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Toxicology Study No. S.0059803-18, July 2023

Effects of Acute and Subacute Oral 2,4,6-Trinitro-3-Bromoanisole (TNBA) Exposure to Rats (*Rattus Norvegicus*), August 2018 – December 2018

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14. ABSTRACT
The acute oral median lethal dose of 2,4,6 Trinitro-3-Bromoanisole (TNBA) in rats was determined to be 2,000 mg/kg. Subacute administration of TNBA result in mortality at doses of 250 mg/kg-d and above that was preceded by weight loss, decreased food consumption, and stomach lesions. TNBA was acutely caustic to the epithelial lining of the stomach, with dose-dependent increase in prevalence and severity. Amount rats that survived to their schedule necropsy at day 14, the most striking observation was dose-dependent splenomegaly, as we as dose-dependent macrocytic anemia and neutrophilia. The lowest observed adverse effect level (LOAEL) was 62.5 mg/kg-d (only tested in females). Benchmark dose (BMD) analysis was conducted using splenomegaly in females as the critical effect. Using a benchmark response of 10%, the BMD was 59 mg/kg-d and the 95% lower confidence level (BMDL10) was 41 mg/kg-d.

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Study Title

TOXICOLOGY STUDY NO. S.0059803-18
EFFECTS OF ACUTE AND SUBACUTE ORAL 2,4,6 TRINITRO-3-BROMOANISOLE EXPOSURE TO RATS (*RATTUS NORVEGICUS*).

Data Requirement

Health Effects Testing Guidelines, Reference Numbers OPPTS 870.1100 and OPPTS 870.3050

Authors

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July 2023

Performing Laboratory

Defense Centers for Public Health-Aberdeen
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Good Laboratory Practice Compliance Statement

The study described in this report was conducted in compliance with Title 40, Code of Federal Regulations (CFR), Part 792, Good Laboratory Practice Standards, except for the following:

1. The test article characterization (purity) was conducted by the manufacturer, and it is not known whether the testing was done in compliance with the above regulation.

2. Statistical analysis was conducted by the study director rather than a statistician. This analysis was not audited for GLP compliance.

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August 10, 2023

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EXECUTIVE SUMMARY
TOXICOLOGY STUDY NO. S.0059803-18
EFFECTS OF ACUTE AND SUBACUTE ORAL 2,4,6-TRINITRO-3-BROMOANISOLE (TNBA) EXPOSURE TO RATS (*RATTUS NORVEGICUS*)
AUGUST 2018 – DECEMBER 2018

1. PURPOSE

The objectives of this study were to determine the oral median lethal dose (LD_{50}), slope, and 95% confidence intervals for 2,4,6-trinitro-3-bromoanisole (TNBA) in female rats and to determine the subacute effects of repetitive oral exposure to TNBA in male and female rats.

2. CONCLUSIONS

The acute oral toxicity of TNBA was evaluated using the Stagewise Adaptive Dose Method (SADM) in female Sprague-Dawley rats. No compound-related mortality was observed up to the limit dose of 2,000 milligrams per kilogram (mg/kg), although gastrointestinal toxicity was noted during necropsy at doses ≥431 mg/kg. Thus, the LD_{50} for TNBA was >2,000 mg/kg.

In the subacute study, male and female rats were dosed daily via oral gavage with 0, 62.5 (female only), 125, 250, 500, 1,000, and 2,000 (male only) mg/kg per day (-d) TNBA in corn oil for 2 weeks. Mortality was observed at 250 mg/kg-d (3/10 female; 0/10 male), 500 mg/kg-d (3/10 female; 7/10 male), 1,000 mg/kg-d (4/10 female; 10/10 male), and 2,000 mg/kg-d (10/10 male). Mortality was commonly preceded by weight loss and decreased food consumptions and was frequently associated with overt lesions in the stomach. Additionally, milder gastrointestinal pathologies were commonly observed in dose-dependent manners at TNBA doses ≥125 mg/kg-d. Thus, TNBA was acutely caustic to the epithelial lining of the stomach, with dose-dependent increases in prevalence and severity.

Among rats that survived to their scheduled necropsy at day 14, the most striking observation was dose-dependent splenomegaly, as well as dose-dependent macrocytic anemia and neutrophilia. The lowest observed adverse effect level (LOAEL) for these effects was 125 mg/kg-d, and the no observed adverse effect level (NOAEL) was 62.5 mg/kg-d (only tested in females). Benchmark dose (BMD) analysis was conducted using splenomegaly in females as the critical effect. Using a benchmark response of 10%, the BMD was 59 mg/kg-d and the 95% lower confidence level ($BMDL_{10}$) was 41 mg/kg-d.

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TOXICOLOGY STUDY NO. S.0059803-18
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AUGUST 2018 – DECEMBER 2018

1. PURPOSE

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2. REFERENCES

See Appendix A for a listing of references cited in this report.

3. AUTHORITY

Funding for this work was provided under Military Interdepartmental Purchase Request (MIPR) No. 11221717. This Final Report addresses, in part, the environment, safety and occupational health (ESOH) requirements outlined in:

- Army Regulation (AR) 200-1, *Environmental Protection and Enhancement*, 2007 (reference 1).
- AR 40-5, *Preventive Medicine*, 2007 (reference 2).
- AR 70-1, *Army Acquisition Policy*, 2018 (reference 3).
- Department of Defense Instruction (DoDI) 4715.1E, *Environment, Safety, and Occupational Health (ESOH)*, 2005, Change 1, 2018 (reference 4).
- Army Environmental Requirement and Technology Assessment (AERTA) requirement PP-3-02-05, *Compliant Ordnance Lifecycle for Readiness of the Transformation and Objective Forces* (reference 5).

The Sponsor is the Joint Enhanced Munitions Technology Program (JEMTP), formerly the Joint Insensitive Munitions Technology Program (JIMTP). The Program Manager is Mr. Lawrence Fan, JEMTP, 4103 Fowler Road, Suite 107, Indian Head, MD 20640-5106.

4. BACKGROUND

Composition B, which consists of 2,4,6-trinitrotoluene (TNT) and 1,3,5-trinitro-1,3,5-triazinane (RDX), is widely used by the Department of Defense (DoD) in artillery and mortar rounds. However, TNT and RDX have known toxicity concerns associated with their manufacture, handling and use, and Composition B does not meet current performance requirements regarding sensitivity to detonation. Historically, military munitions and propellants have been developed and fielded based solely on their effectiveness on the battlefield. Any potential toxicity associated with the manufacture and use of these munitions was not investigated until after the material had been fielded and the contamination already occurred. However, current

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munitions development efforts place greater emphasis on toxicity testing as an integrated part of the development pipeline that occurs in concert with performance testing. The focus of the Green Insensitive Munitions Explosives (GrIMEx) program is to develop an alternative insensitive munition formulation containing novel replacements for TNT and RDX that present fewer concerns to human health. TNBA has progressed through the GrIMEx program as a potential replacement for TNT based on its improved performance and sensitivity compared to Composition B. Toxicity testing of TNBA consists of a phased approach that initially consists of *in silico* (quantitative-structure activity relationship (QSAR)) and *in vitro* analyses, followed by *in vivo* testing of lead candidates. The *in silico* and *in vitro* data for TNBA includes a predicted oral LD_{50} of 885.8 milligrams per kilogram (mg/kg) based on TOPKAT structure-toxicity computer modeling and an estimated LD_{50} of 170 mg/kg based on *in vitro* cytotoxicity data from the Human Cell Line Activation Test (HCLAT) conducted previously at the Defense Centers for Public Health-Aberdeen (DCPH-A) (formerly U.S. Army Public Health Center (APHC)) (references 6 and 7). Additionally, TNBA showed mutagenic responses in the Ames assay, and exhibited high toxicity to aquatic organisms in the microtox acute toxicity assay (Median (50%) Effect Concentration (EC_{50}) = 0.4 milligrams per liter (mg/L) (references 8 and 9). Furthermore, TNBA is predicted to be a skin sensitizer and a developmental or reproductive toxicant, based on TOPKAT modeling (reference 10).

Research, development, testing, and training with explosives and pyrotechnics potentially less hazardous to human health and the environment is vital to the readiness of the U.S. Army. Through reduced environmental compliance constraints, a safer, more environmentally benign formulation can increase life-cycle cost effectiveness. Current formulations that use TNT have contributed to substantial environmental contamination. It is imperative that the Department of the Army develop weapons containing alternative/replacement energetics. Toxicity assessments such as this study are necessary for safeguarding the health of Soldiers, civilians, and the environment and, if begun early in the research, development, testing, and evaluation process, can save significant time and effort by identifying unacceptable replacement compounds (reference 11).

Table 1 identifies the critical dates of this study.

Table 1. Critical Study Events

Critical Event	Date of Event
Animal Use Protocol Approved	11 July 2018
Study Initiation/Animals Ordered	12 July 2018
Acute Study Animal Arrival	1 August 2018
Acute Study Dosing	7, 14 August 2018
Acute Study Necropsies	7–21 August 2018
14-Day Study Animal Arrival	7 November 2018
14-Day Study Dosing Initiation	20 November 2018
14-Day Study Scheduled Necropsies	4–6, 11–13 December 2018
Live Animal Experimental Completion	13 December 2018
Study Completion	July 2023

5. MATERIALS

5.1 Test Substance

TNBA (CASRN 107342-31-4) is a yellow-orange powdered solid with no detectable odor. The chemical formula is $BrC_7H_4N_3O_7$ and the molecular weight is 322.03 grams per mole (g/mol). TNBA was synthesized by BAE Systems at Holston Army Ammunition Plant, Kingsport, Tennessee and identified as lot number BAE-1144-140. The compound purity was determined by the manufacturer and reported as 99.54%. Due to the storage/shipping requirements for energetic materials, the TNBA may have been wetted with up to 15% water by weight but was not indicated on the Safety Data Sheet. For safety reasons, the TNBA for these studies was used on a "as received" basis. For the acute study, fresh dosing suspensions were prepared the day before use for each round of dosing.

During acute dosing, it was noted that the gavage tube occasionally experienced some resistance when administering the 2,000 mg/kg dose. While this did not present an issue during acute dosing, there was concern that repeated dosing of a larger number of animals during subacute dosing may increase the risk for more significant issues. Therefore, during preparation of the test material used for the subacute dosing, all concentrations of the test material were wetted and ground with a mortar and pestle. It is likely that this resulted in an increased surface area:volume ratio of the suspended particulates in the dose preparations compared to the material used during acute dosing, which may have altered bioabsorption of the test material.

For the subacute study, a separate dosing suspension was prepared for each dose group at targeted concentrations of 12.5, 25, 50, 100, and 200 milligrams per milliliter (mg/mL). Dosing suspensions were prepared in volumes sufficient for approximately 2 weeks of dosing, and a single preparation was used throughout dosing. All concentrations of dosing suspensions were sampled and analyzed by the DCPH-A, Laboratory Sciences Directorate via high performance liquid chromatography (HPLC). In addition, the homogeneity of the suspensions was verified by determining the concentration of samples taken from the top, middle, and bottom of the highest concentration (200 mg/mL) suspension. Prior to initiation of subacute dosing, samples were collected from two suspensions of 10 mg/mL TNBA (prepared 1 week apart) at weekly intervals for a 4- to 5-week period to determine the stability of TNBA in corn oil.

5.2 Animals

Each phase of this study was conducted using young adult Sprague-Dawley (Crl:CD(SD)CD®) rats obtained from Charles River Laboratories. Acute dosing was performed only on females, which are generally presumed to be the more sensitive sex, and subacute dosing was conducted on both male and female rats. All animals were housed in temperature-, relative humidity-, and light-controlled rooms with the target conditions of 68–79 degrees Fahrenheit (ºF), 30–70% humidity, and a 12:12 light/dark cycle. Temperature and relative humidity measurements were made continuously. Table 2 summarizes the mean temperature and humidity during both phases of the study, as well as any readings detected out of range. This one deviation in humidity is not considered to have compromised the integrity or validity of the study results. A certified pesticide-free rodent chow (Envigo Teklad®, 2016C Certified Rodent

Diet) and drinking quality water were available *ad libitum* except during overnight fasting prior to acute dosing and final blood collection for the subacute study. Acute study rats were individually housed while subacute rats were same sex pair housed by dosage group. All rats were housed in suspended polycarbonate boxes with Sani-Chip® bedding. Each rat was uniquely identified by number via cage card and tail marking.

Research was conducted in compliance with DoD and federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council. National Academy Press, Washington, DC 2011. The studies reported herein were performed in animal facilities fully accredited by the AAALAC International.

Table 2. Animal Room Environmental Conditions

Acute Study	**8/1/2018-8/21/2018**	**Mean Temp. 69.93 ºF**	**Mean RH 51.73%**
Out of Range	**Date**	**Temperature ºF**	**Relative Humidity %**
	None		
Subacute Study	**11/7/2018-12/13/2018**	**Mean Temp. 71.46 ºF**	**Mean RH 45.08%**
Out of Range	**Date**	**Temperature ºF**	**Relative Humidity %**
	11/23/2018		29.33

Legend:
°F = degrees Fahrenheit
RH = relative humidity

5.3 Quality Assurance

The DCPH-A Compliance, Accreditation, and Monitoring Office (formerly the Quality Systems and Regulatory Compliance Office) audited critical phases of this study. Appendix B provides the phases audited and the dates of these audits.

5.4 Study Personnel

Appendix C contains the names of persons contributing to the performance of this study.

6. METHODS

6.1 Acute Study

Acute oral toxicity of TNBA was assessed via the Stagewise Adaptive Dose Method (SADM) in female rats, as described by ASTM International Guideline E1163-10 (reference 12). Female rats are the preferred sex for acute testing because historical data indicate that females in most instances have lower LD_{50} values than males. Thus, use of females may reduce the overall number of animals required. The SADM proceeds in stages in which small groups of rats are fasted overnight and then dosed via oral gavage, with each group receiving a different dose.

Dosing of stages is separated by a post-dosing observation period of up to 7 days in which animals are weighed daily and observed for signs of toxicity, morbidity, and mortality. Moribund animals that are unlikely to recover may be humanely euthanized. During the first stage, a dose range is recommended that would be predicted to span 0% to 100% mortality, with a limit dose of 2,000 mg/kg. A probit analysis is then used to guide dose selection for subsequent stages. The analysis uses the results from each stage to calculate the LD_{50}, 95% confidence interval, and slope of the dose response curve. The staged dosing typically continues until the variation around the LD_{50} was less than 0.40 (95% upper confidence limit minus 95% lower confidence limit/2x the LD_{50}). Although mortality from the 7-day observation period is the criterion used for the analysis, time to death may also be used to make decisions regarding doses for subsequent stages.

For the current acute study, a total of 10 female rats were dosed over 2 stages, with each stage separated by 7 days. The rats were approximately 8–9 weeks of age at the time of dosing and weighed 185.4 ± 16.5 grams. All doses were administered according to body mass measured on the day of dosing. Oral dosing was performed using a stainless steel 16-gauge x 2-inch gavage needle. All animals were observed for a period of seven days during which clinical observations and body mass were measured daily. Animals that were euthanized prior to the end of the observation period were submitted for gross pathological evaluation. Following the observation period, all surviving animals were euthanized with carbon dioxide (CO_2) and submitted for gross pathological evaluation.

The first stage consisted of five rats, each dosed with a different dose (93, 200, 431, 928, and 2,000 mg/kg). Constant concentration dosing could not be achieved for the first stage due to the large range of doses and dosage volume limits for rats. Three stock suspensions were prepared in corn oil for stage 1, and dosing volumes ranged from 4–10 milliliters per kilogram (mL/kg). Fresh stock suspensions were prepared prior to stage 2, and concentrations were verified analytically. Stage 2 consisted of 5 rats that received 2,000 mg/kg TNBA at a dose volume of 10 mL/kg.

6.2 Subacute Study

Upon evaluating the results of the SADM, a 14-day subacute oral toxicity study was performed to determine the toxicity of repetitive daily dosing with TNBA. Sixty male and 60 female Sprague Dawley rats, approximately 8–9 weeks old, were used for the subacute portion of the study. Initially six male rats were ordered to serve as untreated and positive controls for the micronucleus assay. Six female rats were subsequently ordered to serve as micronucleus assay controls when the mortality observed in the TNBA-treated male rats prevented their use in the assay. Rats were pair-housed throughout the subacute study except in the event of premature death by a rat's cage-mate, and cage-mates were assigned to the same dose group. Male rats weighed 280.2 ± 18.6 grams at initiation of dosing while female rats weighed 208.7 ± 15.0 grams. Animals were acclimated for a period of 13 days upon arrival in the facility before initiation of dosing.

6.2.1 Dose Selection and Test Substance Administration

For each sex, body weights were determined on the day prior to initiation of dosing, and the 30 cages of each sex were stratified according to the combined body weight of both rats. Each cage was then randomized into five TNBA treatment groups and a corn oil negative control group (N=10 males and 10 females per dose group). Body mass did not differ among dose groups prior to initiation of dosing.

Dose selection was initially based on the results of the acute study, with the highest dose corresponding to test limit dose for male rats. Doses were set at 2,000, 1,000, 500, 250, and 125 mg/kg-d. To accommodate necropsy schedules (maximum of 20 necropsies per day), dosing start dates were staggered such that dosing was initiated for males one week prior to females, with dosing of each sex starting over a 3-day period. Furthermore, initiation of dosing was arranged to include 2–4 rats per dose group per day. As a result of this staggered dosing, males began to experience substantial toxicity and mortality prior to initiation of dosing among females. Due to this toxicity, the doses in females were reduced by half. Males were dosed at a volume of 10 mL/kg and females were dosed at 5 mL/kg. Thus, the dose groups in females consisted of 1,000, 500, 250, 125 and 62.5 mg/kg-d.

All TNBA and control doses were administered based on the most recent body mass obtained. Animals were dosed daily (7 days/week) for 14 days at a similar time using a stainless steel 16-gauge x 2-inch gavage needle.

6.2.2 Body Mass, Food Consumption, and Observations

All animals were weighed once prior to study onset, once prior to randomization, and on study days 0, 1, 3, 7, 13, and 14. Rats were fasted overnight (evening of day 13) prior to necropsy on day 14. Feed was provided *ad libitum* 7 days per week in weighed feeder bins with the exception of overnight fasting prior to necropsy. Feeder bins were reweighed on study days 0, 1, 3, 7, and 13. Grams of food consumption for each period were calculated by subtracting the mass of the empty feeder from the mass of the full feeder. Because rats were pair housed and individual food consumption could not be determined, food consumption was reported as grams of food consumed per pair of animals. Food consumption data was collected even after death of one rat in the cage, and the data was adjusted to daily consumption per rat based on estimated time of death. Food conversion efficiency was also calculated as a ratio of food consumed to body mass gained for the pair of animals.

A thorough physical examination of each animal was performed each day concurrently with the dosing procedure. Observations for mortality and signs of toxic effects were made twice daily, once in the morning during dosing and once in the afternoon, except on weekends or holidays when observations were only performed in the morning. Observations included, but were not limited to, evaluation of the skin and fur, eyes and mucous membranes, respiratory and circulatory effects, autonomic effects (e.g., salivation), central nervous system effects (e.g., tremors and convulsions), changes in the level of activity, gait, and posture, reactivity to handling or sensory stimuli, altered strength, and stereotypes or changes in behavior (e.g., self-mutilation).

6.2.3 Necropsy

Rats that survived until scheduled necropsy were rendered unconscious with CO_2, followed by blood collection via intracardiac puncture and euthanasia using CO_2. Necropsies were scheduled over 6 days based on the staggered experimental start dates.

A macroscopic examination was conducted on all terminal animals at necropsy, noting all lesions and abnormal observations. The following organs were removed, trimmed in a uniform manner, and weighed: adrenals, brain, heart, kidneys, epididymides, liver, ovaries, spleen, testes, and thymus. Any observed lesions were retained for processing. All organs, with the exception of the testes and epididymides from each animal were placed in 10% buffered formalin for at least 24 hours for fixation. The testes and epididymides were placed in modified Davidson's fixative overnight (no longer than 24 hours), rinsed, and placed in 70% ethanol. In addition to the organs listed above, samples of the pituitary, thyroid (with attached portion of trachea), lung, trachea, nose, femur bone marrow, salivary glands, gastrointestinal tract, urinary bladder, representative lymph node, peripheral nerve, sternum with bone marrow, accessory sex organs, mammary gland, thigh musculature, eye with optic nerve, femur (including articular surface), spinal cord at three levels (cervical, midthoracic, and lumbar), and exorbital lachrymal glands were collected and placed in 10% buffered formalin.

Rats that were either found dead or euthanized prior to their scheduled necropsy were subjected to a gross necropsy and fixation of tissues for histopathology. However, blood was not collected, and tissues were not routinely weighed.

6.2.4 Clinical Chemistry and Hematology

At the termination of the study, blood was obtained from surviving animals via intracardiac puncture that were rendered unconscious with CO_2. Blood for clinical chemistry analyses was transferred to tubes free of additives, allowed to clot for at least 20 minutes, and centrifuged to obtain serum. Blood for hematology analyses was transferred immediately to tubes containing tripotassium ethylenediamine-tetraacetic acid (K_3EDTA). Blood for average activated prothrombin time was transferred to a tube containing sodium citrate, centrifuged, and analyzed using the MCA 210 Microsample Coagulation Analyzer (BioData Corporation). Animals were fasted overnight prior to blood collection.

Clinical Chemistry parameters including: albumin (ALB), alkaline phosphatase (ALKP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), calcium (Ca), cholesterol (CHOL), creatinine (CREA), glucose (non-fasting) (GLU), globulin (GLOB), lactate dehydrogenase (LDH), inorganic phosphorous (PHOS), total bilirubin (TBIL), total protein (TP), sodium (Na), potassium (K), and chloride (Cl) were determined using the VetTest 8008 Chemistry Analyzer and VetLyte Electrolyte Analyzer (IDEXX Laboratories, Inc) on all valid serum samples.

Hematology parameters including: white blood cell count (WBC), WBC differential (% neutrophils (NEU %N), % lymphocytes (LYM %L), % monocytes (MONO %M), % eosinophils (EOS %E), % basophils (BASO %B)), red blood cell count (RBC), hemoglobin (HGB),

hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelets (PLT), and mean platelet volume (MPV) were determined using the Cell-Dyn 3700 Hematology Analyzer (Abbott Laboratories) on all valid samples.

6.2.5 Micronucleus Assay (MNA)

On study day 14, the surviving female rats from the three lowest dose groups (62.5 (N=10), 125 (N=10), and 250 (N=7) mg/kg-d) and the vehicle control group (N=10) were biosampled from the tail vein for the MNA. Prior to rendering rats unconscious with CO_2, approximately 60–120 microliters (µL) of blood was withdrawn from the tail vein using a 23-gauge thin wall needle attached to a 1-mL syringe preloaded with anticoagulant. Samples were processed and fixed using the MicroFlow Basic Kit® (Litron Laboratories) according to the manufacturer's instructions. Processed samples were shipped to Litron Laboratories for analysis. Additionally, six female rats were used as negative and positive controls for the MNA. Approximately 1 week prior to scheduled necropsies, 60–120 µL of blood was drawn from the six MNA control rats via the tail vein and processed as recommended by the manufacturer. Starting at 5–7 days after initial blood draw, these same MNA control rats were dosed three times (-48, -24, -4 hours) via oral gavage with 200 mg/kg of the genotoxic agent ethyl methanesulfonate (EMS) dissolved in water at 20 mg/mL. At 4 hours after the third dose, 60–120 µL of blood was drawn from the tail vein and processed as recommended to serve as the positive control. EMS dosing was staggered so that two positive control samples were collected on each day of necropsy.

6.3 Histopathology

Tissues were fixed in formalin, trimmed into cassettes, processed, embedded in paraffin, sectioned via a microtome to a thickness of 4–5 microns, and stained with hematoxylin and eosin using a routine automatic stainer. Additionally the testis and epididymis were initially fixed in modified Davidson's solution and additionally stained with the Periodic Acid Schiff (PAS) stain.

The liver, spleen, kidney, adrenal gland, stomach, thymus, and bone marrow aspirate from the high dose groups (1,000 and 2,000 mg/kg-d), and vehicle control were processed and analyzed. Tissues from lower dose groups were evaluated when a significant difference was observed in lesion incidence or organ weight between the high dose group compared to the control group. The general criteria for establishing histologic scores included the following: Score: '0' = the tissue is essentially normal or observed in <1% of the sampled tissue; '1' = minimum (<5% of tissue affected); '2' = mild (6-20% of tissue affected); '3' = moderate (21-40%); '4' = marked (41–79% of tissue affected); '5' = severe (>80% of tissue affected). Absent= not present for microscopic examination due to sampling error, out of the plane of section, or other similar reason.

6.4 Statistical Analysis

Experimental data generated during the course of this study were recorded by hand and tabulated, summarized, and/or statistically analyzed using Microsoft® Excel and IBM SPSS® Statistics Version 21. Environmental data were automatically recorded using the MetaSys® Building Management System.

Data from the subacute study were analyzed based on the type of data collected and the frequency of collection. Prior to analysis of variance, normality was tested using Kolmogorov-Smirnov test with Lilliefors Significance Correction. Data that lacked a normal distribution were log transformed and retested for normality. If necessary, data was rank transformed. Variance equality was determined by a Levene's test.

Body mass at days 0, 3, 7, 13 and 14, as well as changes in body mass at each interval and net change, were analyzed using a repeated measures Analysis of Variance (ANOVA). Dose groups were also compared with respect to food consumption and food efficiency. If data was rank transformed, significance was determined using a one-way ANOVA. If rank transformed data lacked normality, a Kruskal-Wallis test was performed. If the ANOVA or Kruskal-Wallis test was significant ($p<0.05$), an appropriate post hoc test was used to compare dose groups.

For variables measured only at the end of the study, the dose groups were compared using a one-factor ANOVA. If necessary, data was log- or rank-transformed to achieve normal distribution of data. If the assumption of normally distributed data was violated, a Kruskal-Wallis test was conducted on rank-transformed data. If the dose group effect was significant ($p<0.05$), an appropriate post hoc test was used to compare pairs of dose groups and dose groups to the control group.

For absolute organ mass, comparison of the dose groups was made using an Analysis of Covariance (ANCOVA), with body mass at the end of the study being the covariate used. Even though the dose groups were assigned at day 0 to keep the average mass for each dose group similar, body mass can change during the study dependent on the dose group. The ANCOVA adjusted for any differences in body mass among the dose groups at the end of the study since heavier animals would tend to have heavier organs. If the dose group effect was significant ($p<0.05$), an appropriate post hoc test was used to compare pairs of dose groups and dose groups to the control group. Organ to brain and organ to body mass ratios were calculated and analyzed similarly to the other parameters measured at the end of the study using a one-way ANOVA.

Histopathological data from control and TNBA-treated animals was analyzed using a Fisher's Exact Test with the data compared on a 0-1 scale to determine if the lesion existed or not. Histopathology data were analyzed using Minitab® Statistical Software with a p-value <0.05 indicating a significant result.

7. RESULTS

7.1 Analytical Results

The analytical chemistry results are summarized in Tables 3–5. During acute dosing, TNBA was suspended in corn oil 1 day prior to dosing, and the samples were maintained on stir plates for several hours prior to dosing. Samples were submitted for concentration verification at the time of dosing (Table 3). Additionally, two 10-mg/mL samples (prepared 1 week apart) were repeatedly measured approximately weekly for up to 4–5 weeks. Initially, the samples provided to Laboratory Sciences for concentration verification were sub-sampled prior to analysis. Due to low solubility of the compound and concerns that the sub-samples may not accurately reflect the original sample, the sample collection/analysis methodology was altered so as to avoid sub-sampling. The samples analyzed on 7 August 2018 were sub-sampled, which may have resulted in slightly lower concentrations than predicted. All samples analyzed after 7 August 2018, including samples used for the subacute study were not sub-sampled. The analytic results of the samples used for acute dosing differed slightly from their nominal concentrations, with higher deviations as the sample concentrations increased. This may have been due to low solubility of the suspensions, especially at higher concentrations. The results of stability testing indicated that the TNBA concentration in corn oil remained stable for at least 5 weeks. Weekly recovery percentages ranged from 92–110% throughout the sampling period (Table 4).

One week prior to initiation of subacute dosing, TNBA samples were prepared and concentrations were verified analytically. Homogeneity testing of the most concentrated TNBA/corn oil suspension (200 mg/mL) yielded 85% recovery at the top, 85% at the middle and 100% at the bottom of the container (Table 4). Verification of the dosing solution/suspension concentrations prior to use yielded recovery percentages ranging from 85–104% of the nominal concentrations (Table 5). Given the limited solubility and acceptable limits of the analytical laboratory control samples for this method, these analytical results were considered acceptable. All of the dosage levels are reported using the nominal concentrations.

Table 3. Acute Dosing Concentrations

Nominal Concentration (mg/mL)	Analytical Concentration (mg/mL)
Stage 1 (7 August 2018)	
10	9.4 (94%)
50	43 (86%)
200	160 (80%)
Stage 2 (14 August 2018)	
10	10 (100%)
200	180(90%)

Legend:
mg/mL = milligrams per milliliter

Table 4. Stability and Homogeneity Results

Nominal Concentration (mg/mL)	Analytical Concentration (mg/mL)
10 (day 0 stability) 08/07/2018	9.4 (94%)
10 (day 7 stability) 08/14/2018	10 (100%)
10 (day 15 stability) 08/22/2018	10 (100%)
10 (day 21 stability) 08/28/2018	11 (110%)
10 (day 29 stability) 09/05/2018	10 (100%)
10 (day 35 stability) 09/11/2018	11 (110%)
10 (day 0 stability) 08/14/2018	10 (100%)
10 (day 8 stability) 08/22/2018	11 (110%)
10 (day 14 stability) 08/28/2018	10 (100%)
10 (day 22 stability) 09/05/2018	9.9 (99%)
10 (day 28 stability) 09/11/2018	9.2 (92%)
200 (homogeneity top) 11/09/2018	170 (85%)
200 (homogeneity middle) 11/09/2018	170 (85%)
200 (homogeneity (bottom) 11/09/2018	200 (100%)

Legend:
mg/mL = milligrams per milliliter

Table 5. Subacute Dosing Solution/Suspension Concentrations

Nominal Concentration (mg/mL)	Analytical Concentration (mg/mL)
12.5	13 (104%)
25	24 (96%)
50	46 (92%)
100	93 (93%)
200	170 (85%)

Legend:
mg/mL = milligrams per milliliter

7.2 Stagewise Adaptive Dose Method (SADM) Acute Study

In stage 1 of the SADM, five rats each received a different oral dose of TNBA/corn oil suspension (93, 200, 431, 928, and 2,000 mg/kg) and were monitored for up to 7 days. The rat dosed at 200 mg/kg began gasping shortly after dosing, which persisted for >24 hours. Additional clinical signs, including wheezing, squinting, and lethargy were also noted, and bubbles were occasionally observed around the nostrils at approximately 24 hours after dosing. In consultation with a veterinarian, we determined that this rat was suffering due to aspiration of compound, and it was euthanized approximately 28 hours after dosing. All other rats dosed during stage 1 showed little to no clinical signs of toxicity. In stage 2, five rats received 2,000

mg/kg TNBA. Several of these rats showed temporary signs of toxicity shortly after dosing, such as hunched posture, unsteady movement, and squinting. However, these clinical signs resolved within hours of first appearance, and no deaths were observed. The combined results of both stages of dosing resulted in 1 death out of 10 rats dosed (Table 6); although this death was attributed to dosing error. Thus, the LD_{50} for TNBA exceeded the test limit concentration of 2,000 mg/kg.

No weight loss or clinical signs were observed in the rat dosed at 93 mg/kg. Weight loss was observed in rats dosed at 431 or 928 mg/kg at 24 hours after dosing; while weight loss continued for up to 48 hours among some of the rats dosed at 2,000 mg/kg. Clinical signs included squinting, hunched posture, altered gait, dark feces, and yellow-orange urine. Most clinical signs resolved within 2–3 days; however, the orange discoloration of their urine persisted and was noted at necropsy on day 7. Additional gross pathological findings included enlarged/congested gastric vessels and thickening of the stomach mucosa in the non-glandular portion with a roughened surface. See Appendix D for details of clinical signs, body weight, mortality and pathological findings.

Table 6. Combined Mortality Results

Dose (mg/kg)	Total Number Dosed	Number of Mortalities	Notes
93	1	0	
200	1	1	Death was attributed to dosing error
431	1	0	
928	1	0	
2,000	6	0	

Legend:
mg/kg = milligrams per kilogram

7.3 Subacute Study

7.3.1 Clinical Observations and Mortality

Substantial compound-related toxicity and mortality was observed. In males, 100% mortality was observed in rats dosed with 1,000 or 2,000 mg/kg-d TNBA, and 70% mortality was observed in rats dosed with 500 mg/kg-d TNBA (Figure 1). Due to this high rate of mortality in the top three doses, most of which occurred prior to initiation of dosing of the females, the study design was altered such that the females received half the doses that the males received (5 mL/kg of each dosing suspension instead of 10 mL/kg). In females, partial mortality was observed in rats dosed with 250, 500, and 1,000 mg/kg-d TNBA; while no mortality was observed in rats dosed with 62.5 or 125 mg/kg-d TNBA (Figure 1).

Clinical signs associated with TNBA dosing primarily included gastrointestinal effects, such as dark and/or very loose stool and orange/yellow perianal staining. Other general signs of toxicity,

such as weight loss, hunched posture, lethargy, ruffled fur, and walking on toes (rather than on feet), were observed more frequently at higher doses. Additionally, respiratory distress, such as labored breathing and/or gasping often preceded death/euthanasia. Numerous rats also made an intermittent low-pitched sound that resembled rattled breathing or wheezing but was likely a distinct sound. This may have been a vocalization of distress or discomfort by the rat. Numerous rats exhibited orange discoloration of their urine, which may have been due to either a pathological effect of TNBA or the presence of a brominated metabolite of TNBA in the urine. Appendix E provides a summary of clinical observations.

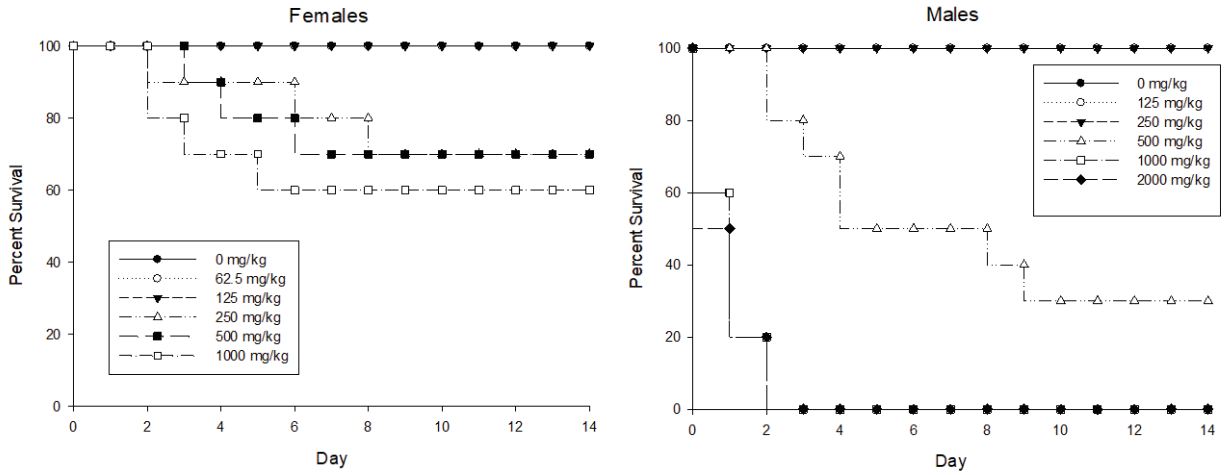


Figure 1. Subacute Survival

7.3.2 Body Mass and Food Consumption

Absolute body mass was assessed on study days 0, 3, 7, 13, and 14 (fasted). Body mass change and food consumption were assessed from days 0–3, 3–7, 7–13, and 0–13. Interpretations of this data were complicated by the numerous TNBA-related deaths observed among both sexes. The female rats that died during the study frequently showed weight loss shortly before death; however, those female rats that survived until scheduled necropsy at day 13 did not significantly differ from the controls for either absolute body mass at day 13 or change in body mass from days 0–13.

Among the female dose groups that displayed no TNBA-related mortality (62.5 and 125 mg/kg-d), absolute body mass did not differ significantly from controls at any time point, and change in body mass differed significantly from controls only from days 0–3 among the rats receiving 62.5 mg/kg-d. Among the female dose groups where mortality was observed (250, 500, and 1,000 mg/kg-d), all three groups exhibited statistically significant decreases in body mass change from days 0–3, followed by deaths from days 2–8 and statistically significant increases in body mass change among survivors from days 7–13. Among the groups of females where deaths were

observed, the only statistically significant difference in absolute body mass was for 1,000 mg/kg-d from days 0–3.

Among males, the complete lethality among rats dosed at 1,000 and 2,000 mg/kg-d precluded interpretations of their body mass. The 500 mg/kg-d dose group exhibited 70% mortality and statistically significant reductions in body mass change from days 0–3 and 0–13 and significant reductions in absolute body mass at all time points, compared to controls. Males dosed at 250 mg/kg-d showed statistically significant reductions in body mass change from days 0–3 but did not differ significantly from controls with respect to absolute body mass at any time point. Neither absolute body mass nor change in body mass differed between 125 mg/kg-d TNBA and controls at any time point. See Appendices F and G for details of body mass and body mass change.

Food consumption was measured for each cage of paired rats, and food consumption monitoring continued for individual rats after the deaths of their cage mates. To account for these deaths, food consumption was reported as daily food consumption per rat (assuming equal consumption by paired rats). The highest three dose groups of female rats (250, 500, and 1,000 mg/kg-d) showed statistically significant decreases in food consumption from days 0–3, and the highest two dose groups (500 and 1,000 mg/kg-d) showed statistically significant decreases in food consumption from days 0–13. Food efficiency, which is defined as weight gain divided by food consumption, was significantly decreased among the highest three dose groups of female rats versus controls from days 0–3 but did not differ from controls over the entire duration of the study (days 0–13).

Among male rats, food consumption and food efficiency decreased significantly compared to controls among the 250 and 500 mg/kg-d TNBA dose groups between days 0–3 and days 0–13. Additionally, male rats dosed at 125 mg/kg-d TNBA showed a significant decrease in food consumption but not food efficiency between days 0–3. See Appendices H and I for details of food consumption and efficiency.

7.3.3 Organ Mass and Ratios

Organs were weighed during necropsy at day 14 and were reported as absolute organ mass and as ratios of organ mass to either fasted body mass or brain mass. Organ mass was only included in analyses if the rat survived to day 14; thus no organ mass data were collected for males dosed at 1,000 or 2,000 mg/kg-d. The most striking and consistent finding was a dose-dependent increase spleen mass, which was significant for both sexes and at all doses compared to controls except at 62.5 mg/kg-d. This difference was also statistically significant for both absolute and relative spleen masses. Spleens from the highest dose group (females dosed at 1,000 mg/kg-d) were 4–5 times larger than those of controls.

Kidneys, livers, and adrenals were also larger among TNBA-dosed groups compared to controls. Females showed significant increases in body weight-normalized kidney mass (250, 500, and 1,000 mg/kg-d) and brain-normalized kidney mass (250 and 500 mg/kg-d); while males showed significant increases in absolute kidney mass (125, 250, and 500 mg/kg-d), body weight-normalized kidney mass (250 and 500 mg/kg-d), and brain-normalized kidney mass (250

mg/kg-d). Females showed significant increases in absolute liver mass (62.5, 125, 250, 500, 1,000 mg/kg-d), body weight-normalized liver mass (62.5 250, 500, 1,000 mg/kg-d), and brain-normalized liver mass (250, 500, 1,000 mg/kg-d); while in males, absolute liver masses were slightly smaller than controls (250 and 500 mg/kg-d), but body-weight normalized liver masses were significantly larger than controls (500 mg/kg-d). Absolute and normalized adrenal masses were significantly enlarged in males at the two highest doses (250 and 500 mg/kg-d), but no significant effect was observed in females.

Other organs showed either no significant differences from controls or differences that were difficult to interpret. Brain masses showed no clear pattern, with females showing a slight decrease in absolute brain mass (500 and 1,000 mg/kg-d) but no difference when normalized to body weight; while males showed no change in absolute brain mass, but a significant increase in body weight-normalized brain mass at the highest dose (500 mg/kg-d). Thymus mass normalized to brain was significantly smaller than controls for males at 500 mg/kg-d, but this effect did not follow an apparent dose response, was not observed for absolute or body weight-normalized masses and was not observed in females. Absolute mass of the epididymides was significantly decreased at the highest dose (500 mg/kg-d), but this effect was not observed when normalized to either brain or body weight, did not follow an apparent dose response, and was based on only three surviving males. No differences were observed for heart, ovaries, uterus, or testes. See Appendix J for details of organ mass and organ mass ratios.

7.3.4 Hematology

The total number of white blood cells (WBCs) did not significantly differ between controls and TNBA-dosed animals; however, the number of neutrophils were increased in both sexes in a dose-dependent manner ($p<0.05$: 500, 1,000 mg/kg-d (females); 125, 250, 500 mg/kg-d (males)). Statistically significant increases in monocytes and basophils were also observed among TNBA-dosed animals; however, these increases did not follow dose-dependent patterns. Statistically significant decreases in lymphocytes were observed; however, these decreases also failed to follow dose-dependent patterns.

Numerous alterations in the red blood cell (RBC) profile were observed that are consistent with macrocytic anemia. The numbers of RBCs were significantly decreased in both sexes in dose-dependent manners ($p<0.05$: 125, 250, 500, 1,000 mg/kg-d (females); 125, 250, 500 mg/kg-d (males)). Similarly, hematocrit (HCT; relative volume of RBCs in blood) and hemoglobin (HGB) were decreased in both sexes in dose-dependent manners ($p<0.05$: 500, 1,000 mg/kg-d (females); 125, 250, 500 mg/kg-d (males)). Parameters of individual RBCs, including mean corpuscular volume (MCV), mean corpuscular HGB (MCH; defined as HGB/RBC), and relative distribution width (RDW; defined as standard deviation/MCV x 100), were significantly increased in both sexes in dose-dependent manners. The MCH concentration (MCHC; defined as MCH/MCV) was similar between TNBA-dosed animals and controls but differed significantly between high-dose females and controls.

The number of platelets among TNBA-dosed animals did not exhibit any statistically significant differences from controls; however, the mean platelet volume (MPV) was increased in both

sexes ($p<0.05$: 250, 500, 1,000 mg/kg-d (females); 125 mg/kg-d (males)). See Appendix K for details of hematology parameters.

7.3.5 Clinical Chemistry and Prothrombin Time

Numerous serum biomarkers were statistically significantly altered; however, the effects generally lacked consistency across sexes and/or dose-groups. Females exhibited statistically significant increases in triglycerides (TRIG; 250, 500, 1,000 mg/kg-d) and potassium (K; 125, 250, 500, 1,000 mg/kg-d) and decreases in sodium (Na; 250, 500, 1,000 mg/kg-d); however, these markers were not altered in males. Other sporadic changes in females included increased albumin (ALB; 125 mg/kg-d) and total protein (TP; 125 mg/kg-d) and decreased urea (1,000 mg/kg-d), blood urea nitrogen (BUN; 1,000 mg/kg-d), and chloride (CL; 125 mg/kg-d). Sporadic changes in males consisted of decreased alanine aminotransferase (ALT; 500 mg/kg-d) and alkaline phosphatase (ALK P; 250 mg/kg-d). No other statistically significant changes were observed. See Appendix L for details of clinical chemistry parameters.

Prothrombin time did not significantly differ between TNBA-dosed rats and controls for either sex. See Appendix M for details of prothrombin time.

7.3.6 Pathology

Among rats that were either found dead or were euthanized prior to their scheduled necropsy, gastrointestinal pathologies were most commonly noted, including blistering and sloughing of the gastric epithelium, red and/or hemorrhagic contents in the stomach and cecum, decreased material in the gastrointestinal tract, and soft, dark feces with a mucus-like consistency. Enlarged spleens were occasionally noted among the rats that died after day 7 but were generally not noted among those rats that died within the first week of dosing.

The most prominent pathological features among the rats that survived until scheduled necropsy at day 14 were dose-dependent splenomegaly and gastrointestinal pathologies (hemorrhaging, blistering, and sloughing of the gastric epithelium; thickening, roughening and yellow (mucoid) or white (fibrous) discoloration of the non-glandular gastric mucosa). Numerous other pathological observations were noted, although these were sporadic and scattered across the dose groups and were thus not considered to be compound-related. See Appendix N for details regarding pathology.

7.3.7 Histopathology

Treatment-related microscopic findings were observed in the kidney, stomach, spleen, and thymus of male and female rats dosed with TNBA compared to controls. Acute kidney injury, consisting of necrosis and degeneration/regeneration of the tubular epithelium, was observed in both male and female rats at doses $\geq$ 250 mg/kg-d. The incidence of acute necrosis was increased in all male and female dose groups $\geq$ 250 mg/kg-d. Tubular epithelial cell degeneration in female rats was elevated in all dose groups $\geq$ 250 mg/kg-d but was only elevated in the 250 and 500 mg/kg-d male dose groups. Additionally, tubular cell regeneration was elevated in the male and female 250 mg/kg-d dose groups. The incidence of cytoplasmic

vacuolation of the tubular epithelium was increased in the female 1,000 mg/kg-d group while the presence intracytoplasmic eosinophilic crystals and globules was elevated in the 125 mg/kg-d males only.

The stomach of all male rats in the 500, 1,000, and 2,000 mg/kg-d groups and female rats in the 500 and 1,000 mg/kg-d groups had an increased incidence of treatment-related microscopic findings. These changes consisted of squamous epithelial hyperplasia and hyperkeratosis in the non-glandular mucosa that was accompanied by submucosal edema, congestion, and neutrophilic and lymphocytic infiltrates as well as ulceration in the most severe cases. Granulation of the submucosal tissue was additionally observed with increased frequency in the male and female 500 mg/kg-d groups.

Significant microscopic changes in the spleen were primarily observed in the 500 and 1,000 mg/kg-d females and the 500 mg/kg-d males. Increased histiocytic pigment accumulation and increased extramedullary hematopoiesis was observed in these three dose groups. The 500 mg/kg-d males also had an increased incidence of red pulp congestion.

Within the thymus, an increased incidence cortical lymphocyte apoptosis and necrosis with increased tingible body macrophages and variably follicular atrophy was observed in all male dose groups $\geq$ 500 mg/kg-d. Only increased cortical lymphocyte apoptosis was observed in the female 500 and 1,000 mg/kg-d groups. The tingible body macrophages were of similar incidence in the dosed females compared to the control group but tended to be slightly more severe in the dosed groups

Various abnormalities were sporadically observed in the liver of male and female rats dosed with TNBA. These changes were only observed in increased frequency compared to control animals for single-cell hepatocellular necrosis (2,000 mg/kg-d males), hepatocellular hypertrophy (1,000 and 2,000 mg/kg-d males), and intrahistiocytic pigment (500 mg/kg-d females). See Appendix O for the full histopathological report.

7.3.8 Genotoxicity

The frequency of micronucleated reticulocytes (%MN-RET) was 0.09 in the baseline samples while in treated animals the frequency was 0.13, 0.17, and 0.22 in rats dosed with 62.5, 125, and 250 mg/kg-d TNBA, respectively. The frequency of MN-RET was significantly elevated in the 125 ($p=0.012$) and 250 mg/kg-d ($p=0.000$) TNBA dose groups. The frequency of reticulocytes (1.62, 4.83, and 3.88, respectively) generally increased with dose and was elevated in the 125 ($p<.0001$) and 250 mg/kg-d ($p<.0001$) dose groups relative to the corn oil control group (0.86). See Appendix P for details of the rat micronucleus assay.

7.4 Determination of Benchmark Dose

Spleen enlargement showed a clear dose-dependent response in both sexes and was identified as the critical effect. Both sexes showed similar susceptibility; however, female rats were selected as the preferred sex to model due to their increased number of dose groups (62.5, 125, 250, 500, and 1,000 mg/kg-d) compared to males (125, 250, 500 mg/kg-d). Additionally, males only had three data points for the 500 mg/kg-d dose. Thus, the data from females was considered more reliable. Furthermore, while splenomegaly was evident based on both absolute and relative spleen mass, brain-normalized spleen mass was considered to be the most reliable indicator tissue pathology. Benchmark Dose Software (BMDS 2.7.0.4) was used to fit mathematical models to the brain-normalized spleen mass in female rats and calculate a lower-bound confidence limit on a dose corresponding to a 10% response rate ($BMDL_{10}$) (reference 13). Due to a lack of constant variance between the dose groups, the variance was modeled by the software. Additionally, the software restricted the curve fitting models to only consider dose-response curves that were biologically plausible. The criteria used to select the best-fitting model included p-value < 0.1, lowest Akaike Information Criterion (AIC) value, and smallest residuals at baseline and at the benchmark dose (BMD). On the basis of these criteria, the Hill model was identified as the closest representation of the data, with a BMD of 59 mg/kg-d and a $BMDL_{10}$ of 41 mg/kg-d. See Appendix Q for details of the BMD determination.

8. DISCUSSION

TNBA is being evaluated as a potential replacement for TNT in munitions, and the current study was designed to determine acute and subacute oral toxicity of TNBA in rats. Historically, female rats tend to show equal or greater susceptibility to acute toxicants than male rats and were thus the preferred sex for this acute test (reference 14). No mortality was observed among the six rats dosed with 2,000 mg/kg TNBA, and the lone death that occurred during acute dosing (200 mg/kg) was attributed to dosing error. Since 2,000 mg/kg was the predetermined test limit dose, the LD_{50} was determined to exceed this dose. The LD_{50} of TNBA estimated in the current study was higher than the values predicted by QSAR modeling (885.8 mg/kg) and the Human Cell Line Activation Test *in vitro* cytotoxicity assay (170 mg/kg) but is comparable to the mouse oral LD_{50} values of the related compounds 2-bromoanisole (2,466 mg/kg) and 4-bromoanisole (2,200 mg/kg) (no data was found for 3-bromoanisole) (references 6, 7, 15 and 16). By comparison, the oral LD_{50} of TNT is estimated at 607 mg/kg in rats and 660 mg/kg in mice (reference 15). Thus, the acute toxicity of TNBA is similar to that of other bromoanisoles but is less toxic than TNT.

Based on the results of acute testing, dose selection for subacute testing of TNBA consisted of 0, 125, 250, 500, 1,000, and 2,000 mg/kg-d. Due to a staggered start, subacute dosing in males preceded that in females by 1 week. During the first week of dosing, all males in the two highest dose groups, as well as several males in the middle dose group, were either found dead or were euthanized. Due to this high rate of mortality, the experimental design was altered such that females received half the dose of males, effectively eliminating the 2,000 mg/kg-d dose group and adding a 62.5 mg/kg-d dose group. Female rats displayed lower mortality than males at 1,000 mg/kg-d (4/10 vs. 10/10) and 500 mg/kg-d (3/10 vs. 7/10); however, females also displayed mortality at 250 mg/kg-d (3/10), which was a dose with no mortality in males. Of note,

two of the three female rats in the 250 mg/kg-d dose groups that were euthanized prematurely were gasping just prior to euthanasia; and thus it is possible that these two rats had aspirated some of the compound during dose administration.

In addition to the unexpected high mortality rate, the number of doses required to induce mortality was also unexpected. Several male rats in the 1,000 and 2,000 mg/kg-d dose groups were found dead or euthanized within 24-hours of dosing, which contrasted with the lack of mortality observed in the acute study of six female rats at 2,000 mg/kg-d. One potential explanation for this could be a sex-based difference in susceptibility, which is possible based on the differences in mortality described above. However, the most likely explanation for this discrepancy between mortality observed in the acute and subacute studies is that the TNBA used for subacute testing was ground with a mortar and pestle during dose preparation, while the TNBA used during acute dosing was not. Grinding of the TNBA into a fine powder may have inadvertently increased the absorption of the test material.

Gastrointestinal pathologies were noted in both the acute and subacute studies. Pathological examination of rats dosed acutely with TNBA revealed thickening of the non-glandular stomach mucosa at doses ≥ 431 mg/kg. During subacute dosing, clinical signs of gastrointestinal toxicity became evident in rats receiving ≥ 250 mg/kg-d TNBA shortly after onset of dosing, which manifested as loose stool with a mucus-like consistency and was often accompanied by rapid weight loss, decreased food consumption, and death/morbidity. Pathologic observations of the rats receiving ≥ 250 mg/kg-d TNBA frequently included sloughing or erosion of the gastric mucosa and separation of the mucosal and sub-mucosal layers of the stomach resulting in fluid-filled blisters or cysts. Thickening of the stomach mucosa was also observed in a dose-dependent manner at TNBA doses ≥ 125 mg/kg-d. The fluid-filled gastric blisters observed during necropsy were most commonly observed in rats that were found dead or euthanized prior to scheduled necropsy and were noted as early as one day after onset of dosing. Thus, at these concentrations, TNBA was severely and acutely caustic to the epithelial lining of the stomach.

