Defense Centers for Public Health – Aberdeen
(formerly U.S. Army Public Health Center)

8252 Blackhawk Road, Aberdeen Proving Ground, Maryland 21010-5403

Toxicology Study No. S. 0058222-18, September 2023,
Toxicology Directorate

Effects of Acute and Subacute Oral 3,4-Dinitropyrazole (DNP) Exposure to Rats (*Rattus Norvegicus*), August 2018 – October 2018

Prepared by: Thomas E. Sussan, Ph.D., Health Effects Division

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support of David Kurk of the Laboratory Sciences Directorate, Defense Centers for Public Health–Aberdeen (DCPH-A, formerly the U.S. Army Public Health Center) for his efforts in analyzing the dosing suspensions used for this study. We would also like to thank Alicia Shiflett, Shannon Rodriguez, and Theresa Hanna (retired) from the Toxicology Directorate, DCPH-A, for their efforts in tissue processing.

REPORT DOCUMENTATION PAGE	Form Approved OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
04-09-2023	Technical Report	August - October 2018

4. TITLE AND SUBTITLE

Effects of Acute and Subacute Oral 3,4-Dinitropyrazole (DNP) Exposure to Rats

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Thomas E. Sussan, Allison M. Narizzano, Lee C.B. Crouse, Matthew A. Bazar, Valerie H. Adams, and Michael J. Quinn

5d. PROJECT NUMBER

S.0058222-18

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Defense Centers for Public Health-Aberdeen
Toxicology Directorate (DCPH-ATS-TOX)
8252 Blackhawk Road
Aberdeen Proving Ground, Maryland 21010-5403

8. PERFORMING ORGANIZATION REPORT NUMBER

S.0058222-18

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Office of the Under Secretary of Defense, Research and Engineering
Joint Enhanced Munitions Technology Program (JEMTP/Lawrence Fan)
4103 Fowler Road, Suite 107
Indian Head, Maryland 20640-5106

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The acute oral median lethal dose, 95% confidence intervals, and slope of 3,4-dinitropyrazole (DNP) in female rats were determined to be 444 mg/kg, 364-542 mg/kg, and 9.77, respectively. Subacute administration of DNP in male and female rats did not result in mortality at doses up to 222 mg/kg-d with only minor, sporadic clinical signs. Body mass gain was reduced in the male 222 mg/kg-d group throughout the study. Numerous subtle, sporadic clinical pathology parameters were observed throughout all male and female DNP dose groups along with significant decreases in absolute and normalized masses of adrenals, heart, kidneys, and spleen. Despite the consistent absence of a dose response for nearly every parameter measured and the relatively minor magnitudes of effect, the rats were determined to have developed mild kidney injury and immunological abnormalities at all doses tested resulting in a lowest observed adverse effect level (LOAEL) of 14 mg/kg-d. Benchmark dose analysis was not determined due to the absence of a monotonic dose response relationship.

15. SUBJECT TERMS

oral toxicity, explosives, insensitive munitions, 3,4-dinitropyrazole, DNP, prerenal azotemia

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Thomas E. Sussan
U	U	U	SAR	142	19b. TELEPHONE NUMBER *(Include area code)* 410-436-3980

Sponsor

Office of the Under Secretary of Defense, Research and Engineering, Joint Enhanced Munitions Technology Program (JEMTP/Lawrence Fan), 4103 Fowler Road, Suite 107, Indian Head, Maryland 20640-5106

Study Title

TOXICOLOGY STUDY NO. S.0058222-18
EFFECTS OF ACUTE AND SUBACUTE ORAL 3,4-DINITROPYRAZOLE (DNP) EXPOSURE TO RATS (*RATTUS NORVEGICUS*)

Data Requirement

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

Authors

Thomas E. Sussan, Allison M. Narizzano, Lee C.B. Crouse, Matthew A. Bazar, Valerie H. Adams, and Michael J. Quinn

Study Completion Date

September 2023

Performing Laboratory

Defense Centers for Public Health-Aberdeen
Toxicology Directorate (DCPH-ATS-TOX)
8252 Blackhawk Road
Aberdeen Proving Ground, MD 21010-5403

Laboratory Project ID

Protocol No. 30-18-07-01

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 (GLP) 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.

