		Male Animals												
1	(N)	10	10	10	10	10	10	10	10	10	10	10	10	10
	Means	25.83	25.95	26.29	26.63	27.06	27.68	27.72	27.84	28.55	28.75	28.57	28.95	29.05
	Sdevs	1.32	1.33	1.43	1.58	1.76	1.84	1.97	1.92	1.96	1.99	2.11	2.30	2.31
2	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	26.49	26.05	26.30	26.63	26.88	27.04	27.19	27.15	28.02	27.91	27.85	28.15	28.48
	Sdevs	2.44	2.54	2.46	2.76	2.64	2.18	2.44	2.36	2.71	2.49	2.63	2.63	2.82
3	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	25.78	24.07	25.04	25.40	25.43	25.96	25.92	26.57	27.56	27.94	28.35	28.82	28.64
	Sdevs	1.32	1.33	1.57	1.89	1.55	1.73	2.00	2.25	2.29	2.37	2.31	2.52	2.37
4	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	25.67	22.38+	22.78+	22.41+	21.73+	21.34+	21.00+	21.01+	21.84+	21.75+	21.78+	21.93+	21.72+
	Sdevs	1.17	1.30	1.45	1.52	2.01	2.19	2.27	2.39	2.56	2.73	2.66	2.95	2.92
5	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	26.37	23.08*	23.00*	22.27+	21.40+	20.82+	20.31+	24.76	25.33	26.71	28.03	28.78	29.17
	Sdevs	1.94	2.12	2.04	1.93	1.91	1.95	1.95	2.12	1.95	1.82	2.20	2.57	3.06
6	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	25.75	21.84+	21.46+	20.47+	18.77+	23.74+	24.20*	25.83	27.19	28.22	28.91	29.06	29.11
	Sdevs	2.62	2.67	2.57	2.47	1.94	2.23	2.67	2.87	2.93	3.06	3.04	3.44	3.33
7	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	25.65	24.76	25.96	26.20	26.32	26.85	26.82	28.28	27.72	27.58	27.71	27.91	28.00
	Sdevs	1.56	1.48	1.53	1.81	1.87	2.04	2.14	1.30	2.09	2.18	2.36	2.36	2.21
8	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	26.17	23.65	24.03	23.62*	23.45+	23.77+	23.78+	23.89+	24.53*	24.90*	25.01*	25.04*	25.16*
	Sdevs	1.82	1.90	2.00	1.77	1.55	1.69	1.49	1.61	1.79	1.75	1.93	2.19	2.50
9	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	25.90	23.94	24.51	24.16	24.36	24.85	24.76	25.59	26.67	26.92	27.18	27.39	27.49
	Sdevs	1.18	1.09	1.10	0.97	1.29	1.35	1.22	1.35	1.41	1.20	1.13	1.15	1.26
10	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	25.57	22.28+	21.84+	20.82+	19.84+	19.51+	18.73+	20.05+	23.80+	24.44+	26.41	27.44	28.49
	Sdevs	1.80	1.88	1.81	1.87	2.16	2.17	2.36	2.58	2.16	2.72	2.16	2.34	2.17
11	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	25.83	21.61+	20.35+	19.12+	22.86+	23.76+	25.24	26.82	27.96	28.35	28.64	28.96	29.37
	Sdevs	1.15	1.48	1.57	1.59	1.82	1.59	1.47	1.46	1.44	1.16	1.28	1.12	1.28

Note: ! = Quarantine/Acclimation; " = Exposure phase
*(+) = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance

%($) = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

Group(s)		Day of Phase 14
		Male Animals
1	(N)	10
	Means	29.53
	Sdevs	2.46
2	(N)	5
	Means	28.67
	Sdevs	2.99
3	(N)	5
	Means	29.55
	Sdevs	2.69
4	(N)	5
	Means	21.71+
	Sdevs	3.09
5	(N)	5
	Means	29.51
	Sdevs	2.97
6	(N)	5
	Means	29.36
	Sdevs	2.97
7	(N)	5
	Means	28.55
	Sdevs	2.29
8	(N)	5
	Means	25.42*
	Sdevs	2.47
9	(N)	5
	Means	27.90
	Sdevs	1.34
10	(N)	5
	Means	28.76
	Sdevs	2.10
11	(N)	5
	Means	29.61
	Sdevs	1.22

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

Mean Animal Body Weights in (g)
Study number: TOX213B
Dosing start date: 26-May-08

Male Animals

Group(s)		Day of Phase 6!	2"	3	4	5	6	7	8	9	10	11	12	13
1	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	26.70	27.12	27.57	28.15	28.48	29.17	29.08	29.43	29.50	29.95	30.04	30.42	30.87
	Sdevs	0.98	1.19	1.32	1.38	1.64	1.84	1.89	1.70	1.62	1.70	1.95	1.94	2.07
2	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	27.98	27.03	27.24	27.21	27.15	27.97	27.97	28.57	28.31	29.03	28.93	29.09	29.56
	Sdevs	2.78	2.70	3.07	2.80	2.95	3.08	3.53	3.68	3.91	3.95	3.89	3.82	4.19
3	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	26.51	24.37	23.53$	22.94+	22.36+	22.33+	22.01+	21.82+	21.95+	21.73+	21.89+	22.23+	23.06+
	Sdevs	0.72	0.85	0.87	0.76	0.95	0.96	0.73	0.80	0.95	1.03	1.10	1.10	1.32
4	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	26.89	24.28	23.22	22.29+	21.28+	21.05+	20.39+	20.85+	24.57*	25.40*	26.45	27.72	28.18
	Sdevs	3.56	3.48	3.51	3.29	2.92	2.95	2.64	2.54	2.75	2.65	3.15	3.24	4.05
5	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	27.44	23.76	22.10$	21.11+	19.91+	25.03*	24.94*	26.89	28.44	29.17	29.79	30.12	30.68
	Sdevs	1.84	1.39	0.96	1.02	0.88	0.78	1.32	1.58	1.57	1.63	1.68	1.67	1.60
6	(N)													
	Means													
	Sdevs													

Note: ! = Quarantine/Acclimation; " = 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

Mean Animal Body Weights in (g)
Study number: TOX213B
Dosing start date: 26-May-08

Group(s)		Day of Phase 14
		Male Animals
1	(N)	5
	Means	31.11
	Sdevs	1.88
2	(N)	5
	Means	29.79
	Sdevs	3.91
3	(N)	5
	Means	23.26+
	Sdevs	1.25
4	(N)	5
	Means	29.02
	Sdevs	3.60
5	(N)	5
	Means	30.94
	Sdevs	1.89
6	(N)	
	Means	
	Sdevs	

Note: Data for Exposure phase
*(+) = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance
%($) = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

Mean Animal Body Weights in (g)
Study number: TOX213A
Dosing start date: 27-May-08

FEEDING STUDY/PALATABILITY

Group(s)		Day of Phase 6!	2"	3	4	5	6	7	8	9	10	11	12	13
		Male Animals												
1	(N)	10	10	10	10	10	10	10	10	10	10	10	10	10
	Means	25.83	25.95	26.29	26.63	27.06	27.68	27.72	27.84	28.55	28.75	28.57	28.95	29.05
	Sdevs	1.32	1.33	1.43	1.58	1.76	1.84	1.97	1.92	1.96	1.99	2.11	2.30	2.31
2	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	26.49	26.05	26.30	26.63	26.88	27.04	27.19	27.15	28.02	27.91	27.85	28.15	28.48
	Sdevs	2.44	2.54	2.46	2.76	2.64	2.18	2.44	2.36	2.71	2.49	2.63	2.63	2.82
3	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	25.78	24.07	25.04	25.40	25.43	25.96	25.92	26.57	27.56	27.94	28.35	28.82	28.64
	Sdevs	1.32	1.33	1.57	1.89	1.55	1.73	2.00	2.25	2.29	2.37	2.31	2.52	2.37
4	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	25.67	22.38+	22.78+	22.41+	21.73+	21.34+	21.00+	21.01+	21.84+	21.75+	21.78+	21.93+	21.72+
	Sdevs	1.17	1.30	1.45	1.52	2.01	2.19	2.27	2.39	2.56	2.73	2.66	2.95	2.92
5	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	26.37	23.08*	23.00*	22.27+	21.40+	20.82+	20.31+	24.76	25.33	26.71	28.03	28.78	29.17
	Sdevs	1.94	2.12	2.04	1.93	1.91	1.95	1.95	2.12	1.95	1.82	2.20	2.57	3.06
6	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	25.75	21.84+	21.46+	20.47+	18.77+	23.74+	24.20*	25.83	27.19	28.22	28.91	29.06	29.11
	Sdevs	2.62	2.67	2.57	2.47	1.94	2.23	2.67	2.87	2.93	3.06	3.04	3.44	3.33
7	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	25.65	24.76	25.96	26.20	26.32	26.85	26.82	28.28	27.72	27.58	27.71	27.91	28.00
	Sdevs	1.56	1.48	1.53	1.81	1.87	2.04	2.14	1.30	2.09	2.18	2.36	2.36	2.21
8	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	26.17	23.65	24.03	23.62*	23.45+	23.77+	23.78+	23.89+	24.53*	24.90*	25.01*	25.04*	25.16*
	Sdevs	1.82	1.90	2.00	1.77	1.55	1.69	1.49	1.61	1.79	1.75	1.93	2.19	2.50
9	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	25.90	23.94	24.51	24.16	24.36	24.85	24.76	25.59	26.67	26.92	27.18	27.39	27.49
	Sdevs	1.18	1.09	1.10	0.97	1.29	1.35	1.22	1.35	1.41	1.20	1.13	1.15	1.26
10	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	25.57	22.28+	21.84+	20.82+	19.84+	19.51+	18.73+	20.05+	23.80+	24.44+	26.41	27.44	28.49
	Sdevs	1.80	1.88	1.81	1.87	2.16	2.17	2.36	2.58	2.16	2.72	2.16	2.34	2.17
11	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	25.83	21.61+	20.35+	19.12+	22.86+	23.76+	25.24	26.82	27.96	28.35	28.64	28.96	29.37
	Sdevs	1.15	1.48	1.57	1.59	1.82	1.59	1.47	1.46	1.44	1.16	1.28	1.12	1.28

Note: ! = Quarantine/Acclimation; " = Exposure phase
*(+) = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance

%($) = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

Group(s)		Day of Phase 14
		Male Animals
1	(N)	10
	Means	29.53
	Sdevs	2.46
2	(N)	5
	Means	28.67
	Sdevs	2.99
3	(N)	5
	Means	29.55
	Sdevs	2.69
4	(N)	5
	Means	21.71+
	Sdevs	3.09
5	(N)	5
	Means	29.51
	Sdevs	2.97
6	(N)	5
	Means	29.36
	Sdevs	2.97
7	(N)	5
	Means	28.55
	Sdevs	2.29
8	(N)	5
	Means	25.42*
	Sdevs	2.47
9	(N)	5
	Means	27.90
	Sdevs	1.34
10	(N)	5
	Means	28.76
	Sdevs	2.10
11	(N)	5
	Means	29.61
	Sdevs	1.22

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

Mean Animal Body Weights in (g)
Study number: TOX213B
Dosing start date: 26-May-08