Among the rats that survived to their scheduled necropsy at day 14, the most striking pathological observation was dose-dependent splenomegaly at ≥ 125 mg/kg-d TNBA. At 125 mg/kg-d TNBA, relative spleen mass (normalized to brain) was increased by 40% and 46% in males and females, respectively. Relative spleen mass was increased 2.2-fold in both sexes at 250 mg/kg-d TNBA and increased 3.2–3.8-fold in males and females at 500 mg/kg-d, respectively. Females dosed at 1000 mg/kg-d TNBA showed a 4.5-fold enlargement in relative spleen mass. Splenomegaly was not noted among those rats that died or were euthanized prior to day 7; although splenomegaly was occasionally noted among rats that were euthanized between days 7 and 13. An enlarged spleen may store excess numbers of RBCs, resulting in anemia. Indeed, macrocytic anemia was observed in a dose-dependent manner in both sexes. Splenic dysfunction may also lead to destruction of WBCs. Lymphocytes were decreased in TNBA-dosed rats; however this response did not show a dose-dependent pattern.

Another noteworthy effect of TNBA exposure was a dose-dependent increase in blood neutrophils that was observed in both sexes. There are numerous potential causes of elevated neutrophil counts, including hemolytic anemia, although this is likely reflective of a general stress response.

Treatment with TNBA increased the frequency of micronucleated reticulocytes in the peripheral blood of female rats at dosages of 125 and 250 mg/kg-d. The frequency of micronucleated reticulocytes in the peripheral blood of female rats in the 62.5 mg/kg-d group did not differ from controls. These results indicate that TNBA is genotoxic at the highest doses tested (125 and 250 mg/kg-d). DCPH-A previously determined that TNBA showed a mutagenic response at concentrations above 1 microgram per milliliter (µg/mL) in the Ames assay tester strain TA100 without S9 activation. However, TNBA was determined to not be mutagenic in the S9+ treated TA100 (reference 8).

TNBA is currently being considered as a potential replacement for TNT. The health effects associated with environmental and occupational exposure to TNT are well documented. TNT has been shown in animals and humans to cause a variety of toxicities to the liver, blood, immune system, eyes, and reproductive system (reference 17). Notably, subchronic oral dosing with TNT causes hepatomegaly (≥125 mg/kg-d), splenomegaly (≥125 mg/kg-d), histologic brain lesions (300 mg/kg-d), anemia (≥125 mg/kg-d), and serious male reproductive effects (≥125 mg/kg-d) (references 18–20). Additionally, the U.S. Environmental Protection Agency (EPA) has identified TNT as a possible human carcinogen (Group C), while the International Agency for Research on Cancer (IARC; World Health Organization) considers TNT to be unclassifiable as to its carcinogenicity to humans (Group 3) (references 21 and 22). The Occupational Safety and Health Administration (OSHA) has established a maximum allowable occupational exposure of 1.5 milligrams per cubic meter (mg/m^3) for a 40-hour work week, and the EPA established an oral reference dose of $5x10^{-4}$ mg/kg-d based on liver effects observed in dogs (lowest observed adverse effect level (LOAEL) 0.5 mg/kg-d) (references 21 and 23). Subchronic or chronic dosing with TNBA is required to determine how the toxicity of TNBA compares to that of TNT.

9. CONCLUSIONS

The acute oral toxicity of TNBA was evaluated using the SADM approach in female Sprague-Dawley rats. The LD_{50} exceeded the limit dose of 2,000 mg/kg.

In the subacute study, mortality was observed in multiple rats at doses ≥ 250 mg/kg-d. Mortality was most strongly associated with clinical signs and pathologies indicative of gastrointestinal toxicity. Gastrointestinal toxicity was also evident among surviving rats at doses ≥125 mg/kg-d. Other dose-dependent adverse effects included splenomegaly, macrocytic anemia, and neutrophilia, which also occurred at doses ≥125 mg/kg-d. Thus, the LOAEL for this study was 125 mg/kg-d and the no observed adverse effect level (NOAEL) was 62.5 mg/kg-d (a dose that was only tested in female rats). The critical effect selected for BMD analysis was splenomegaly in female rats, which resulted in a BMD of 59 mg/kg-d and a $BMDL_{10}$ of 41 mg/kg-d. Generally, TNBA was less toxic than TNT under the same exposure conditions.

10. POINT OF CONTACT

Questions pertaining to this report should be referred to Thomas E. Sussan at commercial 410-436-3980, or by e-mail: usarmy.apg.medcom-phc.mbx.tox-info@health.mil.

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APPENDIX A

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APPENDIX B

QUALITY ASSURANCE STATEMENT

For Protocol No. 30-18-07-02, titled "EFFECTS OF ACUTE AND SUBACUTE ORAL 2,4,6-TRINITRO-3-BROMOANISOLE (TNBA) EXPOSURE TO RATS (*RATTUS NORVEGICUS*)," the following critical phases were inspected/audited by the Quality Assurance Unit:

Critical Phase Inspected/Audited	**Date Inspected /Audited**	**Date Reported to Management**
Study Protocol Good Laboratory Practice Standards and Animal Care Review	6/4/2018	6/4/2018
Acute Study - Test System Facilities, Identification, Husbandry, Feed and Water Supply & Enrichment	8/14/2018	8/17/2018
Acute Oral Toxicity Test-Maintenance and Calibration of Equipment and Good Documentation Practices	8/14/2018	8/17/2018
Acute Study (LD50) -Test Article Storage, Control, Mixing, Labeling & Administration via Oral Gavage	8/14/2018	8/17/2018
Acute Study (LD50) - Pre and Post-procedural Provisions, Observations and Body Weight Determination	8/14/2018	8/17/2018
Acute Oral Toxicity Test - Sub-study Endpoint Criteria Compliance	9/5/2018	9/13/2018
Study Personnel Qualifications and Training Records - Pre-study training requirements verification	9/6/2018	9/13/2018
SubAcute Study - Euthanasia, Necropsy, Organ Collection, & Tissue Preservation Procedures	10/4/2018	10/7/2018
SubAcute Study - Pre-Procedures, Anesthesia, and Blood Collection Procedures	10/4/2018	10/7/2018
SubAcute Study - Males - Test Substance Dosing and Initial Observations	10/10/2018	10/14/2018
SubAcute Study - Males - Test System - Facilities, Identification, Husbandry & Food & Water Supply	10/10/2018	10/14/2018
Final Study Animal In-Life Endpoint Criteria Compliance	10/10/2018	10/14/2018
Study Raw Data Good Laboratory Practice Standard Review	10/29/2019	10/29/2019
Final Study Good Laboratory Practice Standard Report Review	10/29/2019	10/29/2019
Pathology Contributing Scientist Inspection - Quality Assurance Audit of Excel Entered Data	3/25/2020	3/26/2020
Pathology Contributing Scientist Inspection-Interim Pathology Report GLP Standard Regulation Review	3/25/2020	3/26/2020

Study Raw Data Good Laboratory Practice Standard Review	2/22/2023	6/14/023
Final Report Good Laboratory Practice Standard Review	6/8/2023	6/14/023

Note 1: *Any findings were made known to the Study Director and the Program Manager at the time of the audit/inspection. If there were no findings during the inspection, the inspection was reported to Management and the Study Director on the date shown in the table.*

Note 2: *This report has been audited by the Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.*

Note 3: *In addition to the study specific critical phase inspections listed here, general facility and process based inspections not specifically related to this study are also listed here.*

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Allison Seyfert
GLP Quality Assurance Specialist

Date

APPENDIX C

ARCHIVES AND STUDY PERSONNEL

C-1. ARCHIVES

All raw data, documentation, records, protocol, and a copy of the final report generated as a result of this study will be archived in room [redacted] DCPH-A, for a minimum of 10 years following submission of the final report to the Sponsor.

Records on animal receipt, diet, and facility environmental parameters will be archived by the Veterinary Support Office, Directorate of Technical Services, for a minimum of 10 years following submission of the final report to the Sponsor.

Some ancillary records pertaining to this study, such as instrument maintenance logs, animal room observation logs, etc., will not be archived until those logbooks have been completed. Once complete they will be archived in room [redacted] DCPH-A.

Wet tissues, histology slides, and paraffin blocks are stored in building [redacted].

C-2. PERSONNEL

C-2.1 Management

Management (In-Life): Dr. Mark S. Johnson, Ph.D., Director, Toxicology; Arthur J. O'Neill, Division Chief, Toxicity Evaluation Division (TEV); Dr. Michael J. Quinn, Ph.D., Division Chief, Health Effects Division (HEF); Christopher Moses (VSO).

Management (Report): Dr. Mark S. Johnson, Ph.D., Portfolio Director, Toxicology; Arthur J. O'Neill, Division Chief, Toxicity Evaluation Division (TEV); Dr. Michael J. Quinn, Ph.D., Division Chief, Health Effects Division (HEF).

C-2.2 Study Director

Thomas Sussan, Ph.D., Biologist, HEF.

C-2.3 Quality Assurance

In-Life: Michael P. Kefauver, Quality Assurance Specialist, Quality Systems and Regulatory Compliance (QSARC).

Report: Allison M. Seyfert, Compliance, Accreditation, and Monitoring Office.

C-2.4 Veterinary Support and Animal Care

MAJ Alicia Gehling, DVM, MPH, DACVPM, Attending Veterinarian.

Rebecca Kilby, Animal Health Technician; Lindsey Ward, Animal Health Technician.

C-2.5 Pathology Lab Coordinator

Alicia Shiflett, Histotechnician, DTP.

C-2.6 Histopathology

Keith Koistinen, DVM, ACVP, Pathologist, DTP.

C-2.7 In-Life Support

Allison Narizzano, Biologist, TEV.

Lee Crouse, Biologist, TEV.

C-2.8 Hematology, Clinical Chemistry

Matthew A. Bazar, Biologist, TEV.

C-2.9 Archivist

Lee Crouse, Biologist, TEV.

APPENDIX D

ACUTE DOSING - STAGEWISE ADAPTIVE DOSE METHOD (SADM)

Table D-1. Acute Oral Toxicity of TNBA in Rats

Protocol No.: 30-18-07-02 **Species:** Rat **Sex:** Female **Route:** Oral Gavage
Test Article: TNBA **Diluent:** Corn Oil
Date Dosed: 08/07/2018 (Stage 1), 08/14/2018 (Stage 2)

Stage	Animal ID	Weight (kg)	Nominal Dose (mg/kg)	Stock Concentration (mg/mL)	Time Dosed	Clinical Signs (time noted)	Outcome
1	18-0581	0.1727	93	10	0845	Normal (0929, 1142, 1445)	Scheduled necropsy
1	18-0582	0.1690	200	50	0849	Minor gasping (0902); major gasping, dried liquid around mouth (0930, 1015, 1120); cold to the touch and lethargic (1148); wheeze, squinting, mobile and active (1445); wheeze, squinting, gasping, mobile, holding head up as if to aid in breathing (1500); no gasping or wheeze, dried orange liquid around nose and occasional bubble from nostrils (8/8/18- 0824); gasping, wheezing (8/8/18- 1326)	Euthanized (8/8/18-1352)
1	18-0583	0.1654	431	50	0852	Normal (0930, 1152, 1445); orange-yellow urine stain (8/11/18, 8/12/18)	Scheduled necropsy
1	18-0584	0.1947	928	200	0857	Normal (0930, 1154, 1445); reddish urine (8/8/18); orange urine stain (8/9/18); dark feces (8/10/18); orange-yellow urine stain (8/11/18, 8/12/18)	Scheduled necropsy
1	18-0585	0.1660	2000	200	0900	Piloerection (0930); normal (1157, 1445); orange stain around vagina, slightly hunched posture, walking slightly on toes (8/8/18); orange urine stain, slightly hunched (8/9/18); orange-yellow urine stain (8/11/18, 8/12/18)	Scheduled necropsy
2	18-0586	0.1917	2000	200	0844	Normal (1144, 1542); hunched, orange urine stain (8/15/18); orange urine stain (8/16/18, 8/17/18, 8/18/18, 8/19/18, 8/20/18)	Scheduled necropsy
2	18-0587	0.2113	2000	200	0847	Squinting (0929); normal (1146); normal (1542); hunched, orange urine stain (8/15/18); orange urine stain (8/16/18, 8/17/18, 8/18/18, 8/19/18, 8/20/18)	Scheduled necropsy
2	18-0588	0.2053	2000	200	0849	Squinting (0929); sleeping but normal (1149); normal (1542); hunched, orange urine stain (8/15/18); orange urine stain (8/16/18, 8/17/18, 8/18/18, 8/19/18, 8/20/18)	Scheduled necropsy
2	18-0589	0.1866	2000	200	0852	Squinting (0929); alert, eyes open, wobbly gait (1152); normal (1542); hunched, orange urine stain (8/15/18); orange urine stain (8/16/18, 8/17/18, 8/18/18, 8/19/18, 8/20/18)	Scheduled necropsy
2	18-0590	0.1916	2000	200	0855	Hunched posture (1156); normal (1542); hunched, orange urine stain (8/15/18); orange urine stain, feces are small, slightly dark, shiny, and connected by a mucus-like strand (8/16/18); orange urine stain (8/17/18, 8/18/18, 8/19/18, 8/20/18)	Scheduled necropsy

Note: Unless noted otherwise, rats showed no adverse clinical signs beyond 8 hours after dosing. Clinical signs noted for rat 18-0582 were determined to be due to dosing error.

Table D-2. Individual Body Weights (grams) after Acute Dosing

Protocol No.: 30-18-07-02 **Species:** Rat **Sex:** Female **Route:** Oral Gavage
Test Article: TNBA **Diluent:** Corn Oil
Date Dosed: 08/07/2018 (Stage 1), 08/14/2018 (Stage 2)

Stage	Animal ID	Nominal Dose (mg/kg)	Day 0 (fasted)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	18-0581	93	172.7	185.7	189.3	195.4	199.5	206.0	205.5	211.5
1	18-0582	200	169.0	149.4	Euthanized					
1	18-0583	431	165.4	162.5	168.6	169.2	173.3	181.8	189.4	195.4
1	18-0584	928	194.7	173.0	198.9	204.4	207.8	212.7	222.6	224.2
1	18-0585	2000	166.0	161.5	159.8	167.4	172.2	175.3	184.9	185.8
2	18-0586	2000	191.7	180.6	185.9	194.2	200.4	198.7	205.9	212.0
2	18-0587	2000	211.3	204.5	198.5	213.9	223.6	228.4	233.0	239.1
2	18-0588	2000	205.3	192.6	196.1	205.3	213.1	214.6	224.5	225.4
2	18-0589	2000	186.6	180.8	182.7	191.7	199.1	194.7	205.8	209.9
2	18-0590	2000	191.6	184.5	188.0	201.7	207.3	204.6	216.4	220.0

Table D-3. Acute Gross Pathological Findings

Protocol No.: 30-18-07-02 **Species:** Rat **Sex:** Female **Route:** Oral Gavage
Test Article: TNBA **Diluent:** Corn Oil
Date Dosed: 08/07/2018 (Stage 1), 08/14/2018 (Stage 2)

Stage	Animal ID	Nominal Dose (mg/kg)	Outcome (hours after dosing)	Gross Pathological Findings
1	18-0581	93	Scheduled necropsy	Yellow staining around the anus.
1	18-0582	200	Euthanized (28h)	Dark yellow and red staining and matting around mouth, ventral jaw, nose, and whiskers; dark yellow staining around vulva; stomach and intestines are diffusely distended with gas secondary to abdominal breathing; air trapped under the surface layer of the stomach mucosa in the non-glandular portion.
1	18-0583	431	Scheduled necropsy	Large amount of intense yellow-orange staining around vulva and anus; enlarged and congested gastric vessels; slightly thickened and roughened non-glandular stomach mucosa.
1	18-0584	928	Scheduled necropsy	Yellow-orange staining around vulva and anus; enlarged and congested gastric vessels; minimally thickened non-glandular stomach mucosa.
1	18-0585	2000	Scheduled necropsy	Large amount of yellow staining around vulva and anus; congested gastric vessels; slightly thickened and roughened non-glandular stomach mucosa.
2	18-0586	2000	Scheduled necropsy	Large amount of yellow-orange staining around vulva and anus; thickened non-glandular stomach mucosa.
2	18-0587	2000	Scheduled necropsy	Large amount of yellow-orange staining around vulva and anus; thickened non-glandular stomach mucosa.
2	18-0588	2000	Scheduled necropsy	Mild-moderate yellow staining around vulva and anus; thickened non-glandular stomach mucosa.
2	18-0589	2000	Scheduled necropsy	Small amount of yellow staining around vulva and anus; thickened non-glandular stomach mucosa.
2	18-0590	2000	Scheduled necropsy	Large amount of orange-yellow staining around vulva and anus; thickened non-glandular stomach mucosa.

APPENDIX E

SUMMARY OF CLINICAL OBSERVATIONS

Table E-1. Daily Clinical Observations during Subacute Dosing of Female Rats

Dose Group	Animal ID	Observation	Day(s) Observed
0 mg/kg	19-0125		
	19-0126		
	19-0075		
	19-0076		
	19-0115		
	19-0116		
	19-0107		
	19-0108		
	19-0091		
	19-0092		
62.5 mg/kg	19-0071		
	19-0072	Salivating	9, 13
	19-0105		
	19-0106		
	19-0073		
	19-0074	Salivating	9
	19-0119	Low pitch vocalization/purring/rattling	5
	19-0120		
	19-0117		
	19-0118		
125 mg/kg	19-0101	Dark feces	1-5
	19-0102	Dark feces	1-5
		Salivating	4-5, 9-12
	19-0095	Dark feces	1-5
	19-0096	Dark feces	1-5
		Vocalization	8
	19-0079	Dark feces	4
		Congested breathing	13
		Light yellow urine stain	13
	19-0080	Dark feces	4
		Light yellow urine stain	13
	19-0081	Dark feces	1, 3
		Light yellow urine stain	12
	19-0082	Dark feces	1, 3
		Light yellow urine stain	12
	19-0129	Dark feces	3
		Light yellow urine stain	12
	19-0130	Dark feces	3

Table E-1. Daily Clinical Observations during Subacute Dosing of Female Rats (cont.)

Dose Group	Animal ID	Observation	Day(s) Observed
250 mg/kg	19-0121	Dark feces	1-5, 7
		Vocalization/distress	12
		Perianal staining	13-14
	19-0122	Dark feces	1-5, 7
		Salivating	5, 7-11
		Slightly hunched	8
		Perianal staining	13-14
	19-0113	Dark feces	1, 3-5, 7
		Slight gasp/labored breathing; staining around mouth	8
		Perianal staining	13-14
		Severely congested breathing	14
	19-0114	Dark feces	1, 3-5, 7
		Yellow-orange urine stain	1
		Salivating	8
		Gasping, labored breathing, loose stool, 13% weight loss; euthanized	9
	19-0099	Dark feces	1-4, 6
		Gasping	6-7
		Labored breathing; euthanized	7
	19-0100	Dark feces	1-4, 6
		Vocalization	7
		Perianal staining	12-13
	19-0089	Dark feces	1-3, 5
		Loose stool	1
		Slightly hunched	5
		Walking on toes	5-6
		Perianal staining	11-12
	19-0090	Dark feces	1-3, 5
		Loose stool	1
		Slightly hunched	6
		Perianal staining	11-12
	19-0127	Dark feces	1-6, 8
		Slightly hunched	6
		Perianal staining	11-12
		Mild alopecia on back of neck	11-12
	19-0128	Dark feces	1
		Loose stool	2-3
		Hunched, rattled and labored breathing, 19% weight loss; euthanized	3

Table E-1. Daily Clinical Observations during Subacute Dosing of Female Rats (cont.)

Dose Group	Animal ID	Observation	Day(s) Observed
500 mg/kg	19-0111	Gasping, labored breathing	1
		Dark feces	1
		Loose/mucusy stool	1-4
		Hunched	1-4
		Porphyrin staining	1
		Eyes slightly sunken	1
		Rubbing nose/irritated	1
		Perianal staining	2
		Walking on toes	2
		15% weight loss	2
		Low pitch vocalization/ wheeze/rattle/distress	3-4
		22% weight loss; euthanized	4
	19-0112	Hunched	3-5
		Walking on toes	3, 7
		Perianal staining	3, 13-14
		Dark feces	4-5, 8
		Ruffled fur	6
		Low pitch vocalization/congestion/wheeze/ rattle/distress	3-4, 12-14
	19-0093	Dark feces	1-3, 5-6, 8
		Hunched	3-5, 7-9
		Perianal staining	3, 7, 13-14
		Weight loss	4
		Ruffled fur	5
	19-0094	Orange urine stain	1
		Dark feces	1-3, 5-6, 8
		Perianal staining	2-3, 7, 13-14
		Walking on toes	2-3
		Hunched	3-8
		Weight loss	5
		Ruffled fur	5
		Severely congested breathing	14
	19-0077	Dark feces	0-4
		Perianal staining	1, 9, 12-13
		Walking on toes	1, 4-5, 9
		Loose stool	1-2, 9
		Ruffled fur	1
		Weight loss	2
		Low pitch vocalization/wheeze/rattle	2, 6
		Hunched	2, 6-9
		Salivating	11

Table E-1. Daily Clinical Observations during Subacute Dosing of Female Rats (cont.)

Dose Group	Animal ID	Observation	Day(s) Observed
500 mg/kg (cont.)	19-0078	Dark feces	0-1, 3-4
		Perianal staining	1-2, 4-5, 12-13
		Walking on toes	2, 6
		Slightly hunched	5-9
		Salivating	8, 11
	19-0123	Dark feces	1-4
		Perianal staining	1-2, 4, 12-13
		Loose stool	1
		Hunched	3, 6-7
		Walking on toes	5-6
	19-0124	Dark feces	1-4, 6-7
		Perianal staining	1-2, 4
		Loose stool	1, 5-7
		Hunched	3, 5-8
		Walking on toes	5-6
		16% weight loss; euthanized	7
	19-0083	Dark feces	1-3
		Walking on toes	1, 3
		Hunched	3-4
		Perianal staining	3
		Gasping	4-5
		Loose stool, 14% weight loss; euthanized	5
	19-0084	Dark feces	1-3, 6, 8, 13
		Hunched	1, 3-8
		Walking on toes	3, 5
		Perianal staining	3, 11-12
		Ruffled fur	5-6
		6% weight loss	5
1000 mg/kg	19-0085	Orange urine	1
		Dark feces	1-5, 8, 10
		Loose/mucusy stool	1, 6
		Weight loss	2, 7
		Perianal staining	2, 9, 13-14
		Walking on toes	2-3, 7, 10
		Ruffled fur	2
		Low pitch vocalization/wheeze/rattle	3, 8-10
		Hunched	3-9
		Split lip	6
		Labored breathing	6
		Buries face in bedding	7
		Salivating	11

Table E-1. Daily Clinical Observations during Subacute Dosing of Female Rats (cont.)

Dose Group	Animal ID	Observation	Day(s) Observed
1000 mg/kg (cont.)	19-0086	Orange urine	1
		Dark feces	1-5, 8, 10
		Loose stool	1
		Perianal staining	1-2, 13-14
		Weight loss	2
		Low pitch vocalization/purring	2
		Slightly hunched	3-5, 7-8
		Buries face in bedding	7
		Salivating	12
	19-0087	Dark feces	0-3, 5, 7-8
		Orange urine	1
		Perianal staining	1-2, 13-14
		Loose stool	1
		Weight loss	2, 7
		Low pitch vocalization/wheeze/rattle	3
		Walking on toes	3, 6-7, 9
		Hunched	3, 5, 7-9
		Wheezing	4
		Ruffled fur	5
		Salivating	10-11
	19-0088	Dark feces	0-3
		Loose/mucusy stool	1, 3-4
		Perianal staining	1-2
		Red-brown urine stain	2
		Weight loss	2
		Hunched	2-3
		Wheeze, labored breathing	3
		Gasping	4
		19% weight loss; euthanized	4
	19-0097	Low pitch vocalization/purring/rattling	1
		Weight loss	1
		Hunched	1-3
		Walking on toes	1, 3
		Perianal staining	1
		Dark feces	1, 3
		Loose/mucusy stool	2-3
		Ruffled fur, lethargic	3
		16% weight loss; euthanized	3
	19-0098	Orange urine stain	1, 13
		Slightly hunched	2-4, 6
		Vocalization/distress	7-8
		Lethargic	13
		Perianal staining	13
		Slightly congested breathing	13

Table E-1. Daily Clinical Observations during Subacute Dosing of Female Rats (cont.)

Dose Group	Animal ID	Observation	Day(s) Observed
1000 mg/kg (cont.)	19-0103	Dark feces	1-2
		Hunched	1, 4-5
		Ruffled fur	1, 5
		Walking on toes	1
		Loose/mucusy stool	2
		Perianal staining	2, 4, 12
		Walking on toes	3
		Low pitch vocalization/wheeze/rattle	4, 6
		Labored breathing	5
		18% weight loss; euthanized	6
	19-0104	Dark feces	1-2, 4-5, 13
		Hunched	1, 4-8
		Walking on toes	1, 3, 6-8
		Weight loss	1, 6
		Perianal staining	2, 4, 6, 12-13
		Loose stool	5
		Ruffled fur	8
		Hyperactive when stimulated	13
	19-0109	Hunched	1, 3, 6-7
		Dark feces	2, 4, 13
		2% weight loss	5
		Walking on toes	5, 7
		Ruffled fur	8
		Loose stool	8
		Perianal staining	11-13
		Congested breathing	12
	19-0110	Low pitch vocalization/wheeze/rattle	1
		Hunched	1
		Dark feces	2
		Intermittent cough/sneeze, loose stool, 18% weight loss; euthanized	3

Note: Animals with no remarkable observations, a.k.a “normal,” were not noted. Occasionally, small amounts (<10%) of compound/corn oil flowed out of the mouth during dosing. This was not considered a clinical sign unless the rat aspirated some of the compound.

Table E-2. Daily Clinical Observations during Subacute Dosing of Male Rats

Dose Group	Animal ID	Observation	Day(s) Observed
0 mg/kg	19-0019		
	19-0020		
	19-0031		
	19-0032		
	19-0061		
	19-0062	Salivating	8
	19-0011		
	19-0012		
	19-0049		
	19-0050		
125 mg/kg	19-0045	Yellow-orange urine stain	2
		Salivating	7-9, 11
	19-0046		
	19-0065	Low pitched vocalization/purring/rattling	7
	19-0066	Dark feces	2
		Salivating	7
	19-0047	Dark feces	7-8
		Low pitched vocalization/purring/rattling	13
	19-0048	Dark feces	1, 7-8
		Salivating	7, 11
	19-0015	Dark feces	7
		Salivating	13
	19-0016	Salivating	4, 6-11
		Dark feces	7
	19-0039	Dark feces	6
	19-0040	Salivating	4, 6-8, 10, 12-13
		Dark feces	6
250 mg/kg	19-0041	Orange urine stain	1-2, 10
		Dark feces	2-5, 8-10
		Perianal staining	3, 8-9
		Salivating	7-12
		Ruffled fur	11-12
		Slightly hunched	12
	19-0042	Orange urine stain	1, 10
		Salivating	7-8, 10
		Dark feces	8-10
		Ruffled fur	11-12
		Slightly hunched	12
	19-0059	Dark feces	3, 5, 7-12
		Salivating	7
		Orange urine stain	10

Table E-2. Daily Clinical Observations during Subacute Dosing of Male Rats (cont.)

Dose Group	Animal ID	Observation	Day(s) Observed
250 mg/kg (cont.)	19-0060	Orange urine stain	1, 10
		Dark feces	5, 7-12
	19-0053	Dark feces	3-4, 7-10
		Low pitched vocalization/purring/rattling	8
	19-0054	Dark feces	4, 7-10
		Salivating	6-8, 10
	19-0021	Dark feces	3, 6, 8-9
	19-0022	Dark feces	3, 6, 8-9
		Gasping, wheezing, labored breathing	4
		Slightly hunched	4
		Salivating	5, 10, 12
		Intense perianal staining	5
		7% weight loss	6
	19-0055	Dark feces	3, 6-9
	19-0056	Dark feces	3, 6-8
		Salivating	5, 9
500 mg/kg	19-0067	Porphyrin stain	1
		Orange urine stain	1-2
		Mild gasping	1
		Dark, loose stool	2
		23% weight loss since day 0, euthanized	3
	19-0068	Red urine stain	1
		Very runny/loose stool	1, 9-10
		Dark feces	3-5, 7, 11-12
		Lethargic	3
		Slightly hunched	3
		Ruffled fur	3
		Perianal staining	4-7, 9
		Low pitched vocalization/purring/rattling	7-9
		Walking on toes	9
		Hunched	10-13
		Gasping, labored breathing	13

Table E-2. Daily Clinical Observations during Subacute Dosing of Male Rats (cont.)

Dose Group	Animal ID	Observation	Day(s) Observed
500 mg/kg (cont.)	19-0025	Porphyrin stain	1
		Red/Orange urine stain	1-3
		Dark feces	1-5, 7-8
		Runny stool	1-2
		Ruffled fur	3
		Wobbly gait	3
		Perianal staining	4, 6-9
		Intense yellow staining of fur	5
		Loose/mucusy stool	7, 9-10
		Low pitched vocalization/purring/rattling	8
		Salivating	8
		Walking on toes	9
		Slightly hunched	9
		Weight loss; euthanized	10
	19-0026	Orange urine stain	1,3
		Very runny/loose stool	1-2
		Low pitched vocalization/purring	1
		Ruffled fur	3
		Dark feces	3-4, 7-8, 10-11
		Hunched	3, 9, 11, 13
		Wobbly gait	3
		Perianal staining	4, 6-10
		Intense yellow staining of fur	5
		Salivating	5, 7-10, 12
		Walking on toes	12
	19-0005	Dark feces	2
		Wide gait	2
		Perianal staining	2
		Low pitched vocalization/purring/rattling	3
		Loose stool	3
		24.5% weight loss	3
		Aspirated some compound during dosing resulting in gasping; resolved within 45 min	4
		Found dead	5
	19-0006	Dark feces	2-4, 6-7, 10, 13
		Perianal staining	2-3, 5-7, 10-11
		Slightly hunched	3
		Walking on toes	5, 8-9
		Salivating	6-7
		Low pitched vocalization/purring	8

Table E-2. Daily Clinical Observations during Subacute Dosing of Male Rats (cont.)

Dose Group	Animal ID	Observation	Day(s) Observed
500 mg/kg (cont.)	19-0043	Perianal staining	1
		Low pitched vocalization/purring/rattling	2
		Loose stool	2-4
		Slightly hunched	3
		19% weight loss	3
		26% weight loss; euthanized	4
	19-0044	Low pitched vocalization/purring/rattling	0-2
		Perianal staining	1
		Orange urine stain	2
		Found dead	3
	19-0009	Perianal staining	1
		Slightly hunched	1
		Dark feces	2
		Loose stool	2-3, 5
		Spit up <10% of compound during dosing; slight gasp, wheeze, briefly labored breathing	4
		Wheeze	5
		Lost 13.5% body weight in 1 day (24.8% overall); euthanized	5
	19-0010	Perianal staining	1, 4-5, 7
		Slightly hunched	1
		Dark feces	2, 5-8
		Loose stool	2, 6
		Ruffled fur	4
		Low pitched vocalization/purring/rattling	7
		Slight wheeze	8
		Hunched; 5% weight loss; euthanized	9
1000 mg/kg	19-0051	Orange urine stain	1
		Runny/mucusy stool	1
		Very runny/mucusy stool	2
		Dark feces	2
		Lethargic	2
		22% weight loss; euthanized	3
	19-0052	Orange urine stain	1
		Runny/mucusy stool	1-2
		Slightly lethargic	2
		Lethargic, ruffled fur; euthanized	3
	19-0023	Very lethargic, hunched; euthanized	2
	19-0024	Hunched, ruffled fur, 15% weight loss; euthanized	2

Table E-2. Daily Clinical Observations during Subacute Dosing of Male Rats (cont.)

Dose Group	Animal ID	Observation	Day(s) Observed
1000 mg/kg (cont.)	19-0027	Lethargic, hunched, wobbly gait; found dead	2
	19-0028	Orange urine stain	1
		Lethargic; euthanized	2
	19-0033	Found dead	1
	19-0034	Hunched, ruffled fur, 12% weight loss; euthanized	1
	19-0063	Gasping and liquid around mouth-first observed 30 min after dosing	0
		Found dead	1
	19-0064	Very loose/mucusy stool; euthanized	1
2000 mg/kg	19-0017	Slightly Lethargic	0
		Slightly hunched	1
		Dark feces	1-2
		Red urine stain	1
		Red staining around mouth	2
		Hunched	2
		Very loose stool	2
		25% weight loss; euthanized	3
	19-0018	Slightly hunched	1
		Low pitched vocalization/purring/rattling	1-2
		Lethargic	2
		Dark, runny/mucusy stool	2
		Found dead	3
	19-0029	Orange/red urine stain	1
		Very loose/mucusy stool	1
		Lethargic, hunched, 18% weight loss; euthanized	2
	19-0030	Found dead	1
	19-0013	Slightly lethargic	1
		Lethargic, hunched, labored breathing; found dead	2
	19-0014	Slightly lethargic	1
		Found dead	2
	19-0037	Low pitched vocalization/purring/rattling; hunched, 10% weight loss; euthanized	1
	19-0038	Low pitched vocalization/purring	0-1
		Hunched, 13% weight loss; euthanized	1
	19-0007	Low pitched vocalization/purring, staining around eyes, hunched, 15% weight loss; euthanized	1
	19-0008	Hunched, ruffled fur, labored breathing, 13% weight loss; euthanized	1

Note: Animals with no remarkable observations, a.k.a "normal," were not noted. Occasionally, small amounts (<10%) of compound/corn oil flowed out of the mouth during dosing. This was not considered a clinical sign unless the rat aspirated some of the compound.

APPENDIX F

INDIVIDUAL AND SUMMARY OF BODY MASS DATA

Table F-1. Individual and Mean Body Weights of Female Rats (grams)

Dose Group	Animal ID	Day 0	Day 3	Day 7	Day 13	Day 14[1]
0 mg/kg	19-0125	245.1	265.0	284.4	298.9	289.9
	19-0126	209.7	228.6	243.7	262.4	248.7
	19-0075	185.0	207.2	215.3	234.6	219.0
	19-0076	214.3	220.2	231.9	260.6	241.3
	19-0115	214.3	219.2	236.0	258.2	245.9
	19-0116	199.6	212.9	227.6	242.3	228.7
	19-0107	197.6	201.8	219.3	230.2	223.5
	19-0108	205.8	212.9	221.2	239.9	230.2
	19-0091	203.6	214.9	227.9	248.2	234.0
	19-0092	196.1	209.0	221.2	227.4	222.1
	Mean	**207.1**	**219.2**	**232.9**	**250.3**	**238.3**
	SD	**16.1**	**17.8**	**20.0**	**21.2**	**20.7**
62.5 mg/kg	19-0071	206.9	207.6	222.1	247.4	236.9
	19-0072	239.6	226.7	259.2	297.7	277.4
	19-0105	219.1	227.1	234.3	252.7	240.7
	19-0106	217.4	225.5	237.4	243.4	238.0
	19-0073	193.3	192.9	203.1	232.7	214.9
	19-0074	226.3	230.5	249.9	276.7	265.2
	19-0119	206.0	216.9	226.0	243.1	231.1
	19-0120	203.8	212.2	225.1	243.0	228.0
	19-0117	187.6	191.1	205.1	231.3	216.6
	19-0118	210.6	209.2	222.9	248.0	235.4
	Mean	**211.1**	**214.0**	**228.5**	**251.6**	**238.4**
	SD	**15.3**	**14.1**	**17.6**	**20.5**	**19.6**
125 mg/kg	19-0101	208.8	224.0	240.1	259.8	246.7
	19-0102	227.0	233.2	255.7	282.8	272.0
	19-0095	164.2	180.9	196.9	207.4	199.0
	19-0096	251.4	253.2	267.8	296.4	280.0
	19-0079	221.1	235.9	254.5	278.1	255.8
	19-0080	200.5	207.1	215.4	234.0	220.2
	19-0081	217.9	221.6	241.5	251.2	237.4
	19-0082	192.4	206.7	221.7	232.6	222.0
	19-0129	210.9	212.2	223.9	244.4	224.7
	19-0130	208.2	213.1	219.6	244.2	231.3
	Mean	**210.2**	**218.8**	**233.7**	**253.1**	**238.9**
	SD	**22.8**	**19.8**	**21.9**	**26.8**	**25.0**
250 mg/kg	19-0121	238.8	251.4	281.4	321.7	296.5
	19-0122	199.9	195.8	215.8	239.0	231.9
	19-0113	215.9	217.7	243.3	260.3	245.0
	19-0114	199.2	201.9	211.5	ND	ND
	19-0099	216.4	220.7	195.0	ND	ND
	19-0100	191.9	206.3	219.9	236.6	223.9
	19-0089	205.6	202.7	211.5	245.2	237.7
	19-0090	211.3	214.5	214.8	243.3	229.5
	19-0127	203.1	207.8	228.4	244.1	237.5
	19-0128	207.3	168.6	ND	ND	ND
	Mean	**208.9**	**208.7**	**224.6**	**255.7**	**243.1**
	SD	**13.0**	**20.9**	**25.0**	**30.1**	**24.5**
500 mg/kg	19-0111	217.4	188.1	ND	ND	ND
	19-0112	221.9	221.6	237.9	276.7	264.2
	19-0093	209.9	204.2	214.6	262.7	247.4
	19-0094	204.5	203.0	214.7	246.8	232.0
	19-0077	209.7	202.0	207.3	242.0	231.2
	19-0078	198.5	203.1	215.3	250.6	242.9
	19-0123	180.7	181.6	189.6	217.5	214.0
	19-0124	226.1	228.8	189.2	ND	ND
	19-0083	195.7	195.6	ND	ND	ND
	19-0084	207.3	203.0	214.0	242.7	238.2
	Mean	**207.2**	**203.1**	**210.3**	**248.4**	**238.6**
	SD	**13.4**	**13.9**	**15.7**	**18.4**	**15.6**
1000 mg/kg	19-0085	211.0	193.4	200.3	242.0	235.0
	19-0086	226.8	224.8	233.7	270.8	252.8
	19-0087	216.9	212.6	213.5	255.9	240.3
	19-0088	202.5	181.1	ND	ND	ND
	19-0097	208.8	175.6	ND	ND	ND
	19-0098	201.3	193.6	213.1	219.4	208.0
	19-0103	203.9	204.1	ND	ND	ND
	19-0104	196.2	191.6	184.6	225.3	211.9
	19-0109	194.6	189.2	202.4	232.2	218.8
	19-0110	213.7	174.7	ND	ND	ND
	Mean	**207.6**	**194.1***	**207.9**	**240.9**	**227.8**
	SD	**9.9**	**16.0**	**16.5**	**19.5**	**17.7**

Legend: ND = No data analyzed

Notes:

[1]Fasted overnight.

Data from days 13 and 14 showed slight deviations from normality. All data showed equal variance. Due to deaths, which resulted in missing data, repeated measures ANOVA was not performed. Instead, one-way ANOVAs were conducted for each time point. No effect of dose was observed on days 13 or 14 regardless of whether or not data was log/rank transformed.

*$p<0.05$ based on one-way ANOVA and Dunnett t (2-sided) post hoc test versus controls.

Table F-2. Individual and Mean Body Weights of Male Rats (grams)

Dose Group	Animal ID	Day 0	Day 3	Day 7	Day 13	Day 14[1]	Dose Group	Animal ID	Day 0	Day 3	Day 7	Day 13	Day 14[1]
0 mg/kg	19-0019	258.9	283.2	308.8	355.7	338.1	**500 mg/kg**	19-0067	262.7	201.1	ND	ND	ND
	19-0020	261.4	283.6	318.9	360.5	346.7		19-0068	258.7	247.4	281.0	310.6	270.1
	19-0031	263.7	293.4	327.1	377.9	357.6		19-0025	269.4	249.2	276.6	ND	ND
	19-0032	268.2	288.4	309.8	348.6	338.9		19-0026	256.2	215.9	263.0	314.4	303.5
	19-0061	277.7	306.8	333.7	382.2	369.8		19-0005	274.2	207.1	ND	ND	ND
	19-0062	275.4	309.4	342.4	391.4	373.1		19-0006	283.1	263.8	293.1	340.5	334.1
	19-0011	276.2	306.0	332.4	365.6	356.8		19-0043	287.1	232.0	ND	ND	ND
	19-0012	281.5	313.2	344.1	384.8	380.1		19-0044	306.1	ND	ND	ND	ND
	19-0049	303.4	332.2	374.5	433.3	416.7		19-0009	323.3	275.8	ND	ND	ND
	19-0050	299.1	323.1	348.0	387.1	377.3		19-0010	293.2	264.8	288.1	ND	ND
	Mean	**276.6**	**303.9**	**334.0**	**378.7**	**365.5**		**Mean**	**281.4**	**239.7***	**280.4***	**321.8***	**302.6***
	SD	**15.0**	**16.6**	**19.7**	**24.0**	**23.6**		**SD**	**21.7**	**27.0**	**11.6**	**16.3**	**32.0**
125 mg/kg	19-0045	266.7	267.6	304.0	350.1	335.7	**1000 mg/kg**	19-0051	269.9	211.3	ND	ND	ND
	19-0046	255.8	267.1	293.4	340.0	326.7		19-0052	248.0	210.1	ND	ND	ND
	19-0065	262.7	274.1	299.7	337.5	319.2		19-0023	259.8	ND	ND	ND	ND
	19-0066	269.3	301.3	329.5	361.7	345.3		19-0024	274.4	ND	ND	ND	ND
	19-0047	263.7	285.7	312.8	350.3	339.2		19-0027	290.0	ND	ND	ND	ND
	19-0048	286.7	312.7	350.8	380.7	361.5		19-0028	280.6	ND	ND	ND	ND
	19-0015	279.4	307.2	338.8	372.8	362.6		19-0033	286.2	ND	ND	ND	ND
	19-0016	288.4	322.2	355.4	412.5	395.0		19-0034	306.0	ND	ND	ND	ND
	19-0039	313.7	323.7	366.7	420.3	393.3		19-0063	283.6	ND	ND	ND	ND
	19-0040	296.6	320.9	353.7	401.4	389.0		19-0064	312.2	ND	ND	ND	ND
	Mean	**278.3**	**298.3**	**330.5**	**372.7**	**356.8**		**Mean**	**281.1**	**210.7***	**ND**	**ND**	**ND**
	SD	**18.1**	**22.8**	**26.4**	**30.1**	**28.1**		**SD**	**19.5**	**0.8**	**ND**	**ND**	**ND**
250 mg/kg	19-0041	255.4	247.2	280.0	330.6	312.7	**2000 mg/kg**	19-0017	259.6	196.0	ND	ND	ND
	19-0042	267.4	266.1	295.3	345.2	329.5		19-0018	266.5	ND	ND	ND	ND
	19-0059	265.0	267.9	306.9	361.4	343.7		19-0029	261.6	ND	ND	ND	ND
	19-0060	265.0	277.6	310.1	356.0	340.3		19-0030	274.9	ND	ND	ND	ND
	19-0053	286.8	284.3	309.2	332.1	322.2		19-0013	265.6	ND	ND	ND	ND
	19-0054	276.1	280.8	308.5	368.7	347.3		19-0014	287.4	ND	ND	ND	ND
	19-0021	315.9	313.0	338.2	392.8	348.7		19-0037	283.6	ND	ND	ND	ND
	19-0022	286.8	299.2	287.6	342.5	323.9		19-0038	292.1	ND	ND	ND	ND
	19-0055	312.4	308.8	345.6	391.3	369.2		19-0007	324.4	ND	ND	ND	ND
	19-0056	302.6	298.8	332.5	380.9	368.8		19-0008	292.4	ND	ND	ND	ND
	Mean	**283.3**	**284.4**	**311.4**	**360.2**	**340.6**		**Mean**	**280.8**	**196.0**	**ND**	**ND**	**ND**
	SD	**21.2**	**20.8**	**21.6**	**23.0**	**19.0**		**SD**	**19.7**	**ND**	**ND**	**ND**	**ND**

Legend: ND = No data analyzed
Note:
[1]Fasted overnight.
Data showed a normal distribution and equal variance. Due to deaths, which resulted in missing data, repeated measures ANOVA was not performed. Instead, one-way ANOVAs were conducted for each time point.
*p<0.05 based on one-way ANOVA and Dunnett t (2-sided) post hoc test versus controls.

APPENDIX G

INDIVIDUAL AND SUMMARY OF BODY MASS CHANGE DATA

Table G-1. Individual and Mean Body Mass Changes in Females (grams)

Dose Group	Animal ID	Day 0-3	Day 3-7	Day 7-13	Day 0-13	Dose Group	Animal ID	Day 0-3	Day 3-7	Day 7-13	Day 0-13
0 mg/kg	19-0125	19.9	19.4	14.5	53.8	**250 mg/kg**	19-0121	12.6	30.0	40.3	82.9
	19-0126	18.9	15.1	18.7	52.7		19-0122	-4.1	20.0	23.2	39.1
	19-0075	22.2	8.1	19.3	49.6		19-0113	1.8	25.6	17.0	44.4
	19-0076	5.9	11.7	28.7	46.3		19-0114	2.7	9.6	ND	ND
	19-0115	4.9	16.8	22.2	43.9		19-0099	4.3	-25.7	ND	ND
	19-0116	13.3	14.7	14.7	42.7		19-0100	14.4	13.6	16.7	44.7
	19-0107	4.2	17.5	10.9	32.6		19-0089	-2.9	8.8	33.7	39.6
	19-0108	7.1	8.3	18.7	34.1		19-0090	3.2	0.3	28.5	32.0
	19-0091	11.3	13.0	20.3	44.6		19-0127	4.7	20.6	15.7	41.0
	19-0092	12.9	12.2	6.2	31.3		19-0128	-38.7	ND	ND	ND
	Mean	**12.1**	**13.7**	**17.4**	**43.2**		**Mean**	**-0.2***	**11.4**	**25.0**	**46.2**
	SD	**6.6**	**3.8**	**6.2**	**8.1**		**SD**	**14.7**	**16.7**	**9.5**	**16.7**
62.5 mg/kg	19-0071	0.7	14.5	25.3	40.5	**500 mg/kg**	19-0111	-29.3	ND	ND	ND
	19-0072	-12.9	32.5	38.5	58.1		19-0112	-0.3	16.3	38.8	54.8
	19-0105	8.0	7.2	18.4	33.6		19-0093	-5.7	10.4	48.1	52.8
	19-0106	8.1	11.9	6.0	26.0		19-0094	-1.5	11.7	32.1	42.3
	19-0073	-0.4	10.2	29.6	39.4		19-0077	-7.7	5.3	34.7	32.3
	19-0074	4.2	19.4	26.8	50.4		19-0078	4.6	12.2	35.3	52.1
	19-0119	10.9	9.1	17.1	37.1		19-0123	0.9	8.0	27.9	36.8
	19-0120	8.4	12.9	17.9	39.2		19-0124	2.7	-39.6	ND	ND
	19-0117	3.5	14.0	26.2	43.7		19-0083	-0.1	ND	ND	ND
	19-0118	-1.4	13.7	25.1	37.4		19-0084	-4.3	11.0	28.7	35.4
	Mean	**2.9***	**14.5**	**23.1**	**40.5**		**Mean**	**-4.1***	**4.4**	**35.1***	**43.8**
	SD	**6.9**	**7.1**	**8.8**	**8.8**		**SD**	**9.6**	**18.1**	**6.9**	**9.4**
125 mg/kg	19-0101	15.2	16.1	19.7	51.0	**1000 mg/kg**	19-0085	-17.6	6.9	41.7	31.0
	19-0102	6.2	22.5	27.1	55.8		19-0086	-2.0	8.9	37.1	44.0
	19-0095	16.7	16.0	10.5	43.2		19-0087	-4.3	0.9	42.4	39.0
	19-0096	1.8	14.6	28.6	45.0		19-0088	-21.4	ND	ND	ND
	19-0079	14.8	18.6	23.6	57.0		19-0097	-33.2	ND	ND	ND
	19-0080	6.6	8.3	18.6	33.5		19-0098	-7.7	19.5	6.3	18.1
	19-0081	3.7	19.9	9.7	33.3		19-0103	0.2	ND	ND	ND
	19-0082	14.3	15.0	10.9	40.2		19-0104	-4.6	-7.0	40.7	29.1
	19-0129	1.3	11.7	20.5	33.5		19-0109	-5.4	13.2	29.8	37.6
	19-0130	4.9	6.5	24.6	36.0		19-0110	-39.0	ND	ND	ND
	Mean	**8.5**	**14.9**	**19.4**	**42.9**		**Mean**	**-13.5***	**7.1**	**33.0***	**33.1**
	SD	**6.0**	**5.0**	**7.0**	**9.2**		**SD**	**13.7**	**9.3**	**13.9**	**9.2**

Legend: ND = No data analyzed
Note: Data was rank-transformed to achieve a normal distribution. Due to deaths, which resulted in missing data, repeated measures ANOVA was not performed. Instead, one-way ANOVAs were conducted for each time point.
*p<0.05 based on one-way ANOVA and Dunnett t (2-sided) post hoc test versus controls.

Table G-2. Individual and Mean Body Mass Changes in Males (grams)

Dose Group	Animal ID	Day 0-3	Day 3-7	Day 7-13	Day 0-13	Dose Group	Animal ID	Day 0-3	Day 3-7	Day 7-13	Day 0-13
0 mg/kg	19-0019	24.3	25.6	46.9	96.8	**500 mg/kg**	19-0067	-61.6	ND	ND	ND
	19-0020	22.2	35.3	41.6	99.1		19-0068	-11.3	33.6	29.6	51.9
	19-0031	29.7	33.7	50.8	114.2		19-0025	-20.2	27.4	ND	ND
	19-0032	20.2	21.4	38.8	80.4		19-0026	-40.3	47.1	51.4	58.2
	19-0061	29.1	26.9	48.5	104.5		19-0005	-67.1	ND	ND	ND
	19-0062	34.0	33.0	49.0	116.0		19-0006	-19.3	29.3	47.4	57.4
	19-0011	29.8	26.4	33.2	89.4		19-0043	-55.1	ND	ND	ND
	19-0012	31.7	30.9	40.7	103.3		19-0044	ND	ND	ND	ND
	19-0049	28.8	42.3	58.8	129.9		19-0009	-47.5	ND	ND	ND
	19-0050	24.0	24.9	39.1	88.0		19-0010	-28.4	23.3	ND	ND
	Mean	**27.4**	**30.0**	**44.7**	**102.2**		**Mean**	**-39.0***	**32.1**	**42.8**	**55.8***
	SD	**4.4**	**6.2**	**7.4**	**14.9**		**SD**	**20.2**	**9.1**	**11.6**	**3.4**
125 mg/kg	19-0045	0.9	36.4	46.1	83.4	**1000 mg/kg**	19-0051	-58.6	ND	ND	ND
	19-0046	11.3	26.3	46.6	84.2		19-0052	-37.9	ND	ND	ND
	19-0065	11.4	25.6	37.8	74.8		19-0023	ND	ND	ND	ND
	19-0066	32.0	28.2	32.2	92.4		19-0024	ND	ND	ND	ND
	19-0047	22.0	27.1	37.5	86.6		19-0027	ND	ND	ND	ND
	19-0048	26.0	38.1	29.9	94.0		19-0028	ND	ND	ND	ND
	19-0015	27.8	31.6	34.0	93.4		19-0033	ND	ND	ND	ND
	19-0016	33.8	33.2	57.1	124.1		19-0034	ND	ND	ND	ND
	19-0039	10.0	43.0	53.6	106.6		19-0063	ND	ND	ND	ND
	19-0040	24.3	32.8	47.7	104.8		19-0064	ND	ND	ND	ND
	Mean	**20.0**	**32.2**	**42.3**	**94.4**		**Mean**	**-48.3***	ND	ND	ND
	SD	**10.9**	**5.7**	**9.3**	**14.2**		**SD**	**14.6**	ND	ND	ND
250 mg/kg	19-0041	-8.2	32.8	50.6	75.2	**2000 mg/kg**	19-0017	-63.6	ND	ND	ND
	19-0042	-1.3	29.2	49.9	77.8		19-0018	ND	ND	ND	ND
	19-0059	2.9	39.0	54.5	96.4		19-0029	ND	ND	ND	ND
	19-0060	12.6	32.5	45.9	91.0		19-0030	ND	ND	ND	ND
	19-0053	-2.5	24.9	22.9	45.3		19-0013	ND	ND	ND	ND
	19-0054	4.7	27.7	60.2	92.6		19-0014	ND	ND	ND	ND
	19-0021	-2.9	25.2	54.6	76.9		19-0037	ND	ND	ND	ND
	19-0022	12.4	-11.6	54.9	55.7		19-0038	ND	ND	ND	ND
	19-0055	-3.6	36.8	45.7	78.9		19-0007	ND	ND	ND	ND
	19-0056	-3.8	33.7	48.4	78.3		19-0008	ND	ND	ND	ND
	Mean	**1.0***	**27.0**	**48.8**	**76.8***		**Mean**	**-63.6**	ND	ND	ND
	SD	**7.0**	**14.3**	**10.1**	**15.9**		**SD**	ND	ND	ND	ND

Legend: ND = No data analyzed

Notes: Data was rank-transformed to achieve a normal distribution. Due to deaths, which resulted in missing data, one-way ANOVAs were conducted for each time point. If equal error variances were detected by a Levene's test, Dunnett t (2-sided) post hoc test was performed; if error variances were not equal, Dunnett T3 post hoc test was performed.