3. Section V.1.2. of the study protocol states that, for the subacute study, terminal blood samples will be collected for hematology, clinical chemistry, and prothrombin time analysis. Upon review of the study data, the study director at the time of reporting could not locate prothrombin time data. The clinical pathologist had noted in his study records that the sodium citrate tubes needed for prothrombin time analysis were backordered at the time of necropsy, so the analysis could not be performed. Prior to subacute necropsies, no protocol modifications were found documenting that prothrombin time samples would not be collected. Prothrombin time samples were collected for the subsequent subchronic study under a separate study protocol using the same test material. The lack of prothrombin time samples for the subacute study did not affect the quality or integrity of the additional clinical pathology data collected.

SUSSAN.THOMAS.EDWARD.JR.1537772626 Digitally signed by SUSSAN.THOMAS.EDWARD.JR.1537772626 Date: 2023.08.31 13:22:30 -04'00'

THOMAS E. SUSSAN
Biologist
Health Effects Division

August 31, 2023
Date

EXECUTIVE SUMMARY
TOXICOLOGY STUDY NO. S.0058222-18
EFFECTS OF ACUTE AND SUBACUTE ORAL 3,4-DINITROPYRAZOLE (DNP) EXPOSURE TO RATS (*RATTUS NORVEGICUS*)
AUGUST 2018 – OCTOBER 2018

1 PURPOSE

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

2 CONCLUSIONS

The acute oral toxicity of DNP was evaluated in female Sprague-Dawley rats using the Stagewise Adaptive Dose Method. The estimated LD_{50} and 95% confidence intervals, as determined via probit regression using SAS software, were 444 (364–542) mg/kg. The slope of the dose response (predicted probability dead versus log dose (mg/kg)) was 9.77. Gross pathological observations of those rats that died after DNP administration frequently included dark swollen/enlarged livers and spleens as well as white discoloration of the mucosal surface of the stomach with signs of erosion and ulceration and sloughing of the gastric epithelium.

In the subacute study, male and female rats were dosed daily via oral gavage with 0, 14, 28, 56, 111, or 222 mg/kg per day (-d) DNP in corn oil for 2 weeks. No compound-related mortality was observed, and no statistically significant differences in body weight were observed at any time point between individual dose groups versus controls. However, body mass gain over the duration of the study was significantly decreased among males dosed at 222 mg/kg-d DNP versus controls. Food consumption of the paired rats of either sex was not significantly altered between dose groups at any time point measured. Clinical signs noted throughout dosing in either sex were intermittent, minor in severity, and not dose-dependent.

Gross pathological findings that were attributed to DNP dosing included pink/purple discoloration of the thymus (more common in males) and statistically significant decreases in absolute and normalized masses of adrenals, heart, kidneys, and spleen (both sexes). None of these effects showed an apparent dose response, as effect levels were similar across all dose levels. Additionally, yellow staining of the fur around the perineum was noted among rats dosed at 222 mg/kg-d (more common in females) and in one rat dosed at 111 mg/kg-d.

Rats dosed with DNP showed statistically significant changes in numerous hematological and clinical chemistry parameters although these effects tended to be subtle, sporadic, and not dose dependent. The most consistent hematological effects in females were neutropenia (all doses) and mild reductions in red blood cells ($p<0.05$: 28, 56, 222 mg/kg-d), hemoglobin ($p<0.05$: 28, 56, 111, 222 mg/kg-d), and hematocrit ($p<0.05$: 14, 28, 56,

222 mg/kg-d), which did not follow a dose response. The most consistent hematological effects in males were slight reductions in lymphocytes ($p<0.05$: 28, 56, 111 mg/kg-d) and eosinophils ($p<0.05$: 14, 28, 56 mg/kg-d). Statistically significant changes in clinical measurements included mild increases in markers of kidney injury (blood urea nitrogen (BUN), urea, and creatinine) and protein levels (total protein, albumin, and globulin) among both sexes and at multiple doses, which may be attributed to prerenal azotemia, likely due to hemoconcentration/dehydration. Liver enzymes were slightly altered, but not in a consistent manner. Significant decreases in triglycerides were noted among both sexes at nearly all doses, as well as significant increases in total cholesterol (males: ≥111 mg/kg-d; females: 222 mg/kg-d). Additionally, multiple electrolytes showed mild but statistically significant changes. DNP was not genotoxic, based on the *in vivo* rat micronucleus assay.