Male Animals

Group(s)		Day of Phase 6!	2"	3	4	5	6	7	8	9	10	11	12	13
1	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	26.70	27.12	27.57	28.15	28.48	29.17	29.08	29.43	29.50	29.95	30.04	30.42	30.87
	Sdevs	0.98	1.19	1.32	1.38	1.64	1.84	1.89	1.70	1.62	1.70	1.95	1.94	2.07
2	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	27.98	27.03	27.24	27.21	27.15	27.97	27.97	28.57	28.31	29.03	28.93	29.09	29.56
	Sdevs	2.78	2.70	3.07	2.80	2.95	3.08	3.53	3.68	3.91	3.95	3.89	3.82	4.19
3	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	26.51	24.37	23.53$	22.94+	22.36+	22.33+	22.01+	21.82+	21.95+	21.73+	21.89+	22.23+	23.06+
	Sdevs	0.72	0.85	0.87	0.76	0.95	0.96	0.73	0.80	0.95	1.03	1.10	1.10	1.32
4	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	26.89	24.28	23.22	22.29+	21.28+	21.05+	20.39+	20.85+	24.57*	25.40*	26.45	27.72	28.18
	Sdevs	3.56	3.48	3.51	3.29	2.92	2.95	2.64	2.54	2.75	2.65	3.15	3.24	4.05
5	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	27.44	23.76	22.10$	21.11+	19.91+	25.03*	24.94*	26.89	28.44	29.17	29.79	30.12	30.68
	Sdevs	1.84	1.39	0.96	1.02	0.88	0.78	1.32	1.58	1.57	1.63	1.68	1.67	1.60
6	(N)													
	Means													
	Sdevs													

Note: ! = Quarantine/Acclimation; " = 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

Mean Animal Body Weights in (g)
Study number: TOX213B
Dosing start date: 26-May-08

Group(s)		Day of Phase 14
		Male Animals
1	(N)	5
	Means	31.11
	Sdevs	1.88
2	(N)	5
	Means	29.79
	Sdevs	3.91
3	(N)	5
	Means	23.26+
	Sdevs	1.25
4	(N)	5
	Means	29.02
	Sdevs	3.60
5	(N)	5
	Means	30.94
	Sdevs	1.89
6	(N)	
	Means	
	Sdevs	

Note: Data for Exposure phase
*(+) = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance
%($) = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213A
Exposure phase (Day 2) (Reference Day -1)
Dosing start date: 27-May-08

M a l e A n i m a l s
Data nonhomogeneous by Bartlett's test Modified T test of significance

Group	Control	2	3	4	5	6	7	8	9	10	11
Number/group	10	5	5	5	5	5	5	5	5	5	5
Mean	0.11	-0.44	-1.71	-3.30	-3.30	-3.91	-0.90	-2.52	-1.96	-3.29	-4.22
Standard deviation	0.37	0.40	0.05	0.14	0.58	0.48	0.55	0.34	0.29	0.26	0.45
Group diff.@ P=.05		0.56	0.27*	0.31*	0.76*	0.64*	0.73*	0.49*	0.44*	0.41*	0.61*
Group diff.@ P=.01		0.91	0.39*	0.47*	1.25*	1.04*	1.20	0.78*	0.70*	0.64*	0.99*

Analysis of variance: F ratio = 88.99 Df = 10/ 49 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213A
Exposure phase (Day 3) (Reference Day -1)
Dosing start date: 27-May-08

M a l e A n i m a l s
Data homogeneous by Bartlett's test Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11
Number/group	10	5	5	5	5	5	5	5	5	5	5
Mean	0.46	-0.19	-0.74	-2.90	-3.37	-4.29	0.31	-2.13	-1.39	-3.73	-5.48
Standard deviation	0.62	0.46	0.40	0.36	0.78	0.67	0.36	0.50	0.48	0.50	0.91
Group diff.@ P=.05		0.89	0.89*	0.89*	0.89*	0.89*	0.89	0.89*	0.89*	0.89*	0.89*
Group diff.@ P=.01		1.09	1.09*	1.09*	1.09*	1.09*	1.09	1.09*	1.09*	1.09*	1.09*

Analysis of variance: F ratio = 68.28 Df = 10/ 49 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213A
Exposure phase (Day 4) (Reference Day -1)
Dosing start date: 27-May-08

M a l e A n i m a l s

Data homogeneous by Bartlett's test Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11
Number/group	10	5	5	5	5	5	5	5	5	5	5
Mean	0.79	0.14	-0.38	-3.26	-4.10	-5.28	0.54	-2.55	-1.74	-4.75	-6.71
Standard deviation	0.81	0.65	0.96	0.37	0.82	0.84	0.46	0.52	0.55	0.74	1.03
Group diff.@ P=.05		1.14	1.14*	1.14*	1.14*	1.14*	1.14	1.14*	1.14*	1.14*	1.14*
Group diff.@ P=.01		1.39	1.39	1.39*	1.39*	1.39*	1.39	1.39*	1.39*	1.39*	1.39*

Analysis of variance: F ratio = 68.94 Df = 10/ 49 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213A
Exposure phase (Day 5) (Reference Day -1)
Dosing start date: 27-May-08

M a l e A n i m a l s
Data homogeneous by Bartlett's test Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11
Number/group	10	5	5	5	5	5	5	5	5	5	5
Mean	1.22	0.39	-0.35	-3.95	-4.98	-6.98	0.67	-2.72	-1.54	-5.73	-2.97
Standard deviation	0.94	0.53	0.55	0.88	0.79	0.80	0.61	0.99	0.81	0.89	0.84
Group diff.@ P=.05		1.26	1.26*	1.26*	1.26*	1.26*	1.26	1.26*	1.26*	1.26*	1.26*
Group diff.@ P=.01		1.53	1.53*	1.53*	1.53*	1.53*	1.53	1.53*	1.53*	1.53*	1.53*

Analysis of variance: F ratio = 67.28 Df = 10/ 49 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213A
Exposure phase (Day 6) (Reference Day -1)
Dosing start date: 27-May-08

M a l e A n i m a l s
Data homogeneous by Bartlett's test Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11
Number/group	10	5	5	5	5	5	5	5	5	5	5
Mean	1.85	0.55	0.18	-4.33	-5.55	-2.01	1.19	-2.40	-1.05	-6.06	-2.07
Standard deviation	1.28	0.80	0.88	1.03	0.78	0.98	0.69	0.91	1.13	0.98	0.71
Group diff.@ P=.05		1.52	1.52*	1.52*	1.52*	1.52*	1.52	1.52*	1.52*	1.52*	1.52*
Group diff.@ P=.01		1.84	1.84	1.84*	1.84*	1.84*	1.84	1.84*	1.84*	1.84*	1.84*

Analysis of variance: F ratio = 43.44 Df = 10/ 49 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213A
Exposure phase (Day 7) (Reference Day -1)
Dosing start date: 27-May-08

Male Animals
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
Number/group	10	5	5	5	5	5	5	5	5	5	5
Mean	1.88	0.70	0.14	-4.67	-6.07	-1.55	1.17	-2.38	-1.14	-6.84	-0.59
Standard deviation	1.33	0.68	1.07	1.16	1.21	0.58	0.66	1.20	1.23	1.37	0.58
Group diff.@ P=.05		1.67	1.67*	1.67*	1.67*	1.67*	1.67	1.67*	1.67*	1.67*	1.67*
Group diff.@ P=.01		2.03	2.03	2.03*	2.03*	2.03*	2.03	2.03*	2.03*	2.03*	2.03*

Analysis of variance: F ratio = 42.15 Df = 10/ 49 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213A
Exposure phase (Day 8) (Reference Day -1)
Dosing start date: 27-May-08

M a l e A n i m a l s
Data homogeneous by Bartlett's test Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11
Number/group	10	5	5	5	5	5	5	5	5	5	5
Mean	2.00	0.66	0.79	-4.66	-1.61	0.08	2.63	-2.28	-0.31	-5.52	0.99
Standard deviation	1.23	0.65	1.30	1.27	0.99	0.65	1.93	1.32	1.29	1.59	0.74
Group diff.@ P=.05		1.91	1.91	1.91*	1.91*	1.91*	1.91	1.91*	1.91*	1.91*	1.91
Group diff.@ P=.01		2.33	2.33	2.33*	2.33*	2.33	2.33	2.33*	2.33	2.33*	2.33

Analysis of variance: F ratio = 24.35 Df = 10/ 49 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213A
Exposure phase (Day 9) (Reference Day -1)
Dosing start date: 27-May-08

M a l e A n i m a l s
Data homogeneous by Bartlett's test Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11
Number/group	10	5	5	5	5	5	5	5	5	5	5
Mean	2.72	1.53	1.78	-3.84	-1.04	1.44	2.06	-1.64	0.77	-1.77	2.13
Standard deviation	1.32	0.87	1.38	1.51	0.79	0.66	0.60	1.46	1.13	0.81	0.57
Group diff.@ P=.05		1.69	1.69	1.69*	1.69*	1.69	1.69	1.69*	1.69*	1.69*	1.69
Group diff.@ P=.01		2.06	2.06	2.06*	2.06*	2.06	2.06	2.06*	2.06	2.06*	2.06

Analysis of variance: F ratio = 20.62 Df = 10/ 49 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213A
Exposure phase (Day 10) (Reference Day -1)
Dosing start date: 27-May-08

M a l e A n i m a l s
Data homogeneous by Bartlett's test Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11
Number/group	10	5	5	5	5	5	5	5	5	5	5
Mean	2.91	1.42	2.16	-3.92	0.34	2.47	1.92	-1.27	1.02	-1.13	2.52
Standard deviation	1.23	0.93	1.47	1.72	0.64	0.70	0.80	1.31	0.86	1.17	0.51
Group diff.@ P=.05		1.71	1.71	1.71*	1.71*	1.71	1.71	1.71*	1.71*	1.71*	1.71
Group diff.@ P=.01		2.08	2.08	2.08*	2.08*	2.08	2.08	2.08*	2.08	2.08*	2.08

Analysis of variance: F ratio = 19.79 Df = 10/ 49 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213A
Exposure phase (Day 11) (Reference Day -1)
Dosing start date: 27-May-08

M a l e A n i m a l s
Data homogeneous by Bartlett's test Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11
Number/group	10	5	5	5	5	5	5	5	5	5	5
Mean	2.73	1.36	2.57	-3.89	1.66	3.16	2.05	-1.16	1.28	0.84	2.81
Standard deviation	1.36	1.19	1.40	1.59	0.87	0.74	0.88	1.41	0.96	0.70	0.61
Group diff.@ P=.05		1.76	1.76	1.76*	1.76	1.76	1.76	1.76*	1.76	1.76*	1.76
Group diff.@ P=.01		2.14	2.14	2.14*	2.14	2.14	2.14	2.14*	2.14	2.14	2.14

Analysis of variance: F ratio = 17.49 Df = 10/ 49 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213A
Exposure phase (Day 12) (Reference Day -1)
Dosing start date: 27-May-08

M a l e A n i m a l s
Data homogeneous by Bartlett's test Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11
Number/group	10	5	5	5	5	5	5	5	5	5	5
Mean	3.12	1.66	3.04	-3.74	2.41	3.31	2.26	-1.13	1.49	1.87	3.13
Standard deviation	1.48	1.14	1.36	1.84	0.95	1.03	0.95	1.79	0.90	0.75	0.52
Group diff.@ P=.05		1.93	1.93	1.93*	1.93	1.93	1.93	1.93*	1.93	1.93	1.93
Group diff.@ P=.01		2.35	2.35	2.35*	2.35	2.35	2.35	2.35*	2.35	2.35	2.35

Analysis of variance: F ratio = 15.63 Df = 10/ 49 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213A
Exposure phase (Day 13) (Reference Day -1)
Dosing start date: 27-May-08