*p<0.05 based on ANOVA and appropriate post hoc test versus controls.

APPENDIX H

INDIVIDUAL AND SUMMARY OF FOOD CONSUMPTION

Table H-1. Paired Food Consumption (grams per rat per day) for Female Rats

Dose Group	Animal Pair ID	Day 0-3	Day 3-7	Day 7-13	Day 0-13
0 mg/kg	19-0125/0126	21.4	21.3	20.3	20.9
	19-0075/0076	18.0	16.9	17.0	17.2
	19-0115/0116	17.5	18.1	17.8	17.8
	19-0107/0108	15.5	17.4	16.1	16.3
	19-0091/0092	16.2	17.1	16.8	16.8
	Mean	17.7	18.1	17.6	17.8
	SD	2.3	1.8	1.6	1.8
62.5 mg/kg	19-0071/0072	15.2	17.8	22.1	19.2
	19-0105/0106	15.9	17.6	17.3	17.0
	19-0073/0074	16.8	18.6	18.7	18.2
	19-0119/0120	15.8	17.1	17.5	16.9
	19-0117/0118	15.4	17.7	17.3	17.0
	Mean	15.8	17.7	18.6	17.7
	SD	0.6	0.6	2.1	1.0
125 mg/kg	19-0101/0102	18.8	19.6	19.9	19.6
	19-0095/0096	16.9	19.1	17.7	17.9
	19-0079/0080	13.8	17.4	17.8	16.8
	19-0081/0082	16.5	18.5	17.9	17.8
	19-0129/0130	13.9	16.3	16.4	15.8
	Mean	16.0	18.2	17.9	17.6
	SD	2.1	1.3	1.3	1.4
250 mg/kg	19-0121/0122	15.0	18.3	24.1	20.2
	19-0113/0114	12.6	17.9	18.6	16.7
	19-0099/0100	13.0	15.5	17.7	15.4
	19-0089/0090	10.8	15.2	21.1	16.9
	19-0127/0128	7.0	18.0	21.9	15.3
	Mean	11.7*	17.0	20.6	16.9
	SD	3.0	1.5	2.6	2.0
500 mg/kg	19-0111/0112	7.8	13.5	25.3	15.7
	19-0093/0094	9.2	11.1	24.9	17.0
	19-0077/0078	9.2	14.8	25.8	18.6
	19-0123/0124	9.9	10.1	21.0	13.3
	19-0083/0084	8.4	12.5	23.1	14.7
	Mean	8.9*	12.4	24.0	15.9*
	SD	0.8	1.9	2.0	2.1
1000 mg/kg	19-0085/0086	9.3	14.8	25.8	18.6
	19-0087/0088	8.6	10.4	24.5	14.7
	19-0097/0098	5.4	19.0	19.6	14.2
	19-0103/0104	8.0	8.0	20.3	11.9
	19-0109/0110	5.3	17.9	21.2	14.4
	Mean	7.3*	14.0	22.3	14.8*
	SD	1.8	4.7	2.7	2.4

Notes: Food consumption was normalized per rat-days within each interval to account for deaths that occurred during the study, assuming equal consumption by paired rats. Data was rank-transformed to achieve a normal distribution.
*p<0.05 based on one-way ANOVA and Dunnett t (2-sided) post hoc test versus controls.

Table H-2. Paired Food Consumption (grams per rat per day) for Male Rats

Dose Group	Animal Pair ID	Day 0-3	Day 3-7	Day 7-13	Day 0-13
0 mg/kg	19-0019/0020	25.6	25.9	25.2	25.5
	19-0031/0032	25.1	26.0	24.2	25.0
	19-0061/0062	25.3	27.2	26.9	26.6
	19-0011/0012	24.8	26.0	23.2	24.4
	19-0049/0050	27.3	27.9	25.5	26.7
	Mean	25.6	26.6	25.0	25.6
	SD	1.0	0.9	1.4	1.0
125 mg/kg	19-0045/0046	17.2	24.3	23.1	22.1
	19-0065/0066	22.7	25.8	23.1	23.9
	19-0047/0048	22.7	25.5	21.9	23.2
	19-0015/0016	25.5	27.5	26.2	26.4
	19-0039/0040	21.2	29.5	26.7	26.3
	Mean	21.8*	26.5	24.2	24.4
	SD	3.0	2.0	2.1	1.9
250 mg/kg	19-0041/0042	13.7	23.8	25.3	22.1
	19-0059/0060	17.9	27.1	26.2	24.6
	19-0053/0054	13.7	24.5	25.6	22.5
	19-0021/0022	16.8	18.5	28.2	22.6
	19-0055/0056	14.0	28.6	27.4	24.7
	Mean	15.2*	24.5	26.5	23.3*
	SD	2.0	3.9	1.3	1.2
500 mg/kg	19-0067/0068	5.5	24.6	20.9	16.0
	19-0025/0026	6.1	23.9	22.2	18.6
	19-0005/0006	4.5	17.5	27.3	16.4
	19-0043/0044	2.3	ND	ND	ND
	19-0009/0010	5.8	18.8	ND	ND
	Mean	4.8*	21.2	23.5	17.0*
	SD	1.6	3.6	3.4	1.4
1000 mg/kg	19-0051/0052	3.4	ND	ND	ND
	19-0023/0024	ND	ND	ND	ND
	19-0027/0028	ND	ND	ND	ND
	19-0033/0034	ND	ND	ND	ND
	19-0063/0064	ND	ND	ND	ND
	Mean	3.4	ND	ND	ND
	SD	ND	ND	ND	ND
2000 mg/kg	19-0017/0018	1.8	ND	ND	ND
	19-0029/0030	ND	ND	ND	ND
	19-0013/0014	ND	ND	ND	ND
	19-0037/0038	ND	ND	ND	ND
	19-0007/0008	ND	ND	ND	ND
	Mean	1.8	ND	ND	ND
	SD	ND	ND	ND	ND

Legend: ND = No data analyzed

Notes: Food consumption was normalized per rat-days within each interval to account for deaths that occurred during the study, assuming equal consumption by paired rats. Data was rank-transformed to achieve a normal distribution.

*p<0.05 based on one-way ANOVA and Dunnett t (2-sided) post hoc test versus controls.

APPENDIX I

INDIVIDUAL AND SUMMARY OF FOOD EFFICIENCY DATA

Table I-1. Food Efficiency (weight gain/food consumed) of Paired Female Rats

Dose Group	Animal Pair ID	Day 0-3	Day 3-7	Day 7-13	Day 0-13
0 mg/kg	19-0125/0126	0.302	0.203	0.136	0.196
	19-0075/0076	0.261	0.147	0.235	0.215
	19-0115/0116	0.173	0.217	0.173	0.187
	19-0107/0108	0.122	0.186	0.153	0.157
	19-0091/0092	0.249	0.184	0.132	0.174
	Mean	0.221	0.187	0.166	0.186
	SD	0.073	0.026	0.042	0.022
62.5 mg/kg	19-0071/0072	-0.133	0.330	0.240	0.198
	19-0105/0106	0.169	0.136	0.118	0.135
	19-0073/0074	0.038	0.199	0.251	0.189
	19-0119/0120	0.204	0.161	0.167	0.173
	19-0117/0118	0.023	0.196	0.247	0.184
	Mean	0.060	0.204	0.205	0.176
	SD	0.134	0.075	0.060	0.025
125 mg/kg	19-0101/0102	0.190	0.246	0.196	0.210
	19-0095/0096	0.182	0.201	0.184	0.189
	19-0079/0080	0.259	0.193	0.197	0.208
	19-0081/0082	0.182	0.235	0.096	0.159
	19-0129/0130	0.075	0.139	0.229	0.169
	Mean	0.178	0.203	0.180	0.187
	SD	0.066	0.042	0.050	0.023
250 mg/kg	19-0121/0122	0.094	0.341	0.220	0.232
	19-0113/0114	0.060	0.246	0.114	0.121
	19-0099/0100	0.239	-0.098	0.158	0.145
	19-0089/0090	0.005	0.075	0.246	0.163
	19-0127/0128	-0.804	0.287	0.120	0.167
	Mean	-.081*	0.170	0.172	0.166
	SD	0.413	0.180	0.059	0.041
500 mg/kg	19-0111/0112	-0.630	0.242	0.255	0.206
	19-0093/0094	-0.130	0.248	0.268	0.215
	19-0077/0078	-0.056	0.148	0.226	0.174
	19-0123/0124	0.061	-0.391	0.221	0.138
	19-0083/0084	-0.088	0.147	0.207	0.134
	Mean	-0.169*	0.079	0.236	0.173
	SD	0.267	0.267	0.025	0.037
1000 mg/kg	19-0085/0086	-0.351	0.134	0.255	0.155
	19-0087/0088	-0.498	0.017	0.288	0.156
	19-0097/0098	-1.255	0.256	0.053	0.080
	19-0103/0104	-0.092	-0.124	0.334	0.129
	19-0109/0110	-1.383	0.185	0.234	0.163
	Mean	-0.716*	0.094	0.233	0.136
	SD	0.571	0.150	0.107	0.034

Notes: If an individual rat died within a cage, data for subsequent days was calculated based on the one surviving rat. Data was rank-transformed to achieve a normal distribution.
*$p<0.05$ based on one-way ANOVA and Dunnett t (2-sided) post hoc test versus controls.

Table I-2. Food Efficiency (weight gain/food consumed) of Paired Male Rats

Dose Group	Animal Pair ID	Day 0-3	Day 3-7	Day 7-13	Day 0-13
0 mg/kg	19-0019/0020	0.302	0.294	0.293	0.295
	19-0031/0032	0.332	0.265	0.308	0.300
	19-0061/0062	0.415	0.276	0.303	0.319
	19-0011/0012	0.413	0.276	0.266	0.304
	19-0049/0050	0.323	0.301	0.319	0.314
	Mean	0.357	0.282	0.298	0.306
	SD	0.053	0.015	0.020	0.010
125 mg/kg	19-0045/0046	0.118	0.323	0.334	0.291
	19-0065/0066	0.319	0.260	0.252	0.270
	19-0047/0048	0.352	0.319	0.256	0.299
	19-0015/0016	0.403	0.295	0.290	0.317
	19-0039/0040	0.270	0.321	0.317	0.310
	Mean	0.293	0.304	0.290	0.297
	SD	0.109	0.027	0.036	0.018
250 mg/kg	19-0041/0042	-0.116	0.325	0.331	0.266
	19-0059/0060	0.144	0.329	0.319	0.293
	19-0053/0054	0.027	0.268	0.271	0.236
	19-0021/0022	0.094	0.092	0.323	0.226
	19-0055/0056	-0.088	0.308	0.286	0.245
	Mean	0.012*	0.265	0.306	0.253*
	SD	0.113	0.100	0.026	0.027
500 mg/kg	19-0067/0068	-2.223	0.341	0.236	0.202
	19-0025/0026	-1.644	0.389	0.258	0.136
	19-0005/0006	-3.188	0.279	0.289	0.194
	19-0043/0044	-4.022	ND	ND	ND
	19-0009/0010	-2.175	0.207	ND	ND
	Mean	-2.650*	0.304	0.261	0.177*
	SD	0.948	0.079	0.027	0.036
1000 mg/kg	19-0051/0052	-4.730	ND	ND	ND
	19-0023/0024	ND	ND	ND	ND
	19-0027/0028	ND	ND	ND	ND
	19-0033/0034	ND	ND	ND	ND
	19-0063/0064	ND	ND	ND	ND
	Mean	-4.730	ND	ND	ND
	SD	ND	ND	ND	ND
2000 mg/kg	19-0017/0018	-6.057	ND	ND	ND
	19-0029/0030	ND	ND	ND	ND
	19-0013/0014	ND	ND	ND	ND
	19-0037/0038	ND	ND	ND	ND
	19-0007/0008	ND	ND	ND	ND
	Mean	-6.057	ND	ND	ND
	SD	ND	ND	ND	ND

Legend: ND = No data analyzed

Notes: If an individual rat died within a cage, data for subsequent days was calculated based on the one surviving rat. Data was rank-transformed to achieve a normal distribution.

*p<0.05 based on one-way ANOVA and Dunnett t (2-sided) post hoc test versus controls.

APPENDIX J

INDIVIDUAL AND SUMMARY OF ORGAN MASS AND MASS RATIOS

Table J-1. Individual and Mean Absolute Organ Mass (grams) in Females

Dose Group	Animal ID	Fasted Body Weight	Brain	Kidneys (2)	Adrenals (2)	Liver	Heart	Spleen	Thymus	Ovaries (2)	Uterus
0 mg/kg	19-0125	289.9	2.098	2.290	0.072	10.986	1.754	0.609	0.589	0.174	0.420
	19-0126	248.7	1.992	1.958	0.054	8.627	1.334	0.449	0.560	0.153	0.364
	19-0075	219.0	1.869	1.807	0.050	7.512	0.898	0.417	0.688	0.116	0.353
	19-0076	241.3	1.932	2.067	0.064	9.335	1.069	0.572	0.562	0.136	0.556
	19-0115	245.9	1.945	1.714	0.060	8.454	1.053	0.482	1.772	0.110	0.762
	19-0116	228.7	1.846	1.709	0.062	8.601	1.031	0.493	0.650	0.101	0.666
	19-0107	223.5	1.868	1.646	0.050	8.327	0.967	0.551	0.504	0.117	0.291
	19-0108	230.2	1.922	2.043	0.076	9.214	0.842	0.640	0.473	0.156	0.429
	19-0091	234.0	1.960	1.890	0.059	8.519	1.037	0.728	0.707	0.112	0.468
	19-0092	222.1	1.926	2.102	0.058	8.470	0.921	0.549	0.627	0.147	0.372
	Mean	238.3	1.936	1.923	0.061	8.805	1.091	0.549	0.713	0.132	0.468
	SD	20.7	0.073	0.207	0.009	0.913	0.269	0.094	0.380	0.024	0.150
62.5 mg/kg	19-0071	236.9	1.936	1.750	0.067	9.557	1.017	0.575	0.518	0.140	0.555
	19-0072	277.4	1.932	1.961	0.055	11.965	1.130	0.577	0.986	0.142	0.652
	19-0105	240.7	1.958	1.789	0.070	8.758	0.918	0.521	0.669	0.133	0.533
	19-0106	238.0	1.977	1.944	0.060	9.707	1.196	0.610	0.475	0.098	0.333
	19-0073	214.9	1.791	1.618	0.059	8.300	0.921	0.424	0.580	0.124	0.618
	19-0074	265.2	1.841	1.917	0.066	10.254	1.008	0.543	0.532	0.119	0.539
	19-0119	231.1	1.991	1.915	0.055	8.841	0.992	0.502	0.586	0.116	0.279
	19-0120	228.0	1.847	1.972	0.070	9.263	0.938	0.636	0.450	0.108	0.513
	19-0117	216.6	1.885	1.802	0.055	8.841	0.811	0.665	0.435	0.114	0.664
	19-0118	235.4	1.994	1.789	0.067	9.405	0.978	0.595	0.710	0.167	0.490
	Mean	238.4	1.915	1.846	0.062	9.489*	0.991	0.565	0.594	0.126	0.518
	SD	19.6	0.071	0.115	0.006	1.034	0.110	0.070	0.164	0.020	0.126
125 mg/kg	19-0101	246.7	1.988	1.976	0.067	8.580	1.012	1.156	0.532	0.134	0.305
	19-0102	272.0	1.997	2.034	0.055	10.164	1.193	0.898	0.575	0.135	0.802
	19-0095	199.0	1.848	1.779	0.065	8.440	0.748	0.576	0.457	0.122	1.010
	19-0096	280.0	2.046	2.058	0.083	9.757	1.114	0.984	0.682	0.130	0.287
	19-0079	255.8	2.027	2.023	0.078	10.756	1.024	0.794	0.711	0.130	0.384
	19-0080	220.2	1.933	1.609	0.060	8.555	0.818	0.690	0.475	0.103	0.267
	19-0081	237.4	1.865	1.929	0.058	9.472	1.048	0.497	0.637	0.140	0.498
	19-0082	222.0	1.840	1.796	0.067	9.759	0.911	0.712	0.572	0.106	0.381
	19-0129	224.7	1.912	1.869	0.078	8.929	1.021	0.864	0.681	0.146	0.606
	19-0130	231.3	1.987	1.894	0.050	10.152	0.929	0.877	0.501	0.146	0.439
	Mean	238.9	1.944	1.897	0.066	9.456*	0.982	0.805*	0.582	0.129	0.498
	SD	25.0	0.076	0.140	0.011	0.799	0.133	0.195	0.092	0.015	0.243
250 mg/kg	19-0121	296.5	1.921	2.651	0.078	13.432	1.321	1.517	0.622	0.161	0.554
	19-0122	231.9	1.807	2.111	0.049	9.629	0.983	1.191	0.552	0.186	0.382
	19-0113	245.0	1.917	2.580	0.096	11.170	1.058	1.056	0.864	0.174	0.461
	19-0114†	ND	ND	ND	ND	ND	ND	0.874†	ND	ND	ND
	19-0099†	ND	ND	ND	ND	ND	ND	1.253†	ND	ND	ND
	19-0100	223.9	1.830	1.995	0.062	8.978	1.094	0.939	0.480	0.135	0.392
	19-0089	237.7	2.011	2.699	0.051	11.223	0.956	1.629	0.468	0.123	0.296
	19-0090	229.5	1.824	1.999	0.074	8.941	0.935	1.147	0.516	0.132	0.407
	19-0127	237.5	1.929	2.243	0.073	10.116	1.042	0.843	0.577	0.155	0.362
	19-0128	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	243.1	1.891	2.325*	0.069	10.498*	1.056	1.189*	0.583	0.152	0.408
	SD	24.5	0.074	0.311	0.016	1.593	0.130	0.289	0.135	0.023	0.081

Table J-1. Individual and Mean Absolute Organ Mass (grams) in Females (cont.)

Dose Group	Animal ID	Fasted Body Weight	Brain	Kidneys (2)	Adrenals (2)	Liver	Heart	Spleen	Thymus	Ovaries (2)	Uterus
500 mg/kg	19-0111	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0112	264.2	1.871	2.377	0.085	12.410	1.087	2.438	0.541	0.132	0.298
	19-0093	247.4	1.726	2.225	0.057	11.394	1.082	1.585	0.589	0.118	0.413
	19-0094	232.0	1.793	2.434	0.063	11.015	1.135	2.517	0.720	0.136	0.427
	19-0077	231.2	1.869	2.359	0.074	11.281	1.201	1.448	0.441	0.153	0.312
	19-0078	242.9	1.860	2.444	0.063	13.075	1.260	1.947	0.642	0.149	0.338
	19-0123	214.0	1.756	2.024	0.055	11.073	0.900	2.128	0.451	0.127	0.364
	19-0124	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0083	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0084	238.2	1.860	1.942	0.063	10.901	0.957	1.745	0.567	0.138	0.362
	Mean	238.6	1.819*	2.258*	0.066	11.593*	1.089	1.973*	0.564	0.136	0.359
	SD	15.6	0.060	0.202	0.010	0.825	0.127	0.411	0.100	0.012	0.048
1000 mg/kg	19-0085	235.0	1.821	2.177	0.070	12.550	0.984	2.141	0.603	0.120	0.769
	19-0086	252.8	1.867	2.577	0.067	12.313	1.108	3.351	0.622	0.157	0.730
	19-0087	240.3	1.782	3.035	0.062	12.053	1.088	2.032	0.430	0.153	0.590
	19-0088	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0097	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0098	208.0	1.803	2.050	0.058	10.425	0.905	1.433	0.391	0.148	0.295
	19-0103	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0104	211.9	1.812	1.962	0.064	11.051	1.008	2.438	0.344	0.088	0.236
	19-0109	218.8	1.802	2.084	0.082	11.306	0.984	2.584	0.451	0.112	0.263
	19-0110	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	227.8	1.815*	2.314*	0.067	11.616*	1.013	2.330*	0.474	0.130	0.481
	SD	17.7	0.029	0.413	0.008	0.823	0.075	0.640	0.114	0.027	0.245

Legend: ND = No data analyzed

Notes:

*p<0.05 using ANCOVA with body weight as the covariate, followed by pairwise comparisons versus the control group when a significant main effect was observed. Values were log-or rank-transformed in order to achieve normal distribution of data.

†Data collected on rats that were euthanized prior to the end of the study were listed in this summary but were not included in the calculations of mean, SD, or statistical significance.

Table J-2. Individual and Mean Absolute Organ Mass (grams) in Males

Dose Group	Animal ID	Fasted Body Weight	Brain	Kidneys (2)	Adrenals (2)	Liver	Heart	Spleen	Thymus	Testes (2)	Epididymides
0 mg/kg	19-0019	338.1	1.972	2.996	0.056	14.862	1.557	0.663	0.658	3.320	0.749
	19-0020	346.7	1.951	2.745	0.057	13.699	1.320	0.661	0.717	3.023	0.703
	19-0031	357.6	1.973	2.859	0.052	13.727	1.320	0.584	0.640	3.049	0.651
	19-0032	338.9	2.160	2.608	0.048	11.974	1.465	0.619	0.591	3.139	0.632
	19-0061	369.8	2.054	2.721	0.059	14.619	1.301	0.704	0.581	3.450	0.795
	19-0062	373.1	2.030	2.739	0.065	14.722	1.403	0.655	0.784	3.104	0.649
	19-0011	356.8	2.160	2.886	0.037	12.914	1.306	0.560	0.625	2.929	0.616
	19-0012	380.1	2.039	2.685	0.041	15.486	1.526	0.721	0.550	3.363	0.652
	19-0049	416.7	2.063	2.961	0.064	15.786	1.486	0.631	0.700	2.785	0.662
	19-0050	377.3	2.099	3.368	0.049	15.177	1.487	0.675	0.627	3.595	0.665
	Mean	365.5	2.050	2.857	0.053	14.297	1.417	0.647	0.647	3.176	0.677
	SD	23.6	0.074	0.218	0.009	1.202	0.099	0.050	0.070	0.251	0.056
125 mg/kg	19-0045	335.7	2.015	3.338	0.061	12.166	1.329	0.941	0.764	2.708	0.486
	19-0046	326.7	2.047	2.850	0.052	12.856	1.323	0.768	0.480	3.469	0.745
	19-0065	319.2	1.966	2.692	0.063	13.337	1.396	0.762	0.651	3.108	0.793
	19-0066	345.3	2.055	3.002	0.060	13.164	1.399	0.737	0.663	3.257	0.744
	19-0047	339.2	2.089	2.761	0.047	13.188	1.367	0.804	0.761	3.594	0.748
	19-0048	361.5	1.970	2.723	0.059	14.524	1.339	0.888	0.640	3.025	0.629
	19-0015	362.6	2.175	3.139	0.065	14.041	1.257	0.704	0.560	3.046	0.666
	19-0016	395.0	2.147	3.071	0.048	15.270	1.478	0.808	0.897	3.527	0.624
	19-0039	393.3	2.212	3.278	0.052	16.676	1.756	1.428	0.731	3.180	0.700
	19-0040	389.0	1.859	3.069	0.063	15.113	1.386	1.197	0.673	2.768	0.587
	Mean	356.8	2.054	2.992*	0.057	14.034	1.403	0.904*	0.682	3.168	0.672
	SD	28.1	0.107	0.229	0.007	1.364	0.137	0.233	0.116	0.302	0.093
250 mg/kg	19-0041	312.7	1.927	3.021	0.083	12.305	1.150	1.399	0.470	2.673	0.636
	19-0042	329.5	2.124	3.153	0.068	13.502	1.280	1.390	0.532	3.385	0.654
	19-0059	343.7	1.820	3.243	0.059	13.654	1.257	1.439	0.575	2.841	0.748
	19-0060	340.3	2.031	3.140	0.069	14.935	1.468	1.362	0.571	3.139	0.746
	19-0053	322.2	1.990	2.899	0.056	12.595	1.316	1.088	0.504	3.411	0.669
	19-0054	347.3	2.072	3.138	0.063	14.307	1.379	1.320	0.695	2.780	0.655
	19-0021	348.7	2.087	3.015	0.069	14.725	1.432	1.691	0.546	3.233	0.745
	19-0022	323.9	1.908	3.012	0.075	14.046	1.327	1.200	0.512	3.639	0.778
	19-0055	369.2	2.056	3.364	0.073	16.079	1.665	1.426	0.832	3.727	0.741
	19-0056	368.8	2.113	3.161	0.062	14.785	1.462	1.362	0.540	2.893	0.633
	Mean	340.6	2.013	3.115*	0.068*	14.093*	1.374	1.368*	0.578	3.172	0.701
	SD	19.0	0.100	0.133	0.008	1.133	0.143	0.157	0.108	0.369	0.056
500 mg/kg	19-0067	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0068	270.1	2.020	2.591	0.082	13.029	1.232	1.603	0.359	2.635	0.496
	19-0025†	ND	2.050†	2.363†	0.076†	11.975†	1.471†	1.156†	0.246†	2.679†	0.466†
	19-0026	303.5	1.908	2.808	0.060	13.422	1.208	2.629	0.473	3.012	0.586
	19-0005	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0006	334.1	2.045	3.286	0.081	15.213	1.405	1.765	0.419	3.087	0.516
	19-0043†	ND	1.936†	2.815†	0.117†	11.453†	0.860†	0.234†	0.185†	2.925†	0.459†
	19-0044	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0009†	ND	2.036†	2.764†	0.068†	12.061†	1.036†	0.448†	0.105†	2.486†	0.404†
	19-0010†	ND	ND	ND	ND	ND	ND	2.310†	ND	ND	ND
	Mean	302.6	1.991	2.895*	0.074*	13.888*	1.282	1.999*	0.417	2.911	0.533*
	SD	32.0	0.073	0.356	0.012	1.164	0.107	0.552	0.057	0.242	0.047

Table J-2. Individual and Mean Absolute Organ Mass (grams) in Males (cont.)

Dose Group	Animal ID	Fasted Body Weight	Brain	Kidneys (2)	Adrenals (2)	Liver	Heart	Spleen	Thymus	Testes (2)	Epididymides
1000 mg/kg	19-0051	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0052	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0023	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0024	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0027	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0028	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0033	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0034	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0063	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0064	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	SD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2000 mg/kg	19-0017	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0018	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0029	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0030	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0013	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0014	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0037	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0038	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0007	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0008	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	SD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Legend: ND = No data analyzed

Notes:

*p<0.05 using ANCOVA with body weight as the covariate, followed by pairwise comparisons versus the control group when a significant main effect was observed. Masses of spleen were rank-transformed in order to achieve normal distribution of data.

†Data collected on rats that were euthanized prior to the end of the study were listed in this summary but were not included in the calculations of mean, SD, or statistical significance.

Table J-3. Summary of Mean Organ Masses (grams) ±SD for Males and Females

		TNBA in Corn Oil					
	Sex	0 mg/kg	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
Fasted Body Weight	Females	238.3±20.7	238.4±19.6	238.9±25.0	243.1±24.5	238.6±15.6	227.8±17.7
	N	10	10	10	7	7	6
	Males	365.5±23.6	ND	356.8±28.1	340.6±19.0	302.6±32.0	ND
	N	10	ND	10	10	3	0
Brain	Females	1.936±0.073	1.915±0.071	1.944±0.076	1.891±0.074	1.819±0.060*	1.815±0.029*
	N	10	10	10	7	7	6
	Males	2.050±0.074	ND	2.054±0.107	2.013±0.100	1.991±0.073	ND
	N	10	ND	10	10	3	0
Kidneys (2)	Females	1.923±0.207	1.846±0.115	1.897±0.140	2.325±0.311	2.258±0.202	2.314±0.413
	N	10	10	10	7	7	6
	Males	2.857±0.218	ND	2.992±0.229*	3.115±0.133*	2.895±0.356*	ND
	N	10	ND	10	10	3	0
Adrenals (2)	Females	0.061±0.009	0.062±0.006	0.066±0.011	0.069±0.016	0.066±0.010	0.067±0.008
	N	10	10	10	7	7	6
	Males	0.053±0.009	ND	0.057±0.007	0.068±0.008*	0.074±0.012*	ND
	N	10	ND	10	10	3	0
Liver	Females	8.805±0.913	9.489±1.034*	9.456±0.799*	10.498±1.593*	11.593±0.825*	11.616±0.823*
	N	10	10	10	7	7	6
	Males	14.297±1.202	ND	14.034±1.364	14.093±1.133*	13.888±1.164*	ND
	N	10	ND	10	10	3	0
Heart	Females	1.091±0.269	0.991±0.110	0.982±0.133	1.056±0.130	1.089±0.127	1.013±0.075
	N	10	10	10	7	7	6
	Males	1.417±0.099	ND	1.403±0.137	1.374±0.143	1.282±0.107	ND
	N	10	ND	10	10	3	0
Spleen	Females	0.549±0.094	0.565±0.070	0.805±0.195*	1.189±0.289*	1.973±0.411*	2.330±0.640*
	N	10	10	10	7	7	6
	Males	0.647±0.050	ND	0.904±0.233*	1.368±0.157*	1.999±0.552*	ND
	N	10	ND	10	10	3	0
Thymus	Females	0.713±0.380	0.594±0.164	0.582±0.092	0.583±0.135	0.564±0.100	0.474±0.114
	N	10	10	10	7	7	6
	Males	0.647±0.070	ND	0.682±0.116	0.578±0.108	0.417±0.057	ND
	N	10	ND	10	10	3	0
Ovaries (2)	Females	0.132±0.024	0.126±0.020	0.129±0.015	0.152±0.023	0.136±0.012	0.130±0.027
	N	10	10	10	7	7	6
Uterus	Females	0.468±0.150	0.518±0.126	0.498±0.243	0.408±0.081	0.359±0.048	0.481±0.245
	N	10	10	10	7	7	6
Testes (2)	Males	3.176±0.251	ND	3.168±0.302	3.172±0.369	2.911±0.242	ND
	N	10	ND	10	10	3	0
Epididymides	Males	0.677±0.056	ND	0.672±0.093	0.701±0.056	0.533±0.047*	ND
	N	10	ND	10	10	3	0

Legend: ND = No data analyzed
Note:
*p<0.05 versus 0 mg/kg-d dose group based on ANCOVA and paired post hoc test, using fasted body weight as the covariate.

Table J-4. Individual and Mean Organ Mass (grams) per Kilogram Body Mass in Females

Dose Group	Animal ID	Brain	Kidneys (2)	Adrenals (2)	Liver	Heart	Spleen	Thymus	Ovaries (2)	Uterus
0 mg/kg	19-0125	7.237	7.899	0.248	37.896	6.050	2.101	2.032	0.600	1.449
	19-0126	8.010	7.873	0.217	34.688	5.364	1.805	2.252	0.615	1.464
	19-0075	8.534	8.251	0.228	34.301	4.100	1.904	3.142	0.530	1.612
	19-0076	8.007	8.566	0.265	38.686	4.430	2.370	2.329	0.564	2.304
	19-0115	7.910	6.970	0.244	34.380	4.282	1.960	7.206	0.447	3.099
	19-0116	8.072	7.473	0.271	37.608	4.508	2.156	2.842	0.442	2.912
	19-0107	8.358	7.365	0.224	37.257	4.327	2.465	2.255	0.523	1.302
	19-0108	8.349	8.875	0.330	40.026	3.658	2.780	2.055	0.678	1.864
	19-0091	8.376	8.077	0.252	36.406	4.432	3.111	3.021	0.479	2.000
	19-0092	8.672	9.464	0.261	38.136	4.147	2.472	2.823	0.662	1.675
	Mean	8.152	8.081	0.254	36.939	4.530	2.313	2.996	0.554	1.968
	SD	0.407	0.745	0.032	1.953	0.685	0.412	1.534	0.084	0.621
62.5 mg/kg	19-0071	8.172	7.387	0.283	40.342	4.293	2.427	2.187	0.591	2.343
	19-0072	6.965	7.069	0.198	43.133	4.074	2.080	3.554	0.512	2.350
	19-0105	8.135	7.432	0.291	36.386	3.814	2.165	2.779	0.553	2.214
	19-0106	8.307	8.168	0.252	40.786	5.025	2.563	1.996	0.412	1.399
	19-0073	8.334	7.529	0.275	38.623	4.286	1.973	2.699	0.577	2.876
	19-0074	6.942	7.229	0.249	38.665	3.801	2.048	2.006	0.449	2.032
	19-0119	8.615	8.286	0.238	38.256	4.293	2.172	2.536	0.502	1.207
	19-0120	8.101	8.649	0.307	40.627	4.114	2.789	1.974	0.474	2.250
	19-0117	8.703	8.319	0.254	40.817	3.744	3.070	2.008	0.526	3.066
	19-0118	8.471	7.600	0.285	39.953	4.155	2.528	3.016	0.709	2.082
	Mean	8.074	7.767	0.263	39.759*	4.160	2.381	2.476	0.530	2.182
	SD	0.623	0.541	0.031	1.850	0.370	0.359	0.538	0.084	0.569
125 mg/kg	19-0101	8.058	8.010	0.272	34.779	4.102	4.686	2.156	0.543	1.236
	19-0102	7.342	7.478	0.202	37.368	4.386	3.301	2.114	0.496	2.949
	19-0095	9.286	8.940	0.327	42.412	3.759	2.894	2.296	0.613	5.075
	19-0096	7.307	7.350	0.296	34.846	3.979	3.514	2.436	0.464	1.025
	19-0079	7.924	7.909	0.305	42.048	4.003	3.104	2.780	0.508	1.501
	19-0080	8.778	7.307	0.272	38.851	3.715	3.134	2.157	0.468	1.213
	19-0081	7.856	8.126	0.244	39.899	4.414	2.094	2.683	0.590	2.098
	19-0082	8.288	8.090	0.302	43.959	4.104	3.207	2.577	0.477	1.716
	19-0129	8.509	8.318	0.347	39.737	4.544	3.845	3.031	0.650	2.697
	19-0130	8.591	8.188	0.216	43.891	4.016	3.792	2.166	0.631	1.898
	Mean	8.194	7.971	0.278	39.779	4.102	3.357*	2.440	0.544	2.141
	SD	0.625	0.497	0.047	3.375	0.274	0.680	0.317	0.071	1.209
250 mg/kg	19-0121	6.479	8.941	0.263	45.302	4.455	5.116	2.098	0.543	1.868
	19-0122	7.792	9.103	0.211	41.522	4.239	5.136	2.380	0.802	1.647
	19-0113	7.824	10.531	0.392	45.592	4.318	4.310	3.527	0.710	1.882
	19-0114	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0099	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0100	8.173	8.910	0.277	40.098	4.886	4.194	2.144	0.603	1.751
	19-0089	8.460	11.355	0.215	47.215	4.022	6.853	1.969	0.517	1.245
	19-0090	7.948	8.710	0.322	38.959	4.074	4.998	2.248	0.575	1.773
	19-0127	8.122	9.444	0.307	42.594	4.387	3.549	2.429	0.653	1.524
	19-0128	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	7.828	9.571*	0.284	43.040*	4.340	4.880*	2.399	0.629	1.670
	SD	0.638	0.993	0.064	3.079	0.288	1.048	0.522	0.100	0.225

Table J-4. Individual and Mean Organ Mass (grams) per Kilogram Body Mass in Females (cont.)

Dose Group	Animal ID	Brain	Kidneys (2)	Adrenals (2)	Liver	Heart	Spleen	Thymus	Ovaries (2)	Uterus
500 mg/kg	19-0111	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0112	7.082	8.997	0.322	46.972	4.114	9.228	2.048	0.500	1.128
	19-0093	6.977	8.994	0.230	46.055	4.373	6.407	2.381	0.477	1.669
	19-0094	7.728	10.491	0.272	47.478	4.892	10.849	3.103	0.586	1.841
	19-0077	8.084	10.203	0.320	48.793	5.195	6.263	1.907	0.662	1.349
	19-0078	7.657	10.062	0.259	53.829	5.187	8.016	2.643	0.613	1.392
	19-0123	8.206	9.458	0.257	51.743	4.206	9.944	2.107	0.593	1.701
	19-0124	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0083	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0084	7.809	8.153	0.264	45.764	4.018	7.326	2.380	0.579	1.520
	Mean	7.649	9.480*	0.275	48.662*	4.569	8.290*	2.367	0.573	1.514
	SD	0.466	0.828	0.034	3.046	0.510	1.771	0.408	0.064	0.244
1000 mg/kg	19-0085	7.749	9.264	0.298	53.404	4.187	9.111	2.566	0.511	3.272
	19-0086	7.385	10.194	0.265	48.706	4.383	13.256	2.460	0.621	2.888
	19-0087	7.416	12.630	0.258	50.158	4.528	8.456	1.789	0.637	2.455
	19-0088	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0097	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0098	8.668	9.856	0.279	50.120	4.351	6.889	1.880	0.712	1.418
	19-0103	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0104	8.551	9.259	0.302	52.152	4.757	11.505	1.623	0.415	1.114
	19-0109	8.236	9.525	0.375	51.673	4.497	11.810	2.061	0.512	1.202
	19-0110	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	8.001	10.121*	0.296	51.036*	4.450	10.171*	2.063	0.568	2.058
	SD	0.564	1.281	0.042	1.691	0.193	2.401	0.378	0.108	0.933

Legend: ND = No data analyzed
Note:
*p<0.05 versus 0 mg/kg-d control group, based on one-way ANOVA and Dunnett (2-sided) post hoc test. Ratios of thymus, uterus, kidneys, and liver were log-transformed and heart and spleen were rank-transformed in order to achieve normal distribution of data.

Table J-5. Individual and Mean Organ Mass (grams) per Kilogram Body Mass in Males

Dose Group	Animal ID	Brain	Kidneys (2)	Adrenals (2)	Liver	Heart	Spleen	Thymus	Testes (2)	Epididymides
0 mg/kg	19-0019	5.833	8.861	0.166	43.957	4.605	1.961	1.946	9.820	2.215
	19-0020	5.627	7.918	0.164	39.513	3.807	1.907	2.068	8.719	2.028
	19-0031	5.517	7.995	0.145	38.386	3.691	1.633	1.790	8.526	1.820
	19-0032	6.374	7.695	0.142	35.332	4.323	1.826	1.744	9.262	1.865
	19-0061	5.554	7.358	0.160	39.532	3.518	1.904	1.571	9.329	2.150
	19-0062	5.441	7.341	0.174	39.459	3.760	1.756	2.101	8.319	1.739
	19-0011	6.054	8.089	0.104	36.194	3.660	1.570	1.752	8.209	1.726
	19-0012	5.364	7.064	0.108	40.742	4.015	1.897	1.447	8.848	1.715
	19-0049	4.951	7.106	0.154	37.883	3.566	1.514	1.680	6.683	1.589
	19-0050	5.563	8.927	0.130	40.225	3.941	1.789	1.662	9.528	1.763
	Mean	5.628	7.835	0.145	39.122	3.889	1.776	1.776	8.724	1.861
	SD	0.389	0.663	0.024	2.419	0.346	0.155	0.209	0.891	0.204
125 mg/kg	19-0045	6.002	9.943	0.182	36.241	3.959	2.803	2.276	8.067	1.448
	19-0046	6.266	8.724	0.159	39.351	4.050	2.351	1.469	10.618	2.280
	19-0065	6.159	8.434	0.197	41.783	4.373	2.387	2.039	9.737	2.484
	19-0066	5.951	8.694	0.174	38.123	4.052	2.134	1.920	9.432	2.155
	19-0047	6.159	8.140	0.139	38.880	4.030	2.370	2.244	10.596	2.205
	19-0048	5.450	7.533	0.163	40.177	3.704	2.456	1.770	8.368	1.740
	19-0015	5.998	8.657	0.179	38.723	3.467	1.942	1.544	8.400	1.837
	19-0016	5.435	7.775	0.122	38.658	3.742	2.046	2.271	8.929	1.580
	19-0039	5.624	8.335	0.132	42.400	4.465	3.631	1.859	8.085	1.780
	19-0040	4.779	7.889	0.162	38.851	3.563	3.077	1.730	7.116	1.509
	Mean	5.782	8.412	0.161	39.319	3.940	2.520*	1.912	8.935	1.902
	SD	0.460	0.676	0.024	1.777	0.326	0.517	0.293	1.149	0.357
250 mg/kg	19-0041	6.162	9.661	0.265	39.351	3.678	4.474	1.503	8.548	2.034
	19-0042	6.446	9.569	0.206	40.977	3.885	4.219	1.615	10.273	1.985
	19-0059	5.295	9.436	0.172	39.727	3.657	4.187	1.673	8.266	2.176
	19-0060	5.968	9.227	0.203	43.888	4.314	4.002	1.678	9.224	2.192
	19-0053	6.176	8.998	0.174	39.091	4.084	3.377	1.564	10.587	2.076
	19-0054	5.966	9.035	0.181	41.195	3.971	3.801	2.001	8.005	1.886
	19-0021	5.985	8.646	0.198	42.228	4.107	4.849	1.566	9.272	2.137
	19-0022	5.891	9.299	0.232	43.365	4.097	3.705	1.581	11.235	2.402
	19-0055	5.569	9.112	0.198	43.551	4.510	3.862	2.254	10.095	2.007
	19-0056	5.729	8.571	0.168	40.089	3.964	3.693	1.464	7.844	1.716
	Mean	5.919	9.155*	0.200*	41.346	4.027	4.017*	1.690	9.335	2.061
	SD	0.327	0.361	0.030	1.814	0.262	0.429	0.247	1.174	0.186
500 mg/kg	19-0067	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0068	7.479	9.593	0.304	48.238	4.561	5.935	1.329	9.756	1.836
	19-0025	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0026	6.287	9.252	0.198	44.224	3.980	8.662	1.558	9.924	1.931
	19-0005	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0006	6.121	9.835	0.242	45.534	4.205	5.283	1.254	9.240	1.544
	19-0043	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0044	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0009	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0010	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	6.629*	9.560*	0.248*	45.999*	4.249	6.627*	1.381	9.640	1.771
	SD	0.741	0.293	0.053	2.047	0.293	1.793	0.159	0.357	0.201

Table J-5. Individual and Mean Organ Mass (grams) per Kilogram Body Mass in Males (cont.)

Dose Group	Animal ID	Brain	Kidneys (2)	Adrenals (2)	Liver	Heart	Spleen	Thymus	Testes (2)	Epididymides
1000 mg/kg	19-0051	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0052	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0023	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0024	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0027	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0028	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0033	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0034	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0063	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0064	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	ND	ND	ND	ND	ND	ND	ND	ND	ND
	SD	ND	ND	ND	ND	ND	ND	ND	ND	ND
2000 mg/kg	19-0017	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0018	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0029	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0030	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0013	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0014	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0037	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0038	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0007	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0008	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	ND	ND	ND	ND	ND	ND	ND	ND	ND
	SD	ND	ND	ND	ND	ND	ND	ND	ND	ND

Legend: ND = No data analyzed
Note:
*p<0.05 versus 0 mg/kg-d control group, based on one-way ANOVA and Dunnett (2-sided) post hoc test.
Ratios of spleen were log-transformed in order to achieve normal distribution of data.

Table J-6. Summary of Mean Organ Masses (grams) per Kilogram Body Mass ±SD for Males and Females

			TNBA in Corn Oil					
	Sex		0 mg/kg	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
Brain	Females		8.152±0.407	8.074±0.623	8.194±0.625	7.828±0.638	7.649±0.466	8.001±0.564
		N	10	10	10	7	7	6
	Males		5.628±0.389	ND	5.782±0.460	5.919±0.327	6.629±0.741*	ND
		N	10	ND	10	10	3	0
Kidneys (2)	Females		8.081±0.745	7.767±0.541	7.971±0.497	9.571±0.993*	9.480±0.828*	10.121±1.281*
		N	10	10	10	7	7	6
	Males		7.835±0.663	ND	8.412±0.676	9.155±0.361*	9.560±0.293*	ND
		N	10	ND	10	10	3	0
Adrenals (2)	Females		0.254±0.032	0.263±0.031	0.278±0.047	0.284±0.064	0.275±0.034	0.296±0.042
		N	10	10	10	7	7	6
	Males		0.145±0.024	ND	0.161±0.024	0.200±0.030*	0.248±0.053*	ND
		N	10	ND	10	10	3	0
Liver	Females		36.939±1.953	39.759±1.850*	39.779±3.375	43.040±3.079*	48.662±3.046*	51.036±1.691*
		N	10	10	10	7	7	6
	Males		39.122±2.419	ND	39.319±1.777	41.346±1.814	45.999±2.047*	ND
		N	10	ND	10	10	3	0
Heart	Females		4.530±0.685	4.160±0.370	4.102±0.274	4.340±0.288	4.569±0.510	4.450±0.193
		N	10	10	10	7	7	6
	Males		3.889±0.346	ND	3.940±0.326	4.027±0.262	4.249±0.293	ND
		N	10	ND	10	10	3	0
Spleen	Females		2.313±0.412	2.381±0.359	3.357±0.680*	4.880±1.048*	8.290±1.771*	10.171±2.401*
		N	10	10	10	7	7	6
	Males		1.776±0.155	ND	2.520±0.517*	4.017±0.429*	6.627±1.793*	ND
		N	10	ND	10	10	3	0
Thymus	Females		2.996±1.534	2.476±0.538	2.440±0.317	2.399±0.522	2.367±0.408	2.063±0.378
		N	10	10	10	7	7	6
	Males		1.776±0.209	ND	1.912±0.293	1.690±0.247	1.381±0.159	ND
		N	10	ND	10	10	3	0
Ovaries (2)	Females		0.554±0.084	0.530±0.084	0.544±0.071	0.629±0.100	0.573±0.064	0.568±0.108
		N	10	10	10	7	7	6
Uterus	Females		1.968±0.621	2.182±0.569	2.141±1.209	1.670±0.225	1.514±0.244	2.058±0.933
		N	10	10	10	7	7	6
Testes (2)	Males		8.724±0.891	ND	8.935±1.149	9.335±1.174	9.640±0.357	ND
		N	10	ND	10	10	3	0
Epididymides	Males		1.861±0.204	ND	1.902±0.357	2.061±0.186	1.771±0.201	ND
		N	10	ND	10	10	3	0

Legend: ND = No data analyzed
Note:
*p<0.05 versus 0 mg/kg-d control group, based on one-way ANOVA and Dunnett (2-sided) post hoc test.

Table J-7. Individual and Mean Organ Mass (grams) per Gram Brain Mass in Females

Dose Group	Animal ID	Kidneys (2)	Adrenals (2)	Liver	Heart	Spleen	Thymus	Ovaries (2)	Uterus
0 mg/kg	19-0125	1.092	0.034	5.236	0.836	0.290	0.281	0.083	0.200
	19-0126	0.983	0.027	4.331	0.670	0.225	0.281	0.077	0.183
	19-0075	0.967	0.027	4.019	0.480	0.223	0.368	0.062	0.189
	19-0076	1.070	0.033	4.832	0.553	0.296	0.291	0.070	0.288
	19-0115	0.881	0.031	4.347	0.541	0.248	0.911	0.057	0.392
	19-0116	0.926	0.034	4.659	0.559	0.267	0.352	0.055	0.361
	19-0107	0.881	0.027	4.458	0.518	0.295	0.270	0.063	0.156
	19-0108	1.063	0.040	4.794	0.438	0.333	0.246	0.081	0.223
	19-0091	0.964	0.030	4.346	0.529	0.371	0.361	0.057	0.239
	19-0092	1.091	0.030	4.398	0.478	0.285	0.326	0.076	0.193
	Mean	0.992	0.031	4.542	0.560	0.283	0.369	0.068	0.242
	SD	0.083	0.004	0.344	0.115	0.046	0.195	0.011	0.080
62.5 mg/kg	19-0071	0.904	0.035	4.936	0.525	0.297	0.268	0.072	0.287
	19-0072	1.015	0.028	6.193	0.585	0.299	0.510	0.073	0.337
	19-0105	0.914	0.036	4.473	0.469	0.266	0.342	0.068	0.272
	19-0106	0.983	0.030	4.910	0.605	0.309	0.240	0.050	0.168
	19-0073	0.903	0.033	4.634	0.514	0.237	0.324	0.069	0.345
	19-0074	1.041	0.036	5.570	0.548	0.295	0.289	0.065	0.293
	19-0119	0.962	0.028	4.440	0.498	0.252	0.294	0.058	0.140
	19-0120	1.068	0.038	5.015	0.508	0.344	0.244	0.058	0.278
	19-0117	0.956	0.029	4.690	0.430	0.353	0.231	0.060	0.352
	19-0118	0.897	0.034	4.717	0.490	0.298	0.356	0.084	0.246
	Mean	0.964	0.033	4.958	0.517	0.295	0.310	0.066	0.272
	SD	0.061	0.004	0.542	0.052	0.037	0.083	0.010	0.071
125 mg/kg	19-0101	0.994	0.034	4.316	0.509	0.581	0.268	0.067	0.153
	19-0102	1.019	0.028	5.090	0.597	0.450	0.288	0.068	0.402
	19-0095	0.963	0.035	4.567	0.405	0.312	0.247	0.066	0.547
	19-0096	1.006	0.041	4.769	0.544	0.481	0.333	0.064	0.140
	19-0079	0.998	0.038	5.306	0.505	0.392	0.351	0.064	0.189
	19-0080	0.832	0.031	4.426	0.423	0.357	0.246	0.053	0.138
	19-0081	1.034	0.031	5.079	0.562	0.266	0.342	0.075	0.267
	19-0082	0.976	0.036	5.304	0.495	0.387	0.311	0.058	0.207
	19-0129	0.978	0.041	4.670	0.534	0.452	0.356	0.076	0.317
	19-0130	0.953	0.025	5.109	0.468	0.441	0.252	0.073	0.221
	Mean	0.975	0.034	4.864	0.504	0.412*	0.299	0.066	0.258
	SD	0.056	0.005	0.361	0.060	0.090	0.045	0.007	0.131
250 mg/kg	19-0121	1.380	0.041	6.992	0.688	0.790	0.324	0.084	0.288
	19-0122	1.168	0.027	5.329	0.544	0.659	0.305	0.103	0.211
	19-0113	1.346	0.050	5.827	0.552	0.551	0.451	0.091	0.240
	19-0114	ND	ND	ND	ND	ND	ND	ND	ND
	19-0099	ND	ND	ND	ND	ND	ND	ND	ND
	19-0100	1.090	0.034	4.906	0.598	0.513	0.262	0.074	0.214
	19-0089	1.342	0.025	5.581	0.475	0.810	0.233	0.061	0.147
	19-0090	1.096	0.041	4.902	0.513	0.629	0.283	0.072	0.223
	19-0127	1.163	0.038	5.244	0.540	0.437	0.299	0.080	0.188
	19-0128	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	1.226*	0.036	5.540*	0.559	0.627*	0.308	0.081	0.216
	SD	0.125	0.009	0.723	0.068	0.139	0.070	0.014	0.044

Table J-7. Individual and Mean Organ Mass (grams) per Gram Brain Mass in Females (cont.)