Despite the consistent absence of a dose response for nearly every parameter measured, and the relatively minor magnitudes of effect, the rats were determined to have developed mild kidney injury and immunological abnormalities at all doses tested. Thus, the lowest observed adverse effect level (LOAEL) for this study is 14 mg/kg-d. Benchmark Dose (BMD) analysis was not determined due to the absence of a monotonic dose response relationship.

Table of Contents

TABLES

TOXICOLOGY STUDY NO. S.0058222-18
EFFECTS OF ACUTE AND SUBACUTE ORAL 3,4-DINITROPYRAZOLE (DNP) EXPOSURE TO RATS (*RATTUS NORVEGICUS*)
AUGUST 2018 – OCTOBER 2018

1. PURPOSE

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

2. REFERENCES

See Appendix A for a listing of references, all of which were current at the time of the study.

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 the following:

- 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,* 2003 (reference 3).
- Department of Defense Directive (DoDD) 4715.1E, *Environment, Safety, and Occupational Health (ESOH)*, 2005, Change 1, 2018 (reference 4).
- Army Environmental Requirements and Technology Assessments (AERTA). Requirement PP-3-02-05, *Compliant Ordnance Lifecycle for Readiness of the Transformation and Objective Forces*, 2012 (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, Maryland 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 had already occurred. However, current 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.

DNP has progressed through the GrIMEx program as a potential replacement for TNT based on the improved performance and sensitivity of DNP compared to Composition B. Toxicity testing of DNP consists of a phased approach that is initially comprised of *in silico* (quantitative-structure activity relationship (QSAR)), *in chemico*, and *in vitro* analyses, followed by *in vivo* testing of lead candidates. The *in silico*, *in chemico*, and *in vitro* data for DNP include a predicted oral LD_{50} of 728.2 milligrams per kilogram (mg/kg) based on TOPKAT structure-toxicity computer modeling and an estimated LD_{50} of 800–900 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) (references 6 and 7). Additionally, DNP showed mutagenic responses in the Ames assay and was predicted to be a skin sensitizer based on both *in silico* and *in chemico* assays (references 8, 9, and 10). Furthermore, DNP exhibited mild toxicity to aquatic organisms in the microtox acute toxicity assay (Median Effective Concentration (EC_{50}) = 13.55 milligrams per liter (mg/L)) (reference 11).

The current report describes the acute and subacute oral toxicity of rats exposed to 1 of 5 doses of DNP. Research, development, testing, and training with explosives and pyrotechnics potentially less hazardous to human health and the environment are 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 12).

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, 22 August 2018
Acute Study Necropsies	7–29 August 2018
14-Day Study Animal Arrival	5 September 2018
14-Day Study Dosing Initiation	18 September 2018
14-Day Study Scheduled Necropsies	2–4, 9–11 October 2018
Live Animal Experimental Completion	11 October 2018
Study Completion	September 2023

5. MATERIALS

5.1 Test Substance

DNP (CASRN 38858-92-3) is a yellow, powdered solid with no detectable odor. The chemical formula is $C_3H_2N_4O_4$ and the molecular weight is 158.073 grams per mole (g/mol). DNP was synthesized by BAE Systems at Holston Army Ammunition Plant, Kingsport, Tennessee, and identified as lot number BAE-1150-003-2. The compound purity was determined by the manufacturer and reported as 98.5%. Due to the storage/shipping requirements for energetic materials, the DNP may have been wetted with up to 15% water by weight, which was not indicated on the Safety Data Sheet. For safety reasons, the DNP for these studies was used on an "as received" basis. For the acute study, fresh dosing suspensions were prepared the day before use for each round of dosing. For the subacute study, a separate dosing suspension was prepared for each dose group at targeted concentrations of 1.4, 2.8, 5.6, 11.1, and 22.2 milligrams per milliliter (mg/ml). Dosing suspensions were prepared in volumes sufficient for approximately 2 weeks of dosing, resulting in preparation of two sets of dosing solutions. All concentrations/batches of dosing suspensions were sampled and analyzed by the Defense Centers for Public Health–Aberdeen Laboratory Sciences Directorate (LS) 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 (22.2 mg/ml) suspension. Prior to initiation of subacute dosing, samples were collected from two DNP suspensions (10 and 50 mg/ml) at weekly intervals for a 4- and 5-week period, respectively, to determine the stability of DNP 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 were monitored continuously throughout the study. Table 2 summarizes the mean temperature and humidity during both phases of the study, as well as any readings detected out of range. The deviations from target conditions were brief in duration and were 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/necropsy 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 AAALAC International.