M a l e A n i m a l s
Data homogeneous by Bartlett's test Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11
Number/group	10	5	5	5	5	5	5	5	5	5	5
Mean	3.22	1.99	2.86	-3.96	2.80	3.36	2.35	-1.01	1.59	2.92	3.54
Standard deviation	1.56	1.22	1.35	1.86	1.43	0.91	0.84	1.93	1.22	0.68	0.44
Group diff.@ P=.05		2.06	2.06	2.06*	2.06	2.06	2.06	2.06*	2.06	2.06	2.06
Group diff.@ P=.01		2.50	2.50	2.50*	2.50	2.50	2.50	2.50*	2.50	2.50	2.50

Analysis of variance: F ratio = 15.27 Df = 10/ 49 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213A
Exposure phase (Day 14) (Reference Day -1)
Dosing start date: 27-May-08

M a l e A n i m a l s
Data homogeneous by Bartlett's test Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11
Number/group	10	5	5	5	5	5	5	5	5	5	5
Mean	3.69	2.18	3.77	-3.96	3.13	3.61	2.89	-0.74	2.00	3.19	3.78
Standard deviation	1.68	1.43	1.58	2.05	1.38	0.86	1.01	1.98	1.38	0.84	0.57
Group diff.@ P=.05		2.23	2.23	2.23*	2.23	2.23	2.23	2.23*	2.23	2.23	2.23
Group diff.@ P=.01		2.72	2.72	2.72*	2.72	2.72	2.72	2.72*	2.72	2.72	2.72

Analysis of variance: F ratio = 14.43 Df = 10/ 49 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213B
Exposure phase (Day 2) (Reference Day -1)
Dosing start date: 26-May-08

M a l e A n i m a l s
Data homogeneous by Bartlett's test — Test of significance is Dunnett's test

Group	Control	2	3	4	5	6
Number/group	5	5	5	5	5	
Mean	0.42	-0.95	-2.14	-2.61	-3.68	
Standard deviation	0.84	1.06	0.26	0.43	0.59	
Group diff.@ P=.05		1.17*	1.17*	1.17*	1.17*	
Group diff.@ P=.01		1.50	1.50*	1.50*	1.50*	

Analysis of variance: F ratio = 25.41 Df = 4/ 20 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213B
Exposure phase (Day 3) (Reference Day -1)
Dosing start date: 26-May-08

M a l e A n i m a l s

Data homogeneous by Bartlett's test — Test of significance is Dunnett's test

Group	Control	2	3	4	5	6
Number/group	5	5	5	5	5	
Mean	0.87	-0.73	-2.98	-3.67	-5.34	
Standard deviation	0.94	1.39	0.41	0.38	0.96	
Group diff.@ P=.05		1.51*	1.51*	1.51*	1.51*	
Group diff.@ P=.01		1.93	1.93*	1.93*	1.93*	

Analysis of variance: F ratio = 37.30 Df = 4/ 20 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213B
Exposure phase (Day 4) (Reference Day -1)
Dosing start date: 26-May-08

M a l e A n i m a l s

Data homogeneous by Bartlett's test — Test of significance is Dunnett's test

Group	Control	2	3	4	5	6
Number/group	5	5	5	5	5	
Mean	1.45	-0.77	-3.57	-4.60	-6.34	
Standard deviation	1.12	1.40	0.52	0.46	0.86	
Group diff.@ P=.05		1.58*	1.58*	1.58*	1.58*	
Group diff.@ P=.01		2.02*	2.02*	2.02*	2.02*	

Analysis of variance: F ratio = 54.31 Df = 4/ 20 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213B
Exposure phase (Day 5) (Reference Day -1)
Dosing start date: 26-May-08

M a l e A n i m a l s

Data homogeneous by Bartlett's test — Test of significance is Dunnett's test

Group	Control	2	3	4	5	6
Number/group	5	5	5	5	5	
Mean	1.78	-0.83	-4.15	-5.61	-7.53	
Standard deviation	1.38	1.48	0.68	0.77	1.04	
Group diff.@ P=.05		1.87*	1.87*	1.87*	1.87*	
Group diff.@ P=.01		2.39*	2.39*	2.39*	2.39*	

Analysis of variance: F ratio = 56.17 Df = 4/ 20 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213B
Exposure phase (Day 6) (Reference Day -1)
Dosing start date: 26-May-08

M a l e A n i m a l s

Data homogeneous by Bartlett's test — Test of significance is Dunnett's test

Group	Control	2	3	4	5	6
Number/group	5	5	5	5	5	
Mean	2.47	-0.01	-4.18	-5.84	-2.41	
Standard deviation	1.52	1.36	0.93	1.00	1.15	
Group diff.@ P=.05		2.03*	2.03*	2.03*	2.03*	
Group diff.@ P=.01		2.60	2.60*	2.60*	2.60*	

Analysis of variance: F ratio = 37.05 Df = 4/ 20 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213B
Exposure phase (Day 7) (Reference Day -1)
Dosing start date: 26-May-08

M a l e A n i m a l s

Data homogeneous by Bartlett's test — Test of significance is Dunnett's test

Group	Control	2	3	4	5	6
Number/group	5	5	5	5	5	
Mean	2.38	0.00	-4.50	-6.51	-2.51	
Standard deviation	1.65	1.60	0.79	1.36	0.66	
Group diff.@ P=.05		2.15*	2.15*	2.15*	2.15*	
Group diff.@ P=.01		2.75	2.75*	2.75*	2.75*	

Analysis of variance: F ratio = 37.89 Df = 4/ 20 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213B
Exposure phase (Day 8) (Reference Day -1)
Dosing start date: 26-May-08

M a l e A n i m a l s

Data homogeneous by Bartlett's test — Test of significance is Dunnett's test

Group	Control	2	3	4	5	6
Number/group	5	5	5	5	5	
Mean	2.73	0.60	-4.69	-6.04	-0.55	
Standard deviation	1.46	1.51	0.99	1.54	0.62	
Group diff.@ P=.05		2.14	2.14*	2.14*	2.14*	
Group diff.@ P=.01		2.73	2.73*	2.73*	2.73*	

Analysis of variance: F ratio = 41.51 Df = 4/ 20 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213B
Exposure phase (Day 9) (Reference Day -1)
Dosing start date: 26-May-08

M a l e A n i m a l s

Data homogeneous by Bartlett's test — Test of significance is Dunnett's test

Group	Control	2	3	4	5	6
Number/group	5	5	5	5	5	
Mean	2.80	0.34	-4.56	-2.33	1.00	
Standard deviation	1.26	1.88	1.02	3.58	0.86	
Group diff.@ P=.05		3.33	3.33*	3.33*	3.33	
Group diff.@ P=.01		4.26	4.26*	4.26*	4.26	

Analysis of variance: F ratio = 10.63 Df = 4/ 20 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213B
Exposure phase (Day 10) (Reference Day -1)
Dosing start date: 26-May-08

M a l e A n i m a l s

		Data nonhomogeneous by Bartlett's test			Modified T test of significance	
Group	Control	2	3	4	5	6
Number/group	5	5	5	5	5	
Mean	3.25	1.05	-4.78	-1.49	1.73	
Standard deviation	1.34	1.72	1.12	3.65	0.75	
Group diff.@ P=.05		2.71	2.16*	4.83	1.91	
Group diff.@ P=.01		4.51	3.60*	8.05	3.18	

Analysis of variance: F ratio = 12.49 Df = 4/ 20 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213B
Exposure phase (Day 11) (Reference Day -1)
Dosing start date: 26-May-08

M a l e A n i m a l s

Data homogeneous by Bartlett's test — Test of significance is Dunnett's test

Group	Control	2	3	4	5	6
Number/group	5	5	5	5	5	
Mean	3.34	0.95	-4.62	-0.45	2.35	
Standard deviation	1.70	1.66	1.27	0.89	0.92	
Group diff.@ P=.05		2.24*	2.24*	2.24*	2.24	
Group diff.@ P=.01		2.86	2.86*	2.86*	2.86	

Analysis of variance: F ratio = 27.06 Df = 4/ 20 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213B
Exposure phase (Day 12) (Reference Day -1)
Dosing start date: 26-May-08

M a l e A n i m a l s

Data homogeneous by Bartlett's test — Test of significance is Dunnett's test

Group	Control	2	3	4	5	6
Number/group	5	5	5	5	5	
Mean	3.72	1.12	-4.28	0.83	2.67	
Standard deviation	1.76	1.40	1.32	0.86	1.04	
Group diff.@ P=.05		2.20*	2.20*	2.20*	2.20	
Group diff.@ P=.01		2.81	2.81*	2.81*	2.81	

Analysis of variance: F ratio = 27.60 Df = 4/ 20 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213B
Exposure phase (Day 13) (Reference Day -1)
Dosing start date: 26-May-08

M a l e A n i m a l s

Data homogeneous by Bartlett's test — Test of significance is Dunnett's test

Group	Control	2	3	4	5	6
Number/group	5	5	5	5	5	
Mean	4.17	1.58	-3.45	1.29	3.23	
Standard deviation	1.95	1.71	1.51	2.22	1.56	
Group diff.@ P=.05		3.03	3.03*	3.03	3.03	
Group diff.@ P=.01		3.88	3.88*	3.88	3.88	

Analysis of variance: F ratio = 13.20 Df = 4/ 20 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Summary by Dose Group of Absolute Weight Gain (g)
Study number: TOX213B
Exposure phase (Day 14) (Reference Day -1)
Dosing start date: 26-May-08

M a l e A n i m a l s

Data homogeneous by Bartlett's test — Test of significance is Dunnett's test

Group	Control	2	3	4	5	6
Number/group	5	5	5	5	5	
Mean	4.41	1.82	-3.25	2.12	3.49	
Standard deviation	1.81	1.38	1.46	1.07	1.07	
Group diff.@ P=.05		2.33*	2.33*	2.33	2.33	
Group diff.@ P=.01		2.97	2.97*	2.97	2.97	

Analysis of variance: F ratio = 22.92 Df = 4/ 20 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Mean Animal Weight Gains per Day in (g)
Study number: TOX213A
Dosing start date: 27-May-08