Dose Group	Animal ID	Kidneys (2)	Adrenals (2)	Liver	Heart	Spleen	Thymus	Ovaries (2)	Uterus
500 mg/kg	19-0111	ND	ND	ND	ND	ND	ND	ND	ND
	19-0112	1.270	0.045	6.633	0.581	1.303	0.289	0.071	0.159
	19-0093	1.289	0.033	6.601	0.627	0.918	0.341	0.068	0.239
	19-0094	1.358	0.035	6.143	0.633	1.404	0.402	0.076	0.238
	19-0077	1.262	0.040	6.036	0.643	0.775	0.236	0.082	0.167
	19-0078	1.314	0.034	7.030	0.677	1.047	0.345	0.080	0.182
	19-0123	1.153	0.031	6.306	0.513	1.212	0.257	0.072	0.207
	19-0124	ND	ND	ND	ND	ND	ND	ND	ND
	19-0083	ND	ND	ND	ND	ND	ND	ND	ND
	19-0084	1.044	0.034	5.861	0.515	0.938	0.305	0.074	0.195
	Mean	1.241*	0.036	6.373*	0.598	1.085*	0.311	0.075	0.198
	SD	0.107	0.005	0.405	0.064	0.228	0.057	0.005	0.032
1000 mg/kg	19-0085	1.195	0.038	6.892	0.540	1.176	0.331	0.066	0.422
	19-0086	1.380	0.036	6.595	0.593	1.795	0.333	0.084	0.391
	19-0087	1.703	0.035	6.764	0.611	1.140	0.241	0.086	0.331
	19-0088	ND	ND	ND	ND	ND	ND	ND	ND
	19-0097	ND	ND	ND	ND	ND	ND	ND	ND
	19-0098	1.137	0.032	5.782	0.502	0.795	0.217	0.082	0.164
	19-0103	ND	ND	ND	ND	ND	ND	ND	ND
	19-0104	1.083	0.035	6.099	0.556	1.345	0.190	0.049	0.130
	19-0109	1.156	0.046	6.274	0.546	1.434	0.250	0.062	0.146
	19-0110	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	1.276	0.037	6.401*	0.558	1.281*	0.260	0.071	0.264
	SD	0.233	0.005	0.424	0.039	0.334	0.059	0.015	0.132

Table J-8. Individual and Mean Organ Mass (grams) per Gram Brain Mass in Males

Dose Group	Animal ID	Kidneys (2)	Adrenals (2)	Liver	Heart	Spleen	Thymus	Testes (2)	Epididymides
0 mg/kg	19-0019	1.519	0.028	7.537	0.790	0.336	0.334	1.684	0.380
	19-0020	1.407	0.029	7.022	0.677	0.339	0.368	1.549	0.360
	19-0031	1.449	0.026	6.957	0.669	0.296	0.324	1.545	0.330
	19-0032	1.207	0.022	5.544	0.678	0.287	0.274	1.453	0.293
	19-0061	1.325	0.029	7.117	0.633	0.343	0.283	1.680	0.387
	19-0062	1.349	0.032	7.252	0.691	0.323	0.386	1.529	0.320
	19-0011	1.336	0.017	5.979	0.605	0.259	0.289	1.356	0.285
	19-0012	1.317	0.020	7.595	0.748	0.354	0.270	1.649	0.320
	19-0049	1.435	0.031	7.652	0.720	0.306	0.339	1.350	0.321
	19-0050	1.605	0.023	7.231	0.708	0.322	0.299	1.713	0.317
	Mean	1.395	0.026	6.988	0.692	0.316	0.317	1.551	0.331
	SD	0.113	0.005	0.696	0.054	0.029	0.040	0.133	0.034
125 mg/kg	19-0045	1.657	0.030	6.038	0.660	0.467	0.379	1.344	0.241
	19-0046	1.392	0.025	6.280	0.646	0.375	0.234	1.695	0.364
	19-0065	1.369	0.032	6.784	0.710	0.388	0.331	1.581	0.403
	19-0066	1.461	0.029	6.406	0.681	0.359	0.323	1.585	0.362
	19-0047	1.322	0.022	6.313	0.654	0.385	0.364	1.720	0.358
	19-0048	1.382	0.030	7.373	0.680	0.451	0.325	1.536	0.319
	19-0015	1.443	0.030	6.456	0.578	0.324	0.257	1.400	0.306
	19-0016	1.430	0.022	7.112	0.688	0.376	0.418	1.643	0.291
	19-0039	1.482	0.024	7.539	0.794	0.646	0.330	1.438	0.316
	19-0040	1.651	0.034	8.130	0.746	0.644	0.362	1.489	0.316
	Mean	1.459	0.028	6.843	0.684	0.441*	0.332	1.543	0.328
	SD	0.113	0.004	0.674	0.058	0.115	0.054	0.125	0.046
250 mg/kg	19-0041	1.568	0.043	6.386	0.597	0.726	0.244	1.387	0.330
	19-0042	1.484	0.032	6.357	0.603	0.654	0.250	1.594	0.308
	19-0059	1.782	0.032	7.502	0.691	0.791	0.316	1.561	0.411
	19-0060	1.546	0.034	7.354	0.723	0.671	0.281	1.546	0.367
	19-0053	1.457	0.028	6.329	0.661	0.547	0.253	1.714	0.336
	19-0054	1.514	0.030	6.905	0.666	0.637	0.335	1.342	0.316
	19-0021	1.445	0.033	7.056	0.686	0.810	0.262	1.549	0.357
	19-0022	1.579	0.039	7.362	0.695	0.629	0.268	1.907	0.408
	19-0055	1.636	0.036	7.821	0.810	0.694	0.405	1.813	0.360
	19-0056	1.496	0.029	6.997	0.692	0.645	0.256	1.369	0.300
	Mean	1.551*	0.034*	7.007	0.682	0.680*	0.287	1.578	0.349
	SD	0.100	0.005	0.520	0.060	0.079	0.051	0.189	0.039
500 mg/kg	19-0067	ND	ND	ND	ND	ND	ND	ND	ND
	19-0068	1.283	0.041	6.450	0.610	0.794	0.178	1.304	0.246
	19-0025†	1.153†	0.037†	5.841†	0.718†	0.564†	0.120†	1.307†	0.227†
	19-0026	1.472	0.031	7.035	0.633	1.378	0.248	1.579	0.307
	19-0005	ND	ND	ND	ND	ND	ND	ND	ND
	19-0006	1.607	0.040	7.439	0.687	0.863	0.205	1.510	0.252
	19-0043†	1.454†	0.060†	5.916†	0.444†	0.121†	0.096†	1.511†	0.237†
	19-0044	ND	ND	ND	ND	ND	ND	ND	ND
	19-0009†	1.358†	0.033†	5.924†	0.509†	0.220†	0.052†	1.221†	0.198†
	19-0010	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	1.454	0.037*	6.975	0.643	1.012*	0.210*	1.464	0.268
	SD	0.163	0.005	0.497	0.040	0.319	0.035	0.143	0.034

Table J-8. Individual and Mean Organ Mass (grams) per Gram Brain Mass in Males (cont.)

Dose Group	Animal ID	Kidneys (2)	Adrenals (2)	Liver	Heart	Spleen	Thymus	Testes (2)	Epididymides
1000 mg/kg	19-0051	ND	ND	ND	ND	ND	ND	ND	ND
	19-0052	ND	ND	ND	ND	ND	ND	ND	ND
	19-0023	ND	ND	ND	ND	ND	ND	ND	ND
	19-0024	ND	ND	ND	ND	ND	ND	ND	ND
	19-0027	ND	ND	ND	ND	ND	ND	ND	ND
	19-0028	ND	ND	ND	ND	ND	ND	ND	ND
	19-0033	ND	ND	ND	ND	ND	ND	ND	ND
	19-0034	ND	ND	ND	ND	ND	ND	ND	ND
	19-0063	ND	ND	ND	ND	ND	ND	ND	ND
	19-0064	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	ND	ND	ND	ND	ND	ND	ND	ND
	SD	ND	ND	ND	ND	ND	ND	ND	ND
2000 mg/kg	19-0017	ND	ND	ND	ND	ND	ND	ND	ND
	19-0018	ND	ND	ND	ND	ND	ND	ND	ND
	19-0029	ND	ND	ND	ND	ND	ND	ND	ND
	19-0030	ND	ND	ND	ND	ND	ND	ND	ND
	19-0013	ND	ND	ND	ND	ND	ND	ND	ND
	19-0014	ND	ND	ND	ND	ND	ND	ND	ND
	19-0037	ND	ND	ND	ND	ND	ND	ND	ND
	19-0038	ND	ND	ND	ND	ND	ND	ND	ND
	19-0007	ND	ND	ND	ND	ND	ND	ND	ND
	19-0008	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	ND	ND	ND	ND	ND	ND	ND	ND
	SD	ND	ND	ND	ND	ND	ND	ND	ND

Legend: ND = No data analyzed
Note:
*p<0.05 versus 0 mg/kg-d control group, based on one-way ANOVA and Dunnett t (2-sided) post hoc test.
Ratios of spleen were rank-transformed in order to achieve normal distribution of data.

Table J-9. Summary of Mean Organ Masses (grams) per Gram Brain Mass ±SD for Males and Females

		TNBA in Corn Oil					
	Sex	0 mg/kg	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
Kidneys (2)	Females	0.992±0.083	0.964±0.061	0.975±0.056	1.226±0.125*	1.214±0.107*	1.276±0.233
	N	10	10	10	7	7	6
	Males	1.395±0.113	ND	1.459±0.113	1.551±0.100*	1.454±0.163	ND
	N	10	ND	10	10	3	0
Adrenals (2)	Females	0.031±0.004	0.033±0.004	0.034±0.005	0.036±0.009	0.036±0.005	0.037±0.005
	N	10	10	10	7	7	6
	Males	0.026±0.005	ND	0.028±0.004	0.034±0.005*	0.037±0.005*	ND
	N	10	ND	10	10	3	0
Liver	Females	4.542±0.344	4.958±0.542	4.864±0.361	5.540±0.723*	6.373±0.405*	6.401±0.424*
	N	10	10	10	7	7	6
	Males	6.988±0.696	ND	6.843±0.674	7.007±0.520	6.975±0.497	ND
	N	10	ND	10	10	3	0
Heart	Females	0.560±0.115	0.517±0.052	0.504±0.060	0.559±0.068	0.598±0.064	0.558±0.039
	N	10	10	10	7	7	6
	Males	0.692±0.054	ND	0.684±0.058	0.682±0.060	0.643±0.040	ND
	N	10	ND	10	10	3	0
Spleen	Females	0.283±0.046	0.295±0.037	0.412±0.090*	0.627±0.139*	1.085±0.228*	1.281±0.334*
	N	10	10	10	7	7	6
	Males	0.316±0.029	ND	0.441±0.115*	0.680±0.079*	1.012±0.319*	ND
	N	10	ND	10	10	3	0
Thymus	Females	0.369±0.195	0.310±0.083	0.299±0.045	0.308±0.070	0.311±0.057	0.260±0.059
	N	10	10	10	7	7	6
	Males	0.317±0.040	ND	0.332±0.054	0.287±0.051	0.210±0.035*	ND
	N	10	ND	10	10	3	0
Ovaries (2)	Females	0.068±0.011	0.066±0.010	0.066±0.007	0.081±0.014	0.075±0.005	0.071±0.015
	N	10	10	10	7	7	6
Uterus	Females	0.242±0.080	0.272±0.071	0.258±0.131	0.216±0.044	0.198±0.032	0.264±0.132
	N	10	10	10	7	7	6
Testes (2)	Males	1.551±0.133	ND	1.543±0.125	1.578±0.189	1.464±0.143	ND
	N	10	ND	10	10	3	0
Epididymides	Males	0.331±0.034	ND	0.328±0.046	0.349±0.039	0.268±0.034	ND
	N	10	ND	10	10	3	0

Legend: ND = No data analyzed
Note:
*p<0.05 versus 0 mg/kg-d control group, based on one-way ANOVA and Dunnett post hoc test.

APPENDIX K

INDIVIDUAL AND SUMMARY OF HEMATOLOGY DATA

Table K-1. Individual and Summary Hematology in Females

Dose Group	Animal ID	WBC (K/uL)	NEU (K/uL)	NEU (%N)	LYM (K/uL)	LYM (%L)	MONO (K/uL)	MONO (%M)	EOS (K/uL)	EOS (%E)	BASO (K/uL)	BASO (%B)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)
0 mg/kg	19-0125	13.80	1.120	8.080	11.300	81.700	0.650	4.700	0.088	0.638	0.677	4.890	8.36	17.7	48.9	58.5	21.1	36.1	15.8	1154.0	4.30
	19-0126	11.80	0.987	8.400	9.370	79.700	0.761	6.480	0.131	1.110	0.503	4.280	7.38	15.7	42.9	58.1	21.3	36.6	15.3	1129.0	4.83
	19-0075	12.70	0.573	4.530	11.400	89.900	0.297	2.340	0.067	0.526	0.347	2.740	8.02	16.5	45.1	56.2	20.5	36.6	15.2	880.0	6.08
	19-0076	5.21	0.240	4.610	4.720	90.700	0.123	2.300	0.029	0.550	0.093	1.780	8.35	16.8	47.5	56.9	20.1	35.3	15.8	947.0	4.51
	19-0115	10.60	1.230	11.600	7.730	72.900	0.941	8.870	0.090	0.849	0.608	5.740	7.84	16.7	45.3	57.7	21.3	36.9	16.3	1174.0	4.73
	19-0116	10.70	0.727	6.780	8.700	81.100	0.767	7.160	0.048	0.449	0.479	4.470	7.83	17.3	46.4	59.2	22.1	37.2	15.8	1393.0	4.75
	19-0107	8.81	1.030	11.700	6.820	77.400	0.596	6.760	0.085	0.964	0.275	3.120	7.78	15.9	44.5	57.3	20.4	35.7	15.3	1034.0	4.83
	19-0108	9.02	0.477	5.280	7.620	84.400	0.532	5.900	0.080	0.885	0.317	3.510	7.92	16.2	45.3	57.2	20.4	35.7	15.0	625.0	4.92
	19-0091	5.61	0.284	5.070	4.830	86.200	0.217	3.870	0.040	0.719	0.230	4.090	7.37	15.5	43.7	59.3	21.0	35.5	14.5	1229.0	4.88
	19-0092	4.31	0.496	11.500	2.630	61.100	0.408	9.470	0.027	0.616	0.747	17.300	8.80	17.7	49.4	56.1	20.1	35.8	17.1	1417.0	5.42
	Mean	9.26	0.716	7.755	7.512	80.510	0.529	5.785	0.069	0.731	0.428	5.192	7.97	16.6	45.9	57.7	20.8	36.1	15.6	1098.2	4.93
	SD	3.29	0.356	2.972	2.861	8.732	0.265	2.479	0.033	0.214	0.210	4.401	0.44	0.8	2.1	1.1	0.6	0.6	0.7	239.0	0.50
62.5 mg/kg	19-0071	8.13	1.080	13.300	6.300	77.500	0.288	3.550	0.030	0.371	0.428	5.270	7.95	16.8	47.5	59.7	21.1	35.3	16.2	1336.0	5.09
	19-0072	9.88	0.783	7.930	7.900	80.000	0.484	4.900	0.088	0.889	0.626	6.330	8.13	17.5	48.5	59.7	21.6	36.2	15.7	1302.0	5.13
	19-0105	13.80	0.989	7.170	11.300	82.000	0.837	6.070	0.028	0.205	0.626	4.540	8.63	18.2	50.2	58.2	21.1	36.2	17.6	1255.0	4.75
	19-0106	11.00	1.210	11.100	7.760	70.800	1.200	10.900	0.095	0.867	0.687	6.270	8.11	16.7	44.7	55.1	20.6	37.4	16.3	1366.0	4.76
	19-0073	13.70	1.900	14.000	9.950	72.900	0.647	4.740	0.051	0.375	1.090	8.020	8.30	16.6	46.3	55.8	20.0	35.9	15.8	1270.0	4.96
	19-0074	8.82	0.382	4.340	7.140	81.000	0.594	6.730	0.055	0.621	0.643	7.290	8.14	17.0	47.3	58.0	20.9	36.0	17.2	1269.0	5.88
	19-0119	7.87	0.514	6.540	6.600	83.900	0.373	4.740	0.029	0.369	0.350	4.450	7.83	16.3	44.9	57.3	20.9	36.4	14.8	1229.0	4.37
	19-0120	6.85	0.759	11.100	5.210	76.100	0.455	6.640	0.025	0.360	0.397	5.790	7.07	14.6	39.8	56.4	20.6	36.6	16.2	1462.0	5.12
	19-0117	10.50	0.849	8.100	8.280	79.000	0.807	7.700	0.045	0.433	0.505	4.820	7.99	16.8	47.8	59.8	21.0	35.2	15.7	1250.0	4.93
	19-0118	9.35	0.592	6.330	7.370	78.800	0.732	7.830	0.065	0.690	0.596	6.380	7.90	17.5	47.9	60.7	22.2	36.6	15.0	1332.0	5.16
	Mean	9.99	0.906	8.991	7.781	78.200	0.642	6.380	0.051	0.518	0.595	5.916	8.01	16.8	46.5	58.1	21.0	36.2	16.1	1307.1	5.02
	SD	2.34	0.433	3.206	1.768	4.038	0.268	2.112	0.025	0.234	0.209	1.179	0.40	0.9	2.9	1.9	0.6	0.6	0.9	69.8	0.39
125 mg/kg	19-0101	17.00	1.590	9.360	13.900	82.200	0.744	4.380	0.062	0.366	0.628	3.700	6.26	14.6	42.0	67.1	23.3	34.7	18.2	849.0	5.11
	19-0102	10.70	0.721	6.740	8.500	79.500	0.597	5.580	0.037	0.347	0.843	7.880	7.21	16.0	45.8	63.6	22.2	35.0	18.6	946.0	4.67
	19-0095	11.10	1.210	10.900	9.080	82.000	0.481	4.350	0.031	0.284	0.273	2.460	7.79	16.1	45.4	58.2	20.7	35.5	18.9	1410.0	4.62
	19-0096	7.70	0.944	12.300	6.030	78.300	0.346	4.500	0.054	0.703	0.324	4.200	6.45	15.5	42.2	65.4	24.0	36.7	16.7	1868.0	5.37
	19-0079	11.40	0.730	6.390	8.860	77.500	0.832	7.280	0.140	1.230	0.864	7.560	7.11	16.1	45.2	63.6	22.7	35.6	18.7	483.0	5.96
	19-0080	7.59	0.515	6.780	6.290	82.900	0.385	5.080	0.038	0.499	0.360	4.740	7.30	15.4	42.9	58.8	21.1	35.9	18.0	1452.0	5.18
	19-0081	6.90	0.474	6.860	5.580	80.800	0.333	4.820	0.028	0.405	0.487	7.060	7.50	16.7	46.9	62.5	22.3	35.7	22.8	1348.0	5.24
	19-0082	9.00	0.379	4.210	7.700	85.600	0.379	4.210	0.035	0.388	0.506	5.620	7.42	16.3	45.5	61.4	22.0	35.9	18.1	1552.0	6.13
	19-0129	13.80	1.380	9.980	11.200	81.200	0.651	4.710	0.058	0.421	0.516	3.730	7.37	15.8	44.1	59.8	21.4	35.8	16.8	591.0	5.28
	19-0130	14.60	0.454	3.110	13.200	90.300	0.485	3.320	0.057	0.391	0.429	2.930	7.20	15.4	45.0	62.4	21.1	34.3	20.5	1163.0	4.63
	Mean	10.98	0.840	7.663	9.034	82.030	0.523	4.823	0.054	0.503	0.523	4.988	7.16*	15.8	44.5	62.3*	22.1*	35.5	18.7*	1166.2	5.22
	SD	3.34	0.425	2.921	2.912	3.726	0.175	1.046	0.033	0.279	0.202	1.950	0.47	0.6	1.6	2.8	1.0	0.7	1.8	442.5	0.52
250 mg/kg	19-0121	16.00	0.796	4.970	12.800	80.200	1.480	9.230	0.042	0.262	0.860	5.370	7.22	18.4	52.0	72.0	25.4	35.4	28.5	932.0	5.32
	19-0122	14.70	1.360	9.260	10.800	73.500	0.787	5.350	0.187	1.270	1.570	10.700	6.32	16.1	43.4	68.7	25.5	37.2	24.4	1391.0	5.78
	19-0113	20.20	6.030	29.800	12.500	61.600	1.120	5.540	0.082	0.403	0.548	2.710	5.72	14.7	40.1	70.0	25.6	36.6	25.2	1461.0	5.85
	19-0114	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0099	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0100	14.40	1.680	11.700	10.900	76.000	0.949	6.600	0.091	0.631	0.737	5.130	7.00	16.5	46.7	66.7	23.5	35.2	18.5	1280.0	5.52
	19-0089	4.46	0.833	18.700	2.240	50.300	0.460	10.300	0.030	0.675	0.898	20.100	5.43	13.7	38.3	70.6	25.2	35.8	23.9	748.0	6.11
	19-0090	8.38	0.792	9.450	6.950	82.900	0.326	3.890	0.046	0.551	0.270	3.230	5.48	14.1	39.7	72.6	25.8	35.6	16.7	1219.0	4.83
	19-0127	16.50	1.750	10.600	11.600	70.100	1.320	7.990	0.049	0.294	1.820	11.000	6.44	16.7	44.9	69.7	25.9	37.1	23.9	1522.0	5.44
	19-0128	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	13.52	1.892	13.497	9.684	70.657	0.920	6.986	0.075	0.584	0.958*	8.320	6.23*	15.7	43.6	70.0*	25.3*	36.1	23.0*	1221.9	5.55*
	SD	5.33	1.871	8.282	3.806	11.360	0.428	2.292	0.054	0.342	0.551	6.163	0.72	1.7	4.8	2.0	0.8	0.8	4.1	285.1	0.42

Table K-1. Individual and Summary Hematology in Females (cont.)

Dose Group	Animal ID	WBC (K/uL)	NEU (K/uL)	NEU (%N)	LYM (K/uL)	LYM (%L)	MONO (K/uL)	MONO (%M)	EOS (K/uL)	EOS (%E)	BASO (K/uL)	BASO (%B)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)
500 mg/kg	19-0111																				
	19-0112	8.97	2.080	23.200	5.710	63.600	0.518	5.780	0.095	1.060	0.569	6.340	5.51	14.5	40.7	73.8	26.3	35.7	18.4	1528.0	5.91
	19-0093	9.41	0.800	8.500	7.950	84.500	0.264	2.810	0.069	0.738	0.323	3.430	5.39	13.7	39.5	73.2	25.4	34.7	21.9	1228.0	5.41
	19-0094	21.40	4.950	23.200	14.000	65.600	1.230	5.760	0.057	0.266	1.110	5.190	4.75	12.6	37.0	77.9	26.6	34.2	18.7	1264.0	5.48
	19-0077	2.36	0.256	10.800	1.700	71.900	0.152	6.420	0.076	3.230	0.180	7.590	5.33	16.3	45.1	84.6	30.5	36.0	25.6	1159.0	5.76
	19-0078	4.76	1.160	24.400	3.030	63.600	0.311	6.540	0.020	0.424	0.241	5.060	5.24	14.9	41.5	79.3	28.5	35.9	18.8	1366.0	5.67
	19-0123	11.10	5.080	45.800	3.270	29.500	1.210	10.900	0.085	0.766	1.440	13.000	4.93	12.9	36.4	73.8	26.2	35.4	24.2	1574.0	5.32
	19-0124	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0083	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0084	15.40	2.080	13.500	11.100	72.500	0.909	5.910	0.039	0.255	1.200	7.820	4.96	14.0	38.0	76.6	28.3	36.9	27.4	1556.0	5.73
	Mean	10.49	2.344*	21.343*	6.680	64.457*	0.656	6.303	0.063	0.963	0.723	6.919	5.16*	14.1*	39.7*	77.0*	27.4*	35.5	22.1*	1382.1	5.61*
	SD	6.40	1.939	12.596	4.572	17.062	0.456	2.385	0.026	1.042	0.517	3.087	0.28	1.3	3.0	4.1	1.8	0.9	3.7	171.3	0.21
1000 mg/kg	19-0085	27.40	1.670	6.100	24.200	88.500	0.666	2.430	0.042	0.154	0.765	2.790	4.99	14.3	41.6	83.4	28.6	34.3	15.6	617.0	5.35
	19-0086	11.20	4.390	39.100	5.780	51.500	0.463	4.130	0.164	1.460	0.420	3.750	3.62	10.7	31.7	87.6	29.5	33.7	15.9	1700.0	6.35
	19-0087	9.93	2.730	27.500	3.850	38.700	1.240	12.400	0.104	1.050	2.020	20.300	4.22	12.4	36.0	85.3	29.5	34.5	15.6	1135.0	6.14
	19-0088	11.10	1.170	10.600	8.730	78.900	0.406	3.670	0.077	0.698	0.677	6.120	5.05	13.8	38.1	75.5	27.4	36.3	19.7	1624.0	5.61
	19-0097	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0098	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0103	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0104	65.20	5.540	8.500	58.000	89.000	0.622	0.954	0.057	0.087	0.947	1.450	3.83	11.8	35.3	92.2	30.7	33.4	15.7	1234.0	5.49
	19-0109	31.00	4.120	13.300	25.200	81.100	0.712	2.290	0.073	0.234	0.970	3.130	4.89	13.1	38.7	79.2	26.9	33.9	18.0	1930.0	5.10
	19-0110	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	25.97	3.270*	17.517	20.960	71.283	0.685	4.312	0.086	0.614	0.967*	6.257	4.43*	12.7*	36.9*	83.9*	28.8*	34.4*	16.8	1373.3	5.67*
	SD	21.27	1.697	12.983	20.363	21.061	0.297	4.117	0.043	0.556	0.554	7.049	0.63	1.3	3.4	6.0	1.4	1.0	1.7	474.9	0.48

Legend: ND = No data analyzed

Note:

*p<0.05 versus 0 mg/kg-d control group, based on one-way ANOVA and Dunnett post hoc test. Data for WBC, NEU, %NEU, LYM, EOS, %EOS, BASO, AND %BASO were log-transformed and data for %LYM, RBC, HGB,HCT, MCV, MCH, RDW, and PLT were rank-transformed in order to achieve normal distribution of data.

Table K-2. Individual and Summary Hematology in Males

Dose Group	Animal ID	WBC (K/uL)	NEU (K/uL)	NEU (%N)	LYM (K/uL)	LYM (%L)	MONO (K/uL)	MONO (%M)	EOS (K/uL)	EOS (%E)	BASO (K/uL)	BASO (%B)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)
0 mg/kg	19-0019	20.20	1.490	7.370	16.400	81.100	1.110	5.490	0.101	0.499	1.120	5.540	8.25	18.6	49.4	59.8	22.6	37.7	16.8	716.0	5.14
	19-0020	21.10	1.400	6.640	17.800	84.300	1.100	5.220	0.023	0.109	0.792	3.750	7.87	17.9	48.2	61.3	22.8	37.2	15.9	988.0	4.79
	19-0031	14.00	0.897	6.400	11.800	84.500	0.705	5.030	0.062	0.440	0.514	3.670	7.27	18.3	44.8	61.6	25.1	40.8	16.7	777.0	5.43
	19-0032	16.20	0.984	6.080	13.500	83.700	0.731	4.520	0.043	0.267	0.876	5.410	8.76	19.4	48.7	55.6	22.1	39.7	16.3	1186.0	5.50
	19-0061	8.03	0.967	12.100	5.120	63.700	0.537	6.690	0.070	0.868	1.340	16.600	8.26	18.0	49.2	59.6	21.7	36.5	18.2	879.0	5.40
	19-0062	9.18	1.110	12.100	6.000	65.400	0.623	6.790	0.084	0.918	1.350	14.800	7.64	17.3	46.8	61.3	22.7	37.0	16.7	1115.0	5.61
	19-0011	7.02	1.010	14.400	4.680	66.700	0.346	4.930	0.026	0.368	0.955	13.600	7.49	16.0	43.9	58.6	21.3	36.4	16.9	1396.0	5.63
	19-0012	23.30	1.140	4.900	19.400	83.300	0.917	3.930	0.097	0.418	1.730	7.440	7.54	16.6	45.5	60.3	22.0	36.5	16.9	1058.0	5.75
	19-0049	16.30	0.874	5.350	13.800	84.300	1.020	6.250	0.094	0.577	0.579	3.540	7.58	16.8	46.9	61.9	22.2	35.9	17.2	1175.0	4.72
	19-0050	7.44	0.201	2.700	6.660	89.400	0.351	4.710	0.019	0.254	0.215	2.890	7.99	16.4	46.3	57.9	20.5	35.4	17.6	1194.0	5.05
	Mean	14.28	1.007	7.804	11.516	78.640	0.744	5.356	0.062	0.472	0.947	7.724	7.87	17.5	47.0	59.8	22.3	37.3	16.9	1048.4	5.30
	SD	6.10	0.349	3.763	5.542	9.479	0.287	0.949	0.032	0.259	0.453	5.235	0.45	1.1	1.9	2.0	1.2	1.7	0.6	210.3	0.36
125 mg/kg	19-0045	7.69	1.640	21.300	3.670	47.800	1.200	15.600	0.017	0.221	1.160	15.000	6.60	15.8	42.8	64.9	24.0	37.0	16.6	1336.0	6.69
	19-0046	14.70	1.120	7.630	11.300	76.900	0.857	5.840	0.046	0.311	1.370	9.320	7.42	17.2	46.9	63.2	23.2	36.6	16.9	1255.0	6.52
	19-0065	11.80	1.590	13.400	7.190	60.800	1.270	10.700	0.027	0.228	1.750	14.800	7.07	16.3	44.4	62.8	23.0	36.6	16.7	1569.0	6.48
	19-0066	11.90	1.600	13.500	6.760	57.000	0.959	8.080	0.029	0.245	2.510	21.200	7.62	17.1	45.9	60.2	22.4	37.2	16.8	1470.0	7.41
	19-0047	11.00	1.310	11.900	5.930	53.800	1.680	15.300	0.174	1.580	1.920	17.400	7.52	15.9	44.1	58.6	21.2	36.1	16.9	441.0	7.86
	19-0048	14.50	2.410	16.600	8.760	60.400	1.360	9.390	0.054	0.374	1.910	13.200	6.87	15.7	43.8	63.8	22.8	35.8	18.3	1230.0	6.35
	19-0015	10.00	1.240	12.300	6.750	67.300	0.592	5.900	0.067	0.668	1.380	13.800	6.99	15.0	40.8	58.3	21.5	36.8	16.4	1361.0	5.96
	19-0016	11.00	3.850	35.000	4.450	40.500	1.020	9.250	0.030	0.274	1.640	14.900	7.12	15.4	42.5	59.7	21.6	36.2	17.2	1387.0	6.74
	19-0039	21.70	1.810	8.350	17.500	80.400	1.130	5.220	0.036	0.165	1.280	5.910	5.97	14.6	41.0	68.7	24.5	35.7	32.0	1004.0	5.82
	19-0040	14.30	2.430	17.000	8.020	56.000	2.310	16.100	0.061	0.429	1.500	10.500	7.49	16.0	45.0	60.1	21.3	35.4	17.7	1179.0	4.94
	Mean	12.86	1.900*	15.698*	8.033	60.090*	1.238*	10.138*	0.054	0.450	1.642*	13.603*	7.07*	15.9*	43.7*	62.0	22.6	36.3	18.6	1223.2	6.48*
	SD	3.80	0.816	7.900	3.960	12.240	0.479	4.197	0.045	0.422	0.400	4.276	0.50	0.8	2.0	3.3	1.2	0.6	4.8	316.6	0.81
250 mg/kg	19-0041	17.10	1.390	8.120	14.300	83.500	0.686	4.010	0.017	0.099	0.723	4.230	6.84	17.2	42.9	62.7	25.1	40.1	20.3	805.0	4.76
	19-0042	16.10	1.820	11.300	12.200	75.800	1.220	7.620	0.076	0.475	0.759	4.730	6.48	17.2	44.3	68.4	26.5	38.8	18.7	1064.0	4.88
	19-0059	13.10	2.370	18.100	5.240	40.000	2.960	22.600	0.104	0.797	2.430	18.500	5.90	15.5	42.7	72.4	26.3	36.3	22.4	469.0	7.83
	19-0060	14.00	2.870	20.500	6.830	48.800	2.140	15.300	0.090	0.645	2.050	14.700	6.08	16.2	44.0	72.3	26.6	36.8	23.6	1408.0	7.07
	19-0053	21.20	2.450	11.600	15.500	73.400	1.210	5.730	0.041	0.194	1.920	9.060	7.02	15.9	44.8	63.8	22.6	35.4	17.5	1206.0	6.56
	19-0054	14.50	1.210	8.410	11.100	77.000	0.559	3.870	0.040	0.278	1.510	10.500	6.02	15.6	42.5	70.6	26.0	36.8	19.6	1545.0	6.48
	19-0021	18.60	2.010	10.800	14.600	78.300	0.683	3.670	0.040	0.215	1.310	7.040	7.81	17.8	50.2	64.3	22.8	35.5	20.7	1162.0	5.24
	19-0022	17.00	2.950	17.300	10.500	61.700	1.810	10.700	0.055	0.323	1.710	10.000	6.29	13.3	38.4	61.2	21.2	34.7	19.6	1477.0	4.76
	19-0055	14.20	1.550	11.000	11.200	78.900	0.876	6.190	0.040	0.281	0.515	3.640	6.62	14.6	42.0	63.4	22.1	34.8	20.9	1434.0	5.02
	19-0056	11.80	1.750	14.800	8.360	70.700	1.170	9.880	0.052	0.442	0.487	4.120	6.35	14.5	42.9	67.6	22.9	33.9	17.5	1094.0	4.90
	Mean	15.76	2.037*	13.193*	10.983	68.810	1.331*	8.957	0.056	0.375	1.341	8.652	6.54*	15.8*	43.5*	66.7*	24.2	36.3	20.1*	1166.4	5.75
	SD	2.81	0.604	4.245	3.389	14.256	0.762	6.041	0.027	0.216	0.693	4.968	0.57	1.4	2.9	4.1	2.1	1.9	2.0	334.0	1.13
500 mg/kg	19-0067	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0068	13.40	8.690	64.700	2.610	19.400	1.440	10.700	0.056	0.415	0.635	4.730	5.63	16.1	42.7	75.9	28.6	37.8	19.5	1798.0	13.30
	19-0025	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0026	23.50	2.010	8.540	20.100	85.500	0.583	2.480	0.021	0.091	0.799	3.400	5.10	16.2	40.3	78.9	31.8	40.2	15.6	412.0	5.33
	19-0005	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0006	15.80	2.230	14.100	11.500	72.900	0.519	3.290	0.029	0.181	1.500	9.530	5.08	14.1	38.6	75.9	27.8	36.6	16.2	1807.0	7.12
	19-0043	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0044	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0009	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0010	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	17.57	4.310*	29.113	11.403	59.267	0.847	5.490	0.035	0.229	0.978	5.887	5.27*	15.5*	40.5*	76.9*	29.4*	38.2	17.1	1339.0	8.58
	SD	5.28	3.795	30.944	8.745	35.096	0.514	4.530	0.018	0.167	0.459	3.225	0.31	1.2	2.1	1.7	2.1	1.8	2.1	802.8	4.18

Table K-2. Individual and Summary Hematology in Males (cont.)

Dose Group	Animal ID	WBC (K/uL)	NEU (K/uL)	NEU (%N)	LYM (K/uL)	LYM (%L)	MONO (K/uL)	MONO (%M)	EOS (K/uL)	EOS (%E)	BASO (K/uL)	BASO (%B)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)
1000 mg/kg	19-0051	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0052	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0023	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0024	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0027	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0028	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0033	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0034	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0063	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0064	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	SD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2000 mg/kg	19-0017	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0018	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0029	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0030	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0013	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0014	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0037	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0038	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0007	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0008	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	SD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Legend: ND = No data analyzed
Note:
*p<0.05 versus 0 mg/kg-d control group, based on one-way ANOVA and Dunnett post hoc test. Data for NEU, %NEU, MONO, %MONO, %EOS, and MPV were log-transformed and data for MCV, MCH, MCHC, and RDW were rank-transformed in order to achieve normal distribution of data.

Table K-3. Hematology Summary in Females and Males

			TNBA in Corn Oil					
		Sex	0 mg/kg	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
WBC	(K/uL)	Females	9.26±3.29	9.99±2.34	10.98±3.34	13.52±5.33	10.49±6.40	25.97±21.27
		N	10	10	10	7	7	6
		Males	14.28±6.10	ND	12.86±3.80	15.76±2.81	17.57±5.28	ND
		N	10	ND	10	10	3	0
NEU	(K/uL)	Females	0.716±0.356	0.906±0.433	0.840±0.425	1.892±1.871	2.344±1.939*	3.270±1.697*
		N	10	10	10	7	7	6
		Males	1.007±0.349	ND	1.900±0.816*	2.037±0.604*	4.310±3.795*	ND
		N	10	ND	10	10	3	0
	(%N)	Females	7.755±2.972	8.991±3.206	7.663±2.921	13.497±8.282	21.343±12.596*	17.517±12.983
		N	10	10	10	7	7	6
		Males	7.804±3.763	ND	15.698±7.900*	13.193±4.245*	29.113±30.944	ND
		N	10	ND	10	10	3	0
LYM	(K/uL)	Females	7.512±2.861	7.781±1.768	9.034±2.912	9.684±3.806	6.680±4.572	20.960±20.363
		N	10	10	10	7	7	6
		Males	11.516±5.542	ND	8.033±3.960	10.983±3.389	11.403±8.745	ND
		N	10	ND	10	10	3	0
	(%L)	Females	80.510±8.732	78.200±4.038	82.030±3.726	70.657±11.360	64.457±17.062*	71.283±21.061
		N	10	10	10	7	7	6
		Males	78.640±9.479	ND	60.090±12.240*	68.810±14.256	59.267±35.096	ND
		N	10	ND	10	10	3	0
MONO	(K/uL)	Females	0.529±0.265	0.642±0.268	0.523±0.175	0.920±0.428	0.656±0.456	0.685±0.297
		N	10	10	10	7	7	6
		Males	0.744±0.287	ND	1.238±0.479*	1.331±0.762*	0.847±0.514	ND
		N	10	ND	10	10	3	0
	(%M)	Females	5.785±2.479	6.380±2.112	4.823±1.046	6.986±2.292	6.303±2.385	4.312±4.117
		N	10	10	10	7	7	6
		Males	5.356±0.949	ND	10.138±4.197*	8.957±6.041	5.490±4.530	ND
		N	10	ND	10	10	3	0
EOS	(K/uL)	Females	0.069±0.033	0.051±0.025	0.054±0.033	0.075±0.054	0.063±0.026	0.086±0.043
		N	10	10	10	7	7	6
		Males	0.062±0.032	ND	0.054±0.045	0.056±0.027	0.035±0.018	ND
		N	10	ND	10	10	3	0
	(%E)	Females	0.731±0.214	0.518±0.234	0.503±0.279	0.584±0.342	0.963±1.042	0.614±0.556
		N	10	10	10	7	7	6
		Males	0.472±0.259	ND	0.450±0.422	0.375±0.216	0.229±0.167	ND
		N	10	ND	10	10	3	0
BASO	(K/uL)	Females	0.428±0.210	0.595±0.209	0.523±0.202	0.958±0.551*	0.723±0.517	0.967±0.554*
		N	10	10	10	7	7	6
		Males	0.947±0.453	ND	1.642±0.400*	1.341±0.693	0.978±0.459	ND
		N	10	ND	10	10	3	0
	(%B)	Females	5.192±4.401	5.916±1.179	4.988±1.950	8.320±6.163	6.919±3.087	6.257±7.049
		N	10	10	10	7	7	6
		Males	7.724±5.235	ND	13.603±4.276*	8.652±4.968	5.887±3.225	ND
		N	10	ND	10	10	3	0
RBC	(M/uL)	Females	7.97±0.44	8.01±0.40	7.16±0.47*	6.23±0.72*	5.16±0.28*	4.43±0.63*
		N	10	10	10	7	7	6
		Males	7.87±0.45	ND	7.07±0.50*	6.54±0.57*	5.27±0.31*	ND
		N	10	ND	10	10	3	0
HGB	(g/dL)	Females	16.6±0.8	16.8±0.9	15.8±0.6	15.7±1.7	14.1±1.3*	12.7±1.3*
		N	10	10	10	7	7	6
		Males	17.5±1.1	ND	15.9±0.8*	15.8±1.4*	15.5±1.2*	ND
		N	10	ND	10	10	3	0

Table K-3. Hematology Summary in Females and Males (cont.)

			TNBA in Corn Oil					
		Sex	0 mg/kg	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
HCT	(%)	Females	45.9±2.1	46.5±2.9	44.5±1.6	43.6±4.8	39.7±3.0*	36.9±3.4*
		N	10	10	10	7	7	6
		Males	47.0±1.9	ND	43.7±2.0*	43.5±2.9*	40.5±2.1*	ND
		N	10	ND	10	10	3	0
MCV	(fL)	Females	57.7±1.1	58.1±1.9	62.3±2.8*	70.0±2.0*	77.0±4.1*	83.9±6.0*
		N	10	10	10	7	7	6
		Males	59.8±2.0	ND	62.0±3.3	66.7±4.1*	76.9±1.7*	ND
		N	10	ND	10	10	3	0
MCH	(pg)	Females	20.8±0.6	21.0±0.6	22.1±1.0*	25.3±0.8*	27.4±1.8*	28.8±1.4*
		N	10	10	10	7	7	6
		Males	22.3±1.2	ND	22.6±1.2	24.2±2.1	29.4±2.1*	ND
		N	10	ND	10	10	3	0
MCHC	(g/dL)	Females	36.1±0.6	36.2±0.6	35.5±0.7	36.1±0.8	35.5±0.9	34.4±1.0*
		N	10	10	10	7	7	6
		Males	37.3±1.7	ND	36.3±0.6	36.3±1.9	38.2±1.8	ND
		N	10	ND	10	10	3	0
RDW	(%)	Females	15.6±0.7	16.1±0.9	18.9±1.8*	23.0±4.1*	22.1±3.7*	16.8±1.7
		N	10	10	10	7	7	6
		Males	16.9±0.6	ND	18.6±4.8	20.1±2.0*	17.1±2.1	ND
		N	10	ND	10	10	3	0
PLT	(K/uL)	Females	1098.2±239.0	1307.1±69.8	1166.2±442.5	1221.9±285.1	1382.1±171.3	1373.3±474.9
		N	10	10	10	7	7	6
		Males	1048.4±210.3	ND	1223.2±316.6	1166.4±334.0	1339.0±802.8	ND
		N	10	ND	10	10	3	0
MPV	(fL)	Females	4.93±0.50	5.02±0.39	5.22±0.52	5.55±0.42*	5.61±0.21*	5.67±0.48*
		N	10	10	10	7	7	6
		Males	5.30±0.36	ND	6.48±0.81*	5.75±1.13	8.58±4.18	ND
		N	10	ND	10	10	3	ND

Note:
*p<0.05 versus 0 mg/kg-d control group, based on one-way ANOVA and Dunnett post hoc test.

APPENDIX L

INDIVIDUAL AND SUMMARY OF CLINICAL CHEMISTRY DATA

Table L-1. Individual and Summary Clinical Chemistry in Females

Dose Group	Animal ID	ALB (g/dL)	ALB / GLOB ratio (g/dL)	ALT (U/L)	ALKP (U/L)	AST (U/L)	BUN (md/dL)	BUN / CREA ratio (mg/dL)	Ca (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (g/dL)	GLU (mg/dL)	PHOS (mg/dL)	TP (g/dL)	TRIG (g/dL)	UREA (mg/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
0 mg/kg	19-0125	4.12	0.96	47.0	103.9	117.8	15.23	43.53	12.24	88	0.35	4.31	106.93	15.24	8.43	72	32.6	146	9.3	106
	19-0126	3.62	0.96	55.6	160.8	82.0	24.02	70.64	12.25	70	0.34	3.76	157.25	12.19	7.38	69	51.4	148	8.5	105
	19-0075	3.92	0.99	48.5	196.4	120.8	22.24	48.36	12.24	98	0.46	3.94	223.60	14.83	7.86	35	47.6	148	8.0	105
	19-0076	3.54	1.01	50.4	226.5	100.6	15.75	37.50	11.47	69	0.42	3.51	135.26	12.81	7.05	34	33.7	151	7.4	108
	19-0115	3.62	1.01	38.3	119.6	106.3	15.28	38.20	12.33	85	0.40	3.60	116.54	16.48	7.22	39	32.7	147	10.1	108
	19-0116	3.79	0.92	48.2	180.9	99.7	15.42	39.54	12.70	88	0.39	4.10	216.58	18.58	7.89	49	33.0	148	11.3	109
	19-0107	3.66	1.06	66.0	119.2	119.1	19.11	44.45	12.46	101	0.43	3.45	176.56	16.07	7.11	31	40.9	148	9.1	107
	19-0108	3.65	1.05	64.0	156.3	99.2	16.12	36.64	11.71	60	0.44	3.47	180.47	19.03	7.12	39	34.5	152	8.4	107
	19-0091	3.75	1.10	55.2	160.8	114.6	16.59	36.06	11.82	80	0.46	3.41	258.94	17.04	7.16	38	35.5	151	9.9	108
	19-0092	3.97	0.97	45.4	178.7	117.8	14.63	40.63	11.94	62	0.36	4.10	67.22	16.35	8.07	28	31.3	153	8.8	108
	Mean	3.76	1.00	51.9	160.3	107.8	17.44	43.56	12.12	80	0.41	3.77	163.94	15.86	7.53	43	37.3	149.2	9.1	107
	SD	0.19	0.05	8.5	38.0	12.5	3.27	10.28	0.37	14	0.04	0.33	59.17	2.20	0.49	15	7.0	2.3	1.1	1
62.5 mg/kg	19-0071	3.72	1.09	43.1	121.2	97.8	22.57	46.06	11.84	67	0.49	3.42	137.74	21.00	7.14	28	48.3	149	9.6	108
	19-0072	3.68	0.97	35.0	112.6	92.8	25.14	48.35	11.83	90	0.52	3.78	138.39	16.48	7.46	42	53.8	149	10.6	108
	19-0105	3.78	1.04	49.2	140.0	113.0	18.93	57.35	11.03	64	0.33	3.65	99.66	16.02	7.43	42	40.5	148	10.2	109
	19-0106	3.73	0.96	85.4	178.0	195.1	19.67	40.99	11.82	110	0.48	3.90	119.43	16.59	7.63	53	42.1	147	10.7	107
	19-0073	3.67	0.81	49.9	129.9	89.9	19.25	64.18	11.72	109	0.30	4.55	133.92	17.28	8.22	43	41.2	152	8.2	106
	19-0074	3.64	0.95	30.8	125.6	86.6	13.32	32.48	12.42	117	0.41	3.85	178.03	17.88	7.49	54	28.5	146	10.8	106
	19-0119	4.00	1.07	61.7	141.4	120.7	15.75	44.99	11.35	107	0.35	3.75	165.13	16.27	7.75	46	33.7	149	7.7	105
	19-0120	4.00	0.93	64.6	169.4	242.7	17.34	50.99	13.05	109	0.34	4.32	257.92	18.11	8.32	58	37.1	148	10.2	106
	19-0117	3.97	0.90	47.9	103.9	115.1	13.97	37.76	12.56	80	0.37	4.43	141.91	19.46	8.40	37	29.9	149	12.5	108
	19-0118	3.86	0.91	40.7	130.0	84.8	15.23	40.09	12.41	109	0.38	4.24	150.90	15.37	8.10	58	32.6	148	11.2	107
	Mean	3.81	0.96	50.8	135.2	123.9	18.12	46.32	12.00	96	0.40	3.99	152.30	17.45	7.79	46	38.8	148.5	10.2	107
	SD	0.14	0.08	16.1	23.3	52.8	3.78	9.43	0.60	19	0.08	0.37	43.06	1.72	0.44	10	8.1	1.6	1.4	1
125 mg/kg	19-0101	3.87	1.00	35.2	172.9	108.6	21.12	62.12	11.96	85	0.34	3.86	123.71	14.89	7.73	51	45.2	144	8.6	104
	19-0102	4.28	0.92	42.0	105.9	85.4	16.40	52.91	13.08	95	0.31	4.64	201.19	17.04	8.92	96	35.1	146	10.9	105
	19-0095	3.97	1.11	31.5	137.2	107.1	12.85	33.82	11.95	91	0.38	3.57	146.81	17.85	7.54	44	27.5	147	11.3	107
	19-0096	3.94	0.89	43.8	122.0	123.0	22.71	48.32	12.40	95	0.47	4.41	143.69	18.10	8.35	46	48.6	146	10.6	104
	19-0079	3.77	0.95	33.0	118.8	110.8	18.13	41.21	12.45	94	0.44	3.95	142.25	17.13	7.72	42	38.8	145	9.8	104
	19-0080	4.02	0.85	38.0	178.7	111.0	22.66	58.11	12.51	109	0.39	4.74	196.56	18.66	8.76	40	48.5	148	10.6	106
	19-0081	4.38	1.00	45.3	99.4	88.6	13.41	33.53	11.86	93	0.40	4.36	123.39	16.77	8.74	48	28.7	149	11.7	107
	19-0082	3.86	0.99	29.3	97.8	98.2	24.30	44.18	12.38	117	0.55	3.91	130.54	20.32	7.77	34	52.0	151	12.7	106
	19-0129	3.90	1.05	47.1	136.1	140.0	13.41	37.25	12.50	108	0.36	3.73	203.15	18.06	7.63	67	28.7	149	9.4	106
	19-0130	4.01	1.08	71.0	152.7	129.5	16.96	67.85	11.63	91	0.25	3.70	160.42	15.65	7.71	56	36.3	150	10.0	107
	Mean	4.00*	0.98	41.6	132.2	110.2	18.20	47.93	12.27	98	0.39	4.09	157.17	17.45	8.09*	52	38.9	147.5	10.6*	106*
	SD	0.19	0.08	12.0	28.9	17.2	4.29	12.05	0.42	10	0.08	0.42	31.79	1.53	0.54	18	9.2	2.3	1.2	1
250 mg/kg	19-0121	3.66	0.88	47.6	111.3	118.8	19.44	55.54	12.35	75	0.35	4.16	131.59	17.94	7.82	89	41.6	146	12.8	106
	19-0122	3.71	1.04	76.0	80.4	159.3	12.85	36.72	12.45	90	0.35	3.58	232.42	17.62	7.29	77	27.5	144	11.6	106
	19-0113	3.98	0.93	63.3	138.1	85.0	13.50	50.02	13.96	96	0.27	4.27	341.05	18.73	8.25	57	28.9	144	11.3	103
	19-0114	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0099	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0100	4.06	1.06	38.4	201.2	102.7	15.84	41.69	13.32	104	0.38	3.83	240.23	18.31	7.89	63	33.9	147	10.5	106
	19-0089	3.57	0.90	31.6	154.4	113.6	15.00	38.46	12.52	98	0.39	3.97	315.21	16.73	7.54	93	32.1	141	14.7	105
	19-0090	3.60	1.11	50.7	153.8	111.5	12.57	33.08	11.57	83	0.38	3.23	179.84	13.53	6.83	65	26.9	149	9.7	109
	19-0127	3.87	1.06	57.5	149.7	103.4	13.46	70.83	12.73	119	0.19	3.66	137.21	17.13	7.53	73	28.8	148	12.1	107
	19-0128	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	3.78	1.00	52.2	141.3	113.5	14.67	46.62	12.70	95	0.33	3.81	225.36	17.14	7.59	74*	31.4	145.6*	11.8*	106
	SD	0.19	0.09	15.0	37.9	23.0	2.41	13.23	0.76	14	0.07	0.36	82.02	1.73	0.46	13	5.2	2.8	1.6	2

Table L-1. Individual and Summary Clinical Chemistry in Females (cont.)