Table 2. Animal Room Environmental Conditions

Acute Study	**8/1/2018–8/29/2018**	**Mean Temp. 69.93 ºF**	**Mean R.H. 51.68%**
Out of Range	**Date**	**Temperature ºF**	**Relative Humidity %**
	8/21/2018		99.94
	8/27/2018	66.54	73.40
Subacute Study	**9/5/2018–10/11/2018**	**Mean Temp. 71.48 ºF**	**Mean RH 49.73%**
Out of Range	**Date**	**Temperature ºF**	**Relative Humidity %**
	10/1/2018		82.14
Note: Out-of-range periods were <1 hour in duration. Out-of-range readings listed above are the highest humidity/lowest temperature detected during that event.			

Legend:
ºF = 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 who contributed to the performance of this study.

6. METHODS

6.1 Acute Study

Acute oral toxicity of DNP was assessed via the Stagewise Adaptive Dose Method (SADM) in female rats, as described by ASTM International Guideline E1163-10 (reference 13). 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, the use of only females reduces 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 continues until the variation around

the LD_{50} is 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 is also used in decision-making regarding doses for subsequent stages.

For the current acute study, a total of 20 female rats were dosed over 3 stages, with each stage separated by 7 days. The rats were approximately 8–10 weeks of age at the time of dosing and weighed 208.3 ± 27.6 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 7 days during which clinical observations and body mass were measured daily. Animals that were found dead or 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 5 rats, each dosed with a different dose (200, 356, 632, 1,125, 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 each subsequent stage, and concentrations were verified analytically. Stage 2 consisted of 2 rats per group at dose levels of 356, 474, and 632 mg/kg at dose volumes of 6.3–9.5 ml/kg. Stage 3 consisted of 3 rats per group at doses of 200, 474, and 632 mg/kg at dose volumes of 7.5–8.0 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 DNP. Sixty male and 60 female Sprague Dawley rats, approximately 8 weeks old, were used for the subacute portion of the study. Additionally, six male rats were used as positive controls for the micronucleus assay. Rats were pair-housed throughout the subacute study except in the event of the premature death of a rat's cage-mate. Both rats within each cage were assigned to the same dose group. Male rats weighed 318.3 ± 21.7 grams at initiation of dosing while female rats weighed 175.3 ± 11.2 grams. Upon their arrival, animals were acclimated in the facility for a period of 13 days 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 DNP 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 based on the results of the acute study, with the highest dose corresponding to 50% of the LD_{50}. Doses were set at 222, 111, 56, 28, and 14 mg/kg-d (i.e., 50%, 25%, 12.6%, 6.3%, and 3.2% of the LD_{50}). To accommodate necropsy schedules (maximum of 20 necropsies per day), dosing start dates were staggered over 6 days (3 days per sex) in a manner that included 2–4 rats per dose group per day.

All DNP and control doses were administered based on the most recent body mass obtained at a volume of 10 mL/kg. 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 during 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 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 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), and central nervous system effects (e.g., tremors and convulsions); changes in gait, posture, and the level of activity; reactivity to handling or sensory stimuli; altered strength; and stereotypes or changes in behavior (e.g., self-mutilation).

6.2.3 Necropsy

Following the appropriate treatment period, all surviving rats were anesthetized with CO_2, blood was collected via intracardiac puncture, and rats were euthanized 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 were 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 termination of the study, blood was obtained via intracardiac puncture in animals 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). 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

On study day 14, male rats from the three highest dose groups with no compound-related mortality (222, 111, and 56 mg/kg-d) and the vehicle control group were biosampled from the tail vein for the micronucleus assay (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 male 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 6 MNA control rats via the tail vein and processed as recommended by the manufacturer. Starting at 4–6 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 group (222 mg/kg-d) and vehicle control were processed and analyzed. 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 MetaSys® Building Management System. Data from the SADM were analyzed according to the methods of Feder et al. to determine the LD_{50}, 95% confidence interval, and slope of the dose response curve (references 14 and 15). LD_{50} probit analysis was conducted using SAS® (SAS Institute, Inc.).