FEEDING STUDY/PALATABILITY

Group(s)		Day of Phase 6!	2"	3	4	5	6	7	8	9	10	11	12	13
		Male Animals												
1	(N)	10	10	10	10	10	10	10	10	10	10	10	10	10
	Means	0.714	0.057	0.343	0.334	0.430	0.628	0.035	0.119	0.714	0.196	-0.181	0.383	0.100
	Sdevs	0.392	0.183	0.420	0.520	0.332	0.485	0.247	0.222	0.209	0.305	0.350	0.313	0.247
2	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	0.722	-0.220	0.250	0.332	0.252	0.156	0.146	-0.038	0.874	-0.116	-0.058	0.298	0.338
	Sdevs	0.378	0.202	0.238	0.393	0.267	0.651	0.357	0.129	0.560	0.334	0.353	0.106	0.400
3	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	0.438	-0.854$	0.972	0.360	0.024	0.532	-0.040	0.650%	0.994	0.380	0.410	0.462	-0.172
	Sdevs	0.173	0.027	0.421	0.756	0.488	0.408	0.284	0.266	0.389	0.275	0.151	0.728	0.483
4	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	0.574	-1.648$	0.398	-0.362*	-0.686+	-0.388$	-0.340	0.012	0.824	-0.086	0.034	0.146	-0.212
	Sdevs	0.160	0.069	0.252	0.274	0.522	0.307	0.408	0.163	0.404	0.283	0.264	0.581	0.737
5	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	0.653	-1.649$	-0.074	-0.732+	-0.872+	-0.576$	-0.516	4.456$	0.572	1.378+	1.322+	0.748	0.388
	Sdevs	0.468	0.290	0.540	0.267	0.166	0.055	0.481	0.608	0.249	0.381	0.483	0.487	0.627
6	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	0.745	-1.954$	-0.382*	-0.986+	-1.706+	4.972$	0.462	1.632$	1.358	1.026+	0.696+	0.146	0.052
	Sdevs	0.384	0.238	0.401	0.208	0.651	0.488	0.824	0.511	0.660	0.169	0.482	0.736	0.412
7	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	1.089	-0.448%	1.204+	0.234	0.126	0.524	-0.024	1.460	-0.564	-0.142	0.132	0.206	0.088
	Sdevs	0.468	0.276	0.508	0.392	0.160	0.303	0.280	2.280	2.172	0.387	0.360	0.324	0.287
8	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	0.778	-1.260$	0.386	-0.414*	-0.170	0.318	0.016	0.108	0.638	0.372	0.110	0.024	0.120
	Sdevs	0.323	0.169	0.264	0.254	0.532	0.224	0.374	0.161	0.326	0.191	0.273	0.453	0.382
9	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	0.543	-0.978$	0.568	-0.354*	0.198	0.496	-0.094	0.828$	1.088	0.244	0.258	0.216	0.098
	Sdevs	0.077	0.145	0.414	0.388	0.504	0.384	0.334	0.211	0.395	0.337	0.241	0.449	0.421
10	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	0.709	-1.647$	-0.436*	-1.020+	-0.980+	-0.334$	-0.780+	1.328$	3.742$	0.648	1.962+	1.036	1.044+
	Sdevs	0.394	0.129	0.479	0.253	0.352	0.145	0.433	0.348	0.861	0.657	0.620	0.389	0.478
11	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	0.877	-2.110$	-1.256+	-1.236+	3.744+	0.894	1.486+	1.578$	1.138	0.388	0.298	0.318	0.408
	Sdevs	0.564	0.224	0.613	0.235	0.778	0.306	0.486	0.238	0.352	0.392	0.482	0.262	0.488

Note: ! = Quarantine/Acclimation; " = Exposure phase
*(+) = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance

%($) = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

Group(s)		Day of Phase 14
		Male Animals
1	(N)	10
	Means	0.476
	Sdevs	0.347
2	(N)	5
	Means	0.182
	Sdevs	0.348
3	(N)	5
	Means	0.908
	Sdevs	0.564
4	(N)	5
	Means	-0.004
	Sdevs	0.470
5	(N)	5
	Means	0.338
	Sdevs	0.313
6	(N)	5
	Means	0.246
	Sdevs	0.529
7	(N)	5
	Means	0.544
	Sdevs	0.347
8	(N)	5
	Means	0.268
	Sdevs	0.405
9	(N)	5
	Means	0.408
	Sdevs	0.333
10	(N)	5
	Means	0.278
	Sdevs	0.383
11	(N)	5
	Means	0.244
	Sdevs	0.359

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

Mean Animal Weight Gains per Day in (g)
Study number: TOX213B
Dosing start date: 26-May-08

FEEDING STUDY/PALATABILITY

Group(s)		Day of Phase 6!	2"	3	4	5	6	7	8	9	10	11	12	13
		Male Animals												
1	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	0.420	0.208	0.458	0.572	0.336	0.692	-0.096	0.354	0.066	0.452	0.088	0.384	0.444
	Sdevs	0.203	0.421	0.431	0.226	0.359	0.268	0.180	0.219	0.242	0.190	0.407	0.398	0.222
2	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	0.476	-0.475*	0.216	-0.032+	-0.060	0.818	0.006	0.600	-0.260	0.716	-0.100	0.164	0.464
	Sdevs	0.372	0.531	0.478	0.391	0.284	0.292	0.511	0.329	0.734	0.448	0.266	0.504	0.498
3	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	0.419	-1.072+	-0.840+	-0.584+	-0.586+	-0.030+	-0.312	-0.198*	0.138	-0.222*	0.162	0.340	0.830
	Sdevs	0.234	0.132	0.395	0.245	0.236	0.356	0.488	0.241	0.289	0.252	0.225	0.309	0.423
4	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	0.678	-1.305+	-1.060+	-0.930+	-1.012+	-0.228+	-0.668	0.466	3.714	0.834	1.048	1.276+	0.460
	Sdevs	0.262	0.216	0.152	0.251	0.395	0.357	0.411	0.329	3.361	0.251	3.628	0.565	1.551
5	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	0.780	-1.841+	-1.662+	-0.994+	-1.192+	5.120+	-0.096	1.956+	1.546$	0.732	0.620	0.324	0.562
	Sdevs	0.324	0.297	0.453	0.196	0.213	0.317	0.691	0.444	0.245	0.419	0.195	0.172	0.585
6	(N)													
	Means													
	Sdevs													

Note: ! = Quarantine/Acclimation; " = 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

Mean Animal Weight Gains per Day in (g)
Study number: TOX213B
Dosing start date: 26-May-08

Group(s)		Day of Phase 14
		Male Animals
1	(N)	5
	Means	0.246
	Sdevs	0.420
2	(N)	5
	Means	0.234
	Sdevs	0.359
3	(N)	5
	Means	0.192
	Sdevs	0.434
4	(N)	5
	Means	0.834
	Sdevs	1.385
5	(N)	5
	Means	0.258
	Sdevs	0.734
6	(N)	
	Means	
	Sdevs	

Note: Data for Exposure phase
*(+) = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance
%($) = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

Appendix VIII

Data Used for Preparation of Body Weight Figures

TOX213 A & B Mouse Data

Tobacco Blend

Body Weight (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	40.0 (2A)	SD	80.0 (3A)	SD	160.0 (4A)	SD	240.0 (5A)	SD	400.0 (6A)	SD
0	25.83	1.32	26.49	2.44	25.78	1.32	25.67	1.17	26.37	1.94	25.75	2.62
1	25.95	1.33	26.05	2.54	24.07	1.33	22.38	1.30	23.08	2.12	21.84	2.67
2	26.29	1.43	26.30	2.46	25.04	1.57	22.78	1.45	23.00	2.04	21.46	2.57
3	26.63	1.58	26.63	2.76	25.40	1.89	22.41	1.52	22.27	1.93	20.47	2.47
4	27.06	1.76	26.88	2.64	25.43	1.55	21.73	2.01	21.40	1.91	18.77	1.94
5	27.68	1.84	27.04	2.18	25.96	1.73	21.34	2.19	20.82	1.95	23.74	2.23
6	27.72	1.97	27.19	2.44	25.92	2.00	21.00	2.27	20.31	1.95	24.20	2.67
7	27.84	1.92	27.15	2.36	26.57	2.25	21.01	2.39	24.76	2.12	25.83	2.87
8	28.55	1.96	28.02	2.71	27.56	2.29	21.84	2.56	25.33	1.95	27.19	2.93
9	28.75	1.99	27.91	2.49	27.94	2.37	21.75	2.73	26.71	1.82	28.22	3.06
10	28.57	2.11	27.85	2.63	28.35	2.31	21.78	2.66	28.03	2.20	28.91	3.04
11	28.95	2.30	28.15	2.63	28.82	2.52	21.93	2.95	28.78	2.57	29.06	3.44
12	29.05	2.31	28.48	2.82	28.64	2.37	21.72	2.92	29.17	3.06	29.11	3.33
13	29.53	2.46	28.67	2.99	29.55	2.69	21.71	3.09	29.51	2.97	29.36	2.97

Removed from study; NTP-2000 DIET

Tobacco Extract

Body Weight (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	40.0 (7A)	SD	80.0 (8A)	SD	160.0 (9A)	SD	240.0 (10A)	SD	400.0 (11A)	SD
0	25.83	1.32	25.65	1.56	25.90	1.18	26.17	1.82	25.57	1.80	25.83	1.15
1	25.95	1.33	24.76	1.48	23.94	1.09	23.65	1.90	22.28	1.88	21.61	1.48
2	26.29	1.43	25.96	1.53	24.51	1.10	24.03	2.00	21.84	1.81	20.35	1.57
3	26.63	1.58	26.20	1.81	24.16	0.97	23.62	1.77	20.82	1.87	19.12	1.59
4	27.06	1.76	26.32	1.87	24.36	1.29	23.45	1.55	19.84	2.16	22.86	1.82
5	27.68	1.84	26.85	2.04	24.85	1.35	23.77	1.69	19.51	2.17	23.76	1.59
6	27.72	1.97	26.82	2.14	24.76	1.22	23.78	1.49	18.73	2.36	25.24	1.47
7	27.84	1.92	28.28	1.30	25.59	1.35	23.89	1.61	20.05	2.58	26.82	1.46
8	28.55	1.96	27.72	2.09	26.67	1.41	24.53	1.79	23.80	2.16	27.96	1.44
9	28.75	1.99	27.58	2.18	26.92	1.20	24.90	1.75	24.44	2.72	28.35	1.16
10	28.57	2.11	27.71	2.36	27.18	1.13	25.01	1.93	26.41	2.16	28.64	1.28
11	28.95	2.30	27.91	2.36	27.39	1.15	25.04	2.19	27.44	2.34	28.96	1.12
12	29.05	2.31	28.00	2.21	27.49	1.26	25.16	2.50	28.49	2.17	29.37	1.28
13	29.53	2.46	28.55	2.29	27.90	1.34	25.42	2.47	28.76	2.10	29.61	1.22

Reversed Data

Nicotine Hydrogen Tartrate

Body Weight (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	~4.0 (1B)	SD	80.0 (2B)	SD	160.0 (3B)	SD	240.0 (4B)	SD	400.0 (5B)	SD
0	25.83	1.32	26.70	0.98	27.98	2.78	26.51	0.72	26.89	3.56	27.44	1.84
1	25.95	1.33	27.12	1.19	27.03	2.70	24.37	0.85	24.28	3.48	23.76	1.39
2	26.29	1.43	27.57	1.32	27.24	3.07	23.53	0.87	23.22	3.51	22.10	0.96
3	26.63	1.58	28.15	1.38	27.21	2.80	22.94	0.76	22.29	3.29	21.11	1.02
4	27.06	1.76	28.48	1.64	27.15	2.95	22.36	0.95	21.28	2.92	19.91	0.88
5	27.68	1.84	29.17	1.84	27.97	3.08	22.33	0.96	21.05	2.95	25.03	0.78
6	27.72	1.97	29.08	1.89	27.97	3.53	22.01	0.73	20.39	2.64	24.94	1.32
7	27.84	1.92	29.43	1.70	28.57	3.68	21.82	0.80	20.85	2.54	26.89	1.58
8	28.55	1.96	29.50	1.62	28.31	3.91	21.95	0.95	24.57	2.75	28.44	1.57
9	28.75	1.99	29.95	1.70	29.03	3.95	21.73	1.03	25.40	2.65	29.17	1.63
10	28.57	2.11	30.04	1.95	28.93	3.89	21.89	1.10	26.45	3.15	29.79	1.68
11	28.95	2.30	30.42	1.94	29.09	3.82	22.23	1.10	27.72	3.24	30.12	1.67
12	29.05	2.31	30.87	2.07	29.56	4.19	23.06	1.32	28.18	4.05	30.68	1.60
13	29.53	2.46	31.11	1.88	29.79	3.91	23.26	1.25	29.02	3.60	30.94	1.89