Dose Group	Animal ID	ALB (g/dL)	ALB / GLOB ratio (g/dL)	ALT (U/L)	ALKP (U/L)	AST (U/L)	BUN (md/dL)	BUN / CREA ratio (mg/dL)	Ca (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (g/dL)	GLU (mg/dL)	PHOS (mg/dL)	TP (g/dL)	TRIG (g/dL)	UREA (mg/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
500 mg/kg	19-0111	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0112	3.71	0.90	22.6	110.1	81.6	16.59	46.08	12.79	98	0.36	4.10	153.59	16.26	7.81	142	35.5	142	12.2	106
	19-0093	3.73	1.07	38.4	117.4	93.0	13.60	31.62	12.06	110	0.43	3.47	236.69	13.53	7.20	98	29.1	145	9.5	108
	19-0094	3.67	0.98	18.4	136.4	75.1	13.55	46.73	13.29	113	0.29	3.73	283.41	17.44	7.40	103	29.0	141	12.2	108
	19-0077	3.79	1.10	41.2	175.1	113.4	9.16	28.62	11.69	72	0.32	3.44	83.61	14.74	7.23	78	19.6	145	10.0	107
	19-0078	3.84	1.01	90.0	140.1	120.9	13.88	39.65	12.08	88	0.35	3.82	90.27	15.64	7.66	96	29.7	145	10.8	107
	19-0123	3.51	0.94	45.3	185.3	109.5	13.22	35.74	12.67	76	0.37	3.72	167.69	15.90	7.23	90	28.3	144	11.1	107
	19-0124	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0083	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0084	3.92	1.02	48.0	158.5	126.3	14.63	52.24	13.07	73	0.28	3.83	171.40	16.93	7.75	72	31.3	147	11.7	108
	Mean	3.74	1.00	43.4	146.1	102.8	13.52	40.10	12.52	90	0.34	3.73	169.52	15.78	7.47	97*	28.9	144.1*	11.1*	107
	SD	0.13	0.07	23.4	28.2	19.8	2.23	8.66	0.59	17	0.05	0.23	72.35	1.32	0.27	23	4.8	2.0	1.1	1
1000 mg/kg	19-0085	3.55	1.03	29.2	143.1	104.8	14.49	42.61	12.03	99	0.34	3.46	144.55	18.26	7.01	112	31.0	145	12.0	109
	19-0086	3.56	0.87	13.0	164.6	90.4	15.93	39.84	12.31	92	0.40	4.10	299.75	15.68	7.66	148	34.1	143	11.8	109
	19-0087	3.44	0.95	13.4	140.7	102.3	8.88	30.62	12.87	85	0.29	3.62	280.22	17.14	7.06	90	19.0	142	12.2	109
	19-0088	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0097	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0098	3.53	0.98	34.5	61.0	93.6	10.28	28.56	11.93	63	0.36	3.62	238.19	15.99	7.15	47	22.0	145	11.1	107
	19-0103	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0104	3.55	1.10	33.2	167.1	135.9	9.77	34.88	11.31	81	0.28	3.23	74.66	16.81	6.78	183	20.9	143	11.9	109
	19-0109	3.81	0.96	41.2	50.4	110.1	14.11	32.07	12.95	73	0.44	3.97	223.85	21.46	7.78	143	30.2	141	ND	106
	19-0110	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	3.57	0.98	27.4	121.2	106.2	12.24*	34.76	12.23	82	0.35	3.67	210.20	17.56	7.24	121*	26.2*	143.2*	11.8*	108
	SD	0.12	0.08	11.7	51.9	16.3	2.95	5.48	0.62	13	0.06	0.32	85.53	2.12	0.39	48	6.3	1.6	0.4	1

Legend: ND = No data analyzed
Note:
*p<0.05 versus 0 mg/kg-d control group, based on one-way ANOVA and Dunnett post hoc test for normally distributed data and Kruskal-Wallis and Mann-Whitney U post hoc test for data that is not normally distributed. Data for ALT, BUN, GLU, TRIG, AND UREA were log-transformed and data for AST was rank-transformed in order to achieve normal distribution of data. Data for Cl was not normally distributed after transformation; so ranked data was analyzed by Kruskal-Wallis followed by Mann-Whitney U post hoc test.

Table L-2. Individual and Summary Clinical Chemistry in Males

Dose Group	Animal ID	ALB (g/dL)	ALB / GLOB ratio (g/dL)	ALT (U/L)	ALKP (U/L)	AST (U/L)	BUN (md/dL)	BUN / CREA ratio (mg/dL)	Ca (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (g/dL)	GLU (mg/dL)	PHOS (mg/dL)	TP (g/dL)	TRIG (g/dL)	UREA (mg/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
0 mg/kg	19-0019	3.68	1.43	62.4	341.7	95.5	10.70	46.53	13.31	62	0.23	2.58	161.75	20.27	6.26	113	22.9	150	10.7	105
	19-0020	3.40	1.37	60.8	247.9	88.3	12.52	50.09	13.23	85	0.25	2.49	308.43	15.66	5.89	88	26.8	149	9.1	107
	19-0031	3.41	1.23	51.4	283.9	100.3	8.22	34.27	12.25	76	0.24	2.77	197.77	14.95	6.18	98	17.6	150	7.5	106
	19-0032	3.57	1.41	99.3	559.6	92.1	12.94	40.45	12.87	93	0.32	2.54	301.89	18.95	6.11	100	27.7	150	11.3	105
	19-0061	3.55	1.16	73.9	456.0	111.5	9.72	44.18	12.52	96	0.22	3.07	150.21	16.78	6.62	78	20.8	148	8.9	105
	19-0062	3.61	1.30	54.7	343.9	114.5	12.85	47.60	12.68	95	0.27	2.77	272.79	16.42	6.38	95	27.5	148	10.6	104
	19-0011	3.24	1.26	69.7	524.8	77.9	8.55	27.59	12.99	74	0.31	2.58	180.94	16.77	5.82	71	18.3	148	8.4	107
	19-0012	3.60	1.30	77.1	351.8	82.0	9.91	35.38	12.71	99	0.28	2.76	276.58	15.26	6.36	100	21.2	148	9.1	107
	19-0049	3.63	0.93	43.8	237.1	73.9	9.67	26.87	12.59	82	0.36	3.90	376.25	16.35	7.53	252	20.7	146	9.8	104
	19-0050	3.53	0.82	65.2	380.8	95.4	11.36	37.85	12.22	85	0.30	4.30	189.03	14.26	7.83	187	24.3	ND	ND	ND
	Mean	3.52	1.22	65.8	372.8	93.1	10.64	39.08	12.74	85	0.28	2.98	241.56	16.57	6.50	118	22.8	148.6	9.5	106
	SD	0.13	0.20	15.6	110.1	13.4	1.72	8.14	0.37	12	0.04	0.62	75.66	1.83	0.67	57	3.7	1.3	1.2	1
125 mg/kg	19-0045	3.45	1.39	75.4	198.1	105.3	9.91	36.69	12.81	81	0.27	2.48	284.16	16.27	5.93	64	21.2	147	10.3	105
	19-0046	3.49	1.35	93.1	356.1	104.6	13.50	46.57	13.33	76	0.29	2.58	140.26	16.28	6.07	72	28.9	149	9.8	107
	19-0065	3.54	1.37	83.2	218.2	128.8	10.09	32.56	12.84	101	0.31	2.58	70.62	18.04	6.12	89	21.6	152	9.0	106
	19-0066	3.76	1.37	80.5	262.5	146.8	7.29	40.50	12.49	76	0.18	2.75	120.57	15.60	6.51	100	15.6	149	9.6	106
	19-0047	3.39	1.33	82.9	258.8	170.1	8.22	48.38	13.11	69	0.17	2.55	199.64	19.16	5.94	77	17.6	145	11.3	108
	19-0048	3.40	1.42	83.4	323.9	118.3	10.51	43.81	12.93	76	0.24	2.39	217.38	17.46	5.79	147	22.5	148	8.9	105
	19-0015	3.47	1.40	53.9	269.2	86.2	10.56	35.20	13.19	72	0.30	2.47	298.43	16.64	5.94	91	22.6	147	11.0	107
	19-0016	3.51	1.48	84.6	257.4	119.6	8.46	29.17	12.97	100	0.29	2.37	254.87	16.61	5.88	185	18.1	149	8.9	106
	19-0039	3.63	1.03	60.5	265.6	118.6	11.12	34.76	12.22	92	0.32	3.53	223.05	15.74	7.16	176	23.8	147	9.8	106
	19-0040	3.68	0.89	60.5	204.2	96.2	10.37	30.51	12.69	70	0.34	4.12	387.94	17.55	7.80	157	22.2	146	10.4	105
	Mean	3.53	1.30	75.8	261.4	119.5	10.00	37.82	12.86	81	0.27	2.78	219.69	16.94	6.31	116	21.4	147.9	9.9	106
	SD	0.12	0.19	13.0	49.6	24.7	1.74	6.70	0.33	12	0.06	0.58	93.50	1.11	0.66	46	3.7	2.0	0.8	1
250 mg/kg	19-0041	3.57	1.47	64.6	216.1	102.0	8.27	68.93	12.40	69	0.12	2.43	162.10	13.96	6.00	107	17.7	149	8.8	107
	19-0042	3.61	1.66	41.1	224.8	88.4	11.50	41.06	12.75	84	0.28	2.17	285.79	17.20	5.78	82	24.6	147	11.4	108
	19-0059	3.37	1.23	55.6	104.8	166.2	11.31	45.23	12.88	81	0.25	2.75	191.02	17.49	6.12	78	24.2	148	8.0	106
	19-0060	3.55	1.43	78.4	390.3	112.7	13.22	48.98	12.91	76	0.27	2.49	305.65	18.58	6.04	69	28.3	146	12.9	106
	19-0053	3.23	1.30	73.2	291.3	135.2	11.64	43.10	12.40	72	0.27	2.49	202.14	16.65	5.72	88	24.9	146	10.4	106
	19-0054	3.75	1.51	39.8	260.1	96.1	11.92	47.66	13.34	81	0.25	2.48	222.42	18.11	6.23	218	25.5	149	9.4	106
	19-0021	3.50	1.29	83.7	185.1	115.5	10.98	28.90	13.07	106	0.38	2.71	332.81	17.88	6.21	122	23.5	145	10.9	103
	19-0022	3.12	1.38	69.5	252.3	128.4	7.48	27.69	12.94	90	0.27	2.26	205.80	17.16	5.38	67	16.0	147	10.4	107
	19-0055	3.32	0.90	53.8	122.7	106.0	7.85	23.09	11.99	75	0.34	3.70	198.25	16.59	7.02	75	16.8	143	11.7	103
	19-0056	3.58	1.03	53.9	338.1	94.3	12.34	31.63	11.88	78	0.39	3.47	201.50	13.38	7.05	93	26.4	147	8.0	107
	Mean	3.46	1.32	61.4	238.6*	114.5	10.65	40.63	12.66	81	0.28	2.70	230.75	16.70	6.16	100	22.8	146.7	10.2	106
	SD	0.19	0.23	15.0	89.0	23.5	2.02	13.50	0.47	11	0.08	0.50	56.54	1.72	0.53	45	4.3	1.8	1.6	2
500 mg/kg	19-0067	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0068	2.97	1.38	46.5	232.5	132.1	8.79	38.20	12.47	114	0.23	2.16	250.61	17.24	5.13	56	18.8	151	9.0	103
	19-0025	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0026	3.47	1.24	24.1	249.8	93.6	6.40	35.57	12.75	91	0.18	2.80	185.35	14.35	6.27	109	15.0	150	9.0	106
	19-0005	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0006	3.33	1.33	19.5	335.4	85.2	8.79	23.74	12.86	96	0.37	2.50	220.20	18.28	5.83	112	18.8	146	9.5	104
	19-0043	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0044	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0009	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0010	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	3.26	1.32	30.0*	272.6	103.6	7.99	32.50	12.69	100	0.26	2.49	218.72	16.62	5.74	92	17.5	149.0	9.2	104
	SD	0.26	0.07	14.4	55.1	25.0	1.38	7.70	0.20	12	0.10	0.32	32.66	2.04	0.57	32	2.2	2.6	0.3	2

Table L-2. Individual and Summary Clinical Chemistry in Males (cont.)

Dose Group	Animal ID	ALB (g/dL)	ALB / GLOB ratio (g/dL)	ALT (U/L)	ALKP (U/L)	AST (U/L)	BUN (md/dL)	BUN / CREA ratio (mg/dL)	Ca (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (g/dL)	GLU (mg/dL)	PHOS (mg/dL)	TP (g/dL)	TRIG (g/dL)	UREA (mg/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
1000 mg/kg	19-0051	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0052	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0023	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0024	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0027	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0028	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0033	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0034	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0063	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0064	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	SD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2000 mg/kg	19-0017	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0018	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0029	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0030	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0013	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0014	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0037	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0038	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0007	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	19-0008	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	SD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Legend: ND = No data analyzed

Note:

*p<0.05 versus 0 mg/kg-d control group, based on one-way ANOVA and Dunnett post hoc test for normally distributed data and Kruskal-Wallis and Mann-Whitney U post hoc test for data that is not normally distributed. Data for ALKP was log-transformed and data for ALB/GLOB, GLOB, TP, and TRIG were rank-transformed in order to achieve normal distribution of data. Data for Cl was not normally distributed after transformation; so ranked data were analyzed by Kruskal-Wallis test.

Table L-3. Clinical Chemistry Summary in Females and Males

			TNBA in Corn Oil					
		Sex	0 mg/kg	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
Albumin (ALB)	(g/dL)	Females	3.76±0.19	3.81±0.14	4.00±0.19*	3.78±0.19	3.74±0.13	3.57±0.12
		N	10	10	10	7	7	6
		Males	3.52±0.13	ND	3.53±0.12	3.46±0.19	3.26±0.26	ND
		N	10	ND	10	10	3	0
ALB / GLOB		Females	1.00±0.05	0.96±0.08	0.98±0.08	1.00±0.09	1.00±0.07	0.98±0.08
		N	10	10	10	7	7	6
		Males	1.22±0.20	ND	1.30±0.19	1.32±0.23	1.32±0.07	ND
		N	10	ND	10	10	3	0
Alanine aminotrans-ferase (ALT)	(U/L)	Females	51.9±8.5	50.8±16.1	41.6±12.0	52.2±15.0	43.4±23.4	27.4±11.7
		N	10	10	10	7	7	6
		Males	65.8±15.6	ND	75.8±13.0	61.4±15.0	30.0±14.4*	ND
		N	10	ND	10	10	3	0
Alkaline phosphatase (ALKP)	(U/L)	Females	160.3±38.0	135.2±23.3	132.2±28.9	141.3±37.9	146.1±28.2	121.2±51.9
		N	10	10	10	7	7	6
		Males	372.8±110.1	ND	261.4±49.6	238.6±89.0*	272.6±55.1	ND
		N	10	ND	10	10	3	0
Aspartate aminotrans-ferase (AST)	(U/L)	Females	107.8±12.5	123.9±52.8	110.2±17.2	113.5±23.0	102.8±19.8	106.2±16.3
		N	10	10	10	7	7	6
		Males	93.1±13.4	ND	119.5±24.7	114.5±23.5	103.6±25.0	ND
		N	10	ND	10	10	3	0
Blood urea nitrogen (BUN)	(md/dL)	Females	17.44±3.27	18.12±3.78	18.20±4.29	14.67±2.41	13.52±2.23	12.24±2.95*
		N	10	10	10	7	7	6
		Males	10.64±1.72	ND	10.00±1.74	10.65±2.02	7.99±1.38	ND
		N	10	ND	10	10	3	0
BUN / CREA		Females	43.56±10.28	46.32±9.43	47.93±12.05	46.62±13.23	40.10±8.66	34.76±5.48
		N	10	10	10	7	7	6
		Males	39.08±8.14	ND	37.82±6.70	40.63±13.50	32.50±7.70	ND
		N	10	ND	10	10	3	0
Calcium (Ca)	(mg/dL)	Females	12.12±0.37	12.00±0.60	12.27±0.42	12.70±0.76	12.52±0.59	12.27±0.68
		N	10	10	10	7	7	6
		Males	12.74±0.37	ND	12.86±0.33	12.66±0.47	12.69±0.20	ND
		N	10	ND	10	10	3	0
Cholesterol (CHOL)	(mg/dL)	Females	80±14	96±19	98±10	95±14	90±17	82±13
		N	10	10	10	7	7	6
		Males	85±12	ND	81±12	81±11	100±12	ND
		N	10	ND	10	10	3	0
Creatinine (CREA)	(mg/dL)	Females	0.41±0.04	0.40±0.08	0.39±0.08	0.33±0.07	0.34±0.05	0.35±0.06
		N	10	10	10	7	7	6
		Males	0.28±0.04	ND	0.27±0.06	0.28±0.08	0.26±0.10	ND
		N	10	ND	10	10	3	0
Globulin (GLOB)	(g/dL)	Females	3.77±0.33	3.99±0.37	4.09±0.42	3.81±0.36	3.73±0.23	3.67±0.32
		N	10	10	10	7	7	6
		Males	2.98±0.62	ND	2.78±0.58	2.70±0.50	2.49±0.32	ND
		N	10	ND	10	10	3	0
Glucose (GLU)	(mg/dL)	Females	163.94±59.17	152.30±43.06	157.17±31.79	225.36±82.02	169.52±72.35	210.20±85.53
		N	10	10	10	7	7	6
		Males	241.56±75.66	ND	219.69±93.50	230.75±56.54	218.72±32.66	ND
		N	10	ND	10	10	3	0
Phosphate (PHOS)	(mg/dL)	Females	15.86±2.20	17.45±1.72	17.45±1.53	17.14±1.73	15.78±1.32	17.56±2.12
		N	10	10	10	7	7	6
		Males	16.57±1.83	ND	16.94±1.11	16.70±1.72	16.62±2.04	ND
		N	10	ND	10	10	3	0
Total Protein (TP)	(g/dL)	Females	7.53±0.49	7.79±0.44	8.09±.0.54*	7.59±0.46	7.47±0.27	7.24±0.39
		N	10	10	10	7	7	6
		Males	6.50±0.67	ND	6.31±0.66	6.16±0.53	5.74±0.57	ND
		N	10	ND	10	10	3	0

Table L-3. Clinical Chemistry Summary in Females and Males (cont.)

				TNBA in Corn Oil					
		Sex		0 mg/kg	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
Triglycer-ides (TRIG)	(g/dL)	Females		43±15	46±10	52±18	74±13*	97±23*	121±48*
			N	10	10	10	7	7	6
		Males		118±57	ND	116±46	100±45	92±32	ND
			N	10	ND	10	10	3	0
UREA	(mg/dL)	Females		37.3±7.0	38.8±8.1	38.9±9.2	31.4±5.2	28.9±4.8	26.2±6.3*
			N	10	10	10	7	7	6
		Males		22.8±3.7	ND	21.4±3.7	22.8±4.3	17.5±2.2	ND
			N	10	ND	10	10	3	0
Sodium (Na)	(mmol/L)	Females		149±2	149±2	148±2	146±3*	144±2*	143±2*
			N	10	10	10	7	7	6
		Males		149±1	ND	148±2	147±2	149±3	ND
			N	9	ND	10	10	3	0
Potassium (K)	(mmol/L)	Females		9.1±1.1	10.2±1.4	10.6±1.2*	11.8±1.6*	11.1±1.1*	11.8±0.4*
			N	10	10	10	7	7	5
		Males		9.5±1.2	ND	9.9±0.8	10.2±1.6	9.2±0.3	ND
			N	9	ND	10	10	3	0
Chloride (Cl)	(mmol/L)	Females		107±1	107±1	106±1*	106±2	107±1	108±1
			N	10	10	10	7	7	6
		Males		106±1	ND	106±1	106±2	104±2	ND
			N	9	ND	10	10	3	0

Note:
*p<0.05 versus 0 mg/kg-d control group, based on one-way ANOVA and Dunnett post hoc test for normally distributed data and Kruskal-Wallis and Mann-Whitney U post hoc test for data that is not normally distributed.

APPENDIX M

INDIVIDUAL AND SUMMARY OF PROTHROMBIN TIME DATA

Table M-1. Individual and Summary Prothrombin Time in Females

Dose Group	Animal ID	PT (sec)	Dose Group	Animal ID	PT (sec)
0 mg/kg	19-0125	8.4	**250 mg/kg**	19-0121	9.2
	19-0126	9.1		19-0122	ND
	19-0075	8.6		19-0113	7.8
	19-0076	8.9		19-0114	ND
	19-0115	8.7		19-0099	ND
	19-0116	8.7		19-0100	8.3
	19-0107	8.6		19-0089	8.4
	19-0108	8.7		19-0090	8.9
	19-0091	ND		19-0127	8.3
	19-0092	8.6		19-0128	ND
	Mean	8.7		**Mean**	8.5
	SD	0.2		**SD**	0.5
62.5 mg/kg	19-0071	9.2	**500 mg/kg**	19-0111	ND
	19-0072	9.9		19-0112	9.1
	19-0105	9.3		19-0093	8.7
	19-0106	8.5		19-0094	8.3
	19-0073	9.7		19-0077	8.9
	19-0074	9.2		19-0078	8.7
	19-0119	8.4		19-0123	8.3
	19-0120	7.9		19-0124	ND
	19-0117	8.3		19-0083	ND
	19-0118	8.6		19-0084	8.3
	Mean	8.9		**Mean**	8.6
	SD	0.7		**SD**	0.3
125 mg/kg	19-0101	9.2	**1000 mg/kg**	19-0085	8.8
	19-0102	8.7		19-0086	8.9
	19-0095	8.6		19-0087	8.2
	19-0096	8.4		19-0088	ND
	19-0079	7.8		19-0097	ND
	19-0080	8.3		19-0098	9.0
	19-0081	8.4		19-0103	ND
	19-0082	8.7		19-0104	8.5
	19-0129	7.9		19-0109	8.8
	19-0130	8.1		19-0110	ND
	Mean	8.4		**Mean**	8.7
	SD	0.4		**SD**	0.3

Table M-2. Individual and Summary Prothrombin Time in Males

Dose Group	Animal ID	PT (sec)	Dose Group	Animal ID	PT (sec)
0 mg/kg	19-0019	9.3	**500 mg/kg**	19-0067	ND
	19-0020	9.4		19-0068	8.1
	19-0031	9.2		19-0025	ND
	19-0032	9.0		19-0026	9.4
	19-0061	9.3		19-0005	ND
	19-0062	8.7		19-0006	9.2
	19-0011	9.2		19-0043	ND
	19-0012	9.1		19-0044	ND
	19-0049	9.5		19-0009	ND
	19-0050	8.8		19-0010	ND
	Mean	9.1		**Mean**	8.9
	SD	0.3		**SD**	0.7
125 mg/kg	19-0045	8.8	**1000 mg/kg**	19-0051	ND
	19-0046	9.0		19-0052	ND
	19-0065	8.7		19-0023	ND
	19-0066	9.0		19-0024	ND
	19-0047	10.3		19-0027	ND
	19-0048	9.2		19-0028	ND
	19-0015	9.6		19-0033	ND
	19-0016	9.2		19-0034	ND
	19-0039	9.2		19-0063	ND
	19-0040	9.5		19-0064	ND
	Mean	9.2		**Mean**	ND
	SD	0.5		**SD**	ND
250 mg/kg	19-0041	9.9	**2000 mg/kg**	19-0017	ND
	19-0042	9.2		19-0018	ND
	19-0059	8.6		19-0029	ND
	19-0060	9.1		19-0030	ND
	19-0053	8.8		19-0013	ND
	19-0054	8.8		19-0014	ND
	19-0021	8.7		19-0037	ND
	19-0022	9.6		19-0038	ND
	19-0055	9.0		19-0007	ND
	19-0056	9.1		19-0008	ND
	Mean	9.1		**Mean**	ND
	SD	0.4		**SD**	ND

Table M-3. Prothrombin Time Summary in Females and Males

				TNBA in Corn Oil					
		Sex		0 mg/kg	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
Prothrombin Time	(sec)	Females		8.7±0.2	8.9±0.7	8.4±0.4	8.5±0.5	8.6±0.3	8.7±0.3
			N	9	10	10	6	7	6
		Males		9.1±0.3	ND	9.2±0.5	9.1±0.4	8.9±0.7	ND
			N	10	ND	10	10	3	0

APPENDIX N

GROSS PATHOLOGY DATA

Table N-1. Individual Gross Pathological Observations in Males and Females

DOSE	SEX	ANIMAL ID	DEATH	GROSS OBSERVATIONS
0 mg/kg	Female	19-0125	SK (14)	NGLR
		19-0126	SK (14)	NGLR
		19-0075	SK (14)	NGLR
		19-0076	SK (14)	NGLR
		19-0115	SK (14)	NGLR
		19-0116	SK (14)	NGLR
		19-0107	SK (14)	NGLR
		19-0108	SK (14)	NGLR
		19-0091	SK (14)	NGLR
		19-0092	SK (14)	NGLR
	Male	19-0019	SK (14)	NGLR
		19-0020	SK (14)	NGLR
		19-0031	SK (14)	Slight excess body fat
		19-0032	SK (14)	NGLR
		19-0061	SK (14)	Esophagus full of white foamy fluid; lungs diffusely congested with blood
		19-0062	SK (14)	White foam in trachea; lungs diffusely mottled
		19-0011	SK (14)	Stomach small, no food with yellow-clear fluid; liver diffusely mottled tan-burgundy; 1 mm nodule on serosal surface of kidney; cranial edge of thymus has reddish color
		19-0012	SK (14)	NGLR
		19-0049	SK (14)	NGLR
		19-0050	SK (14)	NGLR

Table N-1. Individual Gross Pathological Observations (cont.)

DOSE	SEX	ANIMAL ID	DEATH	GROSS OBSERVATIONS
62.5 mg/kg	Female	19-0071	SK (14)	NGLR
		19-0072	SK (14)	NGLR
		19-0105	SK (14)	NGLR
		19-0106	SK (14)	Faint yellow staining of hair on ventrum
		19-0073	SK (14)	Faint yellow staining of hair on ventrum
		19-0074	SK (14)	Faint yellow staining of hair on ventrum
		19-0119	SK (14)	Faint yellow staining of hair on ventrum
		19-0120	SK (14)	Faint yellow staining of hair on ventrum
		19-0117	SK (14)	Faint yellow staining of hair on ventrum
		19-0118	SK (14)	Faint yellow staining of hair on ventrum
DOSE	SEX	ANIMAL ID	DEATH	GROSS OBSERVATIONS
125 mg/kg	Female	19-0101	SK (14)	Spleen mildly enlarged; minimal congestion of gastric vessels; bone marrow is thin and watery; mild yellow staining of hair on ventral surface
		19-0102	SK (14)	Spleen minimally enlarged; minimal staining on ventral surface
		19-0095	SK (14)	Nonglandular stomach has perforation and hernia of muscular layer; submucosal edema and minimal thickening of nonglandular stomach; mild fluid accumulation in uterus; minimal yellow staining on abdomen
		19-0096	SK (14)	Minor separation of submucosa from nonglandular stomach, minimal thickening and discoloration of mucosa; minimal yellow staining of hair on ventrum
		19-0079	SK (14)	Nonglandular stomach mucosa has minimal thickening and roughened surface; spleen minimally enlarged; decreased stomach contents with mild gas distention; mild yellow staining of hair on ventrum
		19-0080	SK (14)	Mild yellow staining of hair on ventrum
		19-0081	SK (14)	Nonglandular mucosa is mildly thickened; faint yellow staining of hair on ventrum
		19-0082	SK (14)	Faint yellow staining of hair on ventrum
		19-0129	SK (14)	Nonglandular gastric mucosa is mildly thickened; spleen mildly enlarged; mild yellow staining near vulva and anus extending to ventrum
		19-0130	SK (14)	Spleen mildly enlarged; mild yellow staining of hair on ventrum
	Male	19-0045	SK (14)	Spleen enlarged 2x
		19-0046	SK (14)	Spleen mildly enlarged
		19-0065	SK (14)	Spleen mildly enlarged
		19-0066	SK (14)	Spleen mildly enlarged
		19-0047	SK (14)	Lightly yellow staining on abdomen
		19-0048	SK (14)	Yellow staining on abdomen
		19-0015	SK (14)	Light yellow staining of hair
		19-0016	SK (14)	3-4 ,mm hemorrhagic mass at distal edge of stomach; several 1-2 mm petechiae in cranial left lung lobe; light yellow staining of ventral surface
		19-0039	SK (14)	Spleen mildly enlarged; yellow staining of ventral abdomen
		19-0040	SK (14)	Spleen mildly enlarged; very mild yellow staining of fur

Table N-1. Individual Gross Pathological Observations (cont.)

DOSE	SEX	ANIMAL ID	DEATH	GROSS OBSERVATIONS
250 mg/kg	Female	19-0121	SK (14)	Thickened nonglandular mucosa; spleen mildly enlarged; mild yellow staining on abdomen near vulva
		19-0122	SK (14)	nonglandular stomach mucosa is thickened, roughened, and discolored; thymus enlarged; spleen mildly enlarged; severe yellow staining on abdomen centered near vulva
		19-0113	SK (14)	Nonglandular stomach mucosa is thickened, roughened, and discolored; thymus enlarged; bone marrow core is thin and watery; intestines mildly distended with gas; yellow staining on abdomen centered near vulva
		19-0114	MK (9)	Spleen mildly enlarged; GI tract distended with gas; low body fat
		19-0099	MK (7)	Spleen slightly enlarged; distal small intestines and colon are thin/translucent with scattered fecal content; lungs pale in color; yellow rectal staining; yellow-orange fluid around oral cavity
		19-0100	SK (14)	Mild yellow staining of ventrum centered near vulva
		19-0089	SK (14)	Nonglandular gastric mucosa is severely thickened with roughened white surface and areas of yellow discoloration; spleen moderately enlarged; mild yellow staining of hair on ventrum
		19-0090	SK (14)	Nonglandular gastric mucosa is mildly thickened with roughened surface; spleen mildly enlarged; mild yellow staining of hair on ventrum
		19-0127	SK (14)	Nonglandular gastric mucosa is mildly thickened with roughened surface and yellow discoloration; severe yellow staining near vulva and anus extending to ventrum
		19-0128	MK (3)	One 5 mm blister in nonglandular stomach; very little contents in GI tract
	Male	19-0041	SK (14)	Spleen diffusely enlarged 2-3x; caudal left lobe is dark; yellow staining from mouth to scrotum
		19-0042	SK (14)	Spleen enlarged 2x; yellow staining of hair from mouth to scrotum
		19-0059	SK (14)	Spleen slightly enlarged; light yellow hair staining from mouth to scrotum
		19-0060	SK (14)	Enlarged, dark spleen; diffusely mottled red lungs (all lobes); light yellow hair staining from mouth to scrotum
		19-0053	SK (14)	Oral cavity and esophagus full of reflux from stomach; lungs have multifocal points of congestion; dark yellow staining on ventral abdomen
		19-0054	SK (14)	Yellow staining on abdomen- darker around rectum
		19-0021	SK (14)	Spleen enlarged 2-3x; yellow staining on ventrum
		19-0022	SK (14)	Spleen slightly enlarged; yellow staining of ventral abdomen
		19-0055	SK (14)	Spleen enlarged; enlarged right mandibular lymph node with petechiae; yellow staining on abdomen
		19-0056	SK (14)	Spleen enlarged; yellow staining on abdomen

Table N-1. Individual Gross Pathological Observations (cont.)

DOSE	SEX	ANIMAL ID	DEATH	GROSS OBSERVATIONS
500 mg/kg	Female	19-0111	SK (14)	Nonglandular stomach is distended with large focus with yellow discoloration and separation of the superficial surface (blister); gastric vessels congested; GI tract nearly completely empty and distended; severe yellow staining around vulva with matting
		19-0112	SK (14)	Spleen severely enlarged; mild thickening of submucosa with mild roughening and discoloration; congestion of gastric vessels; yellow staining on ventral surface- darker near vulva
		19-0093	SK (14)	Nonglandular stomach has mild thickening of mucosal surface with roughening and yellow discoloration; spleen enlarged; yellow staining around vulva
		19-0094	SK (14)	Nonglandular stomach has submucosal edema and submucosal sruface is thickened with yellow discoloration; spleen severely enlarged; light yellow staining on abdomen-darker near vulva
		19-0077	SK (14)	Nonglandular stomach mucosa is thickened with roughened surface and yellow discoloration; spleen moderately enlarged; yellow staining on abdomen centered near vulva
		19-0078	SK (14)	Nonglandular stomach mucosa is thickened with roughened surface and yellow discoloration; spleen moderately enlarged; severe yellow staining on abdomen centered near vulva
		19-0123	SK (14)	One-third of nonglandular mucosa is thickened with roughened surface and yellow discoloration; spleen mildly enlarged; severe yellow staining of hair near vulva extending to ventrum
		19-0124	MK (7)	Significant thinning of mucosal layer of stomach; entire small intestine is thin/translucent with moderate amount of brown fecal matter; stringy orange feces from rectum; diffusely red mottled lungs; yellow-orange staining darker near vulva; yellow staining of abdomen
		19-0083	MK (5)	Yellow staining of hair near anus
		19-0084	SK (14)	Nonglandular mucosa is thickened with roughened surface; spleen moderately enlarged; yellow staining of hair on ventrum

Table N-1. Individual Gross Pathological Observations (cont.)

DOSE	SEX	ANIMAL ID	DEATH	GROSS OBSERVATIONS
500 mg/kg	Male	19-0067	MK (3)	Sloughing of mucosal lining of stomach*
		19-0068	SK (14)	Multiple 1-2 mm blisters in nonglandular stomach; enlarged spleen 2.5x; GI tract diffusely distended with air; yellow hair from mouth to scrotum- darker near scrotum
		19-0025	MK (10)	No visible ulcers in GI tract; gastric vessels minimally congested; GI contents are decreased throughout with loosely adherent red material on surface; spleen minimally enlarged; lungs are multifocally consolidated; yellow staining on entire body- especially on ventral surface
		19-0026	SK (14)	Spleen enlarged 2-3x; diffuse yellow staining of ventrum- darker near scrotum
		19-0005	FD (5)	Glandular portion of stomach has numerous 1-1.5 mm dark brown ulcerations; dark red stomach contents; cecum enlarged and reddened with serosal vessels; yellow staining of hair from mouth to penis
		19-0006	SK (14)	Spleen enlarged and dark; semi-solid feces around rectum; lungs mottled; yellow hair staining
		19-0043	MK (4)	Layer of white stringy/fibrous material attached to surface of nonglandular stomach lining; 1 mm foci on glandular stomach; dilated mesenteric vessels; stomach distended with air; GI contents mostly empty- small amount of red contents in cecum; large amount of yellow staining of hair near anus and scrotum; wet and matted hair near anus
		19-0044	FD (3)	Red contents in cecum; yellow staining of perineum
		19-0009	MK (5)	Layer of yellow mucoid material attached to surface of nonglandular stomach; red contents in stomach and cecum; intestines are empty and gass filled; large amount of yellow staining of hair near anus and scrotum
		19-0010	MK (9)	Enlarged spleen; severe yellow staining on ventral surface

Table N-1. Individual Gross Pathological Observations (cont.)

DOSE	SEX	ANIMAL ID	DEATH	GROSS OBSERVATIONS
1000 mg/kg	Female	19-0085	SK (14)	Spleen enlarged; nonglandular stomach thickened with yellow discharge; mild congestion of gastric vessels; yellow staining on abdomen
		19-0086	SK (14)	Nonglandular stomach is thickened with submucosal edema and roughened white-yellow surface; spleen severely enlarged; yellow staining on ventral abdomen- severe staining near vulva
		19-0087	SK (14)	Spleen enlarged; nonglandular stomach thickened with bumpy mucosal surface with several ulcerative foci; yellow staining on abdomen- severe near vulva
		19-0088	MK (4)	Several 2-6 mm fluid filled blisters as well as few smaller red hemorrhagic foci in nonglandular stomach; layer of white fibrous material on surface of nonglandular stomach; mesenteric lymph nodes are dark and hemorrhagic; GI tract nearly completely empty except for small amount of red hemorrhagic contents in stomach and cecum
		19-0097	MK (3)	Yellow discoloration of nonglandular stomach with white fibrinous material (mucus) stuck to surface; few 1-2 mm blisters and hemorrhagic foci of nonglandular stomach; GI contents are decreased with dark and reddish material; gastric vessels congested; loose and dark red feces; intense dark orange staining of perianal and vulval area with matting
		19-0098	SK (14)	Nonglandular stomach is mildly thickened roughened surface and yellow discoloration; submucosal gastric edema; spleen mildly enlarged; yellow staining on ventral abdomen- severe staining near vulva
		19-0103		Sloughing of glandular stomach epithelium; dark red contents in cecum; yellow staining around anus
		19-0104	SK (14)	Nonglandular stomach mucosa is mildly thickened; spleen enlarged; severe yellow staining near vulva entending to ventrum
		19-0109	SK (14)	Nonglandular mucosa thickened with roughened surface and yellow discoloration; severely enlarged spleen; stomach distended with ingesta; severe yellow staining near vulva and anus and extending to ventrum
		19-0110	MK (3)	3 5-7 mm blisters of nonglandular stomach epithelium; very little content in GI tract- some in stomach and cecum
	Male	19-0051	MK (3)	Sloughing of mucosal lining of stomach; large cyst in stomach epithelial lining*
		19-0052	MK (3)	NGLR*
		19-0023	MK (2)	Fluid filled cyst in stomach lining*
		19-0024	MK (2)	Fluid filled cyst in stomach lining*
		19-0027	FD (2)	Multiple cysts/blisters on stomach lining*
		19-0028	MK (2)	Multiple small cysts in stomach*
		19-0033	FD (1)	NGLR*
		19-0034	MK (1)	NGLR*
		19-0063	FD (1)	NGLR*
		19-0064	MK (1)	Cyst in lining of glandular stomach; dark red material attached to lining of stomach and mixed into stomach contents*

Table N-1. Individual Gross Pathological Observations (cont.)

DOSE	SEX	ANIMAL ID	DEATH	GROSS OBSERVATIONS
2000 mg/kg	Male	19-0017	MK (3)	Sloughing of mucosal lining of stomach*
		19-0018	FD (3)	NGLR*
		19-0029	MK (2)	NGLR*
		19-0030	FD (1)	NGLR*
		19-0013	FD (2)	Large cyst in stomach epithelial lining*
		19-0014	FD (2)	NGLR*
		19-0037	MK (1)	Multiple fluid filled cysts in stomach*
		19-0038	MK (1)	NGLR*
		19-0007	MK (1)	Fluid filled cyst in stomach lining*
		19-0008	MK (1)	NGLR*

Legend:
NGLR = No gross lesion reported
Death codes:
MK = Moribund Kill
SK = Scheduled Kill
FD = Found Dead (day of death; dosing 0-13, scheduled necropsy 14)
Note:
*21 necropsies were unexpectedly conducted on 11/23/18, which was a day in which no pathologist or veterinarian was present to provide gross findings. Only overt findings were recorded only that day.

Table N-2. Summary of Gross Pathological Observations in Males and Females

	0 mg/kg		62.5 mg/kg	125 mg/kg		250 mg/kg		500 mg/kg		1000 mg/kg		2000 mg/kg
	M	F	F	M	F	M	F	M	F	M	F	M
Necropsy with minimal crew, only overt findings recorded								1		10		10
NGLR	6	10	3							4		6
Skin and hair, yellow staining, perineum and abdomen or around oral cavity			7	6	10	10	8	9	10		8	
Spleen, enlarged				6	5	8	6	5	7		6	
Spleen, dark discoloration						1		1				
Stomach, non-glandular mucosa, thickened					5		6		7		6	
Stomach, non-glandular, mucosa, roughened surface					1		5		6		2	
Stomach, non-glandular portion, mucosa, yellow to white-yellow material					1		4	2	7		6	
Stomach, all locations, mucosal separation, blister, sloughing, hemorrhage or ulceration					2		1	6	3	6	7	4
Stomach, mucosa, fluid filled cyst/blister (separation of layers)					1		1	1	1	6	2	3
Stomach, non-glandular, mucosa, separation of mucosa and submucosa					1			1	1		1	
Stomach, mucosa, sloughing								1		1	1	1
Stomach, non-glandular, submucosal edema					1				2		2	
Stomach, mucosa, thinning									1			
Stomach, non-glandular, mucosa, ulcerative foci											2	
Stomach, glandular portion, focus of red discoloration (ulceration)								2			1	
Stomach, non-glandular, perforation and herniation					1							
Hemorrhagic contents, GI tract, stomach or cecum				1				5		1	3	
Stomach, mucosa, dark-red material (hemorrhage)				1				3		1	1	
GI tract contents, cecum, red (hemorrhagic) contents								4			3	
Stomach, distension									1		1	
Stomach, reduced in size	1											
Vessels, gastric or mesenteric, congested					1			2	2		2	
GI tract contents, rectum, orange stringy									1			
GI tract, gas distension							2	2				
GI tract contents, reduced,							2	3	2		3	
GI tract contents, reduced, stomach	1				1						1	
GI tract contents, fluidic											1	
Small intestine, thin/translucent									1			
Autolysis, GI tract								1			1	

Table N-2. Summary of Gross Pathological Observations in Males and Females (cont.)

	0 mg/kg		62.5 mg/kg	125 mg/kg		250 mg/kg		500 mg/kg		1000 mg/kg		2000 mg/kg
	M	F	F	M	F	M	F	M	F	M	F	M
Thymus, enlarged							2					
Thymus, discoloration, cranial edge	1											
Kidney, nodule, serosal surface	1											
Bone marrow, thin and watery					1		1					
Lungs, congestion, mottled, diffuse, or multifocal	2					3		1	1			
Lungs, consolidated, multifocally								1				
Lungs, petechia				1								
Lungs, pale discoloration							1					
Skin perianal, fecal accumulation								1				
Body as a whole, excess body fat	1											
Body as a whole, reduced fat							1					
Esophagus, white foamy fluid	2											
Esophagus, reflux contents						1						
Liver, discoloration, tan mottled	1											
Uterus, fluid accumulation					1							
Lymph node, mandibular, enlarged						1						
Lymph node, mandibular, petechia						1						
Lymph node, mesenteric, dark and hemorrhagic											1	

Legend:
NGLR = No gross lesion reported

Table N-3. Summary of Gross Pathological Observations for Both Sexes Combined

	0 mg/kg M&F	62.5 mg/kg F	125 mg/kg M&F	250 mg/kg M&F	500 mg/kg M&F	1000 mg/kg M&F	2000 mg/kg M
Necropsy with minimal crew, only overt findings recorded					1	10	10
NGLR	16	3				4	6
Skin and hair, yellow staining, perineum and abdomen or around oral cavity		7	16	18	19	8	
Spleen, enlarged			11	14	12	6	
Spleen, dark discoloration				1	1		
Stomach, non-glandular mucosa, thickened			5	6	7	6	
Stomach, non-glandular, mucosa, roughened surface			1	5	6	2	
Stomach, non-glandular portion, mucosa, yellow to white-yellow material			1	4	9	6	
Stomach, all locations, mucosal separation, blister, sloughing, hemorrhage or ulceration			2	1	9	13	4
Stomach, mucosa, fluid filled cyst/blister (separation of layers)			1	1	2	8	3
Stomach, non-glandular, mucosa, separation of mucosa and submucosa			1		2	1	
Stomach, mucosa, sloughing					1	2	1
Stomach, non-glandular, submucosal edema			1		2	2	
Stomach, mucosa, thinning					1		
Stomach, non-glandular, mucosa, ulcerative foci						2	
Stomach, glandular portion, focus of red discoloration (ulceration)					2	1	
Stomach, non-glandular, perforation and herniation			1				
Hemorrhagic contents, GI tract, stomach or cecum			1		5	4	
Stomach, mucosa, dark-red material (hemorrhage)			1		3	2	
GI tract contents, cecum, red (hemorrhagic) contents					4	3	
Stomach, distension					1	1	
Stomach, reduced in size	1						
Vessels, gastric or mesenteric, congested			1		4	2	
GI tract contents, rectum, orange stringy					1		
GI tract, gas distension				2	2		
GI tract contents, reduced,				2	5	3	
GI tract contents, reduced, stomach	1		1			1	
GI tract contents, fluidic						1	
Small intestine, thin/translucent					1		
Autolysis, GI tract					1	1	
Thymus, enlarged				2			
Thymus, discoloration, cranial edge	1						
Kidney, nodule, serosal surface	1						
Bone marrow, thin and watery			1	1			
Lungs, congestion, mottled, diffuse, or multifocal	2			3	2		
Lungs, consolidated, multifocally					1		
Lungs, petechia			1				
Lungs, pale discoloration				1			
Skin perianal, fecal accumulation					1		
Body as a whole, excess body fat	1						
Body as a whole, reduced fat				1			
Esophagus, white foamy fluid	2						
Esophagus, reflux contents				1			
Liver, discoloration, tan mottled	1						
Uterus, fluid accumulation			1				
Lymph node, mandibular, enlarged				1			
Lymph node, mandibular, petechia				1			
Lymph node, mesenteric, dark and hemorrhagic						1	

APPENDIX O

HISTOPATHOLOGY REPORT

PATHOLOGY REPORT
FOR

Protocol No. 30-18-07-02

Study Title
Effects of Subacute Oral 2,4,6-Trinitro-3-Bromoanisole (TNBA) Exposure to Rats (*Rattus norvegicus*)

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1 Objective

The primary objective of this study is to assess the toxicity of a new experimental explosive, TNBA via oral gavage exposure for 14 days

2 Materials and Methods

2.1 Animals

This study was conducted using male and female Sprague-Dawley rats from Charles River.

2.2 Exposure Methods

The test article was administered via oral gavage for the entire length of the study. The following table provides a summary of the animal exposures by group.

Table 1. Animal Exposure Summary

Group #	Chemical	Route	Targeted Exposure Length (days)	Target Concentration (mg/kg)	# of male rats	# of female rats
0	Vehicle Control	Oral gavage	14	0	10	10
1	TNBA	Oral gavage	14	62.5		10
2	TNBA	Oral gavage	14	125	10	10
3	TNBA	Oral gavage	14	250	10	10
4	TNBA	Oral gavage	14	500	10	10
5	TNBA	Oral gavage	14	1000	10	10
6	TNBA	Oral gavage	14	2000	10	

2.3 Necropsy Procedures and Sample processing

Rats were euthanized with carbon dioxide followed by gross examination, tissue collection and tissue preservation and fixation.

The following tissues were collected and preserved/fixed: mammary gland; heart; lung; thymus; thyroid with parathyroid glands; trachea; adrenal glands; kidneys; liver; lymph nodes (axillary and mesenteric); spleen; urinary bladder; cecum; colon; duodenum; ileum; jejunum; rectum; stomach (forestomach, glandular stomach); prostate ; seminal vesicles with coagulating glands and their fluids (as one unit); ovaries; testes; epididymidis; uterus (with oviducts and cervix); vagina; peripheral nerve; fresh bone marrow aspirate; skeletal muscle; skin; spinal cord (at three

levels: cervical, mid-thoracic, and lumbar); brain; eye; and tissues with an observed gross lesion.

2.4 Histopathology

Tissues were fixed in formalin, trimmed into cassettes, processed, embedded in paraffin, sectioned via a microtome to a thickness of 4-5 um, and stained with hematoxylin and eosin using a routine automatic stainer. Additionally the testis and epididymis were initially fixed in modified Davidson's solution and additionally stained with the Periodic Acid Schiff (PAS) stain.

The **liver, spleen, kidney, adrenal gland, stomach, thymus, and bone marrow aspirate** from the high dose groups (1000 and 2000 mg/kg), and vehicle control were processed and analyzed. Tissues from lower dose groups were evaluated when a significant difference was observed in lesion incidence or organ weight between the high-dose group compared to the control group, see Table 2 regarding tissues examined. The general criteria for establishing a histologic scores included the following: Score: '0' = the tissue is essentially normal or observed in <1% of the sampled tissue; '1' means minimum (<5% of tissue affected); '2' means mild (6-20% of tissue affected); '3' = moderate (21-40%); '4' = marked (41%-79% of tissue affected); '5' = severe (>80% of tissue affected). Absent= not present for microscopic examination due to sampling error, out of the plane of section, or other similar reason.

Table 2. Tissue Processing Summary

	M	F	M	M	F	M	F	M	F	M	F	F
# of animals	10	10	10	10	10	10	10	10	10	10	10	10
Group dose level	0	0	2000	1000	1000	500	500	250	250	125	125	62.5
Liver												
Spleen												
Kidney												
Adrenal gland												
Stomach												
Thymus												
Bone marrow aspirate												

= Tissue processed and microscopically examined.
= Tissues were collected, but not microscopically examined.

3 Results

3.1 Gross Findings

Dose related gross findings were noted in the hair/skin, stomach, and spleen. Yellow urine staining of the hair and skin in the perineum and ventral abdomen was commonly noted in male and female rats from all dose groups, this staining tended to be more severe, but not necessarily more frequent, in the higher dose

groups. Several of the gross findings, including the yellow staining, had significant intraobserver/prosector variation as all findings were not consistently recorded or observed in the high dose group due to the unexpected deaths of a large number of animals that were necropsied with only a minimal crew available. These unscheduled deaths occurred in all of the 1000 and 2000 mg/kg group male rats, 7 of 10 of the 500 mg/kg group male rats, 4 of 10 of the 1000 mg/kg group female rats, and 3 of 10 of the 500 mg/kg group female rats, and only the most overt findings were recorded for these rats. The non-glandular portion of the stomach had macroscopic lesions in a significant number of animals from all dose groups, except the lowest dose group (62.5 mg/kg), and these gastric lesions included thickening of the non-glandular mucosa, separation of the superficial layers forming a fluid-filled cyst/blister, or sloughing of the mucosa. Meanwhile, the glandular portion of the stomach had lesions in only three animals and in these animals the glandular stomach lesions consisted of foci of red discoloration. The GI tract contents were reduced, had orange-stringy material, or hemorrhagic material in the cecum, stomach, or rectum in 2 to 3 animals in several groups. The observation of an enlarged spleen was noted in the majority of animals in all of the compound exposed groups except the female rats at 62.5 mg/kg and the male rats at 2000 and 1000 mg/kg; the absence of a macroscopically enlarged spleen in the highest dose male group animals (2000 and 1000 mg/kg) is likely due to the animals being examined during the minimal crew as noted above for the stomach.

Additional findings were noted in a few or individual animals that were sporadically distributed across dose groups and sexes. These findings included thin and watery bone marrow material (1 female at 250 mg/kg and 1 female at 125 mg/kg); enlarged thymus (1 female at 250 mg/kg); multifocal consolidation, pale discoloration, or congestion of the lungs in 1-3 animals in the control (2 males) and the mid-dose groups (3 males at 250 mg/kg, 2 males at 500 mg/kg, and 1 female at 500 mg/kg); and a renal nodule (1 control male).

3.2 Histopathology

Treatment-related microscopic findings were observed in the 1) kidney, 2) stomach, 3) spleen, and 4) thymus of male and female rats. 1) The kidney of the male and female rats in all dose groups, except the lowest dose groups had evidence of acute kidney injury including acute necrosis and degeneration of the tubular epithelium. The dose groups that did not have acute tubular necrosis were the 125 mg/kg male, 62.5 mg/kg female groups, and 125 mg/kg female groups. The 125 mg/kg male dose group rats had kidney microscopic lesions consisting of sporadic tubular epithelial cell degeneration in 3 of 10, and intracytoplasmic eosinophilic globules and crystals in 9 of 10 rats. All of the female rats in the two lowest dose group had essentially normal kidneys. Additionally tubular cell regeneration was present in 6 of 10 females and 10 of 10 males at 250 mg/kg, 3 of 10 females at 500 mg/kg, and 2 of 10 females at 1000 mg/kg that was variably accompanied by glomerulonephritis. 2) The stomach of all of the male and female rats in the high dose groups (1000 mg/kg) had treatment-related microscopic findings, and these changes consisted of squamous epithelial hyperplasia and

hyperkeratosis in the non-glandular mucosa that was accompanied by submucosal edema, congestion, and neutrophilic and lymphocytic infiltrates. In the most severe cases there was also ulceration and necrosis and proliferation of granulation tissue. The microscopic findings correlate well with gross findings. 3) The spleen of all of the groups examined that were dosed at 500 mg/kg or more were examined. There was treatment related changes in the female high dose groups and the mid-dose male groups; these changes included increased histiocytic pigment accumulation, increased extramedullary hematopoiesis, and red pulp congestion. These changes correlate to the macroscopic finding of enlarged spleen and with dark discoloration in some cases. 4) Within the thymus there were treatment related findings in all examined males at 2000, 1000, and 500 mg/kg groups and all but 1 female at 1000 mg/kg and 3 females at 500 mg/kg. These findings consisted of increased cortical lymphocyte apoptosis, and necrosis with increased tingible body macrophages and variably follicular atrophy was present. The tingible body macrophages were of similar incident in the exposed females compared to the control group, but tended to be slightly more severe in the exposed groups…minimal in controls, versus mild to marked in the exposed groups.

In the liver of male and female rats, various abnormalities including lipid-type vacuolation, single-cell hepatocellular necrosis, hepatocellular hypertrophy, and intrahistiocytic pigment were observed. The changes did not occur in increased incidence in exposed animals compared to the control animals except for single-cell hepatocellular necrosis in males at 2000 mg/kg (7 of 10), hepatocellular hypertrophy in males at 2000 mg/kg (9 of 10) and 1000 mg/kg (8 of 10), and intrahistiocytic pigment in females at 500 mg/kg (9 of 10). The histologic changes suggestive of TNBA-induced toxicity was equally observed in male and female rats. See Tables 3-6 for details.