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 were 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 were rank-transformed, significance of normally distributed data 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 were 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-fasted 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 DNP-treated animals were 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, DNP 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 samples were repeatedly measured approximately weekly for up to 5 weeks. Results of the stability study indicated that the DNP concentration in corn oil remained within acceptable ranges. Weekly recovery percentages ranged from 90–104% throughout the sampling period (Table 4).

One week prior to initiation of subacute dosing, DNP samples were prepared, and concentrations were verified analytically. Homogeneity testing of the most concentrated DNP/corn oil suspension (22.2 mg/mL) yielded 90% recovery at the top, 95% at the middle, and 90% at the bottom of the container (Table 4). Verification of the dosing solution/suspension concentrations prior to use yielded recovery percentages ranging from 88–100% of the nominal concentrations for both batches mixed (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 (08/07/2018)	
50	52 (104%)
100	80 (80%)
200	210 (105%)
Stage 2 (08/14/2018)	
10	9.6 (96%)
50	48 (95%)
100	97 (97%)
Stage 3 (08/22/2018)	
25	25 (100%)
63.2	62 (98%)

Legend:
mg/mL = milligrams per milliliter

Table 4. Stability and Homogeneity Results

Nominal Concentration (mg/mL)	Analytical Concentration (mg/ml)
50 (day 0 stability) 08/07/2018	52 (104%)
50 (day 7 stability) 08/14/2018	48 (96%)
50 (day 15 stability) 08/22/2018	48 (96%)
50 (day 21 stability) 08/28/2018	45 (90%)
50 (day 29 stability) 09/05/2018	49 (98%)
50 (day 35 stability) 09/11/2018	48 (96%)
10 (day 0 stability) 08/14/2018	9.6 (96%)
10 (day 8 stability) 08/22/2018	9.3 (93%)
10 (day 14 stability) 08/28/2018	9.4 (94%)
10 (day 22 stability) 09/05/2018	9.5 (95%)
10 (day 28 stability) 09/11/2018	9.1 (91%)
22.2 (homogeneity top)	20 (90%)
22.2 (homogeneity middle)	21 (95%)
22.2 (homogeneity bottom)	20 (90%)

Legend:
mg/mL = milligrams per milliliter

Table 5. Dosing Solution/Suspension Concentration Results

Nominal Concentration (mg/mL)	Analytical Concentration (mg/mL)	
	Batch 1 09/11/2018	Batch 2 10/02/2018
1.4	1.4 (100%)	1.4 (100%)
2.8	2.5 (89%)	2.6 (93%)
5.6	5.2 (94%)	5.5 (98%)
11.1	9.8 (88%)	11 (99%)
22.2	21 (95%)	21 (95%)

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 DNP/corn oil suspension (200, 356, 632, 1,125, and 2,000 mg/kg) and were monitored for up to 7 days. Mortality was observed at the three highest doses. In the second stage, two rats per dose group received either 356, 474, or 632 mg/kg DNP; mortality was observed in both rats receiving 632 mg/kg and one out of two rats receiving either 356 or 474 mg/kg. For the third stage, three rats per dose group received either 200, 474, or 632 mg/kg DNP; mortality was observed in one of three rats receiving 474 mg/kg and three of three rats receiving 632 mg/kg. Based on the combined results of all stages (Table 6), a probit analysis was used to estimate the LD_{50} and 95% confidence intervals at 444 (364–542) mg/kg. The slope of the dose response (predicted probability dead versus log dose) was 9.78. Clinical signs observed during the period after dosing included squinting, lethargy, labored breathing, pale appearance, wobbly gait, hunched posture, and the animal lying prostrate or on its side. In some cases, these clinical signs resolved within hours of initial appearance; however, in other cases, these signs progressed to death. Weight loss was observed at 24 hours after dosing in one of four rats dosed at 200 mg/kg, two of three of rats dosed at 356 mg/kg, three of three rats dosed at 474 mg/kg, and four of four rats dosed at 632 mg/kg. At 48 hours after dosing, surviving rats generally exhibited weight gain compared to the previous day; this continued throughout the remainder of the observation period. Gross pathological observations of those rats that died due to DNP administration frequently included dark swollen/enlarged livers and spleens as well as white discoloration of the mucosal surface of the stomach with signs of erosion and ulceration and sloughing of the gastric epithelium. Rats that survived until scheduled necropsy at day 7 showed no obvious gross lesions. See Appendix D for details of clinical signs, body weight, mortality, pathological findings, and probit results.