Tobacco Blend

Absolute Body Weight Gain (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	40.0 (2A)	SD	80.0 (3A)	SD	160.0 (4A)	SD	240.0 (5A)	SD	400.0 (6A)	SD
0	0.00		0.00		0.00		0.00		0.00		0.00	
1	0.11	0.37	-0.44	0.40	-1.71	0.05	-3.30	0.14	-3.30	0.58	-3.91	0.48
2	0.46	0.62	-0.19	0.46	-0.74	0.40	-2.90	0.36	-3.37	0.78	-4.29	0.67
3	0.79	0.81	0.14	0.65	-0.38	0.96	-3.26	0.37	-4.10	0.82	-5.28	0.84
4	1.22	0.94	0.39	0.53	-0.35	0.55	-3.95	0.88	-4.98	0.79	-6.98	0.80
5	1.85	1.28	0.55	0.80	0.18	0.88	-4.33	1.03	-5.55	0.78	-2.01	0.98
6	1.88	1.33	0.70	0.68	0.14	1.07	-4.67	1.16	-6.07	1.21	-1.55	0.58
7	2.00	1.23	0.66	0.65	0.79	1.30	-4.66	1.27	-1.61	0.99	0.08	0.65
8	2.72	1.32	1.53	0.87	1.78	1.38	-3.84	1.51	-1.04	0.79	1.44	0.66
9	2.91	1.23	1.42	0.93	2.16	1.47	-3.92	1.72	0.34	0.64	2.47	0.70
10	2.73	1.36	1.36	1.19	2.57	1.40	-3.89	1.59	1.66	0.87	3.16	0.74
11	3.12	1.48	1.66	1.14	3.04	1.36	-3.74	1.84	2.41	0.95	3.31	1.03
12	3.22	1.56	1.99	1.22	2.86	1.35	-3.96	1.86	2.80	1.43	3.36	0.91
13	3.69	1.68	2.18	1.43	3.77	1.58	-3.96	2.05	3.13	1.38	3.61	0.86

Tobacco Extract

Absolute Body Weight Gain (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	40.0 (7A)	SD	80.0 (8A)	SD	160.0 (9A)	SD	240.0 (10A)	SD	400.0 (11A)	SD
0	0.00		0.00		0.00		0.00		0.00		0.00	
1	0.11	0.37	-0.90	0.55	-1.96	0.29	-2.52	0.34	-3.29	0.26	-4.22	0.45
2	0.46	0.62	0.31	0.36	-1.39	0.48	-2.13	0.50	-3.73	0.50	-5.48	0.91
3	0.79	0.81	0.54	0.46	-1.74	0.55	-2.55	0.52	-4.75	0.74	-6.71	1.03
4	1.22	0.94	0.67	0.61	-1.54	0.81	-2.72	0.99	-5.73	0.89	-2.97	0.84
5	1.85	1.28	1.19	0.69	-1.05	1.13	-2.40	0.91	-6.06	0.98	-2.07	0.71
6	1.88	1.33	1.17	0.66	-1.14	1.23	-2.38	1.20	-6.84	1.37	-0.59	0.58
7	2.00	1.23	2.63	1.93	-0.31	1.29	-2.28	1.32	-5.52	1.59	0.99	0.74
8	2.72	1.32	2.06	0.60	0.77	1.13	-1.64	1.46	-1.77	0.81	2.13	0.57
9	2.91	1.23	1.92	0.80	1.02	0.86	-1.27	1.31	-1.13	1.17	2.52	0.51
10	2.73	1.36	2.05	0.88	1.28	0.96	-1.16	1.41	0.84	0.70	2.81	0.61
11	3.12	1.48	2.26	0.95	1.49	0.90	-1.13	1.79	1.87	0.75	3.13	0.52
12	3.22	1.56	2.35	0.84	1.59	1.22	-1.01	1.93	2.92	0.68	3.54	0.44
13	3.69	1.68	2.89	1.01	2.00	1.38	-0.74	1.98	3.19	0.84	3.78	0.57

Nicotine Hydrogen Tartrate

Absolute Body Weight Gain (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	40.0 (1B)	SD	80.0 (2B)	SD	160.0 (3B)	SD	240.0 (4B)	SD	400.0 (5B)	SD
0	0.00		0.00		0.00		0.00		0.00		0.00	
1	0.11	0.37	0.42	0.84	-0.95	1.06	-2.14	0.26	-2.61	0.43	-3.68	0.59
2	0.46	0.62	0.87	0.94	-0.73	1.39	-2.98	0.41	-3.67	0.38	-5.34	0.96
3	0.79	0.81	1.45	1.12	-0.77	1.40	-3.57	0.52	-4.60	0.46	-6.34	0.86
4	1.22	0.94	1.78	1.38	-0.83	1.48	-4.15	0.68	-5.61	0.77	-7.53	1.04
5	1.85	1.28	2.47	1.52	-0.01	1.36	-4.18	0.93	-5.84	1.00	-2.41	1.15
6	1.88	1.33	2.38	1.65	0.00	1.60	-4.50	0.79	-6.51	1.36	-2.51	0.66
7	2.00	1.23	2.73	1.46	0.60	1.51	-4.69	0.99	-6.04	1.54	-0.55	0.62
8	2.72	1.32	2.80	1.26	0.34	1.88	-4.56	1.02	-2.33	3.58	1.00	0.86
9	2.91	1.23	3.25	1.34	1.05	1.72	-4.78	1.12	-1.49	3.65	1.73	0.75
10	2.73	1.36	3.34	1.70	0.95	1.66	-4.62	1.27	-0.45	0.89	2.35	0.92
11	3.12	1.48	3.72	1.76	1.12	1.40	-4.28	1.32	0.83	0.86	2.67	1.04
12	3.22	1.56	4.17	1.95	1.58	1.71	-3.45	1.51	1.29	2.22	3.23	1.56
13	3.69	1.68	4.41	1.81	1.82	1.38	-3.25	1.46	2.12	1.07	3.49	1.07

Tobacco Blend

Feed Consumption (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	40.0 (2A)	SD	80.0 (3A)	SD	160.0 (4A)	SD	240.0 (5A)	SD	400.0 (6A)	SD
0												
1	7.00	0.90	8.00	0.90	7.20	1.20	7.30	3.10	5.90	1.80	4.60	1.30
2	6.70	0.80	7.60	2.40	11.50	3.30	11.10	2.70	9.00	1.10	8.80	2.00
3	6.80	1.20	8.00	2.60	10.90	1.20	9.50	2.70	7.90	0.70	10.40	2.20
4	6.80	0.90	7.90	1.60	12.80	2.50	9.50	2.90	8.20	1.70	9.80	2.20
5	7.40		7.30	1.20	7.20	1.00	5.30	0.80	5.90	1.20	11.00	7.90
6	8.70	3.30	8.90	3.70	9.10	2.60	5.70	0.80	7.10	1.20	8.40	1.50
7	5.70	0.80	7.10	2.00	10.10	1.90	6.10	1.00	34.50	5.30	9.10	2.70
8	6.40	0.80	7.70	1.50	11.90	2.10	9.10	2.90	8.50	0.60	10.10	2.10
9	5.90	0.60	7.60	1.60	10.60	2.30	9.90	3.60	9.30	0.80	9.40	1.50
10	6.00	1.20	7.00	2.20	10.90	0.90	7.40	1.70	8.60	0.60	7.60	1.10
11	6.50	1.00	8.10	2.60	10.80	2.30	9.60	2.00	9.40	1.70	7.60	2.40
12	7.20	1.10	7.40	1.30	6.90	1.40	7.10	1.40	7.90	1.30	7.20	1.60
13	6.10	0.60	5.70	0.80	5.20	0.80	3.50	0.50	5.40	0.50	5.40	1.80
14	6.50	1.20	6.50	1.50	5.80	0.60	5.20	1.10	6.00	0.40	5.70	0.90

Tobacco Extract

Feed Consumption (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	40.0 (7A)	SD	80.0 (8A)	SD	160.0 (9A)	SD	240.0 (10A)	SD	400.0 (11A)	SD
0												
1	7.00	0.90	6.70	0.70	6.90	3.20	5.80	1.30	7.10	2.90	6.80	4.60
2	6.70	0.80	10.20	2.50	7.10	1.90	10.50	2.40	12.20	2.30	8.20	1.70
3	6.80	1.20	9.60	2.00	6.90	1.80	11.30	2.10	10.70	1.40	8.30	1.30
4	6.80	0.90	10.80	0.50	9.00	2.20	12.00	2.60	8.70	2.10	9.30	2.20
5	7.40		7.60	1.30	7.10	1.60	8.60	1.40	6.80	1.20	7.90	2.00
6	8.70	3.30	7.40	1.40	7.60	1.80	10.60	7.70	6.10	1.30	13.00	5.40
7	5.70	0.80	8.30	1.60	7.20	0.90	7.50	1.40	12.10	1.30	11.20	4.30
8	6.40	0.80	7.10	1.20	7.90	1.60	7.30	1.20	7.70	1.20	8.70	1.80
9	5.90	0.60	7.60	1.10	8.10	1.70	8.50	1.80	10.00	0.90	8.90	1.10
10	6.00	1.20	7.50	4.00	7.30	1.10	7.90	1.90	9.80	2.30	7.40	3.30
11	6.50	1.00	8.30	1.70	7.70	0.70	8.30	1.70	10.50	2.40	6.10	3.00
12	7.20	1.10	6.10	0.60	6.80	0.40	6.30	0.70	8.50	0.70	6.90	0.80
13	6.10	0.60	4.10	2.40	4.50	0.70	3.80	0.40	5.70	0.20	5.40	0.60
14	6.50	1.20	6.00	0.70	5.50	0.20	5.70	0.70	6.70	0.70	6.30	0.50

Nicotine Hydrogen Tartrate

Feed Consumption (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	~4.0 (1B)	SD	80.0 (2B)	SD	160.0 (3B)	SD	240.0 (4B)	SD	400.0 (5B)	SD
0												
1	7.00	0.90	6.60	0.40	7.40	3.00	3.90	1.20	5.00	0.80	4.7	3.5
2	6.70	0.80	6.90	0.80	8.10	4.50	5.00	0.80	5.10	2.00	8.1	2.4
3	6.80	1.20	7.20	0.50	7.00	4.00	5.50	1.00	5.70	2.30	6.8	2.5
4	6.80	0.90	7.20	0.30	6.20	1.80	7.70	3.10	7.00	2.20	7.6	3.3
5	7.40		6.80	2.20	7.70	2.10	8.40	1.90	8.60	1.40	20.1	6.3
6	8.70	3.30	6.20	0.50	6.50	1.60	7.20	3.30	7.70	1.20	13.30	5.8
7	5.70	0.80	5.50	0.60	5.70	1.10	6.10	2.30	6.80	1.80	13.30	4.2
8	6.40	0.80	6.00	0.40	5.50	1.20	4.50	0.80	9.7	1.00	12.1	3.4
9	5.90	0.60	6.00	1.10	5.90	1.50	4.70	0.80	11.10	2.70	11.4	4.2
10	6.00	1.20	5.70	0.50	5.30	0.60	5.10	0.90	10.00	1.80	9.8	2.5
11	6.50	1.00	6.80	0.30	6.30	1.70	6.50	1.50	9.20	0.90	9.7	1.3
12	7.20	1.10	6.40	0.70	5.60	0.90	5.80	1.20	7.50	1.60	7.2	0.4
13	6.10	0.60	5.00	0.30	4.90	0.70	5.00	1.10	6.10	0.90	6.1	0.8
14	6.50	1.20	5.80	0.60	6.00	2.00	4.90	0.50	6.90	1.50	8.2	2.3