4 Discussion

The microscopic findings in the kidney likely led to acute renal failure and is the likely cause of unscheduled deaths in the high dose animals. Clinical chemistry, especially BUN and creatinine is needed to confirm the diagnosis of acute renal failure, but clinical chemistry was unable to be performed on these animals due to the unscheduled death of these high dose animals. Azotemia was not present in animals that had clinical chemistry performed, but this may have been due to recovery and compensation after initial insult that could have occurred earlier in the dosing period. The presence of tubular cell regeneration in the males and females at 250 mg/kg, and females at 500 mg/kg and at 1000 mg/kg is supportive of previous insult with recovery. These animals did not have azotemia or evidence of renal failure when they were euthanized which indicates that less than 25% of the renal capacity was affected and/or due to recovery. The BUN and creatinine are relatively insensitive indicators of renal toxicity because loss of more than 75% of the renal capacity must occur before azotemia (increased BUN and creatinine) and renal failure becomes apparent. Eosinophilic (hyaline) cytoplasmic renal

tubular droplets were noted in several high dose males, these cytoplasmic droplets most likely represent induction of alpha 2u-globulin expression that occurs in male rats.

The microscopic findings of increased extramedullary hematopoiesis (EMH) and pigment deposition correlates with the macroscopic finding of enlargement and dark discoloration of the spleen; EMH is normally present in the spleen of rodents, but it is increased in the dosed animals compared to the controls in this study. These findings also correlate with the clinical pathology findings of anemia (decreased erythrocyte mass...RBC, HGB, and HCT) that would have resulted in increased hematopoietic demand specifically erythroid precursors. This anemia was more severe in the higher doses. The anemia was present in absolute number of erythrocytes (RBC) and red blood cell mass (HGB and HCT). The anemia is further classified as macrocytic (increased cell volume (MCV)), hyperchromic (increased mean corpuscular hemoglobin (MCH), and variably affected mean corpuscular hemoglobin concentration (MCHC). The MCHC is a less reliable indicator of hemoglobin content and the changes are not likely significant. The red cell distribution width is also increased, which is an indicator of a larger variation in size between RBC's in this case this increased RDW is due to macrocytosis. This type of anemia (macrocytic) is consistent with a regenerative anemia; new erythrocytes released from the bone marrow tend to have a larger volume which correlates with the splenic observations. Typically regenerative anemia is accompanied by decreased hypochromasia (decreased MCH and MCHC). The loss of RBC's could be due to hemolysis, which is supported by the presence of hyperchromasia, which is an indication of hemolysis. The pigment deposition in the spleen is also indicative of hemolysis, and phagocytosis of liberated iron from erythrocytes by splenic macrophages/histiocytes. The hemolysis is most likely compound related and is responsible for the findings in the spleen and clinical pathology findings.

The renal tubular degeneration and necrosis could be the result of hemolysis, hemoglobinuric nephrosis, or hemoglobin toxicity, and not necessarily be a direct compound effect. But the deposition of hemoglobin within the renal tubules was not microscopically observed, which makes hemoglobinuric nephrosis less likely.

The cause of the thymic lymphocyte apoptosis and necrosis cannot be determined with certainty. Lymphocyte apoptosis is a physiologically normal process that can be increased by stress, and it is difficult to separate a direct acting effect immunotoxicity effect versus stress related thymic apoptosis. The stress of organ (renal) failure and other toxicologic effects likely played a significant role for the thymic changes observed in this study.

The microscopic changes in the stomach observed in the high dose groups correlate with the macroscopic findings in this study. These observations in the stomach are likely attributed to a direct irritant effect due to oral gavage. These lesions were only observed in the non-glandular portion of the stomach. The rat

stomach is composed of a large area of non-glandular mucosa that is lined by keratinized squamous epithelium and lacks the gastric glands that produce acid and enzymes (e.g., pepsin) found in the remainder of the stomach. This portion of the stomach acts as a storage and fermentation chamber for bacterial and protozoal digestion. A similar structural feature of non-glandular mucosa does not exist in humans and therefore classification of this effect as a relevant adverse finding is not warranted.

5 Point of Contact

Questions pertaining to this report should be referred to LTC Keith Koistinen at DSN 584-3980, commercial 410-436-3980, or by e-mail: usarmy.apg.medcom-dcs-ph.mbx.tox-info@mail.mil.

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Table 3: Incidence of Histopathologic Finding by Group

	Sex	F	F	F	F	F	F	M	M	M	M	M	M
	Dose Group	0	62.5	125	250	500	1000	0	125	250	500	1000	2000
	Number of Animals	10	10	10	10	10	10	10	10	10	10	10	10
Liver	# Examined	10				10	10	10			10	10	10
	Essentially normal tissue	2						1			2	1	1
	Rarefaction, (vacuolar change, glycogen type)							3			1	1	
	Vacuolation, lipid-type	2					1	9			2	4	
	Hepatocellular hypertrophy										2	8	9
	Necrosis, hepatocellular, single cell, multifocal	4				9	7				1	4	7
	Infiltrates, lymphocytic										1		
	Pigment, intravascular						2						
	Pigment, intrahistiocytic					9	4						
	Cytoplasmic Inclusions, hepatocyte (congestion droplets)	7				9	3	2			3	1	
	EMH, multifocal, nodular					4	4						
Spleen	Absent										1	1	
	Autolysis												2
	# Examined	10				10	10	10			9	9	10
	Essentially normal tissue	9				2		10			4	7	3
	Increased Intrahistiocytic pigment accumulation, red pulp					7	9				4		
	Lymphoid hyperplasia, follicular	1											
	Red pulp, increased extramedullary hematopoiesis					8	10				4		6
	Lymphocytolysis, increased, follicular										1	2	2
	Congestion, red pulp					7	3						
Kidney	Autolysis				1						2	3	3
	# Examined	10	10	10	10	10	10	10	10	10	10	10	10
	Essentially normal tissue	10	9	9		2	2	10	1		5	2	4
	Infiltrate, interstitial, lymphocytic				7		1			7			
	Tubular epithelial cell degeneration				9	5	8		3	9	5	2	3
	Tubular cell, cytoplasmic vacuolation					4	6			1			
	Necrosis, tubular epithelium, PCT, acute				10	8	7			9	5	6	6
	Intracytoplasmic eosinophilic globules						2		9		1	4	3
	Intracytoplasmic eosinophilic crystals								9				
	Cortical cyst		1										
	Hemmorhage, interstitial										1		
	Glomerular synechia						1						
	Tubular epithelial cell regeneration				6	3	2			10			
	Glomerulornephritis, multifocal				8		1			3			
	Tubular dilatation				1							1	
	Cortical adipose deposition, focal						1						
	Renal pelvic dilation			1									
Adrenal gland	Absent												1
	Autolysis											2	4
	# Examined	10	10	10	10	10	10	10	10	10	10	10	9
	Essentially normal tissue	10	10	10	10	8	10	10	10	8	9	10	8
	Cortical epithelial cells, vacuolation, increased					2				2	1		
Stomach	Absent											1	
	Autolysis										1		1
	# Examined	10				10	10	10			10	9	10
	Essentially normal tissue	8						10					
	Squamous epithelial hyperplasia, Non-glandlr mucosa					10	10				10	9	10
	Hyperkeratosis, non-glandular mucosa					10	10				10	9	10
	Submucosal edema and/or hemorrhage	2				10	10				10	9	10
	Cellular infiltrates, neutrophilic and lymphocytic					10	10				10	9	10
	Congestion, submucosa	1				10	10				10	9	10
	Ulceration/necrosis, mucosal, non-glandular stomach					6	5				9	9	9
	Granulation tissue, submucosal					8	4				7		
Thymus	Absent										1	3	2
	Autolysis												
	# Examined	10			10	10	10	10		10	9	7	8
	Essentially normal tissue	3			7			4		10			
	Atrophy, follicular				1	3	4				3		
	Perifollicular congestion/hemorrhage					4	1	2			1		1
	Apoptosis, incrsd, lymphocyte, cortical, multifocal to diffuse	1			3	7	9				9	7	8
	Necrosis, lymphocyte, cortical, multifocal to diffuse				3	3	4				5	6	4
	Tingible body macrophage, increased	7			3	10	10	5			9	7	8
Bone marrow	# Examined	10				10	10	10			10	10	10
	Essentially normal tissue	10				10	10	10			10	10	8
	Increased blastic cells, likely erythroid												2

Table 4: Incidence of Gross Findings by Group

	Sex	F	F	F	F	F	F	M	M	M	M	M	M
	Dose Group	0	62.5	125	250	500	1000	0	125	250	500	1000	2000
	Number of Animals	10	10	10	10	10	10	10	10	10	10	10	10
Gross Findings	Necropsy with minimal crew, only overt findings recorded											10	10
	NGLR	10	3					6				4	6
	Skin and hair, yellow staining, perineum and abdomen or around ora		7	10	8	10	8		6	10	9		
	Spleen, dark discoloration									1	1		
	Spleen, enlarged			5	6	7	6		6	8	5		
	Stomach, non-glandular mucosa, thickened			5	6	7	6						
	Stomach, non-glandular, mucosa, roughened surface			1	5	6	2						
	Stomach, non-glandular portion, mucosa, yellow to white-yellow material			1	4	7	6				2		
	Vessels, gastric or mesenteric, congested			1		2	2				2		
	Stomach, non-glandular, submucosal edema			1		2	2						
	Stomach, non-glandular, mucosa, ulcerative foci						2						
	Stomach, mucosa, fluid filled cyst/blister (seperation of layers)			1	1	1	2				1	6	3
	Stomach, non-glandular, mucosa, seperation of submucosa			1		1	1				1		
	Stomach, mucosa, sloughing						1				1	1	1
	Stomach, reduced in size							1					
	Stomach, mucosa, thinning					1							
	Stomach, glandular portion, focus of red discoloration (ulceration)						1				2		
	Stomach, mucosa, dark-red material (hemorrhage)						1		1		3	1	
	Stomach, non-glandular mucosa, perforation and herniation			1									
	Stomach, non-glandular, distension					1	1						
	Autolysis, GI tract						1				1		
	Thymus, enlarged				2								
	Thymus, discoloration, cranial edge							1					
	Kidney, nodule, serosal surface							1					
	Bone marrow, thin and watery			1	1								
	Lungs, pale discoloration				1								
	Liver, discoloration, tan mottled							1					
	Lungs, congestion, mottled, diffuse, or multifocal					1		2		3	1		
	Lungs, petechia								1				
	Lungs, consolidated, multifocally										1		
	Skin perianal, fecal accumulation										1		
	Small intestine, thin/translucent					1							
	GI tract contents, reduced,				2	2	3				3		
	GI tract contents, reduced, stomach			1			1	1					
	GI tract contents, rectum, orange stringy					1							
	GI tract contents, cecum, red (hemorrhagic) contents						3				4		
	Body as a whole, excess body fat							1					
	Esophagus, white foamy fluid							2					
	Esophagus, reflux contents									1			
	Uterus, fluid accumulation			1									
	GI tract, gas distension				2						2		
	Body as a whole, reduced fat				1								
	Lymph node, mandibular, enlarged									1			
	Lymph node, mesenteric, dark and hemorrhagic						1						
	Lymph node, mandibular, petechia									1			
	GI tract contents, loose						1						

Table 5a: Statistical Analysis Results (Female)

TNBA Dose Group (mg/kg)		Female					
		0	62.5	125	250	500	1000
Kidney	n	10	10	10	10	10	10
Infiltrate, interstitial, lymphocytic	incidence	0	0	0	7**	0	1
Necrosis, tubular epithelium, PCT, acute	incidence	0	0	0	10**	8**	7**
Tubular epithelial cell degeneration	incidence	0	0	0	9**	5*	8**
Tubular epithelial cell regeneration	incidence	0	0	0	6**	3	2
Tubular epithelium, cytoplasmic vacuolation	incidence	0	0	0	0	4	6**
Intracytoplasmic eosinophilic crystals	incidence	0	0	0	0	0	0
Intracytoplasmic eosinophilic globules	incidence	0	0	0	0	0	2
Stomach	n	10	0	0	0	10	10
Squamous epithelial hyperplasia, Non-glandular stomach	incidence	0	0	0	0	10**	10**
Hyperkeratosis, non-glandular mucosa	incidence	0	0	0	0	10**	10**
Submucosal edema and or hemorrhage	incidence	2	0	0	0	10**	10**
Cellular infiltrates, neutrophilic and lymphocytic	incidence	0	0	0	0	10**	10**
Congestion, submucosa	incidence	1	0	0	0	10**	10**
Ulceration necrosis, mucosal, non-glandular stomach	incidence	0	0	0	0	6**	5*
Granulation tissue, submucosal	incidence	0	0	0	0	8**	4
Thymus	n	10	0	0	10	10	10
Tingible body macrophage, increased	incidence	7	0	0	3	10	10
Apoptosis, increased, lymphocyte, cortical, multifocal to diffuse	incidence	1	0	0	3	7*	9**
Necrosis, lymphocyte, cortical, multifocal to diffuse	incidence	0	0	0	3	3	4
Spleen	n	10	0	0	0	10	10
Increased Intrahistiocytic pigment accumulation, red pulp	incidence	0	0	0	0	7**	9**
Red pulp, increased extramedullary hematopoiesis	incidence	0	0	0	0	8**	10**
Congestion, red pulp	incidence	0	0	0	0	7**	3
Liver	n	10	0	0	0	10	10
Vacuolation, lipid-type	incidence	2	0	0	0	0	1
Necrosis, hepatocellular, single cell, multifocal	incidence	4	0	0	0	9	7
Hepatocellular hypertrophy	incidence	0	0	0	0	0	0
Pigment, intrahistiocytic	incidence	0	0	0	0	9**	4
Gross Findings	n	10	10	10	10	10	10
Necropsy with minimal crew, only overt findings recorded	incidence	0	0	0	0	0	0
Skin and hair, yellow staining, perineum and abdomen	incidence	0	7**	10**	8**	10**	8**
Spleen, enlarged	incidence	0	0	5*	6**	7**	6**
Stomach, mucosa, fluid filled cyst/blister (seperation of layers)	incidence	0	0	1	1	1	2
Stomach, non-glandular mucosa, thickened	incidence	0	0	5*	6*	7**	6*
Stomach, non-glandular, mucosa, roughened surface	incidence	0	0	1	5*	6*	2
Stomach, non-glandular portion, mucosa, yellow to white-yellow material		0	0	1	4	7**	6*

*significantly different from control, p<0.05; **significantly different from control, p<0.01

Table 5b: Statistical Analysis Results (Male)

TNBA Dose Group (mg/kg)		Male					
		0	125	250	500	1000	2000
Kidney	n	10	10	10	10	10	10
Infiltrate, interstitial, lymphocytic	incidence	0	0	7**	0	0	0
Necrosis, tubular epithelium, PCT, acute	incidence	0	0	9**	5*	6*	6*
Tubular epithelial cell degeneration	incidence	0	3	9**	5*	2	3
Tubular epithelial cell regeneration	incidence	0	0	10**	0	0	0
Tubular epithelium, cytoplasmic vacuolation	incidence	0	0	1	0	0	0
Intracytoplasmic eosinophilic crystals	incidence	0	9**	0	0	0	0
Intracytoplasmic eosinophilic globules	incidence	0	9**	0	1	4	3
Stomach	n	10	0	0	10	9	10
Squamous epithelial hyperplasia, Non-glandular stomach	incidence	0	0	0	10**	9**	10**
Hyperkeratosis, non-glandular mucosa	incidence	0	0	0	10**	9**	10**
Submucosal edema and or hemorrhage	incidence	0	0	0	10**	9**	10**
Cellular infiltrates, neutrophilic and lymphocytic	incidence	0	0	0	10**	9**	10**
Congestion, submucosa	incidence	0	0	0	10**	9**	10**
Ulceration necrosis, mucosal, non-glandular stomach	incidence	0	0	0	9**	9**	9**
Granulation tissue, submucosal	incidence	0	0	0	7**	0	0
Thymus	n	10	0	10	9	7	8
Tingible body macrophage, increased	incidence	4	0	0	9*	7**	8*
Apoptosis, increased, lymphocyte, cortical, multifocal to diffuse	incidence	0	0	0	9**	7**	8**
Necrosis, lymphocyte, cortical, multifocal to diffuse	incidence	0	0	0	5*	6**	4*
Spleen	n	10	0	0	9	9	10
Increased Intrahistiocytic pigment accumulation, red pulp	incidence	0	0	0	4*	0	0
Red pulp, increased extramedullary hematopoiesis	incidence	0	0	0	4*	0	6*
Congestion, red pulp	incidence	10	0	0	9	9	10
Liver	n	10	0	0	10	10	10
Vacuolation, lipid-type	incidence	9	0	0	2**	4	0**
Necrosis, hepatocellular, single cell, multifocal	incidence	0	0	0	1	4	7**
Hepatocellular hypertrophy	incidence	0	0	0	2	8**	9**
Pigment, intrahistiocytic	incidence	0	0	0	0	0	0
Gross Findings	n	10	10	10	10	10	10
Necropsy with minimal crew, only overt findings recorded	incidence	0	0	0	0	10**	10**
Skin and hair, yellow staining, perineum and abdomen	incidence	0	6*	10**	9**	0	0
Spleen, enlarged	incidence	0	6*	8	5*	0	0
Stomach, mucosa, fluid filled cyst/blister (seperation of layers)	incidence	0	0	0	1	6*	3
Stomach, non-glandular mucosa, thickened	incidence	0	0	0	0	0	0
Stomach, non-glandular, mucosa, roughened surface	incidence	0	0	0	0	0	0
Stomach, non-glandular portion, mucosa, yellow to white-yellow material		0	0	0	2	0	0

*significantly different from control, p<0.05; **significantly different from control, p<0.01

Table 6a: Control Individual Animal Histopathologic Findings

	Date of death	12/12	12/12	12/13	12/13	12/13	12/13	12/12	12/12	12/11	12/11	12/5	12/5	12/4	12/4	12/4	12/4	12/6	12/6	12/5	12/5
	Timing of death premature sacrifice or end of study	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS
	Manner of death (FD, MK, SK)	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK
	Date of necropsy	12/12	12/12	12/13	12/13	12/13	12/13	12/12	12/12	12/11	12/11	12/5	12/5	12/4	12/4	12/4	12/4	12/6	12/6	12/5	12/5
	Sex	F	F	F	F	F	F	F	F	F	F	M	M	M	M	M	M	M	M	M	M
	Dose Group	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Number of Animals	75	76	91	92	107	108	115	116	125	126	11	12	19	20	31	32	49	50	61	62
Kidney	Autolysis (5=most severe, precludes analysis)																				
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Essentially normal tissue	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Infiltrate, interstitial, lymphocytic																				
	Tubular epithelial cell degeneration																				
	Tubular cell, cytoplasmic vacuolation																				
	Necrosis, tubular epithelium, PCT, acute																				
	Intracytoplasmic eosinophilic globules																				
	Intracytoplasmic eosinophilic crystals																				
	Cortical cyst																				
	Hemmorhage, interstitial																				
	Glomerular synechia																				
	Tubular epithelial cell regeneration																				
	Glomerulornephritis, multifocal																				
	Tubular dilatation																				
	Cortical adipose deposition, focal																				
	Renal pelvic dilation																				
Adrenal gland	Absent (1=true, blank not true)																				
	Autolysis (5=most severe, precludes analysis)																				
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Essentially normal tissue	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Cortical epithelial cells, vacuolation, increased																				
Thymus	Absent (1=true, blank not true)																				
	Autolysis (5=most severe, precludes analysis)											.									
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Essentially normal tissue	0				0			0						0	0	0		0		
	Atrophy, follicular																				
	Perifollicular congestion/hemorrhage											2									1
	Apoptosis, incrsd, lymphocyte, cortical, multifocal to diffuse						1														
	Necrosis, lymphocyte, cortical, multifocal to diffuse																				
	Tingible body macrophage, increased		1	1	1		1	1		1	1		1	1				1		1	1
Liver	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Essentially normal tissue	0		0													0				
	Rarefaction, (vacuolar change, glycogen type)													2	1	1					
	Vacuolation, lipid-type					2		1				1	1	1	1	1		2	1	1	2
	Hepatocellular hypertrophy																				
	Necrosis, hepatocellular, single cell, multifocal	1		1	2						1										
	Infiltrates, lymphocytic																				
	Pigment, intravascular																				
	Pigment, intrahistiocytic																				
	Cytoplasmic Inclusions, hepatocyte (congestion droplet		3	1	2		3	1	1	1				2	1						
	EMH, multifocal, nodular																				
Spleen	Absent (1=true, blank not true)																				
	Autolysis (5=most severe, precludes analysis)																				
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Essentially normal tissue	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0
	Increased Intrahistiocytic pigment accumulation, red pulp																				
	Lymphoid hyperplasia, follicular								1												
	Red pulp, increased extramedullary hematopoiesis																				
	Lymphocytolysis, increased, follicular																				
	Congestion, red pulp																				
	Absent (1=true, blank not true)																				
	Autolysis (5=most severe, precludes analysis)																				
Stomach	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Essentially normal tissue	0	0		0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0
	Squamous epithelial hyperplasia, Non-glandlr mucosa																				
	Hyperkeratosis, non-glandular mucosa																				
	Submucosal edema and/or hemorrhage			1						1											
	Cellular infiltrates, neutrophilic and lymphocytic																				
	Congestion, submucosa									1											
	Ulceration/necrosis, mucosal, non-glandular stomach																				
	Granulation tissue, submucosal																				
Bone marrow, section or	Absent (1=true, blank not true)																				
	Autolysis (5=most severe, precludes analysis)																				
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Essentially normal tissue	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Increased blastic cells, likely erythroid																				

Table 6b: 2000 mg/kg Individual Animal Histopathologic Findings

Date of death		11/23	11/23	11/23	11/22	11/23	11/23	11/23	11/23	11/23	11/23
Timing of death premature sacrifice or end of study		PD	PD	PD	PD	PD	PD	PD	PD	PD	PD
Manner of death (FD, MK, SK)		MK	FD	MK	FD	FD	FD	MK	MK	MK	MK
Date of necropsy		11/23	11/23	11/23	11/22	11/23	11/23	11/23	11/23	11/23	11/23
Sex		M	M	M	M	M	M	M	M	M	M
Dose Group		2000	2000	2000	2000	2000	2000	2000	2000	2000	2000
Number of Animals		17	18	29	30	13	14	37	38	7	8
Kidney	Autolysis (5=most severe, precludes analysis)				3	2	2				
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1
	Essentially normal tissue						0	0	0	0	
	Infiltrate, interstitial, lymphocytic										
	Tubular epithelial cell degeneration		2	2	2						
	Tubular cell, cytoplasmic vacuolation										
	Necrosis, tubular epithelium, PCT, acute	3	2	1	1	3					3
	Intracytoplasmic eosinophilic globules	2	2			1					
	Intracytoplasmic eosinophilic crystals										
	Cortical cyst										
	Hemmorhage, interstitial										
	Glomerular synechia										
	Tubular epithelial cell regeneration										
	Glomerulornephritis, multifocal										
	Tubular dilatation										
	Cortical adipose deposition, focal										
	Renal pelvic dilation										
Adrenal gland	Absent (1=true, blank not true)							1			
	Autolysis (5=most severe, precludes analysis)				2	2	2		1		
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1		0	1	1
	Essentially normal tissue	0	0	0	0	0	0			0	0
	Cortical epithelial cells, vacuolation, increased										
Thymus	Absent (1=true, blank not true)		1				1				
	Autolysis (5=most severe, precludes analysis)										
	Tissue Examined (1=true, blank=not examined)	1		1	1	1		1	1	1	1
	Essentially normal tissue										
	Atrophy, follicular										
	Perifollicular congestion/hemorrhage					2					
	Apoptosis, incrsd, lymphocyte, cortical, multifoc	5		4	2	5		3	3	3	4
	Necrosis, lymphocyte, cortical, multifocal to diff	4		4		5					4
	Tingible body macrophage, increased	3		3	2	2		2	3	2	3
Liver	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1
	Essentially normal tissue						0				
	Rarefaction, (vacuolar change, glycogen type)										
	Vacuolation, lipid-type										
	Hepatocellular hypertrophy	1	1	1	1	2		1	1	2	2
	Necrosis, hepatocellular, single cell, multifocal	1	1	1		1		1	1		1
	Infiltrates, lymphocytic										
	Pigment, intravascular										
	Pigment, intrahistiocytic										
	Cytoplasmic Inclusions, hepatocyte (congestion droplets)										
	EMH, multifocal, nodular										
Spleen	Absent (1=true, blank not true)										
	Autolysis (5=most severe, precludes analysis)				2		2				
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1
	Essentially normal tissue	0							0	0	
	Increased Intrahistiocytic pigment accumulation, red pulp										
	Lymphoid hyperplasia, follicular										
	Red pulp, increased extramedullary hematopoiesis		1	1	1	1		1			1
	Lymphocytolysis, increased, follicular			1	1						
Stomach	Congestion, red pulp Absent (1=true, blank not true)										
	Autolysis (5=most severe, precludes analysis)				4						
	Tissue Examined (1=true, blank=not examined)	1	1	1	4	1	1	1	1	1	1
	Essentially normal tissue										
	Squamous epithelial hyperplasia, Non-glandlr m	3	3	4	3	2	1	3	3	3	2
	Hyperkeratosis, non-glandular mucosa	3	3	4	3	2	1	3	3	3	2
	Submucosal edema and/or hemorrhage	4	4	4	3	5	5	5	4	5	5
	Cellular infiltrates, neutrophilic and lymphocytic	5	5	5	3	5	5	5	5	4	5
	Congestion, submucosa	4	4	3	2	3	3	3	3	3	4
	Ulceration/necrosis, mucosal, non-glandular sto	4	4	4		4	3	4	4	4	4
	Granulation tissue, submucosal										
Bone marrow, section or	Absent (1=true, blank not true)										
	Autolysis (5=most severe, precludes analysis)										
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1
	Essentially normal tissue		0	0	0	0		0	0	0	0
	Increased blastic cells, likely erythroid	2					3				

Table 6c: 1000 mg/kg Individual Animal Histopathologic Findings

	Date of death	12/11	12/11	12/11	12/1	12/1	12/12	12/4	12/12	12/13	12/2	11/23	11/23	11/23	11/23	11/23	11/23	11/23	11/23	11/23	11/23	11/23
	Timing of death premature sacrifice or end of study	EOS	EOS	EOS	PD	PD	EOS	PD	EOS	EOS	PD	PD	PD	PD	PD	PD	PD	PD	PD	PD	PD	PD	PD
	Manner of death (FD, MK, SK)	SK	SK	SK	MK	MK	SK	MK	SK	SK	MK	MK	MK	MK	MK	FD	MK	FD	MK	FD	MK	FD	MK
	Date of necropsy	12/11	12/11	12/11	12/1	12/1	12/12	12/4	12/12	12/13	12/2	11/23	11/23	11/23	11/23	11/23	11/23	11/23	11/23	11/23	11/23	11/23	11/23
	Sex	F	F	F	F	F	F	F	F	F	F	M	M	M	M	M	M	M	M	M	M	M	M
	Dose Group	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
	Number of Animals	85	86	87	88	97	98	103	104	109	110	51	52	23	24	27	28	33	34	63	64		
Kidney	Autolysis (5=most severe, precludes analysis)															1		5		5			
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
	Essentially normal tissue	0	0																0		0		
	Infiltrate, interstitial, lymphocytic			2																			
	Tubular epithelial cell degeneration			2	3	1	2	2	2	1	4		2	2									
	Tubular cell, cytoplasmic vacuolation				3	1		1	2	1	5												
	Necrosis, tubular epithelium, PCT, acute			2	3	1	3	3		1	5	3	3	3	1	2	2						
	Intracytoplasmic eosinophilic globules				1		2					2	1	2			2						
	Intracytoplasmic eosinophilic crystals																						
	Cortical cyst																						
	Hemmorhage, interstitial																						
	Glomerular synechia			1																			
	Tubular epithelial cell regeneration			2				2															
	Glomerulornephritis, multifocal			2																			
	Tubular dilatation													2									
	Cortical adipose deposition, focal							2															
	Renal pelvic dilation																						
Adrenal gland	Absent (1=true, blank not true)																						
	Autolysis (5=most severe, precludes analysis)																	2		3			
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
	Essentially normal tissue	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Cortical epithelial cells, vacuolation, increased																						
Thymus	Absent (1=true, blank not true)											1				1				1			
	Autolysis (5=most severe, precludes analysis)																						
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1		1	1	1		1	1	1		1		
	Essentially normal tissue																						
	Atrophy, follicular				4	4		4			3												
	Perifollicular congestion/hemorrhage										3												
	Apoptosis, incrsd, lymphocyte, cortical, multifoc	2	2	3	3	3	3	2		2	4		4	5	4		4	3	2		4		
	Necrosis, lymphocyte, cortical, multifocal to diff	1			2	2					2		4	4	2		3	1			3		
	Tingible body macrophage, increased	2	2	3	3	3	3	2	1	2	3		3	4	4		5	3	2		3		
Liver	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
	Essentially normal tissue																	0					
	Rarefaction, (vacuolar change, glycogen type)											1											
	Vacuolation, lipid-type										1		1		1				2	1			
	Hepatocellular hypertrophy											1	1	1	1	2	1		1		1		
	Necrosis, hepatocellular, single cell, multifocal				1	3	1	1	1	2	3	1		1	1						1		
	Infiltrates, lymphocytic																						
	Pigment, intravascular		1				2																
	Pigment, intrahistiocytic		1				2		3	2													
	Cytoplasmic Inclusions, hepatocyte (congestion droplets)							2	3	1							1						
	EMH, multifocal, nodular	1	1	1					1														
Spleen	Absent (1=true, blank not true)																			1			
	Autolysis (5=most severe, precludes analysis)																						
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		1		
	Essentially normal tissue											0	0	0	0	0		0	0				
	Increased Intrahistiocytic pigment accumulation	2	3	2	3		3	1	2	3	2												
	Lymphoid hyperplasia, follicular																						
	Red pulp, increased extramedullary hematopoie	2	4	2	2	2	3	1	3	3	1												
	Lymphocytolysis, increased, follicular																1				1		
	Congestion, red pulp						2	1			2												
	Absent (1=true, blank not true)																			1			
Stomach	Autolysis (5=most severe, precludes analysis)																						
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		1		
	Essentially normal tissue																						
	Squamous epithelial hyperplasia, Non-glandlr m	3	3	4	4	4	4	4	4	4	4	4	4	3	3	3	4	3	4		3		
	Hyperkeratosis, non-glandular mucosa	3	3	4	4	4	4	4	4	4	4	4	4	3	3	3	4	3	4		3		
	Submucosal edema and/or hemorrhage	3	3	3	5	5	5	3	3	3	5	5	3	4	4	5	5	4	5		4		
	Cellular infiltrates, neutrophilic and lymphocytic	2	3	3	4	4	4	4	3	2	5	5	5	5	5	5	5	4	5		5		
	Congestion, submucosa	2	2	3	3	3	3	2	3	2	3	3	3	3	3	3	3	2	3		3		
	Ulceration/necrosis, mucosal, non-glandular stomach				4	4	2	3			5	5	5	4	4	4	5	3	5		5		
	Granulation tissue, submucosal			1	3		2	4															
Bone marrow, section or	Absent (1=true, blank not true)																						
	Autolysis (5=most severe, precludes analysis)																						
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
	Essentially normal tissue	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Increased blastic cells, likely erythroid																						

Table 6d: 500 mg/kg Individual Animal Histopathologic Findings

	Date of death	12/12	12/12	12/4	12/13	12/11	12/11	12/1	12/11	12/12	12/5	11/26	12/5	11/27	12/1	11/30	12/4	11/26	11/26	11/23	12/4
	Timing of death premature sacrifice or end of study	EOS	EOS	PD	EOS	EOS	EOS	PD	EOS	EOS	PD	PD	EOS	PD	PD	PD	EOS	PD	PD	PD	EOS
	Manner of death (FD, MK, SK)	SK	SK	MK	SK	SK	SK	MK	SK	SK	MK	FD	SK	MK	MK	MK	SK	MK	FD	MK	SK
	Date of necropsy	12/12	12/12	12/4	12/13	12/11	12/11	12/1	12/11	12/12	12/5	11/26	12/5	11/27	12/1	11/30	12/4	11/26	11/26	11/23	12/4
	Sex	F	F	F	F	F	F	F	F	F	F	M	M	M	M	M	M	M	M	M	M
	Dose Group	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500
	Number of Animals	77	78	83	84	93	94	111	112	123	124	5	6	9	10	25	26	43	44	67	68
Kidney	Autolysis (5=most severe, precludes analysis)											4							3		
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Essentially normal tissue				0					0			0		0	0	0				0
	Infiltrate, interstitial, lymphocytic																				
	Tubular epithelial cell degeneration	2		2		2		2			3	2		2				2	2	3	
	Tubular cell, cytoplasmic vacuolation	2		3				4			3										
	Necrosis, tubular epithelium, PCT, acute	2	1	2		2	1	1	1		2	3		2				3	2	4	
	Intracytoplasmic eosinophilic globules													1							
	Intracytoplasmic eosinophilic crystals																				
	Cortical cyst																				
	Hemmorhage, interstitial																			2	
	Glomerular synechia																				
	Tubular epithelial cell regeneration	2				2	1														
	Glomerulornephritis, multifocal																				
	Tubular dilatation																				
	Cortical adipose deposition, focal																				
	Renal pelvic dilation																				
Adrenal gland	Absent (1=true, blank not true)																				
	Autolysis (5=most severe, precludes analysis)																				
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Essentially normal tissue		0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	
	Cortical epithelial cells, vacuolation, increased	2								2											1
Thymus	Absent (1=true, blank not true)											1									
	Autolysis (5=most severe, precludes analysis)																				
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1		1	1	1	1	1	1	1	1	1
	Essentially normal tissue																				
	Atrophy, follicular			4				4			5							3	4	4	
	Perifollicular congestion/hemorrhage			3		1	1			2									2		
	Apoptosis, incrsd, lymphocyte, cortical, multifoc	1		5		1		2	2	2	4		2	4	2	3	2	4	4	4	3
	Necrosis, lymphocyte, cortical, multifocal to diffuse			2				1			3			1		1		4	3	3	
	Tingible body macrophage, increased	1	2	4	2	2	1	3	2	2	4		2	3	2	3	2	3	2	3	3
Liver	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Essentially normal tissue															0					0
	Rarefaction, (vacuolar change, glycogen type)														1						
	Vacuolation, lipid-type												3		3						
	Hepatocellular hypertrophy													1						1	
	Necrosis, hepatocellular, single cell, multifocal	1		3	2	1	2	2	2	1	1			1							
	Infiltrates, lymphocytic																2				
	Pigment, intravascular																				
	Pigment, intrahistiocytic	1	1	1	1	1	1		1	1	1										
	Cytoplasmic Inclusions, hepatocyte (congestion	2	3	2	2		1	1	3	2	1							2	2	1	
	EMH, multifocal, nodular		1						1	1	2										
Spleen	Absent (1=true, blank not true)											1									
	Autolysis (5=most severe, precludes analysis)																				
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1		1	1	1	1	1	1	1	1	1
	Essentially normal tissue			0				0						0				0		0	0
	Increased Intrahistiocytic pigment accumulation	2	2		1	2			1	1	1		2		2	2	2				
	Lymphoid hyperplasia, follicular																				
	Red pulp, increased extramedullary hematopoie	2	2		1	2	1		1	2	4				2	2	2		1		
	Lymphocytolysis, increased, follicular												2								
	Congestion, red pulp	3	2		2	2	2		1	2											
	Absent (1=true, blank not true)																				
	Autolysis (5=most severe, precludes analysis)											3									
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Essentially normal tissue																				
Stomach	Squamous epithelial hyperplasia, Non-glandlr m	4	5	4	4	4	4	4	5	4	4	3	4	5	5	5	4	5	5	4	5
	Hyperkeratosis, non-glandular mucosa	4	5	4	4	4	4	4	5	4	4	3	4	5	5	5	4	5	5	4	5
	Submucosal edema and/or hemorrhage	3	3	3	4	4	4	4	4	4	2	4	3	4	3	3	2	2	1	2	3
	Cellular infiltrates, neutrophilic and lymphocytic	3	3	5	4	4	4	4	4	4	2	5	3	5	4	3	3	4	3	4	4
	Congestion, submucosa	2	2	2	3	3	3	3	3	3	2	3	3	2	2	2	2	2	2	2	3
	Ulceration/necrosis, mucosal, non-glandular sto	2		4		3		4	3	4		5	2	4	2	3	1	3		4	4
	Granulation tissue, submucosal	4	4	3	3	3		4	4	5			3	3	3	3		3		3	3
Bone marrow, section or	Absent (1=true, blank not true)																				
	Autolysis (5=most severe, precludes analysis)																				
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Essentially normal tissue	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Increased blastic cells, likely erythroid																				

Table 6e: 250 mg/kg Individual Animal Histopathologic Findings

Date of death																							
Timing of death premature sacrifice or end of study	EOS	EOS	PD	EOS	EOS	?	EOS	EOS	EOS	PD	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS
Manner of death (FD, MK, SK)	SK	SK	MK	SK	SK	?	SK	SK	SK	MK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK
Date of necropsy	12/13	12/13	12/5	12/12	12/11	?	12/11	12/11	12/13	12/3	12/6	12/6	12/4	12/4	12/5	12/5	12/6	12/6	12/4	12/4	12/4	12/4	12/4
Sex	F	F	F	F	F	F	F	F	F	F	M	M	M	M	M	M	M	M	M	M	M	M	M
Dose Group	250	250	250	250	250	250	250	250	250	250	250	250	250	250	250	250	250	250	250	250	250	250	250
Number of Animals	89	90	99	100	113	114	121	122	127	128	21	22	41	42	53	54	55	56	59	60			
Kidney																							
Autolysis (5=most severe, precludes analysis)										1													
Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
Essentially normal tissue																							
Infiltrate, interstitial, lymphocytic	2	1	2			1	1	1	1			1			1	2	2	1	1	1			
Tubular epithelial cell degeneration	2	1	3	1	2	2		1	2	2	2		1	1	1	1	1	1	1	1			
Tubular cell, cytoplasmic vacuolation											2												
Necrosis, tubular epithelium, PCT, acute	3	1	3	2	2	2	1	1	2	2	2	1	1		1	1	1	1	1	2			
Intracytoplasmic eosinophilic globules																							
Intracytoplasmic eosinophilic crystals																							
Cortical cyst																							
Hemmorhage, interstitial																							
Glomerular synechia																							
Tubular epithelial cell regeneration	3	1	3		2	2			2		1	1	2	1	1	1	1	1	1	2			
Glomerulornephritis, multifocal		1		3	2	2	1	2	2	2		2				1	1						
Tubular dilatation						1																	
Cortical adipose deposition, focal																							
Renal pelvic dilation																							
Adrenal gland																							
Absent (1=true, blank not true)																							
Autolysis (5=most severe, precludes analysis)																							
Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
Essentially normal tissue	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
Cortical epithelial cells, vacuolation, increased																			3	3			
Thymus																							
Absent (1=true, blank not true)																							
Autolysis (5=most severe, precludes analysis)																							
Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
Essentially normal tissue	0	0		0	0		0	0	0		0	0	0	0	0	0	0	0	0	0			
Atrophy, follicular										2													
Perifollicular congestion/hemorrhage																							
Apoptosis, incrsd, lymphocyte, cortical, multifocal to diffuse			3			2				3													
Necrosis, lymphocyte, cortical, multifocal to diffuse			3			3				3													
Tingible body macrophage, increased			3			3				3													
Liver																							
Tissue Examined (1=true, blank=not examined)																							
Essentially normal tissue																							
Rarefaction, (vacuolar change, glycogen type)																							
Vacuolation, lipid-type																							
Hepatocellular hypertrophy																							
Necrosis, hepatocellular, single cell, multifocal																							
Infiltrates, lymphocytic																							
Pigment, intravascular																							
Pigment, intrahistiocytic																							
Cytoplasmic Inclusions, hepatocyte (congestion droplets)																							
EMH, multifocal, nodular																							
Spleen																							
Absent (1=true, blank not true)																							
Autolysis (5=most severe, precludes analysis)																							
Tissue Examined (1=true, blank=not examined)																							
Essentially normal tissue																							
Increased Intrahistiocytic pigment accumulation, red pulp																							
Lymphoid hyperplasia, follicular																							
Red pulp, increased extramedullary hematopoiesis																							
Lymphocytolysis, increased, follicular																							
Congestion, red pulp																							
Absent (1=true, blank not true)																							
Stomach																							
Autolysis (5=most severe, precludes analysis)																							
Tissue Examined (1=true, blank=not examined)																							
Essentially normal tissue																							
Squamous epithelial hyperplasia, Non-glandlr mucosa																							
Hyperkeratosis, non-glandular mucosa																							
Submucosal edema and/or hemorrhage																							
Cellular infiltrates, neutrophilic and lymphocytic																							
Congestion, submucosa																							
Ulceration/necrosis, mucosal, non-glandular stomach																							
Granulation tissue, submucosal																							
Bone marrow, section or																							
Absent (1=true, blank not true)																							
Autolysis (5=most severe, precludes analysis)																							
Tissue Examined (1=true, blank=not examined)																							
Essentially normal tissue																							
Increased blastic cells, likely erythroid																							

Table 6f: 125 mg/kg Individual Animal Histopathologic Findings

	Date of death																					
	Timing of death premature sacrifice or end of study	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS
	Manner of death (FD, MK, SK)	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK
	Date of necropsy	12/12	12/12	12/13	12/13	12/11	12/11	12/11	12/11	12/13	12/13	12/5	12/5	12/6	12/6	12/4	12/4	12/5	12/5	12/4	12/4	
	Sex	F	F	F	F	F	F	F	F	F	F	M	M	M	M	M	M	M	M	M	M	
	Dose Group	125	125	125	125	125	125	125	125	125	125	125	125	125	125	125	125	125	125	125	125	
	Number of Animals	79	80	81	82	95	96	101	102	129	130	15	16	39	40	45	46	47	48	65	66	
Kidney	Autolysis (5=most severe, precludes analysis)																					
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	Essentially normal tissue	0	0	0	0	0	0	0	0		0							0				
	Infiltrate, interstitial, lymphocytic																					
	Tubular epithelial cell degeneration												2			1	1					
	Tubular cell, cytoplasmic vacuolation																					
	Necrosis, tubular epithelium, PCT, acute																					
	Intracytoplasmic eosinophilic globules											3	3	2	2	2	2		1	2	1	
	Intracytoplasmic eosinophilic crystals											2	3	2	2	2	2		1	1	1	
	Cortical cyst																					
	Hemmorhage, interstitial																					
	Glomerular synechia																					
	Tubular epithelial cell regeneration																					
	Glomerulornephritis, multifocal																					
	Tubular dilatation																					
	Cortical adipose deposition, focal																					
	Renal pelvic dilation									2												
Adrenal gland	Absent (1=true, blank not true)																					
	Autolysis (5=most severe, precludes analysis)																					
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	Essentially normal tissue	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Cortical epithelial cells, vacuolation, increased																					
Thymus	Absent (1=true, blank not true)																					
	Autolysis (5=most severe, precludes analysis)																					
	Tissue Examined (1=true, blank=not examined)																					
	Essentially normal tissue																					
	Atrophy, follicular																					
	Perifollicular congestion/hemorrhage																					
	Apoptosis, incrsd, lymphocyte, cortical, multifocal to diffuse																					
	Necrosis, lymphocyte, cortical, multifocal to diffuse																					
	Tingible body macrophage, increased																					
Liver	Tissue Examined (1=true, blank=not examined)																					
	Essentially normal tissue																					
	Rarefaction, (vacuolar change, glycogen type)																					
	Vacuolation, lipid-type																					
	Hepatocellular hypertrophy																					
	Necrosis, hepatocellular, single cell, multifocal																					
	Infiltrates, lymphocytic																					
	Pigment, intravascular																					
	Pigment, intrahistiocytic																					
	Cytoplasmic Inclusions, hepatocyte (congestion droplets)																					
	EMH, multifocal, nodular																					
Spleen	Absent (1=true, blank not true)																					
	Autolysis (5=most severe, precludes analysis)																					
	Tissue Examined (1=true, blank=not examined)																					
	Essentially normal tissue																					
	Increased Intrahistiocytic pigment accumulation, red pulp																					
	Lymphoid hyperplasia, follicular																					
	Red pulp, increased extramedullary hematopoiesis																					
	Lymphocytolysis, increased, follicular																					
Stomach	Congestion, red pulp Absent (1=true, blank not true)																					
	Autolysis (5=most severe, precludes analysis)																					
	Tissue Examined (1=true, blank=not examined)																					
	Essentially normal tissue																					
	Squamous epithelial hyperplasia, Non-glandlr mucosa																					
	Hyperkeratosis, non-glandular mucosa																					
	Submucosal edema and/or hemorrhage																					
	Cellular infiltrates, neutrophilic and lymphocytic																					
	Congestion, submucosa																					
	Ulceration/necrosis, mucosal, non-glandular stomach																					
	Granulation tissue, submucosal																					
Bone marrow, section or	Absent (1=true, blank not true)																					
	Autolysis (5=most severe, precludes analysis)																					
	Tissue Examined (1=true, blank=not examined)																					
	Essentially normal tissue																					
	Increased blastic cells, likely erythroid																					

Table 6g: 62.5 mg/kg Individual Animal Histopathologic Findings

	Date of death										
	Timing of death premature sacrifice or end of study	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS	EOS
	Manner of death (FD, MK, SK)	SK	SK	SK	SK	SK	SK	SK	SK	SK	SK
	Date of necropsy	12/11	12/11	12/12	12/12	12/12	12/12	12/13	12/13	12/13	12/13
	Sex	F	F	F	F	F	F	F	F	F	F
	Dose Group	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
	Number of Animals	71	72	73	74	105	106	117	118	119	120
Kidney	Autolysis (5=most severe, precludes analysis)										
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1
	Essentially normal tissue	0	0		0	0	0	0	0	0	0
	Infiltrate, interstitial, lymphocytic										
	Tubular epithelial cell degeneration										
	Tubular cell, cytoplasmic vacuolation										
	Necrosis, tubular epithelium, PCT, acute										
	Intracytoplasmic eosinophilic globules										
	Intracytoplasmic eosinophilic crystals										
	Cortical cyst			1							
	Hemmorhage, interstitial										
	Glomerular synechia										
	Tubular epithelial cell regeneration										
	Glomerulornephritis, multifocal										
	Tubular dilatation										
	Cortical adipose deposition, focal										
	Renal pelvic dilation										
Adrenal gland	Absent (1=true, blank not true)										
	Autolysis (5=most severe, precludes analysis)										
	Tissue Examined (1=true, blank=not examined)	1	1	1	1	1	1	1	1	1	1
	Essentially normal tissue	0	0	0	0	0	0	0	0	0	0
	Cortical epithelial cells, vacuolation, increased										
Thymus	Absent (1=true, blank not true)										
	Autolysis (5=most severe, precludes analysis)										
	Tissue Examined (1=true, blank=not examined)										
	Essentially normal tissue										
	Atrophy, follicular										
	Perifollicular congestion/hemorrhage										
	Apoptosis, incrsd, lymphocyte, cortical, multifocal to diffuse										
	Necrosis, lymphocyte, cortical, multifocal to diffuse										
	Tingible body macrophage, increased										
Liver	Tissue Examined (1=true, blank=not examined)										
	Essentially normal tissue										
	Rarefaction, (vacuolar change, glycogen type)										
	Vacuolation, lipid-type										
	Hepatocellular hypertrophy										
	Necrosis, hepatocellular, single cell, multifocal										
	Infiltrates, lymphocytic										
	Pigment, intravascular										
	Pigment, intrahistiocytic										
	Cytoplasmic Inclusions, hepatocyte (congestion droplets)										
	EMH, multifocal, nodular										
Spleen	Absent (1=true, blank not true)										
	Autolysis (5=most severe, precludes analysis)										
	Tissue Examined (1=true, blank=not examined)										
	Essentially normal tissue										
	Increased Intrahistiocytic pigment accumulation, red pulp										
	Lymphoid hyperplasia, follicular										
	Red pulp, increased extramedullary hematopoiesis										
	Lymphocytolysis, increased, follicular										
Stomach	Congestion, red pulp Absent (1=true, blank not true)										
	Autolysis (5=most severe, precludes analysis)										
	Tissue Examined (1=true, blank=not examined)										
	Essentially normal tissue										
	Squamous epithelial hyperplasia, Non-glandlr mucosa										
	Hyperkeratosis, non-glandular mucosa										
	Submucosal edema and/or hemorrhage										
	Cellular infiltrates, neutrophilic and lymphocytic										
	Congestion, submucosa										
	Ulceration/necrosis, mucosal, non-glandular stomach										
	Granulation tissue, submucosal										
Bone marrow, section or	Absent (1=true, blank not true)										
	Autolysis (5=most severe, precludes analysis)										
	Tissue Examined (1=true, blank=not examined)										
	Essentially normal tissue										
	Increased blastic cells, likely erythroid										

APPENDIX P

MICRONUCLEUS ASSAY DATA

Table P-1. Individual and Summary of Micronucleus Assay Results

Treatment	Animal ID	Day*	No. NCE [1]	No. MN-NCE [2]	No. RET [3]	No. MN-RET [4]	%RET [a]	%MN-RET [b]
0	19-0091	3	2967092	409	19974	26	0.67	0.13
	19-0092	3	2800973	518	20325	24	0.72	0.12
	19-0107	3	3051744	356	19988	12	0.65	0.06
	19-0108	3	1828591	193	19968	32	1.08	0.16
	19-0126	1	3537145	356	19982	19	0.56	0.09
	19-0125	1	2072096	150	19972	28	0.96	0.14
	19-0115	2	1622797	204	19983	17	1.22	0.09
	19-0116	2	1433171	47	19979	21	1.38	0.11
	19-0075	2	3505433	685	19984	16	0.57	0.08
	19-0076	2	2422287	357	19994	6	0.82	0.03
62.5	19-0117	3	613834	83	19958	42	3.15	0.21
	19-0118	3	1575374	164	19982	18	1.25	0.09
	19-0119	3	2892248	197	19970	30	0.69	0.15
	19-0120	3	911466	106	19974	26	2.15	0.13
	19-0071	1	923017	60	19975	25	2.12	0.13
	19-0072	1	1249041	256	19964	36	1.58	0.18
	19-0073	2	1702904	122	19978	22	1.16	0.11
	19-0074	2	1203053	174	19989	11	1.64	0.06
	19-0105	2	1555050	164	19987	13	1.27	0.07
	19-0106	2	1684570	95	19976	24	1.17	0.12
125	19-0081	3	435005	232	19962	38	4.39	0.19
	19-0082	3	404801	247	19958	42	4.71	0.21
	19-0129	3	373108	85	19978	22	5.09	0.11
	19-0130	3	474785	281	19976	24	4.04	0.12
	19-0095	1	345669	156	19977	23	5.47	0.12
	19-0096	1	425030	271	19975	25	4.49	0.13
	19-0101	1	252780	275	19967	33	7.32	0.17
	19-0102	1	573832	440	19944	56	3.37	0.28
	19-0079	2	347652	326	19958	42	5.44	0.21
	19-0080	2	478576	150	19973	27	4.01	0.14
250	19-0089	3	339725	1130	19959	41	5.54	0.21
	19-0090	3	NR	NR	NR	NR	NR	NR
	19-0127	3	745882	840	19962	38	2.61	0.19
	19-0113	1	462421	242	19975	25	4.14	0.13
	19-0121	1	464915	1483	19953	47	4.11	0.24
	19-0122	1	641142	2019	19953	47	3.02	0.24
	19-0100	2	498275	1109	19941	59	3.85	0.30
+control prebleed	19-0154	**	1182208	69	19987	13	1.66	0.07
	19-0155	**	848112	65	19968	32	2.30	0.16
	19-0156	**	887236	78	19993	7	2.20	0.04
	19-0157	**	1180292	57	19976	24	1.67	0.12
	19-0158	**	1387988	175	19978	22	1.42	0.11
	19-0159	**	760572	123	19982	18	2.56	0.09
+control	19-0154	1	1659482	38	4965	35	0.30	0.70
	19-0155	1	2420147	254	19732	268	0.82	1.34
	19-0156	2	3315655	176	19956	44	0.60	0.22
	19-0157	2	2422370	90	4963	37	0.21	0.74
	19-0158	3	2447572	105	4931	69	0.20	1.38
	19-0159	3	3503843	161	19807	193	0.57	0.97

*=necropsy day, 1=12/11/2018, 2=12/12/2018, 3=12/13/2018; **=12/4/2018

[1] NCE = normochromatic erythrocytes

[2] MN-NCE = micronucleated normochromatic erythrocytes

[3] RET = young (high CD71-positive) reticulocytes

[4] MN-RET = young (high CD71-positive) micronucleated reticulocytes

[a] % RET = frequency (%) of young (high CD71-positive) reticulocytes; calculated as [(Number of RET + Number of MN-RET)/ (Number of NCE + Number of MN-NCE + Number of RET + Number of MN-RET)] x 100