Tobacco Blend

Group Mean Feed Consumption (g) Normalized per Group Mean (g) Body Weight

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	40.0 (2A)	SD	80.0 (3A)	SD	160.0 (4A)	SD	240.0 (5A)	SD	400.0 (6A)	SD
0												
1	0.27		0.31		0.30		0.33		0.26		0.21	
2	0.25		0.29		0.46		0.49		0.39		0.41	
3	0.26		0.30		0.43		0.42		0.35		0.51	
4	0.25		0.29		0.50		0.44		0.38		0.52	
5	0.27		0.27		0.28		0.25		0.28			
6	0.31		0.33		0.35		0.27		0.35			
7	0.20		0.26		0.38		0.29					
8	0.22		0.27		0.43		0.42					
9	0.21		0.27		0.38		0.46					
10	0.21		0.25		0.38		0.34					
11	0.22		0.29		0.37		0.44					
12	0.25		0.26		0.24		0.33					
13	0.21		0.20		0.18		0.16					

Removed from study; NTP-2000 DIET

Tobacco Extract

Group Mean Feed Consumption (g) Normalized per Group Mean (g) Body Weight

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	40.0 (2A)	SD	80.0 (3A)	SD	160.0 (4A)	SD	240.0 (5A)	SD	400.0 (6A)	SD
0												
1	0.27		0.27		0.29		0.25		0.32		0.31	
2	0.25		0.39		0.29		0.44		0.56		0.40	
3	0.26		0.37		0.29		0.48		0.51		0.43	
4	0.25		0.41		0.37		0.51		0.44			
5	0.27		0.28		0.29		0.36		0.35			
6	0.31		0.28		0.31		0.45		0.33			
7	0.20		0.29		0.28		0.31					
8	0.22		0.26		0.30		0.30					
9	0.21		0.28		0.30		0.34					
10	0.21		0.27		0.27		0.32					
11	0.22		0.30		0.28		0.33					
12	0.25		0.22		0.25		0.25					
13	0.21		0.14		0.16		0.15					

Removed from study; NTP-2000 DIET

Nicotine Hydrogen Tartrate

Group Mean Feed Consumption (g) Normalized per Group Mean (g) Body Weight

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	SD	~4.0 (2A)	SD	80.0 (3A)	SD	160.0 (4A)	SD	240.0 (5A)	SD	400.0 (6A)	SD
0												
1	0.27		0.24		0.27		0.16		0.21		0.20	
2	0.25		0.25		0.30		0.21		0.22		0.37	
3	0.26		0.26		0.26		0.24		0.26		0.32	
4	0.25		0.25		0.23		0.34		0.33			
5	0.27		0.23		0.28		0.38		0.41			
6	0.31		0.21		0.23		0.33		0.38			
7	0.20		0.19		0.20		0.28					
8	0.22		0.20		0.19		0.21					
9	0.21		0.20		0.20		0.22					
10	0.21		0.19		0.18		0.23					
11	0.22		0.22		0.22		0.29					
12	0.25		0.21		0.19		0.25					
13	0.21		0.16		0.16		0.21					

Removed from study; NTP-2000 DIET

Tobacco Blend

Percent Body Weight Gain (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	40.0 (2A)	80.0 (3A)	160.0 (4A)	240.0 (5A)	400.0 (6A)
0	0.00	0.00	0.00	0.00	0.00	0.00
1	0.43	-1.66	-6.63	-12.86	-12.51	-15.18
2	1.78	-0.72	-2.87	-11.30	-12.78	-16.66
3	3.06	0.53	-1.47	-12.70	-15.55	-20.50
4	4.72	1.47	-1.36	-15.39	-18.89	-27.11
5	7.16	2.08	0.70	-16.87	-21.05	-7.81
6	7.28	2.64	0.54	-18.19	-23.02	-6.02
7	7.74	2.49	3.06	-18.15	-6.11	0.31
8	10.53	5.78	6.90	-14.96	-3.94	5.59
9	11.27	5.36	8.38	-15.27	1.29	9.59
10	10.57	5.13	9.97	-15.15	6.30	12.27
11	12.08	6.27	11.79	-14.57	9.14	12.85
12	12.47	7.51	11.09	-15.43	10.62	13.05
13	14.29	8.23	14.62	-15.43	11.87	14.02

Tobacco Extract

Percent Body Weight Gain (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	40.0 (7A)	80.0 (8A)	160.0 (9A)	240.0 (10A)	400.0 (11A)
0	0.00	0.00	0.00	0.00	0.00	0.00
1	0.43	-3.51	-7.57	-9.63	-12.87	-16.34
2	1.78	1.21	-5.37	-8.14	-14.59	-21.22
3	3.06	2.11	-6.72	-9.74	-18.58	-25.98
4	4.72	2.61	-5.95	-10.39	-22.41	-11.50
5	7.16	4.64	-4.05	-9.17	-23.70	-8.01
6	7.28	4.56	-4.40	-9.09	-26.75	-2.28
7	7.74	10.25	-1.20	-8.71	-21.59	3.83
8	10.53	8.03	2.97	-6.27	-6.92	8.25
9	11.27	7.49	3.94	-4.85	-4.42	9.76
10	10.57	7.99	4.94	-4.43	3.29	10.88
11	12.08	8.81	5.75	-4.32	7.31	12.12
12	12.47	9.16	6.14	-3.86	11.42	13.70
13	14.29	11.27	7.72	-2.83	12.48	14.63

Nicotine Hydrogen Tartrate

Percent Body Weight Gain (g)

Dose (mg nicotine/kg BW/day)

Day of Study	Control (1A)	~4.0 (1B)	80.0 (2B)	160.0 (3B)	240.0 (4B)	400.0 (5B)
0	0.00	0.00	0.00	0.00	0.00	0.00
1	0.43	1.57	-3.40	-8.07	-9.71	-13.41
2	1.78	3.26	-2.61	-11.24	-13.65	-19.46
3	3.06	5.43	-2.75	-13.47	-17.11	-23.10
4	4.72	6.67	-2.97	-15.65	-20.86	-27.44
5	7.16	9.25	-0.04	-15.77	-21.72	-8.78
6	7.28	8.91	0.00	-16.97	-24.21	-9.15
7	7.74	10.22	2.14	-17.69	-22.46	-2.00
8	10.53	10.49	1.22	-17.20	-8.66	3.64
9	11.27	12.17	3.75	-18.03	-5.54	6.30
10	10.57	12.51	3.40	-17.43	-1.67	8.56
11	12.08	13.93	4.00	-16.14	3.09	9.73
12	12.47	15.62	5.65	-13.01	4.80	11.77
13	14.29	16.52	6.50	-12.26	7.88	12.72

Appendix IX

Terminal Body Weights

Summary by Dose Group of Animal Body Weights in (g)
Study number: TOX213A
Exposure phase (Day 14)
Dosing start date: 27-May-08

M a l e A n i m a l s
Data homogeneous by Bartlett's test Test of significance is Dunnett's test

Group	Control	2	3	4	5	6	7	8	9	10	11
Number/group	10	5	5	5	5	5	5	5	5	5	5
Mean	29.53	28.67	29.55	21.71	29.51	29.36	28.55	25.42	27.90	28.76	29.61
Standard deviation	2.46	2.99	2.69	3.09	2.97	2.97	2.29	2.47	1.34	2.10	1.22
Group diff.@ P=.05		3.85	3.85	3.85*	3.85	3.85	3.85	3.85*	3.85	3.85	3.85
Group diff.@ P=.01		4.68	4.68	4.68*	4.68	4.68	4.68	4.68	4.68	4.68	4.68

Analysis of variance: F ratio = 4.90 Df = 10/ 49 F probability = 0.000
Note: A * indicates group mean is significantly different from control at level of significance shown.

Mean Animal Body Weights in (g)
Study number: TOX213B
Dosing start date: 26-May-08

Group(s)		Day of Phase 14
		Male Animals
1	(N)	5
	Means	31.11
	Sdevs	1.88
2	(N)	5
	Means	29.79
	Sdevs	3.91
3	(N)	5
	Means	23.26+
	Sdevs	1.25
4	(N)	5
	Means	29.02
	Sdevs	3.60
5	(N)	5
	Means	30.94
	Sdevs	1.89
6	(N)	
	Means	
	Sdevs	

Note: Data for Exposure phase
*(+) = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance
%($) = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

Appendix X

Feed Consumption

Male Animals

Group(s)		Day of Phase 2	3	4	5	6	7	8	9	10	11	12	13	14
1	(N)	10	10	10	10	10	9	10	10	10	10	10	10	10
	Means	7.0	6.7	6.8	6.8	43.6	8.7	5.7	6.4	5.9	6.0	6.5	7.2	6.1
	Sdevs	0.9	0.8	1.2	0.9	114.5	3.3	0.8	0.8	0.6	1.2	1.0	1.1	0.6
2	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	8.0	7.6	8.0	7.9	7.3	8.9	7.1	7.7	7.6	7.0	8.1	7.4	5.7
	Sdevs	0.9	2.4	2.6	1.6	1.2	3.7	2.0	1.5	1.6	2.2	2.6	1.3	0.8
3	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	7.2	11.5+	10.9+	12.8+	7.2	9.1	10.1$	11.9+	10.6%	10.9$	10.8+	6.9	5.2
	Sdevs	1.2	3.3	1.2	2.5	1.0	2.6	1.9	2.1	2.3	0.9	2.3	1.4	0.8
4	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	7.3	11.1+	9.5	9.5	5.3	5.7%	6.1	9.1*	9.9	7.4	9.6*	7.1	3.5$
	Sdevs	3.1	2.7	2.7	2.9	0.8	0.8	1.0	2.9	3.6	1.7	2.0	1.4	0.5
5	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	5.9	9.0	7.9	8.2	5.9	7.1	34.5$	8.5	9.3$	8.6$	9.4	7.9	5.4
	Sdevs	1.8	1.1	0.7	1.7	1.2	1.2	5.3	0.6	0.8	0.6	1.7	1.3	0.5
6	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	4.6%	8.8	10.4+	9.8*	11.0	8.4	9.1	10.1+	9.4$	7.6%	7.6	7.2	5.4
	Sdevs	1.3	2.0	2.2	2.2	7.9	1.5	2.7	2.1	1.5	1.1	2.4	1.6	1.8
7	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	6.7	10.2*	9.6*	10.8+	7.6	7.4	8.3%	7.1	7.6%	7.5	8.3	6.1	4.1
	Sdevs	0.7	2.5	2.0	0.5	1.3	1.4	1.6	1.2	1.1	4.0	1.7	0.6	2.4
8	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	5.8	10.5*	11.3+	12.0+	8.6	10.6	7.5	7.3	8.5%	7.9	8.3	6.3	3.8$
	Sdevs	1.3	2.4	2.1	2.6	1.4	7.7	1.4	1.2	1.8	1.9	1.7	0.7	0.4
9	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	6.9	7.1	6.9	9.0	7.1	7.6	7.2%	7.9	8.1%	7.3	7.7	6.8	4.5%
	Sdevs	3.2	1.9	1.8	2.2	1.6	1.8	0.9	1.6	1.7	1.1	0.7	0.4	0.7
10	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	7.1	12.2+	10.7+	8.7	6.8	6.1	12.1$	7.7	10.0$	9.8%	10.5+	8.5	5.7
	Sdevs	2.9	2.3	1.4	2.1	1.2	1.3	1.3	1.2	0.9	2.3	2.4	0.7	0.2
11	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	6.8	8.2	8.3	9.3	7.9	13.0	11.2%	8.7	8.9$	7.4	6.1	6.9	5.4
	Sdevs	4.6	1.7	1.3	2.2	2.0	5.4	4.3	1.8	1.1	3.3	3.0	0.8	0.6

Note: Data for Exposure phase
*(+) = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance

%($) = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

Group(s)		Day of Phase 15
		Male Animals
1	(N)	10
	Means	6.5
	Sdevs	1.2
2	(N)	5
	Means	6.5
	Sdevs	1.5
3	(N)	5
	Means	5.8
	Sdevs	0.6
4	(N)	5
	Means	5.2
	Sdevs	1.1
5	(N)	5
	Means	6.0
	Sdevs	0.4
6	(N)	5
	Means	5.7
	Sdevs	0.9
7	(N)	5
	Means	6.0
	Sdevs	0.7
8	(N)	5
	Means	5.7
	Sdevs	0.7
9	(N)	5
	Means	5.5%
	Sdevs	0.2
10	(N)	5
	Means	6.7
	Sdevs	0.7
11	(N)	5
	Means	6.3
	Sdevs	0.5

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

Average Feed Consumed/day by Day in (g)
Study number: TOX213B
Dosing start date: 26-May-08

FEEDING STUDY/PALATABILITY

Group(s)		Day of Phase 2	3	4	5	6	7	8	9	10	11	12	13	14
		Male Animals												
1	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	6.6	6.9	7.2	7.2	6.8	6.2	5.5	6.0	6.0	5.7	6.8	6.4	5.0
	Sdevs	0.4	0.8	0.5	0.3	2.2	0.5	0.6	0.4	1.1	0.5	0.3	0.7	0.3
2	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	7.4	8.1	7.0	6.2	7.7	6.5	5.7	5.5	5.9	5.3	6.3	5.6	4.9
	Sdevs	3.0	4.5	4.0	1.8	2.1	1.6	1.1	1.2	1.5	0.6	1.7	0.9	0.7
3	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	3.9$	5.0%	5.5%	7.7	8.4	7.2	6.1	4.5%	4.7	5.1	6.5	5.8	5.0
	Sdevs	1.2	0.8	1.0	3.1	1.9	3.3	2.3	0.8	0.8	0.9	1.5	1.2	1.1
4	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	5.0%	5.1	5.7	7.0	8.6	7.7	6.8	9.7$	11.1%	10.0$	9.2*	7.5	6.1
	Sdevs	0.8	2.0	2.3	2.2	1.4	1.2	1.8	1.0	2.7	1.8	0.9	1.6	0.9
5	(N)	5	5	5	5	5	5	5	5	5	5	5	5	5
	Means	4.7	8.1	6.8	7.6	20.1%	13.3	13.3%	12.1%	11.4%	9.8%	9.7+	7.2	6.1
	Sdevs	3.5	2.4	2.5	3.3	6.3	5.8	4.2	3.4	4.2	2.5	1.3	0.4	0.8
6	(N)													
	Means													
	Sdevs													

Note: Data for Exposure phase
*(+) = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance
%($) = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

Group(s)		Day of Phase 15
		Male Animals
1	(N)	5
	Means	5.8
	Sdevs	0.6
2	(N)	5
	Means	6.0
	Sdevs	2.0
3	(N)	5
	Means	4.9
	Sdevs	0.5
4	(N)	5
	Means	6.9
	Sdevs	1.5
5	(N)	5
	Means	8.2
	Sdevs	2.3
6	(N)	
	Means	
	Sdevs	

Note: Data for Exposure phase
*(+) = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance
%($) = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

Male Animals

Animal	Group		Day of Phase 2	3	4	5	6	7	8	9	10	11	12	13	14
1	1		8.5	6.9	6.8	7.5	369.3		6.6	6.3	6.7	5.9	5.9	8.9	6.7
2			5.6	6.6	5.6	6.0	7.3	7.1	5.7	6.0	5.8	5.0	5.5	6.5	5.5
3			6.5	6.5	7.6	7.7	10.3	15.3	5.4	6.5	5.5	8.6	8.3	8.5	6.3
4			7.0	6.8	7.1	7.6	7.1	4.2	6.1	6.2	5.9	5.6	6.2	6.3	5.8
5			7.3	7.7	7.5	7.6	8.3	12.2	6.8	7.7	7.1	6.8	7.3	8.3	6.6
6			6.6	6.4	6.5	6.3	7.0	6.5	4.8	5.4	5.1	4.6	5.8	5.6	7.1
7			5.9	6.4	5.8	5.8	7.1	7.4	5.1	6.8	5.4	4.9	5.8	6.3	5.7
8			7.8	8.2	9.4	6.6	7.3	9.4	6.6	7.5	5.9	6.4	7.8	7.2	5.9
9			6.9	5.0	5.4	5.2	5.5	7.4	5.8	6.2	5.8	6.4	7.1	7.5	5.3
10			7.4	6.5	6.4	7.3	6.4	8.6	4.3	5.3	5.9	5.5	5.7	6.8	6.1
		(n)	10	10	10	10	10	9	10	10	10	10	10	10	10
		Means	7.0	6.7	6.8	6.8	43.6	8.7	5.7	6.4	5.9	6.0	6.5	7.2	6.1
		Sdevs	0.9	0.8	1.2	0.9	114.5	3.3	0.8	0.8	0.6	1.2	1.0	1.1	0.6
11	2		8.6	11.7	12.3	10.2	7.8	8.3	6.9	9.1	9.2	5.4	8.1	7.8	5.6
12			7.0	6.5	6.4	5.7	5.3	7.1	7.4	9.2	8.7	5.9	5.9	7.8	5.3
13			7.5	5.6	6.4	8.0	8.5	6.8	4.9	5.5	5.1	5.7	5.3	5.1	4.8
14			9.3	6.6	6.1	7.9	7.6	15.5	10.2	7.6	7.6	10.6	11.2	8.4	6.9
15			7.7	7.4	8.8	7.9	7.3	6.9	5.9	7.1	7.3	7.4	10.2	8.0	5.8
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	8.0	7.6	8.0	7.9	7.3	8.9	7.1	7.7	7.6	7.0	8.1	7.4	5.7
		Sdevs	0.9	2.4	2.6	1.6	1.2	3.7	2.0	1.5	1.6	2.2	2.6	1.3	0.8
16	3		7.4	12.8	13.0	17.0	7.1	7.1	12.1	9.4	9.0	11.7	12.4	6.4	4.3
17			6.4	13.8	10.3	10.5	7.9	9.5	12.2	12.1	10.2	11.6	13.7	9.1	5.1
18			6.0	11.4	10.7	12.8	5.6	13.4	8.6	15.2	14.4	10.2	10.9	7.4	5.4
19			7.1	5.8	9.8	12.2	8.0	7.8	8.5	10.8	11.1	11.4	9.0	5.7	6.4
20			9.0	13.5	10.7	11.5	7.3	7.5	8.9	12.0	8.5	9.8	8.2	6.0	4.6
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	7.2	11.5	10.9	12.8	7.2	9.1	10.1	11.9	10.6	10.9	10.8	6.9	5.2
		Sdevs	1.2	3.3	1.2	2.5	1.0	2.6	1.9	2.1	2.3	0.9	2.3	1.4	0.8
21	4		5.2	11.8	13.5	8.9	5.0	5.5	4.7	5.7	5.5	4.9	7.1	6.1	4.2
22			7.3	14.2	11.0	13.9	5.9	6.5	7.2	11.1	13.8	8.5	9.6	6.8	3.1
23			5.5	8.1	8.2	6.4	6.2	5.6	5.9	6.9	9.0	8.0	9.0	7.3	3.8
24			5.7	8.5	6.6	10.4	4.4	4.6	6.8	9.4	7.8	6.5	9.8	6.0	3.1
25			12.6	12.9	8.3	7.9	4.8	6.4	5.7	12.6	13.5	9.0	12.6	9.4	3.2
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	7.3	11.1	9.5	9.5	5.3	5.7	6.1	9.1	9.9	7.4	9.6	7.1	3.5
		Sdevs	3.1	2.7	2.7	2.9	0.8	0.8	1.0	2.9	3.6	1.7	2.0	1.4	0.5

Note: Data for Exposure phase

Animal	Group		Day of Phase 2	3	4	5	6	7	8	9	10	11	12	13	14
							Male Animals								
26	5		3.8	10.4	8.0	8.0	4.8	8.0	37.0	8.1	8.5	8.5	8.0	7.1	5.0
27			8.3	7.3	8.5	6.6	4.5	5.7	30.6	9.0	9.7	9.3	9.8	7.2	5.3
28			5.4	8.9	7.3	10.4	7.3	7.3	29.5	8.9	10.5	8.9	12.0	10.1	6.1
29			7.2	8.9	7.1	9.4	6.5	6.0	42.5	8.7	8.7	7.6	7.8	8.0	5.0
30			4.9	9.3	8.5	6.4	6.2	8.3	33.0	7.6	9.3	8.7	9.6	6.9	5.7
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	5.9	9.0	7.9	8.2	5.9	7.1	34.5	8.5	9.3	8.6	9.4	7.9	5.4
		Sdevs	1.8	1.1	0.7	1.7	1.2	1.2	5.3	0.6	0.8	0.6	1.7	1.3	0.5
31	6		2.8	6.7	10.6	9.9	21.2	8.8	9.2	10.4	9.5	9.1	11.3	9.2	4.4
32			6.2	8.1	7.8	9.2	1.5	7.2	7.0	8.0	8.4	7.1	5.3	5.3	4.2
33			4.0	10.1	9.0	11.4	4.9	9.0	8.8	9.8	10.8	7.6	7.6	8.0	4.6
34			5.0	7.4	13.5	6.4	14.7	6.6	7.0	8.9	7.6	6.2	5.7	5.9	8.5
35			5.2	11.5	10.9	12.2	12.8	10.4	13.7	13.4	10.9	7.9	8.2	7.4	5.5
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	4.6	8.8	10.4	9.8	11.0	8.4	9.1	10.1	9.4	7.6	7.6	7.2	5.4
		Sdevs	1.3	2.0	2.2	2.2	7.9	1.5	2.7	2.1	1.5	1.1	2.4	1.6	1.8
36	7		6.5	9.7	7.4	11.3	7.5	5.3	7.3	7.0	8.0	9.2	9.8	5.9	4.5
37			7.4	14.4	12.3	10.5	5.6	6.8	8.2	7.2	5.9	13.3	6.1	5.9	4.9
38			7.5	9.9	8.4	11.2	8.4	7.7	6.7	7.9	8.5	5.7	8.2	5.3	0.0
39			6.6	8.8	8.9	10.2	8.9	8.5	8.5	8.3	8.5	2.8	7.1	6.5	4.8
40			5.7	8.1	11.2	10.8	7.4	8.7	10.8	5.3	7.3	6.3	10.2	7.0	6.2
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	6.7	10.2	9.6	10.8	7.6	7.4	8.3	7.1	7.6	7.5	8.3	6.1	4.1
		Sdevs	0.7	2.5	2.0	0.5	1.3	1.4	1.6	1.2	1.1	4.0	1.7	0.6	2.4
41	8		4.7	11.5	14.3	15.8	9.5	6.4	7.5	5.3	10.1	7.6	7.7	5.4	4.0
42			5.0	9.3	11.8	11.5	9.1	24.4	9.7	7.5	8.0	11.2	8.9	7.1	3.1
43			6.8	8.2	8.6	12.8	6.1	6.3	6.4	7.8	8.4	6.6	7.0	6.8	4.2
44			7.7	9.3	11.8	8.6	8.9	7.6	7.8	8.6	10.0	7.3	11.0	6.3	3.9
45			4.9	14.1	10.0	11.4	9.6	8.5	6.0	7.4	5.8	6.7	7.1	5.8	4.0
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	5.8	10.5	11.3	12.0	8.6	10.6	7.5	7.3	8.5	7.9	8.3	6.3	3.8
		Sdevs	1.3	2.4	2.1	2.6	1.4	7.7	1.4	1.2	1.8	1.9	1.7	0.7	0.4
46	9		4.6	5.9	5.4	11.8	7.6	7.9	7.5	6.1	6.8	5.9	6.7	7.3	4.4
47			11.2	8.1	8.7	10.3	8.4	7.9	8.5	9.3	7.8	8.0	8.4	6.8	5.6
48			3.7	6.8	6.4	6.6	6.2	5.7	6.4	6.4	7.1	6.5	8.1	6.6	4.9
49			5.7	4.8	5.1	7.1	4.9	6.2	6.5	8.1	7.9	7.6	7.1	6.2	3.8