[b] % MN-RET = frequency (%) of young (high CD71-positive) micronucleated reticulocytes; calculated as [Number of MN-RET/(Number of RET + Number of MN-RET)] x 100

	% RET Summary					
	Controls *		TNBA mg/kg-day **			
	baseline	+EMS	0	62.5	125	250
Mean	1.968	0.45	0.863	1.618	4.833	3.878
SD	0.4469	0.2516	0.2855	0.7002	1.095	1.023
SEM	0.1824	0.1027	0.0903	0.2214	0.3464	0.4175
N	6	6	10	10	10	6
95%CI (L)	1.499	0.186	0.6587	1.117	4.049	2.805
95%CI (H)	2.437	0.714	1.067	2.119	5.617	4.952
p value		<.0001		0.1168	**<0.0001**	**<0.0001**

*=T-Test; **=one way ANOVA, Dunnett's multiple comparison

	% MN-RET Summary					
	Controls *		TNBA mg/kg-day **			
	baseline	+EMS	0	62.5	125	250
Mean	0.09833	0.8917	0.101	0.125	0.168	0.2183
SD	0.04167	0.4374	0.039	0.04673	0.05453	0.05707
SEM	0.01701	0.1786	0.01233	0.01478	0.01724	0.0233
N	6	6	10	10	10	6
95%CI (L)	0.0546	0.4327	0.0731	0.09157	0.129	0.1584
95%CI (H)	0.1421	1.351	0.1289	0.1584	0.207	0.2782
p value		<.0001		0.5723	**0.0122**	**0.0002**

*=T-Test; **=one way ANOVA, Dunnett's multiple comparison

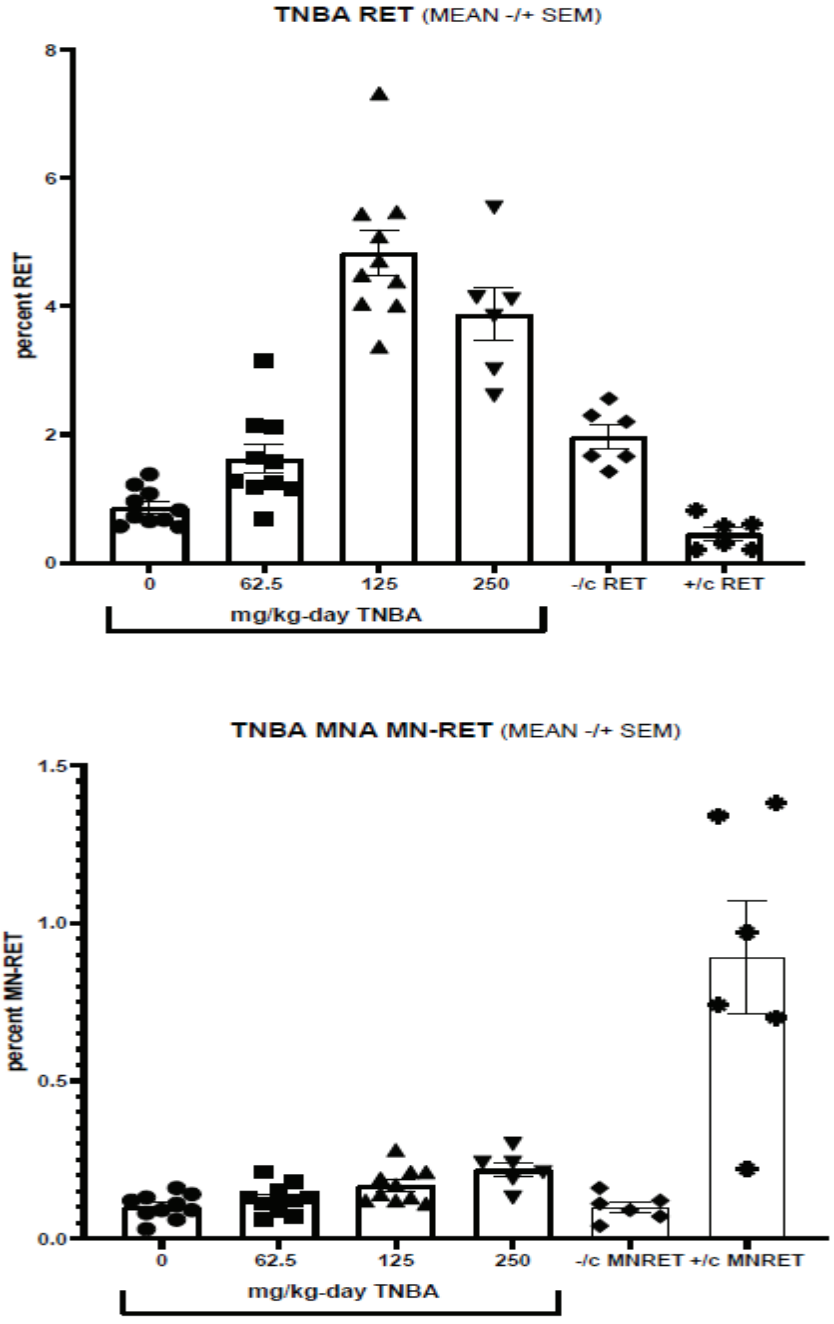


Figure P-1. Summary of Percent Young Reticulocytes (% RET) and Percent Young Micronucleated Reticulocytes (% MN-RET)

APPENDIX Q

DETERMINATION OF BMD AND $BMDL_{10}$

Table Q-1. Benchmark Dose Output for Brain-normalized Female Spleen Mass using a Continuous Benchmark Response of 10% and Modeled Variance in BMDS (2.7.0.4)

Model Name	BMD	BMDL	BMDU	p-value Test 1: Lack dose response?	p-value Test 2: Constant variance?	p-value Test 3: Good variance model?	p-value for fit: Does the model for the mean fit?	AIC	Scaled residual for dose group nearest the BMD	Scaled residual for control group
Exponential2	39.0109	33.8214	46.0455	< 0.0001	< 0.0001	0.7034	< 0.0001	-145.8002	-1.958	-0.2014
Exponential3	39.0109	33.8214	46.5939	< 0.0001	< 0.0001	0.7034	< 0.0001	-145.8002	-1.958	-0.2014
Exponential4	19.2827	14.4962	24.5681	< 0.0001	< 0.0001	0.7034	0.0002486	-162.1981	1.484	1.484
Exponential5	53.4769	37.4054	73.2692	< 0.0001	< 0.0001	0.7034	0.1991	-176.1699	-1.006	0.5658
Hill	58.8023	41.1385	79.0035	<.0001	<.0001	0.7034	0.2398	-176.541658	-0.797	0.48
Linear	20.4616	17.1823	24.8065	<.0001	<.0001	0.7034	0.0006538	-163.991274	1.38	1.38
Polynomial	20.4616	17.1823	26.8425	<.0001	<.0001	0.7034	0.0006538	-163.991274	1.38	1.38
Polynomial	20.4616	17.1823	26.8425	<.0001	<.0001	0.7034	0.0006538	-163.991274	1.38	1.38
Power	28.5296	18.089	43.5347	<.0001	<.0001	0.7034	0.0004325	-163.362443	1.02	1.02

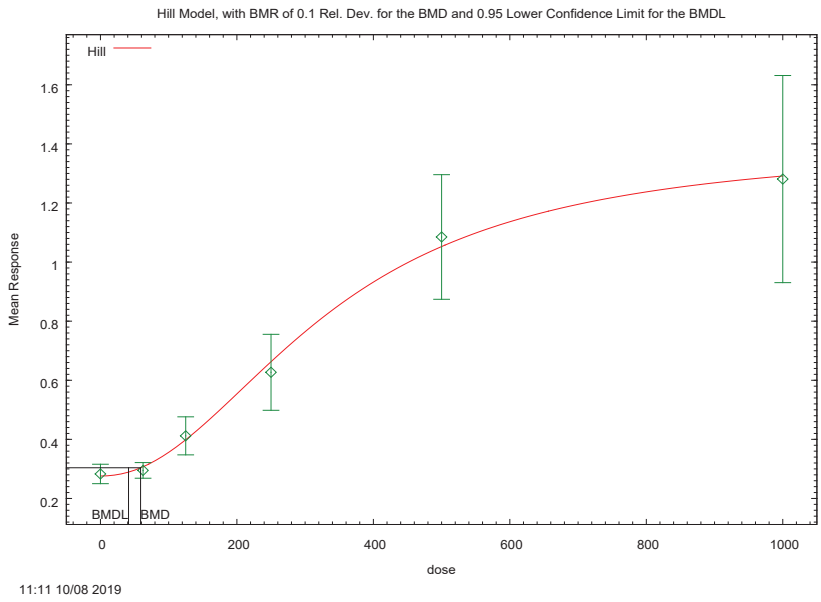


Figure Q-1. Hill Model of the Brain-normalized Spleen Mass in Females, Showing Data Points (green), Model Curve (red), and BMD/BMDL$_{10}$ (black), using BMDS (2.7.0.4)

APPENDIX R

STUDY PROTOCOL WITH MODIFICATIONS

ANIMAL USE PROTOCOL
ARMY PUBLIC HEALTH CENTER
ABERDEEN PROVING GROUND MD 21010-5403

PROTOCOL TITLE: Effects of Acute and Subacute Oral 2,4,6-Trinitro-3-Bromoanisole (TNBA) Exposure to Rats (*Rattus norvegicus*)

PROTOCOL NUMBER: 30-18-07-02

DATE OF APPROVAL: 11 JULY 2018

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PROJECT SPONSOR: Strategic Environmental Research and Development Program (SERDP)

ACRONYMS:
ALB: Albumin
ALK-P: Alkaline phosphatase
ALT: Alanine transaminase
ANCOVA: Analysis of Covariance
ANOVA: Analysis of Variance
APHC: Army Public Health Center
AST: Aspartate transaminase
AV: Attending Veterinarian
BMD: Benchmark dose
BRD: Biomedical Research Database
BUN: Blood urea nitrogen

CA: Calcium
CFR: Code of Federal Regulations
CHOL: Cholesterol
CI: 95% Confidence interval
CO2: Carbon dioxide
CREA: Creatinine
DOD: Department of Defense
DTIC: Defense Technical Information Center
EDTA: Ethylenediaminetetraacetic acid
FIFRA: Federal Insecticide, Fungicide, and Rodenticide Act
GLP: Good Laboratory Practice
GLU: Glucose
GrIMEx: Green Insensitive Munitions Explosive
IACUC: Institutional Animal Care and Use Committee
IAW: In accordance with
IM: Insensitive munitions
LD50: Lethal dose 50%
LOAEL: Lowest Observed Adverse Effect Level
LOEL: Lowest Observed Effect Level
LPS: Lipopolysaccharide
LS: Laboratory Sciences Directorate
MNA: Micronucleus Assay
NOAEL: No Observed Adverse Effect Level
NOEL: No Observed Effect Level
NRC: National Research Council
NSAID: Non-steroidal anti-inflammatory drug
OECD: Organisation for Economic Co-operation and Development
PI/SD: Principal Investigator/Study Director
PHOS: Inorganic phosphate
PPE: Personal Protective Equipment
QSARC: Quality Systems and Regulatory Compliance
RDX: Research Department Explosive
RePORT: Research Portfolio Online Reporting Tools
SERDP: Strategic Environmental Research and Development Program
SOP: Standing Operating Procedure
SADM: Stagewise Adaptive Dose Method
TBD: To Be Determined
TNBA: 2,4,6-Trinitro-3-Bromoanisole
TNF-α: Tumor Necrosis Factor α
TNT: Trinitrotoluene
TOX: Toxicology Directorate
TP: Total protein
TRIG: Triglycerides
TSCA: Toxic Substances Control Act
USDA: United States Department of Agriculture
U.S. EPA: United States Environmental Protection Agency

Animal Use Protocol: Effects of Acute and Subacute Oral 2,4,6-Trinitro-3-Bromoanisole (TNBA) Exposure to Rats (*Rattus norvegicus*)

I. NON-TECHNICAL SYNOPSIS:

The purpose of this study is to assess the toxicity of a new experimental explosive TNBA, which is being considered as a replacement for TNT. This study will be composed of two experiments. In the first experiment, female rats will receive a single oral dose of TNBA and then be observed for 7 days for signs of toxicity. Information learned from this experiment will be used to determine doses for the second experiment in which female and male rats will be orally dosed with TNBA daily for 14 consecutive days. At the end of each experiment, all animals will be euthanized and their tissues will be examined for TNBA related effects. This study will determine how the toxicity of this experimental compound compares to other compounds being considered as replacements for currently used explosives.

II. BACKGROUND

II.1. Background: The DoD utilizes a large amount of Composition B, which consists of TNT and RDX, in artillery and mortar rounds. TNT and RDX have known toxicity concerns and contaminate soil and groundwater. TNT is a possible human carcinogen, and can exhibit a variety of toxicities to the liver, blood, immune system, and reproductive system. Additionally Composition B does not meet current IM requirements mandated by the DoD. As such, the focus of the GrIMEx program is to develop a novel IM Component B replacement formulation containing novel, environmentally friendly favorable TNT and RDX replacements. TNBA has progressed through this program as a potential replacement for TNT. Currently, *in vivo* toxicity data for TNBA is unavailable.

This protocol will assess the acute oral toxicity of TNBA in female Sprague-Dawley rats and the subacute oral toxicity in both sexes. IAW ASTM International SADM E1163-10 [1], female rats are the preferred sex for acute testing because historical data indicate that females in most instances have lower LD50 values than males. Thus, use of females will reduce the overall number of animals required. Additionally, OECD technical guidance was considered for acute toxicity testing (i.e. OECD 423- Acute Oral Toxicity – Acute Toxic Class Method [2]). While this approaches further reduces the number of animals required, this test method is not intended to allow the calculation of a precise LD50. Since the objective of this animal protocol is to compare acute and subacute toxicity of TNBA to those of other established and experimental explosive compounds, the determination of an accurate LD50 is considered a valuable metric. Therefore, the current acute toxicity testing protocol is IAW ASTM International SADM E1163-10 [1], which is designed to determine an LD50 while using as few animals as possible.

II.2. Literature Search for Duplication:

II.2.1. Literature Source(s) Searched: AGRICOLA, TOXNET, CRIS, NIH RePorter. Proquest Collection which includes; ABI/INFORM Collection, Biology Database, Continental Europe Database, Health & Medical Collection, Health Management Database, Military Database, Psychology Database, Public Health Database, Research

Library. AMEDD Collection which includes; Ovid, JAMA Network, CINAHL, MEDLINE, PsycINFO, PubMed.

II.2.2. Date of Search: 20 April 2018

II.2.3. Period of Search: 1900-2018

II.2.4. Key Words of Search: (2,4,6-trinitro-3-bromoanisole or TNBA or 107342-31-4) and (oral or mouth) and (lethal dose 50 or ld 50 or ld50 or ld-50 or median lethal dose or mld) and (toxicity or no-observed-adverse-effect-level or no observed adverse effect level or loael or loel or noael or noel) and (rat or rats).

II.2.5. Results of Search: IAW IACUC SOP 1.3 [3] every effort to uncover literature relating to TNBA toxicity was made by searching the above databases. A total of 5 references were identified from the literature searches; however, none of these pertained to TNBA. Additionally, my own literature search identified one *in vitro* study published in November 2017 by APHC that was not identified in the formal literature search [4]. This study is not an *in vivo* toxicity study. Thus, this proposal does not present a duplication of any effort.

Additionally, a search of DTIC identified the same *in vitro* reference described above. However, searches of PubMed, Web of Science, Scopus, TOXNET, Yale University, FedRIP, NIH RePORT, EBSCO-Host's CINAHL Plus, and The Cochrane Library returned 0 hits. Thus, there is no duplication of effort.

III. OBJECTIVE/HYPOTHESIS:

The objectives of this research are to (1) determine the oral LD50 for TNBA in female rats, along with the 95% confidence interval and slope constant; and (2) determine if health effects, including genotoxicity using the MNA, occur from a 14-day repetitive oral exposure regime of TNBA in male and female rats. Data from the 14-day repetitive dose study may be used, if applicable (3) to identify the subacute NOAEL, LOAEL, and/or BMD.

IV. MILITARY RELEVANCE:

A functional, effective, quality-engineered warhead formulation comprised of environmentally viable alternative substances can make a positive contribution to current and future Army readiness by being less toxic to the environment and human health. Through reduced environmental compliance constraints, a safer, more environmentally benign formulation can increase life-cycle cost effectiveness. Current formulations that use TNT have contributed to substantial environmental contamination. It is imperative that the Department of the Army develop weapons containing alternative/replacement energetics. The acute and sub-acute toxicity tests proposed in this protocol can be used as a useful screening tool to provide support in developing less toxic munition alternatives for TNT.

V. MATERIALS AND METHODS

Test Article: The test material, 2,4,6-trinitro-3-bromoanisole, will be provided by BAE Systems, Inc (see Table 1). Analytical test data will also be provided with respect to purity/percent composition and the method used to determine purity. Verification of concentrations and stability in the vehicle will be conducted prior to study initiation by LS Directorate- Client Services Division- Method Development Section. Method of analysis will be validated prior to the start of the study.

Table 1. TNBA Chemical/Physical Properties

Name	2,4,6-Trinitro-3-Bromoanisole
CASRN	107342-31-4
Molecular Formula	$BrC_7H_4N_3O_7$
Molecular Weight (g/mol)	322.03
Aqueous Solubility @ 25ºC (mg/L)	32.03 (EPI Suite 4.1 estimate)

V.1. Experimental Design and General Procedures: The acute toxicity of TNBA will be determined in female rats. In Experiment 1, TNBA will be administered by oral gavage in a single dose of increasing concentrations via the SADM to determine the LD50. In Experiment 2, rats will be dosed daily by oral gavage at five different doses (determined from analysis of Experiment 1) for 14 days. Vehicle-only treated animals will be included as controls. Positive and negative controls for the MNA will also be included in Experiment 2. The determination for the use of both sexes in the experiments is addressed in V.3.2.The study end-point is euthanasia either when the rats become moribund due to compound toxicity or 14 days after dosing initiation, i.e., at the end of the study. Blood and tissue samples will be collected at the end of the study for analysis of chemistry values and pathology, respectively. Additionally, whenever possible, blood and tissues will be collected from moribund animals at the time of euthanasia. Concurrent with the 14-day subacute experiment, the genotoxicity of TNBA in male rats will be assessed by measuring DNA damage using the erythrocyte MNA.

V.1.1. Experiment 1: The objectives of Experiment 1 are to determine the acute oral LD50 and slope constant of TNBA in the female Sprague-Dawley rat and to set dosage levels for the subacute (14-day) study. The general procedures of this acute study will follow the U.S. EPA Health Effects Test Guidelines for Acute Oral Toxicity OPPTS 870.1100 [5] but use the ASTM SADM E1163-10 [1] instead of the Up/Down Procedure. All oral dosing will be administered as described in section V.4.4.8.1.

The SADM proceeds in stages in which small groups of rats (1-3) are fasted overnight and then dosed via oral gavage with each group receiving a different oral dose. Dosing of stages is separated by a post-dosing observation period of up to 7 days in which animals are observed for signs of toxicity, moribundity and mortality. This period may be reduced (i.e., from 7 days to 24-48 hours) for determination of dosages in subsequent stages if confident in the survival of the animals. ASTM E1163-10 recommends that the first stage include 1-2 animals per group in 3-5 dose groups that are expected to span the range from 0% mortality to 100% mortality. In the absence of historical data or

literature values, doses for the first stage of dosing may be set at the default starting value of 175 mg/kg with half-log dose intervals (3.2 dose progression factor) [1]. The available toxicity data for TNBA includes a predicted oral LD50 of 885.8 mg/kg based on TOPKAT modeling [6] and an estimated LD50 of 170 mg/kg based on *in vitro* cytotoxicity data from the Human Cell Line Activation Test [4]. Based on this data, five female rats will be used in the first stage with each animal receiving a different oral dose set at 1/3 log intervals (e.g., 93, 200, 431, 928, and 2000 mg/kg). As a result of the existing preliminary toxicity data, we are using a narrower dose range than is typical. This will facilitate the use of fewer animals while attempting to bracket the oral toxicity of TNBA in the first stage of acute dosing.

The doses selected for the second stage will be based on the doses from the first stage where lethality is observed. For the second stage of the SADM, 3-4 doses that bracket the lethal dose observed in the first stage will be used and 1-3 animals will be used for each dose. If stage three (or beyond) is needed, a similar approach will be used with an additional 1-4 dose groups. Dosing will stop when either the ratio of the confidence limit interval divided by 2 times the estimated LD50 is less than 0.40 or 30 animals have been used, whichever comes first. The ratio is determined after each stage of dosing. These data may be reviewed with a statistician for confirmation.

If no deaths are observed at the highest dose level (2000 mg/kg), the remainder of the study will be based on the limit test provision. Three consecutive animals must survive at the upper dose limit for the LD50 value to be reported as greater than the upper dose limit of 2000 mg/kg.

Although mortality from the 7-day observation period will be the criterion used for the analysis, time to death or time determined that individuals are unlikely to recover may also be used to make decisions regarding doses for subsequent stages. The results of the SADM method will be used to determine the doses for the longer 14-day sub-acute study.

The LD50 determination will use up to 30 female Sprague Dawley rats (**n=30**, see Table 2). If fewer than 30 rats provide sufficient results, the remaining animals will be humanely euthanized per QSARC 800 [7], transferred for training purposes if requested by the AV, or transferred to another approved study protocol. An appropriate vehicle (e.g., water, corn oil or methylcellulose) will be determined based on the solubility characteristics of TNBA and the requirement to not exceed the maximum dosing volume of 10 mL/kg body weight. All surviving dosed animals will be euthanized and will undergo gross necropsy at the end of the 7-day observation period. Animals that are found dead or are euthanized prior to the end of the observation period will also undergo a gross necropsy. Blood and tissues will not be collected from any animals in Experiment 1.

V.1.2. Experiment 2: The purpose of the 14-day study is to determine if there are adverse effects from short-term repetitive oral exposures to TNBA. This 14-day study will include five test compound-treated groups, a vehicle control group, and an MNA

negative/positive control group (male only) (**n=6 groups X 10M+10F PLUS 6M rats=126,** see Table 2). The test compound-treated and vehicle control groups will be orally dosed for 14 days. Rats will not be fasted for the daily dose. The MNA negative/positive control group will be treated as described in sections V.4.4.8.1. and V.4.4.8.2. The test compound will be dissolved/suspended in an appropriate vehicle, e.g., water, corn oil, polyethylene glycol 200, or methylcellulose, and administered via oral gavage. Dose selection will depend on the results of the SADM done in Experiment 1 (e.g. 0.5x, 0.25x, 0.125x, 0.0625x, 0.03125x the LD50). The appropriate amount of test compound to be delivered to each animal will be calculated based on the most recently collected weight for each animal. The vehicle control group will receive a volume of vehicle equivalent to the volume used for the highest exposure group. For all oral dosing, the maximum volume will not exceed 10 mL/kg.

The PI/study staff will weigh the rats on day minus (-) 1 (used to randomize dose groups), day 0 (starting weight; first dosing day), plus (+) 3, +7, +13 (final weight before fasting; last dosing day) and +14 (terminal weight). Randomization into study groups is based on stratification by weight. Briefly, pair-housed animals of each sex will be organized by increasing weight at day -1, and the cages with the six smallest weights will be randomly assigned to one of the six study groups. This will be repeated until all animals have been assigned. Weight distributions will then be compared by ANOVA to ensure that no statistically significant differences in body weight exist prior to dosing. If body weight of dosed animals is observed to decrease relative to controls, measurements may be done more frequently. Animals that exhibit an excessive decrease in weight (due to either weight loss or failure to gain weight compared to controls) will be evaluated further by the PI and AV (or designee) for possible early removal from the study, IAW Section V.4.5. The weights will be recorded directly into the laboratory notebook.

The PI/study staff will monitor food consumption by weighing the container plus food on day 0, 7, and 13. On day 7 (or earlier if food containers are approaching empty), the container will be weighed, resupplied with food, and a second weight will be recorded. If body weight is observed to decrease, measurements may be done more frequently. All weights will be entered into the laboratory notebook.

The subacute study will have a staggered start over a 3 day period for each sex. The 6 MNA positive/negative rats are subdivided into groups of 2 and scheduled for dosing so that their necropsy dates coincide with the subacute study necropsy dates.

On study day 14, male rats from the three highest dose groups with no mortalities will be biosampled from the tail vein for the MNA as described in V.4.4.3. All surviving animals on the sub-acute study will be rendered unconscious with CO_2 gas, bled, euthanized with CO_2, and necropsied. Animals will be fasted overnight (<20 hrs) prior to necropsy. The fasting of the animals is necessary to reduce the potential variability in the clinical chemistry values due to food intake. The following tissues will be harvested, weighed, and preserved in 10% formalin: brain, heart, kidneys, liver, spleen, ovaries, uterus, testes, left epididymis and thymus. Additional organs/tissues may be collected, if

warranted, based on necropsy observations. All gross pathology changes will be recorded on the necropsy report form (TOX DOC 4). Training records of necropsy personnel will be verified prior to necropsy and the names of personnel participating in necropsy will be documented. A table containing the training record information for personnel performing necropsy procedures will be part of the study records.

Clinical chemistry, hematology, and hemostasis analyses will be conducted at the end of the study, if blood sample volumes are adequate, according to TOX SOP 011 Clinical Chemistry Analysis of Blood Specimens [8], TOX SOP 013 Cell-Dyn 3700 Hematology Analyzer [9], and TOX SOP 079 Prothrombin Time and Activated Partial Thromboplastin Time [10]. Cardiac blood will be collected as described in V.4.4.3, Biosamples; and aliquoted to the appropriate type and number of blood collection tubes for analysis. Samples for clinical chemistry will be collected in serum or serum-gel tubes, and evaluated for: ALB, ALKP, ALT, AST, BUN, CA, CHOL, CREA, GLU, PHOS, TP, TRIG, and electrolytes. Hematology samples will be collected in EDTA tubes and evaluated for total red blood cell and white blood cell counts, packed cell volume, hemoglobin, and five-part leukocyte differential. Samples for hemostasis will be collected in sodium citrate tubes and analyzed for prothrombin time.

Whenever possible, blood and tissues will also be collected from moribund animals at the time of euthanasia. If the pathologist and/or other qualified personnel are unavailable at the time of euthanasia, animals will be bled and euthanized as described in V.4.4.3. Euthanized animals will be placed in the refrigerator until gross necropsy. Animals will be necropsied, and the decision of whether or not to collect tissues for pathological analysis will be at the discretion of the pathologist after assessing the level of tissue autolysis. Animals that are found dead will be refrigerated until necropsy. Blood will not be collected from these animals, and tissue collection will be at the discretion of the pathologist.

Table 2. Summary table of animal use and pain category.

TEST	**ANIMALS**		**PAIN CATEGORY**
ACUTE STUDY	**Females**	**Males**	
LD_{50}	30	0	C=15, E=15
14-DAY STUDY			
Control (0 mg/kg)	10	10	D
Dose TBD	10	10	D
Dose TBD	10	10	E
Dose TBD	10	10	E
Dose TBD	10	10	E
Dose TBD	10	10	E
MNA control	0	6	E
Total	**90**	**66**	**C=15, D=40, E=101**

Study Conduct: The study described will be conducted in a manner consistent with the principles of GLP regulations in the U.S. EPA TSCA: 40 CFR792 and FIFRA 40 CFR160 [11]. The investigators and technicians will adhere to *the Guide for the Care and Use of Laboratory Animals* [12].

Study Time Frame: Estimated experimental initiation date is July 2018, depending on availability of the test compound. Estimated experimental completion date is October 2018.

V.2. Sample Size Evaluation, Data Analysis Plan, and Archiving of Data: With the exception of clinical chemistry, hematology and genotoxicity data, all data will be recorded in the laboratory notebook. The data from the SADM will be analyzed according to the methods of Feder et al. [13, 14] in order to obtain an estimated LD50 value, CI, and slope. Note, an LD50 and slope are not calculated if the LD50 is above the limit dose of 2000 mg/kg. The use of up to 30 animals in the determination of LD50 (Experiment 1) is IAW ASTM Guideline 1163-10 (SADM). While other acute study methods can be used that further reduce the number of animals (i.e. OECD Test Guideline 423), these methods provide only rough estimates of the LD50 that do not allow meaningful comparisons with other compounds. The goal of this study is to compare the toxicity of TNBA to either TNT or other emerging replacement explosive materials. Thus, an accurate LD50, including slope and confidence intervals, is required to make these comparisons.

U.S. EPA guidelines recommend a minimum of 10 animals per group (5 males and 5 females) for sub-acute studies [15]. Experiment 2 will utilize 10 animals per sex per group, which is modestly larger than the minimal sample size recommended to account for (1) potential loss of animals due to high predicted toxicity of DNP and/or experimental error and (2) potential for sex-specific effects. Continuous data will be analyzed using a one-way ANOVA with dose group as the main effect. Organ weights will be analyzed using ANCOVA with terminal body weight as the covariate. When statistically significant main effects are observed ($p<0.05$), an appropriate post hoc test will be used to compare pairs of dose groups and dose groups to the control group. Variance equality will be determined by Levene's test. Data will be tested for normality and may be transformed appropriately prior to ANOVA/ANCOVA, or analyzed using a nonparametric Kruskal-Wallis test. Non-parametric analysis will be the method of last resort since it does not allow analysis of co-variation. The choice of statistical software will be at the discretion of the PI and APHC statistician. For all tests $\alpha = 0.05$ is the level of significance.

Records will be kept in standard APHC laboratory notebooks and/or three ring binders IAW TOX SOP 005 [16]. Daily records will be kept on survival and clinical signs collected on the animals after dosing occurs. Procedures for preparation of any euthanasia solution, drug administration, animal bleeds, observation logs, morbidity/mortality logs, etc., will be stored with the study records. All post mortem procedures not listed in this protocol will be documented in the study records and kept with the study raw data. Records pertaining to this study will be made available to

oversight organizations such as the U.S. EPA and the IACUC. The protocol, protocol amendments, raw data, statistical analysis, tabular calculations, and graphic analysis of the data will be saved with the study records. Additionally, memoranda to the study file, study logs, signature logs, final reports, final report amendments, and test and control articles will be archived at APHC.

V.3. Laboratory Animals Required and Justification

V.3.1. Non-animal Alternatives Considered: The objective of this study is to determine the adverse health effects of oral exposures to TNBA in the rat. The collected LD50 values (CI and slope) and the derived repeated dose LOAEL and NOAEL values for TNBA will be compared to that of TNT and alternative explosives. There are no non-animal alternatives that would provide the necessary toxicological information on TNBA to allow for an accurate comparison with previously performed animal testing on other explosives. The computational and *in vitro* estimated LD50 values described in section V.1.1 provide guidance on acute dosing strategy to reduce animal use. However, these values are currently insufficient to derive an *in vivo* LD50 or subacute NOAEL/LOAEL. Therefore, it is necessary to perform these studies in an animal model.

V.3.2. Animal Model and Species Justification: The U.S. EPA Health Effects Test Guidelines state that the rat is the preferred species for oral toxicity studies in mammals [5]. The Sprague-Dawley rat strain has been used routinely for oral toxicity studies at the APHC and is therefore recommended due to the extensive historical database that is available for this strain. As reviewed by Gad and Chengelis [17], female rats are deemed to be more sensitive to acute toxicants than male rats. For example, when the acute LD50 values for male and female rats exposed to 48 individual chemicals were compared, there were only 3 instances where the male LD50s were lower than the female LD50s. Conversely, there were 13 instances where the male LD50s were, on average, 29% higher than the female LD50s. Thus, female rats will be used preferentially to take advantage of this potential sensitivity. Different aged animals will be used in the acute and 14-day studies to comply with U.S. EPA Guidelines [5, 15]. In the acute study, rats are fasted overnight before the acute oral dose; thus, older rats (8-12 wks) are used because fasting causes undue stress to younger rats. Younger rats (6-10 wks) are used for the 14-day study as they will not be fasted (prior to dosing), and will require lower total doses of TNBA due to their lighter weight. For the subacute study, both males and females are tested so that in addition to the standard battery of endpoints, testicular effects can be evaluated in the males. For the MNA, mutagenic compounds typically show positive results in both sexes, although some evidence suggests that the sensitivity of this response may be slightly higher in males. Thus, if one sex is to be performed, males are generally the preferred sex. A study by the Collaborative Study Group for the Micronucleus Test demonstrated that among 20 mutagenic compounds tested, all compounds induced micronuclei in both sexes, and 15 of 20 showed sensitivities that were comparable between sexes. Among the remaining 5 compounds, 4 showed higher sensitivity in males [18]. The determination of whether a compound is mutagenic/carcinogenic requires multiple different *in vivo* and *in vitro* assays. The MNA offers a means to screen in an *in vivo* system that will add to the

overall weight of evidence, and slight sex differences are unlikely to be meaningful in this larger context. However, detection of any DNA damage via this assay (regardless of sensitivity) will escalate the concern over human exposure to the compound. The use of only one sex will reduce the total number of animals required for Experiment 2.

V.3.3. Laboratory Animals

V.3.3.1. Genus species*: Rattus norvegicus*

V.3.3.2. Strain / Stock / Breed: Sprague-Dawley

V.3.3.3. Source / Vendor: Charles River Laboratories (USDA 14-R-0144) or other APHC-approved vendor.

V.3.3.4. Age: Upon Receipt: Acute: 7-11 weeks
14-Day: 5-9 weeks

V.3.3.5. Weight: Age appropriate

V.3.3.6. Sex: Females (Acute and 14-day subacute); Males (14-day subacute)

V.3.3.7. Special Considerations: None

V.3.4. Number of Animals Required (by Species): rats (156)

V.3.5. Refinement, Reduction, Replacement (3 Rs):

V.3.5.1. Refinement: Animals will be provided an environmental enrichment strategy, consisting of non-food enrichment (e.g. nylabones), as described in section V.5.3. Animals will be appropriately anesthetized prior to collecting blood by cardiac puncture and then immediately euthanized. Animals will be considered for early removal from the study based on clinical signs of morbundity. To minimize distress, any animal defined as moribund (see V.4.5) will be euthanized with CO_2 in accordance with QSARC 800 [7].

V.3.5.2. Reduction:
1) The minimal number of animals needed for statistical significance will be used. The SADM method uses fewer animals than a classic Acute LD50 estimation (5 doses, 10 animals per sex per dose = 100 animals) while providing quantitative estimates of median lethality, slope, and confidence intervals.

2) If a dose of 2,000 mg/kg body weight does not cause mortality or morbidity in the first round of acute dosing, then round two of acute dosing will revert to a U.S. EPA limit test and thus use fewer animals to estimate an LD50.

3) Only female animals will be used for the acute study (Experiment 1). If both male and female rats were used the number of animals used would be doubled.

4) Allowing tissue sharing may also reduce the need for other animal studies to collect additional tissues.

5) Incorporating the MNA genotoxicity test with the subacute experiment reduces the need for initiating a separate genotoxicity study.

6) The MNA positive controls will serve as their own negative controls through early lateral tail vein sampling prior to dosing. This reduces the necessary number of animals by half, and provides a better control for the test itself.

V.3.5.3. Replacement: There are no non-animal alternatives that would provide the necessary toxicological information on TNBA to allow for an accurate comparison with previously performed animal testing on other explosives. Therefore, it is necessary to perform these studies in an animal model. The rat is the least sentient and most preferred animal model according to the U.S. EPA Health Effects Test Guidelines.

V.4. Technical Methods:

V.4.1. Pain / Distress Assessment:

V.4.1.1. APHIS Form 7023 Information:

V.4.1.1.1. Number of Animals

V.4.1.1.1.1. Column B: 0 (animal #)

V.4.1.1.1.2. Column C: 15 (animal #)

V.4.1.1.1.3. Column D: 40 (animal #)

V.4.1.1.1.4. Column E: 101 (animal #)

V.4.1.2. Pain Relief / Prevention

V.4.1.2.1. Anesthesia / Analgesia / Tranquilization: Animals will be rendered unconscious with CO_2 prior to cardiac blood collection. Animals will be brought to the necropsy room in home cage or transport cage. The stainless steel lid will be placed on the cage. If using a transport cage, the grommet will be covered with tape or a magnet. CO_2 will displace 10-30% of the chamber volume per minute IAW QSARC 800. Animals will remain in the cage until they are recumbent and breathing is slow and shallow. Once recumbent, a toe or space between the toes will be pinched to assess appropriate level of unconsciousness. If there is no response to toe pinch, the animal will be removed and blood collected (as described in V.4.4.3). Upon completion of blood collection the animal will be returned to the cage and euthanized as described in section V.4.6.

V.4.1.2.2. Pre- and Post-procedural Provisions:

Fasting: Rats will be fasted overnight prior to dosing for the acute portion of the study (Experiment 1) as per U.S. EPA Acute Oral Guidelines [5]. One day prior to dosing for Experiment 1, the PI/study staff will remove the feed bins at the end of the work day (for example, 4pm) and will return them approximately 3 hours after dosing (not to exceed 20 hours of fasting).
For Experiment 2, rats will not be fasted prior to dosing. However, rats will be fasted overnight prior to necropsy (15-22 hours of fasting). Fasting prior to necropsy is important for assessment of clinical chemistry that will be measured in Experiment 2.

Gavage observations: The rats will be observed after dosing for acute signs of misadministration, e.g., gasping, respiratory distress, etc., and euthanized when appropriate. The rats will be observed for the first 30 minutes after dosing and again approximately 4 hours later, with the possible exception of non-duty days (weekends/holidays), for signs of respiratory distress or gavage injury. The animals will also be observed during the acute and 14-day sub-acute study for signs of compound-induced toxicity based on a weight-of-evidence approach (e.g. a lack of general toxicity or clinical signs) and in consultation with a veterinarian (see Section V.5.2.1).

V.4.1.2.3. Paralytics: N/A

V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures:

V.4.1.3.1. Source(s) Searched: AGRICOLA, TOXNET, CRIS, NIH RePorter. Proquest Collection which includes; ABI/INFORM Collection, Biology Database, Continental Europe Database, Health & Medical Collection, Health Management Database, Military Database, Psychology Database, Public Health Database, Research Library. AMEDD Collection which includes; Ovid, JAMA Network, CINAHL, MEDLINE, PsycINFO, PubMed.

V.4.1.3.2. Date of Search: April 20, 2018

V.4.1.3.3. Period of Search: 1900-2018

V.4.1.3.4. Key Words of Search: (oral or mouth) and (lethal dose 50 or LD 50 or LD50 or LD-50 or median lethal dose or mld) and (toxicity or no-observed-adverse-effect-level or no observed adverse effect level or LOAEL or LOEL or NOAEL or NOEL) and (pain or distress or * or refin* or reduc* or replac* or artificial or vitro or culture or tissue or cell or organ or insect or arachnid or invertebrate or fish or mollusc or cephalopod or simulat* or digital or interactive or mannequin or manikin or model or cardiac or blood or cardiac puncture).

V.4.1.3.5. Results of Search: The search identified one reference pertaining to alternative toxicity testing strategies [19]. The principles pertaining to the 3Rs have been applied in this protocol, as outlined in section V.3.5. Additionally, this reference advocates for using computational methods and *in vitro* studies in lieu of animal studies

whenever possible. These approaches have been used for TNBA, as discussed in section V.1.1, but are currently insufficient to adequately characterize the *in vivo* toxicity. Additionally, *in vitro* to *in vivo* extrapolation models or read-across models are currently unavailable. Lastly, this reference advocates for using the Up/Down method for determining acute toxicity, since this method has the potential to substantially reduce the number of animals used compared to traditional acute toxicity studies that use up to 100 animals. The current protocol will employ the SADM approach, which will reduce the number of animals used to ≤30. The Up/Down method has the potential to further reduce the number of animals used; however, the Up/Down method typically provides less accuracy on the LD50, slope, and confidence intervals. These values have been determined to be of high importance due to the desire to compare the relative toxicity of TNBA to TNT or other novel explosive compounds. Thus, the SADM approach reduces animal use while still providing the necessary data.

Additionally, a detailed literature search for alternative approaches for testing TNBA was conducted, but retrieved 0 hits after searching PubMed, DTIC, FedRIP, EBSCO-Host's CINAHL Plus, and ToxNet.

V.4.1.4. Unalleviated Painful or Distressful Procedure Justification: The nature of the study precludes the use of totally painless procedures. The toxicity of the compound of interest is unknown and if toxic, the mechanism of toxicity is unknown. An attempt to alleviate pain or distress by the administration of anesthetics, analgesics, or other drugs may alter the manifestation of the toxic responses and/or interfere with the mechanism of toxicity, e.g., NSAIDs might mask a compound's toxicity resulting from the induction of systemic inflammation [20]. In addition, morphine and other opioids have been reported to produce a number of immunomodulatory effects in both laboratory animals and humans. Morphine also suppresses T- and B-cells, depresses natural killer cell activity, and decreases primary antibody responses in mice [21, 22]. Animals have been shown to be more susceptible to disease if opioids are used short term or in a single dose [23]. *In vitro* exposure of murine lymphocytes and macrophages to morphine and its metabolites at a wide range of concentrations resulted in suppressed B-cell proliferation, suppression of interleukin 2, 4, and 6, and inhibited cytotoxic T-lymphocyte induction [24]. Additionally, both fentanyl and buprenorphine (opioid analgesics) have shown a dose-dependent attenuation of the serum TNF-α response in mice as a result of exposure to LPS [22]. Previous studies indicate that opioid analgesics, including butorphanol, an opioid commonly used for pain alleviation in laboratory animals, cause substantial respiratory depression in nonhuman primates [25, 26]. Narcotic analgesics can cause histamine release and respiratory depression which could alter the pathogenic and clinical response to immune mediated inflammation [27]. Opioids have a significant effect on thermoregulatory control in humans and concentrations of opioids commonly observed in critical care patients significantly inhibit the manifestation of fever [28]. The cyclooxygenase inhibiting NSAIDs have been shown to produce progressive alterations of parameters of the thrombocyte vessel system of hemostasis, decreased ability of thrombocytes to aggregate, and activation of lipid peroxidation processes in rabbits injected with bacterial endotoxin [29]. Steroidal and nonsteroidal anti-inflammatory drugs are also contraindicated due to their

interference with the inflammatory response, a potentially integral part of the host response during the disease process. The observation of the onset, duration, and/or reversibility of toxic signs is critical to mechanistic interpretation. "Toxic signs" are defined in QSARC 806 [30].

V.4.2. Prolonged Restraint and Restraint Methods: See V.4.4.8.1. for manual restraint details.

V.4.3. Surgery: N/A

V.4.4. Animal Manipulations

V.4.4.1. Injections: N/A

V.4.4.2. Use of Non-pharmaceutical-grade chemicals: TNBA is not available in a pharmaceutical-grade composition. It is under investigation as described in section III. The manufacturer/sponsor will provide a statement of chemical purity, sterility, stability, etc, which will be verified by LS prior to initiation of the acute experiment.

V.4.4.3. Biosamples:

Animals in Experiment 1 are not biosampled.

For Experiment 2, MNA will be conducted on blood collected from male animals from the three highest 14-day dose groups without mortality, as well as from males from the vehicle control group and MNA control rats. The MNA assay is very sensitive to CO_2 and requires peripheral blood from unanesthetized rats. Thus, for these animals, blood will be collected prior to CO_2-induced unconsciousness on the day of euthanasia via a lateral tail venipuncture with an 18-23 gauge needle and syringe pre-loaded with anticoagulant. The needle is entered into the tail vein at a superficial angle nearly parallel to the tail with the bevel side up and approximately 60-120 µL of blood is drawn. This procedure will require two individuals- one person to manually restrain the animal and a second person to perform the venipuncture. Furthermore, the group of six negative/positive MNA control animals will undergo two tail venipunctures ≥72 hours apart- one prior to treatment with a genotoxic agent (negative sample) and a second tail venipuncture after treatment with the genotoxic agent (positive sample), as described in section 4.4.8.3. The rationale for collecting two peripheral blood samples from the control animals is so that one animal can serve as both a positive and negative control and thus reduce the number of animals required. The volume of blood collected by venipuncture can be more precisely controlled than via a tail snip or nick.

Additionally, a terminal cardiac blood draw will be collected from all animals in Experiment 2 (excluding the MNA controls), including those animals used for peripheral blood collection. Animals will be rendered unconscious via CO_2. Upon confirmation of unconsciousness via a toe pinch and just prior to euthanasia, a terminal intra-cardiac blood draw (0.5-7 mL) will be taken using an 18-21 gauge, 1-1.5 inch needle, as outlined in QSARC 805 [31], with the exception that CO_2 is used in place of anesthesia.

Biosampling will be promptly followed by euthanasia via CO_2. The blood collection tubes are as described in V.1.2.

V.4.4.4. Adjuvants: N/A

V.4.4.5. Monoclonal Antibody (MAb) Production: N/A

V.4.4.6. Animal Identification: Individual animals will be identified by cage card for the acute study (Experiment 1) and by cage card and tail markings (indelible ink) for the 14-day study, IAW QSARC 804 [32].

V.4.4.7. Behavioral Studies: N/A

V.4.4.8. Other Procedures:

V.4.4.8.1. Oral Gavage: All oral dosing will be administered using an oral gavage needle (16-18 ga. x 2 in) fitted to a 3-10 mL syringe; maximum volume is not to exceed 10 mL/kg [5]. The materials planned for oral administration include the control vehicle and TNBA. If dosing requires administering a volume greater than 10mL/kg, the total dose will be split into 2 smaller volumes that will be given at least 4 hrs apart (4 hrs is the gastric clearance time for the rat). In general, the distance from the tip of the nose to the last rib will be measured to determine the length of the tube to be inserted.

The method for gavage is as follows: The animal will be gently restrained and the head immobilized with accessibility to the oral cavity [e.g., 1) by "scruffing"- grasping the animal by the loose skin of the neck and back – which immobilizes the head, neck, and torso of the rat; 2) by using a "claw-grip"- positioning the rat's neck between the handler's index and middle finger and using the rest of the hand (palm, thumb, and fingers) to support the body of the rat; or 3) by a modified version of these holds that accomplishes the same purpose]. The animal is maintained in an upright (vertical) and extended position, the gavage needle is visually confirmed to be the appropriate size (i.e., hub-to-bulb distance spans the length between the mouth and last rib of the rat), and then the bulb of the needle is passed into the mouth of the rat with the bulb directed towards the junction of the palate and cheek (palatoglossal arch). Following the roof of the mouth and the dorsal aspect of the throat, the needle is advanced into the esophagus and toward the stomach. To aid insertion into the esophagus, the needle may be used as a lever to gently tip the head of the rat up and back (thus extending the neck) during the act of needle advancement. Additionally, the swallowing reflex of the rat may be used as guide for esophageal insertion. After the needle is advanced to the correct length, the compound is administered with an even, steady depression of the syringe plunger and the needle is withdrawn (the time to evacuate the syringe is approximately 2-4 seconds, depending on volume and vehicle). The advancement of the needle is not forced; if any resistance is encountered, the needle bulb is withdrawn to the front of the soft palate and insertion is attempted again. Note, if the handler's grip (or rat) has shifted during the preceding insertion attempt, the needle is completely removed from the mouth and the restraint hold is reinitiated prior to the next gavage attempt. After the compound is administered, the rat is observed briefly for acute signs

of maladministration (e.g., gasping, respiratory distress, etc.) and euthanized when appropriate.

V.4.4.8.2. Food Consumption Monitoring: The PI/study staff will monitor food consumption of the subacute study rats by weighing the container + food on day 0, 7, and 13. On day 7 (or earlier if the food containers are approaching empty), the container will be resupplied with food and a second weight will be recorded.

V.4.4.8.3 Micronucleus Assay: Biosampling for the MNA will be from the three highest 14-day dose groups without mortality (males only), as well as from the vehicle control and 6 MNA positive/negative control animals.

During Experiment 2, 6 additional male rats will be included as concurrent joint negative and positive controls for the genotoxicity MNA experiment. In brief, the positive control animals will serve as their own negative control, by obtaining a blood sample prior to gavage treatment with a genotoxic agent (e.g. 200 mg/kg EMS; CAS 62-50-0). In order to get a negative control sample, the 6 male rats will be left untreated and will not undergo oral gavage. At least 48 hours prior to euthanasia, approximately 60-120 µL of blood will be collected via a tail venipuncture, as described in section V.4.4.3. The rats will then receive EMS treatment in an appropriate vehicle (e.g. 0.5% weight by volume sodium carboxymethylcellulose solution, polyethylene glycol 200, water, or corn oil; maximum volume 10 mL/kg) three times: approximately 48-hours, 24-hours and 4-hours prior to a second tail venipuncture blood collection of 60-120 µL and euthanasia, as described in section V.4.4.3. The timing of the dosing for the EMS-treated animals will be staggered such that control animals are euthanized concurrently with the 14-day TNBA treated male rats (i.e., six control animals spread evenly over the three days of necropsy for the 14-day treated male rats).

The MNA assay will be conducted using the MicroFlow Basic Kit® (Litron Laboratories, Rochester, NY) following manufacturer's instructions. In brief, 60-120 µL of blood will be used for analysis as described in V.4.4.3. It is most important to consider that blood be free flowing and collected in syringes/tubes/pipette tips coated with the anti-coagulant solution provided in the kit, and that no more than 120 µL of blood are collected per 350 µL of anti-coagulant. Following collection, tubes/syringes will be capped and inverted several times to mix the blood and anti-coagulant solution. This mixture can be stored at room temperature for up to 5 hours prior to fixation, or it may be refrigerated for up to 24 hours. Samples will then be fixed and processed according to the manufacturer's instructions. Processed samples will be shipped to Litron Laboratories for analysis.

V.4.4.9. Tissue Sharing: Tissues from these experiments will be made available to collaborators and other APHC personnel with PI/SD and/or AV coordination, as long as no deviation from the protocol is required for collection.

V.4.5. Study Endpoint: The study endpoint is either death from exposure to TNBA, intervention euthanasia of moribund animals or euthanasia at the end of the experiment, i.e., no more than 7 days after study initiation for Experiment 1 and 14 days for

Experiment 2. The duration of the observational period for the acute test will not exceed 7 days. Any moribund animal will be euthanized humanely according to section V.4.6.

One or more of the following clinical signs will be considered in deciding to remove an animal from study and administering euthanasia: prolonged impaired ambulation which prevents animals from reaching food or water; weight loss or failure to gain weight (> 20% body weight as compared to controls) plus one other clinical sign or excessive weight loss/lack of weight gain (> 25% body weight as compared to controls); prolonged labored breathing; unabated seizure activity lasting longer than 1 hour; inability to urinate or defecate for greater than 24 hours; or a prolonged (greater than 1 hour) inability to remain upright. The AV or designee may be consulted to evaluate animals presenting clinical signs. The veterinarian and PI/SD will determine if euthanasia is indicated for these animals [7].

The time at which toxicity signs appear, their duration, and the time to death are important, especially if there is a tendency for deaths or morbidity to be delayed. At the end of the observation or dosing period, all surviving animals will be euthanized by CO_2 (surviving animals from the 14-day sub-acute study will first be anesthetized for cardiac blood sampling) and necropsied.

The endpoint for the 6 MNA control animals is euthanasia scheduled to coincide with the euthanasia days of the 14-day sub-acute study.

V.4.6. Euthanasia: Rats will be euthanized by CO_2 asphyxiation followed by thoracotomy to ensure death IAW QSARC 800 [7]. Moribund animals will be euthanized by CO_2 asphyxiation followed by thoracotomy to ensure death IAW QSARC 800 [7]. A veterinarian will be consulted to evaluate moribund animals unless the PI/SD plans to immediately euthanize the animal. The PI or Study Staff will perform the euthanasia.Blood and tissues will be collected from euthanized animals whenever possible, as described in V.4.4.3. Blood will not be collected from animals that are found dead, but the dead animals will be refrigerated until a necropsy is performed. Tissue collection from animals found dead will be at the discretion of the pathologist.

V.5. Husbandry & Veterinary Care:

V.5.1. Husbandry Considerations: Rats will be fasted overnight prior to dosing for the acute portion of the study (Experiment 1) as per U.S. EPA Acute Oral Guidelines [5]. Otherwise, they will be given certified rodent feed *ad libitum*. Animal rooms will be maintained at a constant temperature range (68-79°F) and humidity range (30 - 70%) with a 12-hour light/dark cycle. Animals will undergo an acclimation period of no less than 5 days after their arrival into the animal facility. The animal facilities are fully accredited by AAALAC International. Detailed husbandry practices and animal room sanitation schedules are contained in QSARC 707 [33].

V.5.1.1. Study Room: Studies will be conducted at the APHC Toxicology Directorate facilities, [redacted] study room as assigned.