Note: Data for Exposure phase

Animal	Group		Day of Phase 2	3	4	5	6	7	8	9	10	11	12	13	14
							Male Animals								
50	9		9.1	9.7	8.9	9.2	8.6	10.3	7.3	9.5	11.1	8.6	8.0	6.9	3.9
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	6.9	7.1	6.9	9.0	7.1	7.6	7.2	7.9	8.1	7.3	7.7	6.8	4.5
		Sdevs	3.2	1.9	1.8	2.2	1.6	1.8	0.9	1.6	1.7	1.1	0.7	0.4	0.7
51	10		8.9	12.7	8.6	7.9	5.5	5.0	12.4	7.9	9.0	8.7	14.5	9.5	6.0
52			3.8	15.1	12.0	6.3	6.7	7.7	11.5	7.1	9.3	8.2	9.0	8.3	5.5
53			10.9	13.1	9.8	12.0	5.8	4.7	10.9	7.2	9.8	8.7	10.8	8.9	5.5
54			5.1	8.9	11.2	8.8	7.9	7.0	14.2	9.7	11.3	9.5	9.3	7.6	5.7
55			6.8	11.4	11.8	8.7	8.0	6.3	11.5	6.8	10.4	13.8	8.8	8.2	5.6
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	7.1	12.2	10.7	8.7	6.8	6.1	12.1	7.7	10.0	9.8	10.5	8.5	5.7
		Sdevs	2.9	2.3	1.4	2.1	1.2	1.3	1.3	1.2	0.9	2.3	2.4	0.7	0.2
56	11		3.4	5.5	9.1	7.5	7.9	13.6	14.1	11.6	10.0	7.4	9.0	7.9	6.3
57			5.1	8.7	9.6	9.9	5.6	10.1	13.2	8.4	9.0	11.7	6.3	6.2	5.0
58			3.3	10.1	8.9	7.9	7.6	10.3	5.0	7.5	9.8	7.8	7.4	7.3	5.6
59			8.0	8.9	7.9	8.5	7.4	22.1	8.5	6.9	7.4	7.6	1.1	7.0	5.0
60			14.2	7.6	6.2	12.9	11.0	9.0	15.0	9.0	8.3	2.4	6.9	6.1	5.0
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	6.8	8.2	8.3	9.3	7.9	13.0	11.2	8.7	8.9	7.4	6.1	6.9	5.4
		Sdevs	4.6	1.7	1.3	2.2	2.0	5.4	4.3	1.8	1.1	3.3	3.0	0.8	0.6

Note: Data for Exposure phase

Animal	Group		Day of Phase 15
			Male Animals
1	1		6.0
2			6.0
3			4.7
4			5.7
5			7.3
6			7.2
7			6.0
8			6.7
9			9.1
10			5.9
		(n)	10
		Means	6.5
		Sdevs	1.2
11	2		8.3
12			7.6
13			4.6
14			5.7
15			6.1
		(n)	5
		Means	6.5
		Sdevs	1.5
16	3		5.1
17			6.7
18			6.1
19			5.4
20			5.6
		(n)	5
		Means	5.8
		Sdevs	0.6
21	4		5.0
22			3.8
23			5.9
24			4.6
25			6.7
		(n)	5
		Means	5.2
		Sdevs	1.1

Note: Data for Exposure phase

Individual Animal Feed Consumed/day (g)
Study number: TOX213A
Dosing start date: 27-May-08

Animal	Group		Day of Phase 15
			Male Animals
26	5		6.3
27			5.8
28			6.3
29			5.4
30			6.4
		(n)	5
		Means	6.0
		Sdevs	0.4
31	6		4.8
32			4.6
33			6.5
34			6.2
35			6.5
		(n)	5
		Means	5.7
		Sdevs	0.9
36	7		6.2
37			6.4
38			6.9
39			5.1
40			5.5
		(n)	5
		Means	6.0
		Sdevs	0.7
41	8		5.3
42			4.7
43			6.7
44			5.9
45			5.8
		(n)	5
		Means	5.7
		Sdevs	0.7
46	9		5.8
47			5.6
48			5.4
49			5.3

Note: Data for Exposure phase

Animal	Group		Day of Phase 15
			Male Animals
50	9		5.5
		(n)	5
		Means	5.5
		Sdevs	0.2
51	10		7.8
52			6.7
53			6.0
54			6.4
55			6.5
		(n)	5
		Means	6.7
		Sdevs	0.7
56	11		6.2
57			6.1
58			6.3
59			7.1
60			5.9
		(n)	5
		Means	6.3
		Sdevs	0.5

Note: Data for Exposure phase

Animal	Group		Day of Phase 2	3	4	5	6	7	8	9	10	11	12	13	14
							Male Animals								
61	1		6.2	8.1	7.7	7.0	8.7	5.9	6.0	6.4	8.0	6.2	6.6	6.3	5.4
62			7.2	6.5	7.0	7.5	7.6	6.7	5.7	5.4	5.7	6.3	7.4	7.4	5.1
63			6.3	6.3	6.7	7.1	3.0	6.8	6.0	6.1	5.7	5.6	6.6	6.6	4.8
64			6.4	6.4	6.9	6.8	6.7	5.5	4.9	5.9	5.1	5.2	6.8	5.4	5.0
65			6.8	7.4	7.8	7.6	7.9	6.2	4.9	6.0	5.7	5.4	6.7	6.4	4.7
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	6.6	6.9	7.2	7.2	6.8	6.2	5.5	6.0	6.0	5.7	6.8	6.4	5.0
		Sdevs	0.4	0.8	0.5	0.3	2.2	0.5	0.6	0.4	1.1	0.5	0.3	0.7	0.3
66	2		12.7	15.7	14.2	9.1	10.4	7.8	5.8	6.0	8.3	5.2	8.1	6.1	5.5
67			6.1	8.9	5.4	4.4	6.6	7.3	5.8	5.2	5.3	5.9	8.0	6.2	5.1
68			5.8	5.3	5.0	5.2	5.5	4.6	4.0	5.5	4.2	4.8	4.3	4.1	3.8
69			6.0	6.2	5.5	6.4	6.8	7.9	7.1	7.1	6.2	5.9	6.2	6.2	4.7
70			6.5	4.6	5.0	5.9	9.4	4.9	6.0	3.7	5.6	4.6	5.0	5.5	5.3
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	7.4	8.1	7.0	6.2	7.7	6.5	5.7	5.5	5.9	5.3	6.3	5.6	4.9
		Sdevs	3.0	4.5	4.0	1.8	2.1	1.6	1.1	1.2	1.5	0.6	1.7	0.9	0.7
71	3		3.4	4.7	4.8	6.1	7.0	5.0	5.5	5.8	5.5	6.2	8.0	7.1	5.0
72			4.4	5.8	6.4	6.3	8.2	5.6	6.5	4.4	5.0	5.8	8.1	7.2	6.6
73			3.3	4.5	4.7	6.5	7.1	6.9	4.4	3.7	3.9	4.0	4.9	5.4	5.3
74			5.7	5.7	4.8	6.2	8.2	5.6	4.3	4.0	3.8	4.9	5.1	5.0	4.1
75			2.7	4.1	6.8	13.2	11.7	13.0	9.8	4.7	5.3	4.4	6.4	4.5	3.8
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	3.9	5.0	5.5	7.7	8.4	7.2	6.1	4.5	4.7	5.1	6.5	5.8	5.0
		Sdevs	1.2	0.8	1.0	3.1	1.9	3.3	2.3	0.8	0.8	0.9	1.5	1.2	1.1
76	4		5.4	7.6	8.0	7.4	8.5	8.2	6.0	8.2	7.6	6.9	7.6	7.4	4.9
77			4.2	3.1	2.7	4.0	6.4	8.5	4.6	10.8	12.6	9.9	9.8	9.6	6.5
78			4.4	5.6	7.1	7.4	9.9	8.8	9.4	10.4	14.4	11.4	9.7	5.3	6.9
79			5.0	6.2	3.7	10.2	8.9	6.2	7.2	10.0	9.3	11.2	9.7	6.7	5.4
80			6.1	2.9	7.0	6.2	9.5	6.6	6.9	9.2	11.5	10.8	9.1	8.3	6.6
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	5.0	5.1	5.7	7.0	8.6	7.7	6.8	9.7	11.1	10.0	9.2	7.5	6.1
		Sdevs	0.8	2.0	2.3	2.2	1.4	1.2	1.8	1.0	2.7	1.8	0.9	1.6	0.9
81	5		2.3	10.4	8.3	11.5	10.4	9.8	8.7	11.9	10.2	8.2	10.4	7.0	5.0
82			6.2	5.1	4.1	2.9	24.8	10.2	12.5	9.2	8.5	8.3	9.6	7.5	5.5
83			1.4	10.1	8.3	6.0	24.6	8.9	13.8	13.9	10.5	12.0	9.3	7.7	6.5
84			10.0	6.1	4.2	8.5	23.8	22.8	11.7	8.6	9.2	7.6	7.7	6.7	7.0

Note: Data for Exposure phase

Individual Animal Feed Consumed/day (g)
Study number: TOX213B
Dosing start date: 26-May-08

Animal	Group		Day of Phase 2	3	4	5	6	7	8	9	10	11	12	13	14
							Male Animals								
85	5		3.8	8.7	9.2	9.1	16.9	14.6	20.0	16.8	18.8	12.9	11.3	7.0	6.3
		(n)	5	5	5	5	5	5	5	5	5	5	5	5	5
		Means	4.7	8.1	6.8	7.6	20.1	13.3	13.3	12.1	11.4	9.8	9.7	7.2	6.1
		Sdevs	3.5	2.4	2.5	3.3	6.3	5.8	4.2	3.4	4.2	2.5	1.3	0.4	0.8

Note: Data for Exposure phase

Individual Animal Feed Consumed/day (g)
Study number: TOX213B
Dosing start date: 26-May-08

Animal	Group		Day of Phase 15
			Male Animals
61	1		5.2
62			6.1
63			6.2
64			5.1
65			6.4
		(n)	5
		Means	5.8
		Sdevs	0.6
66	2		7.8
67			5.8
68			3.4
69			4.9
70			8.2
		(n)	5
		Means	6.0
		Sdevs	2.0
71	3		5.5
72			5.5
73			4.5
74			4.4
75			4.8
		(n)	5
		Means	4.9
		Sdevs	0.5
76	4		4.8
77			7.9
78			8.8
79			6.5
80			6.5
		(n)	5
		Means	6.9
		Sdevs	1.5
81	5		6.9
82			5.9
83			7.6
84			8.5

Note: Data for Exposure phase

Animal	Group		Day of Phase 15
			Male Animals
85	5		11.9
		(n)	5
		Means	8.2
		Sdevs	2.3

Note: Data for Exposure phase