V.5.1.2. Special Husbandry Provisions: Animals will be pair-housed (same sex) during the acclimation period for all tests. Animals will be pair-housed (same sex) during study conduct for all tests unless behavioral changes (e.g., aggression or hyperactivity) warrant single-housing. For the subacute study, the animals and food in feed containers are weighed and monitored by the PI/study staff to determine dosing volumes and food consumption. This requires restriction of the food enrichment, i.e., no additional food supplements will be provided other than the food in the food bin. To prevent a data entry/management conflict, clean food bins will be provided at day 0 of the subacute study (prior to initial weighing) and food bins will not be exchanged during the study conduct. Animals will be handled and socialized during the acclimation period by the study staff and/or veterinary staff to reduce the stress of handling during study conduct. Handling and socialization conditioning will not occur sooner than 48 hours after animals are received into the animal facility.

V.5.1.3. Exceptions: If warranted, animals may be transferred to single-housing due to behavioral incompatibility.

V.5.2. Veterinary Medical Care

V.5.2.1. Routine Veterinary Medical Care: Animals will routinely be observed no less than once daily by assigned veterinary medical personnel for husbandry conditions, humane care, and general health status. IAW current IACUC guidance, in the event an animal becomes ill or injured, veterinary or toxicology personnel will contact the AV or his/her designated backup who will determine the appropriate course of action. During the observation period of the acute study and for the duration of the 14-day sub-acute study, animals will be observed at least twice daily on duty days and at least once daily on non-duty days by assigned veterinary medical personnel and/or study staff. Observations by study staff will be taken approximately 4 hours or later following dosing. In consultation with a veterinarian and using a weight of evidence approach, the continued necessity of 4-hour post-dose observations on non-duty days will be assessed. Factors that contribute to the weight of evidence evaluation include: 1) a general lack of compound toxicity; 2) a lack of other clinical signs of illness; and 3) no distressed animals that are likely to require some form of intervention in less than a 24-hour period. Only the AV can authorize the suspension of the second observation on non-duty days. If an animal becomes ill or injured, the observer will comply with IACUC policy on notification to the AV and will inform the PI/SD. The animal will be euthanized by the veterinary staff or study staff if it becomes critically injured or moribund. For illnesses unrelated to administration of the test compound, the AV will discuss the plan for care with the PI/SD prior to initiation of any intervention.

V.5.2.2. Emergency Veterinary Medical Care: In the event an animal requires after-hours emergency veterinary care, a veterinarian is available 24 hours a day, 7 days a week. In the case of an emergency health problem, if the PI or co-PI is unavailable or the investigator staff and veterinary staff cannot reach consensus on treatment of a study animal, the veterinarian has the authority to treat the animal, remove it from the

experiment, institute appropriate measures to relieve severe pain or distress, or perform euthanasia if necessary. However, all decisions involving the treatment of a study animal in which a consensus cannot be reached will only be made after the veterinarian or designated backup veterinarian has actually observed and examined the animal in question. To facilitate communication, the animal care staff will maintain an emergency contact roster. In an emergency, the animal care staff will phone the numbers (office, home, and mobile) listed for the PI and co-PI. If the PI or co-PI cannot be reached by phone within 1 hour, then they are considered unavailable.

V.5.3. Environmental Enrichment

V.5.3.1. Enrichment Strategy: All enrichment will be provided in accordance with QSARC 802 [34]. In addition, the PI/SD, co-investigators, or veterinary staff will handle rats several times per week during the acclimation period (after the initial 48 hours in the facility) and prior to test article exposure to acclimate them to handling prior to study start. Rats may be provided with nylabones in their cage for the duration of all studies. Other non-food enrichment items/activities may be added as approved by the AV and PI.

V.5.3.2. Enrichment Restrictions: Food enrichment will be restricted due to food consumption monitoring. Rodent chow blocks will not be placed on the cage floor for animals during the acclimation period of the study, during Experiment 1 (acute study) due to overnight fasting or during the 14-day study (Experiment 2) due to food consumption monitoring.

VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING:

Personnel on Protocol	Activities to be performed on this protocol	Formal Training	Qualifications & Experience
Thomas Sussan	Handling & observations	Small animal handling workshop (9/28/2017, 6/7/2018); Rat handling 4/26-27/2018, 5/1/2018)	Ph.D., Biochemistry, Cell, Molecular Biology 17+ Yrs animal research experience
	Gavage	Rat gavage (5/2,7-9,14-16,21-23,29/2018)	
	CO_2 anesthesia/ euthanasia	Small animal handling workshop (9/28/2017, 6/7/2018)	
	Cardiac blood collection	Small animal handling workshop (9/28/2017, 6/7/2018)	
Allison Jackovitz	Handling & observations	Small animal handling workshop (6/4/09); Rat handling (6/12/12; 12/30/16)	B.S., Biology 7+ Yrs animal research experience
	CO_2 anesthesia/ euthanasia	Small animal handling workshop: euthanasia (6/4/09); Rat CO2 euthanasia (6/29/12)	

	Cardiac blood collection	Cardiac blood collection in rats using CO2 anesthesia (05/02/13; 05/08/13; 05/10/13)	
	Gavage	Rat oral gavage (6/12/12); Rat oral gavage (7/9-18/2014)	
Valerie Adams	Handling & observations	Rat techniques: handling and observations (11/3/08); Small animal handling workshop (5/28/09)	Ph.D., Cell and Structural Biology 15+ Yrs Animal Research Experience
	Cardiac blood collection	Rat techniques: basic bleeding (11/03/08); Small animal handling workshop: Intracardiac bleed (5/28/09); Rat bleeding techniques (11/7/2014, 5/13/2015)	
	Tail venipuncture	Saphenous and Tail Vein Bleeding (5/28/14); Rat bleeding techniques (8/11/14, 9/17/14)	
	Gavage	Rat oral gavage (7/9/2014-8/4/2014 (12 sessions))	
Mark Way	Handling & observations	Rodent and small animal handling workshop (5/17/07); Rat handling (7/19/07; 7/9/09; 12/10/15; 12/2016)	B.S., Biology 17+ Yrs animal research experience
	Gavage	Rat handling and dosing (7/18/07): Rat oral gavage (3/6/08; 5/5/08; 7/14/14)	
	Tail venipuncture	Rat techniques: blood collection (7/19/07); Rat Peripheral Bleeding (7/2009; 6/24/14; 8/11/14; 9/17/14)	
	Cardiac blood collection	Cardiac Bleed (5/13/15)	
	CO_2 anesthesia/ euthanasia	Rat techniques: euthanasia (7/19/07); CO2 euthanasia (7/9/09)	
Lee Crouse	Handling & observations	Rat handling (7/19/07; 12/8/16)	M.S., Environmental Sciences 18+ Yrs animal research experience
	Gavage	Rat gavage (07/19/07); Rat oral gavage (05/05/08); rat oral gavage (03/06/08; 7/14/14); rat oral gavage, 14 day (05/01/09)	
	CO_2 anesthesia/ euthanasia	Rat euthanasia via CO2 (7/19/07; 5/01/09; 10/2014; 11/2014)	
	Cardiac blood collection	Rat blood	

		collection (7/19/07); Terminal cardiac blood draw (5/1/09); Rat cardiac bleeding under CO2 anesthesia (5/10/13; 10/2014; 11/7/2014; 1/12/17)	
	Tail venipuncture	Rat bleeding techniques (12/17/08)	
Theresa Hanna	Handling & observations	Animal handling: rat (3/12/92); rat techniques: handling/observations (11/3/08); Rodent small animal handling workshop (2/25/98; 4/2/04; 11/22/05)	ALAT 18+ Yrs animal research experience
	CO_2 anesthesia/euthanasia	Rat euthanasia CO2 (3/27/09); Rat CO2 euthanasia (5/1/09)	
	Cardiac blood collection	Rat cardiac blood draw (5/1/09; 8/2014)	
	Tail venipuncture	Rat bleeding techniques: tail nick (6/24/14)	
Caroline Procell	Handling & observations	Rat handling (4/30/18)	B.S., Biology 2+ Yrs animal research experience
	Gavage	Rat gavage (4/30-6/4/20/18)	
Emily Lent	Handling & observations	Rat handling (7/19/07)	Ph.D., Natural Resources and Environmental Studies, M.S., Wildlife Biology 18+ Yrs animal research experience
	CO_2 anesthesia/euthanasia	Rat euthanasia via CO2 (7/19/07; 11/18/10; 8/2014)	
	Gavage	Rat handling and dosing (7/19/07); Rat gavage (3/6/08; 5/1/09)	
	Tail venipuncture	Rat handling and dosing- blood collection (7/19/07); Rat bleeding techniques (4/30/08)	
	Cardiac blood collection	Rat cardiac blood draw (8/21/14)	

VII. BIOHAZARD/SAFETY: Risks associated with this protocol include bites/scratches/needle sticks, transmission of zoonotic diseases, and the development of animal allergies. To minimize risk, appropriate handling techniques will be used and appropriate PPE will be worn for all animal handling work. This includes (but may not be limited to) facemask, gloves, and disposable lab coat. Personnel will wash their hands upon completion of animal work. Applicable current SOPs and APHC regulations will be followed (APHC Regs. 385-1 [35] and 385-5 [36] and QSARC 807 [37]). These documents specify hazardous waste disposal, bite/scratch procedures, and zoonotic disease prevention. A sharps container will be present at all times when using sharps and needles will not be recapped after entering animal tissue. Test substances shall be handled IAW TOX SOP 051 [38]. Test material will be stored in sealed containers when

not in use. All manipulations of the test substance, prior to animal treatment, shall be performed in a laboratory (using a fume hood when necessary). Although the precise toxicity of the test substance may not be known, information regarding its chemical family is provided by the sponsor such that a reasonable assessment of its safety can be made.

VIII. ENCLOSURES:

References (Appendix A)

Animal Use Protocol: Effects of Acute and Subacute Oral 2,4,6-Trinitro-3-Bromoanisole (TNBA) Exposure to Rats (*Rattus norvegicus*)

IX. ASSURANCES:

IX.1. As the Principal Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Duplication of Effort: I have made every effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with a qualified individual who evaluated the experimental design with respect to the statistical analysis, and that the minimum number of animals needed for scientific validity will be used.

D. Biohazard/Safety: I have taken into consideration and made the proper coordination regarding all applicable rules and regulations concerning radiation protection, biosafety, recombinant issues, and so forth, in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures / manipulations / observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures / manipulations.

F. Responsibility: I acknowledge the inherent moral, ethical and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R", namely, "Responsibility," which the DOD has embraced for implementing animal use alternatives where feasible and conducting humane and lawful research.

G. Scientific Review: This proposed animal use protocol has received appropriate peer scientific review and is consistent with good scientific research practice.

H. Painful Procedures: (*Applicable if the research being conducted has the potential to cause more than momentary or slight pain or distress even if an anesthetic or analgesic is used to relieve the pain and/or distress.*)

I am conducting biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress WILL / WILL NOT (circle one or both, if applicable) be relieved with the use of anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

I. Unexpected Adverse Events: I acknowledge the responsibility for reporting unexpected adverse events IAW the most current version of IACUC Local Guidance Document 008 "Reportable Events".

Thomas E. Sussan		7/12/2018
(PRINT) Principal Investigator	(Signature)	(Date)

Animal Use Protocol: Effects of Acute and Subacute Oral 2,4,6-Trinitro-3-Bromoanisole (TNBA) Exposure to Rats (*Rattus norvegicus*)

IX.2. As the Primary Co-Investigator on this protocol, I provide the following assurances:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Authority: I understand that, as the Primary Co-Investigator, I am authorized and responsible for performing all procedures and manipulations as assigned to the SD/PI in the SD/PI's absence. This includes euthanasia of distressed animals.

C. Training: I verify that I am technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

D. Responsibility: I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that I will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R", namely "Responsibility," which the DOD has embraced for implementing animal use alternatives where feasible, and conducting humane and lawful research.

E. Painful Procedures: I am conducting biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress WILL or WILL NOT (circle one or both, if applicable) be relieved with the use of anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

F. Unexpected Adverse Events: I acknowledge the responsibility for reporting unexpected adverse events IAW the most current version of IACUC Local Guidance Document 008 "Reportable Events".

Allison M. Jackovitz

(PRINT) First name, MI, Last name of Primary Co-Investigator

A Jackovitz — 12 July 2018

(Signature) (Date)

IX.2. As a Co-Investigator on this protocol, I provide the following assurances:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Authority: I understand that, as a Co-Investigator, I am authorized, responsible for, and willing to perform all procedures and manipulations as assigned to me by the SD/PI.

C. Training: I verify that I am technically competent and have been or will be properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the assigned procedures/manipulations performed by me.

D. Responsibility: I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that I will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to participate in this study in the spirit of the fourth "R", namely "Responsibility," which the DOD has embraced for implementing animal use alternatives where feasible, and conducting humane and lawful research.

E. Painful Procedures: I am participating in biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals. I will follow the direction of the SD/PI relative to potential pain and/or distress and relief by use of anesthetics, analgesics, and/or tranquilizers.

F. Unexpected Adverse Events: I acknowledge the responsibility for reporting unexpected adverse events IAW the most current version of IACUC Local Guidance Document 008 "Reportable Events".

Valerie H. Adams		July 11 2018
(PRINT)	(SIGNATURE)	(DATE)
First name, MI, Last name of Co-Investigator		

(PRINT)	(SIGNATURE)	(DATE)
First name, MI, Last name of Co-Investigator		

(PRINT)	(SIGNATURE)	(DATE)
First name, MI, Last name of Co-Investigator		

(PRINT)	(SIGNATURE)	(DATE)
First name, MI, Last name of Co-Investigator		

APPENDIX A
References (see table at end of references for cross-reference of newly renamed SOPs)

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ARMY PUBLIC HEALTH CENTER
PROTOCOL REVIEW AND APPROVAL FORM

Test Type: **30** IACUC protocol number: **18-07-02**

TITLE: Effects of Acute Oral 2,4,6-Trinitro-3-Bromoanisole (TNBA) Exposure to Rats (*Rattus norvegicus*)

1. PRINCIPAL INVESTIGATOR/ STUDY DIRECTOR

Printed Name (First, MI, Last)	*Title/ Division/ Program/ Directorate*
Thomas E Sussan	Biologist / Health Effects Division / Toxicology Directorate

PHONE: 410-436-6590 EMAIL: thomas.e.sussan2.civ@mail.mil

2018-05-08 SUSSAN.THOMAS.EDWARD.1537772626 Digitally signed by SUSSAN.THOMAS.EDWARD.1537772626 Date: 2018.05.08 11:23:59 -04'00'

Date *Signature* *(SD/PI will sign here before submitting protocol to the IACUC office)*

2. SCIENTIFIC/ PEER REVIEW

Printed Name (First, MI, Last)	*Title/ Division/ Program/ Directorate*
Matthew Bazar	Biologist / Toxicity Evaluation Division / Toxicology Directorate

PHONE: 410-436-7704 EMAIL: matthew.a.bazar.civ@mail.mil

2018-05-07 BAZAR.MATTHEW.A.1241429322 Digitally signed by BAZAR.MATTHEW.A.1241429322 Date: 2018.05.07 07:40:43 -04'00'

Date *Signature*

3. STATISTICAL REVIEW

Printed Name (First, MI, Last)	*Title/ Division/ Program/ Directorate*
Robyn B Lee-Stubbs	Statistician/USAMRICD

PHONE: 410-436-5322 EMAIL: robyn.b.lee2.civ@mail.mil

2018-05-07 LEE.ROBYN.BELLEK.1267390289 Digitally signed by LEE.ROBYN.BELLEK.1267390289 Date: 2018.05.07 08:55:56 -04'00'

Date *Signature*

4. ATTENDING VETERINARIAN REVIEW

Printed Name (First, MI, Last)	*Title/ Division/ Program/ Directorate*
Michael R Bonhage	Attending Veterinarian/VMO/QSARC

PHONE: 410-436-8863 EMAIL: michael.r.bonhage.mil@mail.mil

2018-05-07 BONHAGE.MICHAEL.ROBERT.1014029881 Digitally signed by BONHAGE.MICHAEL.ROBERT.1014029881 Date: 2018.05.07 11:24:02 -04'00'

Date *Signature*

5. IACUC APPROVAL *Date:* 2018-07-11

Printed Name (First, MI, Last) *Signature*

Kristin T. Newkirk NEWKIRK.KRISTIN.TORELL.1014786895 Digitally signed by NEWKIRK.KRISTIN.TORELL.1014786895 Date: 2018.07.11 16:50:55 -04'00'

6. INSTITUTIONAL OFFICIAL REVIEW

Printed Name (First, MI, Last) *Signature*

John J. Resta RESTA.JOHN.J.1229129305 Digitally signed by RESTA.JOHN.J.1229129305 Date: 2018.07.12 10:39:11 -04'00'

ID 007 REV 0 SEPT 2018 Page 1 of 1

APHC IACUC Protocol Modification Review/Approval

Study Director/ Principal Investigator: Thomas Sussan

Phone No.: 410-436-6590 *FAX No.:*

Email: thomas.e.sussan2.civ@mail.mil

PROTOCOL #: **30-18-07-02** *Protocol Approval Date:* 11 July 2018

PROTOCOL TITLE: **Effects of Acute and Subacute Oral 2,4,6-Trinitro-3-Bromoanisole (TNBA) Exposure to Rats (Rattus norvegicus)**

Modification Request #: **1** *Submission Date:* Jul 12, 2018

Study Director/ Principal Investigator Signature: SUSSAN.THOMAS.EDWARD.1537772626 Digitally signed by SUSSAN.THOMAS.EDWARD.1537772626 Date: 2018.07.12 13:20:31 -04'00'

For review use only: Review Process: ADMIN ☒ FCR ☐ DMR ☐ VVC ☐

Received Date: 23 July 2018 AURO receipt: DAWSON.CHELSEA.LEE.1544052116 Digitally signed by DAWSON.CHELSEA.LEE.1544052116 Date: 2018.07.23 12:27:01 -04'00'

IACUC Designated Member Reviewer(s) (*sign when review is completed*):

Attending/ Alternate Veterinarian Review Signature: Approved via VVC: N/A ☐ YES ☐ NO ☐

Date

Safety Review Signature (*if applicable*):

Date

LTACO/GLP Review Signature (*if applicable*):

Date

IACUC Chair Review Signature:

Jul 24, 2018 DECK.ADAM.T.1267159900 Digitally signed by DECK.ADAM.T.1267159900 Date: 2018.07.24 08:09:23 -04'00'

Date

Modification IACUC APPROVED ☒ GLP REVIEWED ☐ Date: Jul 24, 2018

APHC IACUC Animal Use Protocol Modification

Study Director/ Principal Investigator: **Thomas Sussan**

PROTOCOL #: **30-18-07-02** Protocol Approval Date: 11 July 2018

PROTOCOL TITLE: **Effects of Acute and Subacute Oral 2,4,6-Trinitro-3-Bromoanisole (TNBA) Exposure to Rats (Rattus norvegicus)**

Phone No.: 410-436-6590 FAX No.:

Email: thomas.e.sussan2.civ@mail.mil

Modification Request #: 1 Submission Date: 12 July 2018

1. Brief non-technical synopsis of existing protocol or background information pertaining to the modification request.

Add two individuals to the study team who were not originally included. Duties may include any procedure that was described in the original protocol for which they have been trained, although the primary need is for gavage dosing and handling of the rats.

2. Type(s) of Modification Requested: (check all that apply)

	Type Of Change	X
ADMINISTRATIVE – AURO/IQAU/SEMO REVIEW	Administrative Modifications (*e.g., correct typographical errors/grammar, contact information update, change in study start/completion dates, change in references, protocol title change, change that requires review and approval for GLP compliance*)	
	Addition or deletion of a qualified technician, co-investigator, or study staff	**X**
MINOR Modifications *(examples)*	Change in animal usage [*e.g., vendor, sex, age, weight, strain, small increase in # of animals used (less than 10% of overall number of approved animals)*]	
	Need to repeat an experiment without the addition of animals	
	Addition/ change of sample collection times	
	Additional noninvasive sampling	
	Changes in acclimation or recovery period	
	Special housing request or change in husbandry procedures	
	Changes in dosing procedures (e.g., dose, volume, or timing)	
	Other: _____	
MAJOR Modifications *(examples)*	Change in SD/PI	
	Change in objectives of the study	
	Addition of a test article to be evaluated	
	Change that results in greater pain, distress, or degree of invasiveness (changes to pain categories D or E from C require a literature search for alternatives to be completed)	

	Addition of animals to pain Category E (literature search for alternatives required)	
	Addition of or change in Species	
	Addition of more than 10% of the total # of animals originally approved	
	Addition of blood sampling	
	Change in frequency of observations for morbidity	
	Change in study endpoint	
	Changes in housing or use of animals in a location that is not part of the animal program overseen by the IACUC	
	Changes from nonsurvival to survival surgery	
	Change that impacts personnel safety	
	Other change that relates to the specific experimental design and aims of the original protocol	
	Other: _____	

3. PREVIOUSLY APPROVED MODIFICATIONS: (*Hit 'Enter' after description of each modification to list all individually.*)

Mod #	Short Description of the Amendment(s) (Include pain category breakdown of animal usage if changes were made from the original protocol)	No. & Species of Animal Requested	Approval Date

4. CURRENT APPROVED ANIMAL USAGE ON PROTOCOL:

Species/Total: Rat/156 **B:** 0 **C:** 15 **D:** 40 **E:** 101

5. FOR ITEMS A-H BELOW: Describe the requested changes to be implemented and the justification(s) for each.

A. Administrative change: Editorial (typographical, grammar, contact info update, etc), GLP compliance changes, or change in personnel (not change of study director/principal investigator):

I would like to add Michael Quinn and James Cox to the current study for the procedures described in the attached personnel training/qualifications table. At the time of the protocol submission, Mike Quinn had an expired occupational health certificate that prevented him from being included, but this was recently approved. James Cox was originally the PI on a separate study, which prevented him from being included here. However, the funding for that study is now delayed, and he is available to assist on this study.

B. Specific changes or additions to the experimental design of the protocol (Section V.1. Experimental Design).

N/A

C. Change in sample size evaluation, data analysis plan, archiving of data, (Section V.2.).

N/A

D. Change in the animals used, (Section V.3). *(Include any changes in amount used, sex, age/weight, vendor/source, refinement, reduction, and replacement [3Rs]).*

N/A

E. Changes to technical methods (Section V.4.). *(Include changes in USDA pain category classifications [section V.4.1.1.1.1 thru V.4.1.1.1.4.], changes in anesthesia, analgesia, restraint, injections, identification, behavior studies, 'other procedures', study endpoint, euthanasia, etc. Include reference source for basis of dose/treatment change.).*
**** * * * If the modification requested will be adding animals to COLUMN D or E and the protocol did not previously have animals in those categories, a LITERATURE SEARCH FOR ALTERNATIVES TO PAINFUL OR DISTRESSFUL PROCEDURES must be completed and submitted with this modification request.***

N/A

F. Changes to the Husbandry and Veterinary Care procedures (Section V.5.). *(Changes in husbandry considerations, special provisions, exceptions to the Guide, AWAR, or IACUC Policy that have an impact on animal care and use; any changes to the veterinary medical care or environmental enrichment.)*

N/A

G. Changes to the personnel conducting this protocol (Section VI.). *(Include record of completed training for any animal handling or use procedures assigned to new personnel. Signed assurance page(s) must be included with the modification if changing SD/PI, Primary Co-investigoator, or adding a co-investigator.)*

See attached chart.

H. Changes in Biohazard/ Safety (Section VII).

N/A

Personnel on Protocol	Activities to be performed on this protocol	Formal Training	Qualifications & Experience
James Cox	Handling/observations	Small animal handling workshop (9/28/2017); Rat handling 4/30/2018)	M.S., Biology 7+ Yrs animal research experience
	Oral Gavage	Small animal handling workshop (9/28/2017); Rat gavage Hands-on training with Lee Crouse and AV (10/16/17-10/20/17, 10/25/17, 10/26/17, 4/30/2018, 5/2/2018, 5/7/2018, 5/8/2018, 5/14/2018)	
	CO_2 anesthesia/euthanasia	Small animal handling workshop (9/28/2017)	
	Cardiac blood collection	Small animal handling workshop (9/28/2017)	
Michael Quinn	Handling & observations	Rodent small animal handling workshop (6/21/05); Rodent handling techniques (6/30/11)	Ph.D., Animal Science 18+ Yrs animal research experience
	Gavage	Rat oral gavage (6/2012)	

APHC IACUC Protocol Modification Review/Approval

Study Director/ Principal Investigator: Thomas Sussan

Phone No.: 410-436-6590 *FAX No.:*

Email: thomas.e.sussan2.civ@mail.mil

PROTOCOL #: **30-18-07-02** *Protocol Approval Date:* 11 July 2018

PROTOCOL TITLE: **Effects of Acute and Subacute Oral 2,4,6-Trinitro-3-Bromoanisole (TNBA) Exposure to Rats (Rattus norvegicus)**

Modification Request #: **2** *Submission Date:* Jul 27, 2018

Study Director/ Principal Investigator Signature: SUSSAN.THOMAS.EDWARD.1537772626 Digitally signed by SUSSAN.THOMAS.EDWARD.1537772626 Date: 2018.07.27 15:31:43 -04'00'

For review use only: Review Process: ADMIN ☐ FCR ☐ DMR ☒ VVC ☐

Received Date: Jul 30, 2018 AURO receipt: NEWKIRK.KRISTIN.TORELL.1014786895 Digitally signed by NEWKIRK.KRISTIN.TORELL.1014786895 Date: 2018.07.30 14:06:34 -04'00'

IACUC Designated Member Reviewer(s) (*sign when review is completed*):

NEWKIRK.KRISTIN.TORELL.1014786895 Digitally signed by NEWKIRK.KRISTIN.TORELL.1014786895 Date: 2018.07.30 14:06:56 -04'00'

Attending/ Alternate Veterinarian Review Signature: Approved via VVC: N/A ☐ YES ☐ NO ☐

Aug 1, 2018 MOCCIA.KRINON.DODEKA.1167935563 Digitally signed by MOCCIA.KRINON.DODEKA.1167935563 Date: 2018.08.01 15:55:53 -04'00'

Date

Safety Review Signature (*if applicable*):

Date

LTACO/GLP Review Signature (*if applicable*):

Date

IACUC Chair Review Signature:

Aug 2, 2018 NEWKIRK.KRISTIN.TORELL.1014786895 Digitally signed by NEWKIRK.KRISTIN.TORELL.1014786895 Date: 2018.08.02 07:54:35 -04'00'

Date

Modification IACUC APPROVED ☒ GLP REVIEWED ☐ Date: Aug 2, 2018

ID 002 REV 0 JUNE 2017 Page 1 of 1

APHC IACUC Animal Use Protocol Modification

Study Director/ Principal Investigator: **Thomas Sussan**

PROTOCOL #: **30-18-07-02** Protocol Approval Date: 11 July 2018

PROTOCOL TITLE: **Effects of Acute and Subacute Oral 2,4,6-Trinitro-3-Bromoanisole (TNBA) Exposure to Rats (Rattus norvegicus)**

Phone No.: 410-436-6590 FAX No.:

Email: thomas.e.sussan2.civ@mail.mil

Modification Request #: 2 Submission Date: 27 July 2018

1. Brief non-technical synopsis of existing protocol or background information pertaining to the modification request.

As described in the animal protocol, some of the male rats in the 14-day dosing experiment will undergo a tail venipuncture to withdraw 60-120ul of blood. The stated method of restraint for this procedure is a two-person method in which one person manually restrains the animal while a second person performs the venipuncture. This is the method preferred by several of the key personnel; however, other personnel prefer to use a plastic cylindrical restrainer. The time required for the rat to remain in the restrainer is typically 1-2 minutes and will not exceed 5 minutes. The rat can breathe normally while inside the restrainer. The rationale for using a cylindrical restrainer is that it provides less potential opportunity for the animal to jerk suddenly during venipuncture, and is thus safer for the animal and study personnel. This modification request will enable the person conducting the venipuncture to use whichever method of restraint they prefer. Both methods of restraint may cause slight distress to the animal, and both methods require similar amount of time for the animal to remain in the restrained position. Neither method of restraint is painful to the animal.

The original protocol also states that all animals will be pair-housed unless behavioral changes/incompatibility warrant single housing. Animals will be socially housed on this study to the greatest extent possible. However, this modification will add specific exceptions to this procedure.

Acute Study (Experiment 1): Most significantly, the animals used for the acute study will be singly housed after dosing. The rationale for housing dosed animals individually is that each animal may receive a different dose of the test material or may be dosed on a different day and may thus exhibit varying degrees of toxicity. Pair-housing of these animals may result in healthy animals paired with animals displaying signs of toxicity, such as seizures, aggression, or moribundity, which may negatively impact the healthy cage-mate. If these animals display no signs of toxicity after 3-6 days, the rats may be re-paired prior to euthanasia, at the discretion of the PI, or veterinarian after consultation with the PI. The PI or veterinarian after consultation with the PI, may also use discretion to separate animals that have been re-paired if they display delayed signs of toxicity or incompatibility. Additionally, the acute studies may result in the use of odd numbers of animals during each round of dosing, and thus some of the undosed animals may be separated from their cage-mate resulting in single housing. For example, the original protocol states that 5 rats will be dosed in round 1 of the acute study, which will leave 25 animals that have not yet been dosed. If there are multiple singly-housed

rats of the same sex that have yet to be dosed, they may be combined at the discretion of the PI or veterinarian (after consultation with the PI) to minimize the number of singly-housed animals.

The original protocol also states that animals will be handled and socialized during the 5-day acclimation period by the study staff and/or veterinary staff to reduce the stress of handling during study conduct. Handling and socialization conditioning will not occur sooner than 48 hours after animals are received into the animal facility. This modification request will enable the rats to be weighed by the PI or study staff during the handling period (2-5 days after arrival). The rationale for weighing rats is to assess how well the rats are acclimating and maintaining/gaining weight. For example, rats that fail to eat or drink may complicate the study results, and this weight loss may be mistakenly attributed to the test material. Weighing the rats prior to initiation of dosing may alert the study staff to potential issues with the animal's health.

One additional administrative modification is also listed to add a TOX SOP number to a listed reference.

2. Type(s) of Modification Requested: (check all that apply)

	Type Of Change	X
ADMINISTRATIVE – AURO/QAU/SEMO REVIEW	Administrative Modifications (*e.g., correct typographical errors/grammar, contact information update, change in study start/completion dates, change in references, protocol title change, change that requires review and approval for GLP compliance*)	**X**
	Addition or deletion of a qualified technician, co-investigator, or study staff	
MINOR Modifications *(examples)*	Change in animal usage [*e.g., vendor, sex, age, weight, strain, small increase in # of animals used (less than 10% of overall number of approved animals)*]	
	Need to repeat an experiment without the addition of animals	
	Addition/ change of sample collection times	
	Additional noninvasive sampling	**X**
	Changes in acclimation or recovery period	
	Special housing request or change in husbandry procedures	**X**
	Changes in dosing procedures (e.g., dose, volume, or timing)	
	Other: Addition of second method of restraint	**X**
MAJOR Modifications	Change in SD/PI	
	Change in objectives of the study	
	Addition of a test article to be evaluated	

	Change that results in greater pain, distress, or degree of invasiveness (changes to pain categories D or E from C require a literature search for alternatives to be completed)	
	Addition of animals to pain Category E (literature search for alternatives required)	
	Addition of or change in Species	
	Addition of more than 10% of the total # of animals originally approved	
	Addition of blood sampling	
	Change in frequency of observations for morbidity	
	Change in study endpoint	
	Changes in housing or use of animals in a location that is not part of the animal program overseen by the IACUC	
	Changes from nonsurvival to survival surgery	
	Change that impacts personnel safety	
	Other change that relates to the specific experimental design and aims of the original protocol	
	Other: _____	

3. PREVIOUSLY APPROVED MODIFICATIONS: (*Hit 'Enter' after description of each modification to list all individually.*)

Mod #	Short Description of the Amendment(s) (Include pain category breakdown of animal usage if changes were made from the original protocol)	No. & Species of Animal Requested	Approval Date
1	Addition of two study personnel	Rat/156	7/24/2018

4. CURRENT APPROVED ANIMAL USAGE ON PROTOCOL:

Species/Total: Rat/156 **B:** 0 **C:** 15 **D:** 40 **E:** 101

5. FOR ITEMS A-H BELOW: Describe the requested changes to be implemented and the justification(s) for each.

A. Administrative change: Editorial (typographical, grammar, contact info update, etc), GLP compliance changes, or change in personnel (not change of study director/principal investigator):

In the references, number 10 is changed to read, "APHC, TOX SOP 079, Prothrombin Time and Activated…". The SOP number was inadvertently left out of the reference listing.

B. Specific changes or additions to the experimental design of the protocol (Section V.1. Experimental Design).

N/A

C. Change in sample size evaluation, data analysis plan, archiving of data, (Section V.2.).

N/A

D. Change in the animals used, (Section V.3). *(Include any changes in amount used, sex, age/weight, vendor/source, refinement, reduction, and replacement [3Rs]).*

N/A

E. Changes to technical methods (Section V.4.). *(Include changes in USDA pain category classifications [section V.4.1.1.1.1 thru V.4.1.1.1.4.], changes in anesthesia, analgesia, restraint, injections, identification, behavior studies, 'other procedures', study endpoint, euthanasia, etc. Include reference source for basis of dose/treatment change.).*
**** * * * If the modification requested will be adding animals to COLUMN D or E and the protocol did not previously have animals in those categories, a LITERATURE SEARCH FOR ALTERNATIVES TO PAINFUL OR DISTRESSFUL PROCEDURES must be completed and submitted with this modification request.***

This modification enables the study staff to use either of two methods of restraint while performing the tail venipuncture- either manual restraint by a second person or use of a cylindrical restrainer. Both methods of restraint may cause slight distress to the animal, and both methods require similar amount of time for the animal to remain in the restrained position. Neither method of restraint is painful to the animal. The manual restraint is described in the original protocol. The modified procedure is described here. Once the animal is placed in the cylinder with its tail protruding out of the back end of the tube, a donut-shaped restrainer is slid inside the opposite end of the tube and locked in place at a position that enables the rat to move its nose into the donut-hole but prevents the rat from being able to turn around within the tube. Once the rat is restrained, venipuncture of the tail is performed. Immediately after withdrawing blood from the tail, the animal is released from the restraining device. The maximum time in which a rat will be restrained is 5 minutes.

This modification will also enable at least one weight measurement of the animals by study staff during the acclimation period (2-5 days after arrival). Obtaining weights of the animals prior to dosing may improve the ability of the study personnel to accurately assess the dose response by understanding the trends in weight that preceded dosing. A possible scenario for rats that arrive on a Wednesday (acclimation period Wednesday – Sunday) would be to obtain weights on Friday and then again on Monday prior to dosing on Tuesday. Thus under this scenario, a single weight would be obtained during the acclimation period and a follow-up weight would be obtained at day -1 (after completion of acclimation). However, other scenarios may warrant additional weight measurements during the acclimation period.

F. Changes to the Husbandry and Veterinary Care procedures (Section V.5.). *(Changes in husbandry considerations, special provisions, exceptions to the Guide, AWAR, or IACUC Policy that have an impact on animal care and use; any changes to the veterinary medical care or environmental enrichment.)*

The acute study animals will be singly housed after dosing. Animals showing no signs of toxicity during the first 3-6 days of the observation period may be pair housed again at the discretion of the PI or veterinarian after consultation with the PI. Pair housed animals showing signs of delayed toxicity will be returned to single housing. Single housing is necessary because the toxicity of the compound is unknown; therefore the toxic signs expected at each dose are unknown. Thus, pair housing could result in animals being co-housed that may be exhibiting very different toxic signs. This practice may result in undue stress to the healthy animal or loss of data from the moribund animal if aggression or cannibalism occurs. We do not anticipate that single versus paired-housing will impact the quality of the study results. During the sub-acute study, animals will be pair housed. However, single housing may occur as a result of animal deaths/euthanasia or if signs of toxicity (e.g., seizures, aggression, or moribundity) could potentially negatively imact the healthy cage-mate. Separations due to signs of toxicity would be performed only at the discretion of the PI or veterinarian (after consultation with the PI) if a concern can be justified.

G. Changes to the personnel conducting this protocol (Section VI.). *(Include record of completed training for any animal handling or use procedures assigned to new personnel. Signed assurance page(s) must be included with the modification if changing SD/PI, Primary Co-investigoator, or adding a co-investigator.)*

N/A

H. Changes in Biohazard/ Safety (Section VII).

N/A

APHC IACUC Protocol Modification Review/Approval

Study Director/ Principal Investigator: Thomas Sussan

Phone No.: 436-6590 *FAX No.:*

Email: thomas.e.sussan2@civ@mail.mil

PROTOCOL #: **30-18-07-02** *Protocol Approval Date:* 11 July 2018

PROTOCOL TITLE: **Effects of Acute and Subacute Oral 2,4,6-Trinitro-3-Bromoanisole (TNBA) Exposure to Rats (Rattus norvegicus)**

Modification Request #: **3** *Submission Date:* Nov 26, 2018

Study Director/ Principal Investigator Signature: SUSSAN.THOMAS.EDWARD.1537772626 Digitally signed by SUSSAN.THOMAS.EDWARD.1537772626 Date: 2018.11.26 13:30:09 -05'00'

For review use only: Review Process: ADMIN ☐ FCR ☐ DMR ☒ VVC ☐

Received Date: Nov 26, 2018 AURO receipt: NEWKIRK.KRISTIN.TORELL.1014786895 Digitally signed by NEWKIRK.KRISTIN.TORELL.1014786895 Date: 2018.11.26 13:39:50 -05'00'

IACUC Designated Member Reviewer(s) (*sign when review is completed*):

NEWKIRK.KRISTIN.TORELL.1014786895 Digitally signed by NEWKIRK.KRISTIN.TORELL.1014786895 Date: 2018.11.26 13:40:05 -05'00'

Attending/ Alternate Veterinarian Review Signature: Approved via VVC: N/A ☐ YES ☒ NO ☐

GEHLING.ALICIA.MARIE.1364571689 Digitally signed by GEHLING.ALICIA.MARIE.1364571689 Date: 2018.11.26 14:27:44 -05'00'

Date

Safety Review Signature (*if applicable*):

Date

LTACO/GLP Review Signature (*if applicable*):

Date

IACUC Chair Review Signature:

Nov 26, 2018

NEWKIRK.KRISTIN.TORELL.1014786895 Digitally signed by NEWKIRK.KRISTIN.TORELL.1014786895 Date: 2018.11.26 14:37:52 -05'00'

Date

Modification IACUC APPROVED ☒ GLP REVIEWED ☐ Date: Nov 26, 2018

ID 002 REV 0 JUNE 2017

APHC IACUC Animal Use Protocol Modification

Study Director/ Principal Investigator: **Thomas Sussan**

PROTOCOL #: **30-18-07-02** Protocol Approval Date: 11 July 2018

PROTOCOL TITLE: **Effects of Acute and Subacute Oral 2,4,6-Trinitro-3-Bromoanisole (TNBA) Exposure to Rats (Rattus norvegicus)**

Phone No.: 436-6590 FAX No.:

Email: thomas.e.sussan2.civ@mail.mil

Modification Request #: 3 Submission Date: 26 November 2018

1. Brief non-technical synopsis of existing protocol or background information pertaining to the modification request.

The existing protocol is testing the oral toxicity of trinitrobromoanisole (TNBA) in rats. One component of the 14-day study is the micronucleus assay (MNA) which examines the ability of TNBA to damage DNA in immature red blood cells. The protocol study design specified using only males for the MNA test, and included 6 additional males that serve as MNA controls. These 6 control animals are treated with a chemical known to damage DNA. The MNA test design requires blood samples from the three highest dose groups that did not have deaths during the study. To date, lethality has been observed in three of the five dose groups which means that the criteria of the MNA test design will not be met. A suitable alternative is to use the female rats for the MNA test (the MNA test design does not specify which sex has to be used, only that all of the same sex be used for the controls as well as the dose groups evaluated). The female rats begin their dosing regime 11/27-29/2018, and the dose range will be lowered to avoid high lethality in the intermediate doses. In turn, this will provide enough samples for the MNA. Six additional female rats are needed to serve as the positive control samples. If ordered today (11/26/2018) these additional rats will arrive 11/28/2018 and will be in house for the duration of the female 14-day test.

2. Type(s) of Modification Requested: (check all that apply)

	Type Of Change	X
ADMINISTRATIVE – AURO/QAU/SEMO REVIEW	Administrative Modifications (*e.g., correct typographical errors/grammar, contact information update, change in study start/completion dates, change in references, protocol title change, change that requires review and approval for GLP compliance*)	
	Addition or deletion of a qualified technician, co-investigator, or study staff	
MINOR Modifications *(examples)*	Change in animal usage [*e.g., vendor, sex, age, weight, strain, small increase in # of animals used (less than 10% of overall number of approved animals)*]	x
	Need to repeat an experiment without the addition of animals	
	Addition/ change of sample collection times	x

	Additional noninvasive sampling	
	Changes in acclimation or recovery period	
	Special housing request or change in husbandry procedures	
	Changes in dosing procedures (e.g., dose, volume, or timing)	
	Other: _____	
MAJOR Modifications *(examples)*	Change in SD/PI	
	Change in objectives of the study	
	Addition of a test article to be evaluated	
	Change that results in greater pain, distress, or degree of invasiveness (changes to pain categories D or E from C require a literature search for alternatives to be completed)	
	Addition of animals to pain Category E (literature search for alternatives required)	
	Addition of or change in Species	
	Addition of more than 10% of the total # of animals originally approved	
	Addition of blood sampling	
	Change in frequency of observations for morbidity	
	Change in study endpoint	
	Changes in housing or use of animals in a location that is not part of the animal program overseen by the IACUC	
	Changes from nonsurvival to survival surgery	
	Change that impacts personnel safety	
	Other change that relates to the specific experimental design and aims of the original protocol	
	Other: _____	

3. PREVIOUSLY APPROVED MODIFICATIONS: (*Hit 'Enter' after description of each modification to list all individually.*)

Mod #	Short Description of the Amendment(s) (Include pain category breakdown of animal usage if changes were made from the original protocol)	No. & Species of Animal Requested	Approval Date
1	Addition of two study personnel.	N/A	7/24/2018
2	Addition of restrainer use for venipuncture; addition of single-housing during acute study phase; addition of animal weighing during acclimation period.	N/A	8/2/2018

4. CURRENT APPROVED ANIMAL USAGE ON PROTOCOL:

Species/Total: 156 **B:** 0 **C:** 15 **D:** 40 **E:** 101

5. FOR ITEMS A-H BELOW: Describe the requested changes to be implemented and the justification(s) for each.

A. Administrative change: Editorial (typographical, grammar, contact info update, etc), GLP compliance changes, or change in personnel (not change of study director/principal investigator):

None

B. Specific changes or additions to the experimental design of the protocol (Section V.1. Experimental Design).

Section V.1. is to be modified as follows (All instances of males being used for the micronucleus assay (MNA) will be changed to females)-
page 7 line 1-2, change "male only" to "female only", change PLUS 6M to PLUS 6F rats,
page 7 last paragraph, change "On study day 14, male rats" to "On study day 14, female rats",
page 8 Table 2. in the MNA control row, change male animal column from 6 to 0 and change female animal column from 0 to 6; pain category is unchanged.

C. Change in sample size evaluation, data analysis plan, archiving of data, (Section V.2.).

None

D. Change in the animals used, (Section V.3). *(Include any changes in amount used, sex, age/weight, vendor/source, refinement, reduction, and replacement [3Rs]).*

The number of animals used will increase by 6 as 6 additional females need to be ordered. The additional females will be cohort matched (i.e. born on the same day) as the original female rats that were ordered (date of birth 10/2/2018).

E. Changes to technical methods (Section V.4.). *(Include changes in USDA pain category classifications [section V.4.1.1.1.1 thru V.4.1.1.1.4.], changes in anesthesia, analgesia, restraint, injections, identification, behavior studies, 'other procedures', study endpoint, euthanasia, etc. Include reference source for basis of dose/treatment change.).*
**** * * * If the modification requested will be adding animals to COLUMN D or E and the protocol did not previously have animals in those categories, a LITERATURE SEARCH FOR ALTERNATIVES TO PAINFUL OR DISTRESSFUL PROCEDURES must be completed and submitted with this modification request.***

V.4.4.3. Biosamples section. Change all instances refering to males used for the MNA to females.
V.4.4.8.3. Micronucleus Assay, change all instances refering to males used for the MNA to females.
V.4.5. Study Endpoint: The remaining 6 male naïve rats that will not be used as controls for the MNA test will be made available for transfer to another approved protocol, coordinated with

the AV. If there are no requests for these animals, they will be euthanized as described in section V.4.6.

F. Changes to the Husbandry and Veterinary Care procedures (Section V.5.). *(Changes in husbandry considerations, special provisions, exceptions to the Guide, AWAR, or IACUC Policy that have an impact on animal care and use; any changes to the veterinary medical care or environmental enrichment.)*

None.

G. Changes to the personnel conducting this protocol (Section VI.). *(Include record of completed training for any animal handling or use procedures assigned to new personnel. Signed assurance page(s) must be included with the modification if changing SD/PI, Primary Co-investigoator, or adding a co-investigator.)*

None.

H. Changes in Biohazard/ Safety (Section VII).

None.

APHC IACUC Protocol Modification Review/Approval

Study Director/ Principal Investigator: Thomas Sussan

Phone No.: 436-6590 *FAX No.:*

Email: thomas.e.sussan2@civ@mail.mil

PROTOCOL #: **30-18-07-02** *Protocol Approval Date:* 11 July 2018

PROTOCOL TITLE: **Effects of Acute and Subacute Oral 2,4,6-Trinitro-3-Bromoanisole (TNBA) Exposure to Rats (Rattus norvegicus)**

Modification Request #: **4** *Submission Date:* Nov 27, 2019

Study Director/ Principal Investigator Signature: SUSSAN.THOMAS.EDWARD.1537772626 Digitally signed by SUSSAN.THOMAS.EDWARD.1537772626 Date: 2019.11.27 12:42:12 -05'00'

For review use only: Review Process: ADMIN ☒ FCR ☐ DMR ☐ VVC ☐

Received Date: Nov 27, 2019 AURO receipt: DAWSON.CHELSEA.LEE.1544052116 Digitally signed by DAWSON.CHELSEA.LEE.1544052116 Date: 2019.11.27 12:58:51 -05'00'

IACUC Designated Member Reviewer(s) (*sign when review is completed*):

Attending/ Alternate Veterinarian Review Signature: Approved via VVC: N/A ☐ YES ☐ NO ☐

Date

Safety Review Signature (*if applicable*):

Date

LTACO/GLP Review Signature (*if applicable*):

Dec 4, 2019 KEFAUVER.MICHAEL.P.1229209678 Digitally signed by KEFAUVER.MICHAEL.P.1229209678 Date: 2019.12.04 10:52:35 -05'00'

Date

IACUC Chair Review Signature:

Dec 2, 2019 NEWKIRK.KRISTIN.TORELL.1014786895 Digitally signed by NEWKIRK.KRISTIN.TORELL.1014786895 Date: 2019.12.02 10:42:49 -05'00'

Date

Modification IACUC APPROVED ☐ GLP REVIEWED ☒ Date: December 04, 2019

ID 002 REV 0 JUNE 2017

Page 1 of 1

APHC IACUC Animal Use Protocol Modification

Study Director/ Principal Investigator: **Thomas Sussan**

PROTOCOL #: **30-18-07-02** Protocol Approval Date: 11 July 2018

PROTOCOL TITLE: **Effects of Acute and Subacute Oral 2,4,6-Trinitro-3-Bromoanisole (TNBA) Exposure to Rats (Rattus norvegicus)**

Phone No.: 410-436-6590 FAX No.:

Email: thomas.e.sussan2.civ@mail.mil

Modification Request #: 4 Submission Date: 27 November 2019

1. Brief non-technical synopsis of existing protocol or background information pertaining to the modification request.

All live animal procedures are completed and the study report is currently being prepared. The current study director/PI Thomas Sussan is leaving APHC and will be replaced by Dr. Michael Quinn. The submission of this modification is for GLP documentation purposes to indicate change of SD/PI as required for the final report. Animal procedure training records are not provided in this modification as the duties being transferred at this stage in the protocol are for data analysis and final report preparation only.

2. Type(s) of Modification Requested: (check all that apply)

	Type Of Change	X
ADMINISTRATIVE – AURO/QAU/SEMO REVIEW	Administrative Modifications (*e.g., correct typographical errors/grammar, contact information update, change in study start/completion dates, change in references, protocol title change, change that requires review and approval for GLP compliance*)	
	Addition or deletion of a qualified technician, co-investigator, or study staff	
MINOR Modifications *(examples)*	Change in animal usage [*e.g., vendor, sex, age, weight, strain, small increase in # of animals used (less than 10% of overall number of approved animals)*]	
	Need to repeat an experiment without the addition of animals	
	Addition/ change of sample collection times	
	Additional noninvasive sampling	
	Changes in acclimation or recovery period	
	Special housing request or change in husbandry procedures	
	Changes in dosing procedures (e.g., dose, volume, or timing)	
	Other: _____	
MAJOR Mo	Change in SD/PI	**X**
	Change in objectives of the study	

	Addition of a test article to be evaluated	
	Change that results in greater pain, distress, or degree of invasiveness (changes to pain categories D or E from C require a literature search for alternatives to be completed)	
	Addition of animals to pain Category E (literature search for alternatives required)	
	Addition of or change in Species	
	Addition of more than 10% of the total # of animals originally approved	
	Addition of blood sampling	
	Change in frequency of observations for morbidity	
	Change in study endpoint	
	Changes in housing or use of animals in a location that is not part of the animal program overseen by the IACUC	
	Changes from nonsurvival to survival surgery	
	Change that impacts personnel safety	
	Other change that relates to the specific experimental design and aims of the original protocol	
	Other: _____	

3. PREVIOUSLY APPROVED MODIFICATIONS: (*Hit 'Enter' after description of each modification to list all individually.*)

Mod #	Short Description of the Amendment(s) (Include pain category breakdown of animal usage if changes were made from the original protocol)	No. & Species of Animal Requested	Approval Date
1	Addition of two study personnel.	N/A	7/24/2018
2	Addition of restrainer use for venipuncture; addition of single-housing during acute study phase; addition of animal weighing during acclimation period.	N/A	8/2/2018
3	Change MNA from males to females. Addition of 6 female rats to serve as controls for the MNA.	6 rats	11/26/2018

4. CURRENT APPROVED ANIMAL USAGE ON PROTOCOL:

Species/Total:
156 **B:** 0 **C:** 15 **D:** 40 **E:** 101

5. FOR ITEMS A-H BELOW: Describe the requested changes to be implemented and the justification(s) for each.

A. Administrative change: Editorial (typographical, grammar, contact info update, etc), GLP compliance changes, or change in personnel (not change of study director/principal investigator):

None

B. Specific changes or additions to the experimental design of the protocol (Section V.1. Experimental Design).

None

C. Change in sample size evaluation, data analysis plan, archiving of data, (Section V.2.).

None

D. Change in the animals used, (Section V.3). *(Include any changes in amount used, sex, age/weight, vendor/source, refinement, reduction, and replacement [3Rs]).*

None

E. Changes to technical methods (Section V.4.). *(Include changes in USDA pain category classifications [section V.4.1.1.1.1 thru V.4.1.1.1.4.], changes in anesthesia, analgesia, restraint, injections, identification, behavior studies, 'other procedures', study endpoint, euthanasia, etc. Include reference source for basis of dose/treatment change.).* **** * * * If the modification requested will be adding animals to COLUMN D or E and the protocol did not previously have animals in those categories, a LITERATURE SEARCH FOR ALTERNATIVES TO PAINFUL OR DISTRESSFUL PROCEDURES must be completed and submitted with this modification request.***

None

F. Changes to the Husbandry and Veterinary Care procedures (Section V.5.). *(Changes in husbandry considerations, special provisions, exceptions to the Guide, AWAR, or IACUC Policy that have an impact on animal care and use; any changes to the veterinary medical care or environmental enrichment.)*

None

G. Changes to the personnel conducting this protocol (Section VI.). *(Include record of completed training for any animal handling or use procedures assigned to new personnel. Signed assurance page(s) must be included with the modification if changing SD/PI, Primary Co-investigoator, or adding a co-investigator.)*

The Study Director / Principal Investigator will change from Thomas Sussan to Michael Quinn. The live animal portion of this study was completed in FY19, and no further handling or use of animals is required. The SD/PI duties for this stage of the protocol are data analysis and final report preparation only.

H. Changes in Biohazard/ Safety (Section VII).

None