Table 6. Combined Mortality Results

Dose (mg/kg)	Total Number Dosed	Number Dead	Time to Death (m=minutes, h=hours)
200	4	0	
356	3	1	40h
474	5	2	24m, 1.25h
632	6	6	30m, 45m, 26h, 28h, 28h, 40h
1,125	1	1	30m
2,000	1	1	15m

Legend:
mg/kg = milligrams per kilogram

7.3 Subacute Study

7.3.1 Clinical Observations and Mortality

One of the animals in the subacute study died prior to scheduled study termination; however, this death was not considered to be compound-related. Just prior to initiation of dosing (day 0), this rat was observed to have ruffled fur, and it was noted that this rat lost 27.5 grams in body weight between day -3 and day 0. These clinical signs persisted on day 1, with the addition of hunched posture. Prior to dosing on the morning of day 2, this rat was found dead. A gross necropsy identified a 2-cm urinary tract neoplasm, enlarged kidneys, and dilated pelvises (hydronephrosis). The hydronephrosis and enlarged kidneys were most likely the result of ureter obstruction by the neoplasm. This rat was excluded from all subsequent analyses, and it was assumed that this rat did not consume any feed between day 0 and its death. All other rats survived to scheduled necropsy on day 14.

Other signs noted throughout dosing in either sex were intermittent, minor in severity, and not dose-dependent. One male rat in the 222 mg/kg-d dose group displayed congested breathing on day 11, but this was not noted at any other time. Two male rats in the 14 mg/kg-d group displayed minor eye injuries, although one of these injuries was first noted prior to initiation of dosing. A female rat in the 28 mg/kg-d dose group displayed alopecia on its ventral surface on day 4. Occasionally, rats of both sexes and across multiple dose groups displayed temporary signs of gasping (likely aspirated small amounts of test material) and/or exhibited minor bleeding from the mouth (likely bit tongue) immediately after dosing. In all cases, these signs resolved within 30 minutes of dosing. Appendix E provides a summary of clinical observations.

7.3.2 Body Mass and Food Consumption

Body mass did not differ between treated and control groups for female rats when measured at time points throughout the study (i.e., days 0, 3, 7, 13, and 14). Additionally, change in body mass over time (i.e., from days 0–3, 3–7, 7–13, and 0–13) did not differ among dose groups for female rats. Male rats showed no statistically significant difference among dose groups with respect to absolute body mass at any time point; however, statistically significant decreases in

body mass change relative to controls were noted for days 0–3 (56, 111, and 222 mg/kg-d), days 7–13 (28, 111, and 222 mg/kg-d), and days 0–13 (222 mg/kg-d), based on repeated measures ANOVA and Dunnett t (2-sided) post hoc tests. Male rats receiving 222 mg/kg-d DNP gained 36.9% less weight than controls over the duration of dosing (49.0 grams versus 77.6 grams). See Appendices F and G for details of body mass and body mass change.

Food consumption of the paired rats of either sex was not significantly altered among dose groups at any time point measured. Food efficiency, which is defined as paired weight gain divided by paired food consumption, did not differ among females at any dose or time point measured. However, significant effects of DNP on food efficiency were observed among males, with multiple dose groups showing statistically significant decreases in food efficiency from days 0–3 (111 and 222 mg/kg-d) and from days 7–13 (28 and 222 mg/kg-d) compared to controls. Interestingly, multiple dose groups showed statistically significant increases in food efficiency from days 3–7 (14, 56, 111, and 222 mg/kg-d), which may have been a compensatory response to the initial decreases observed from days 0–3. Only the 222 mg/kg-d dose group differed significantly from controls over the entire duration of dosing (days 0–13). Thus, changes in weight may be attributed to food efficiency rather than food consumption. See Appendices H and I for details of food consumption and food efficiency.

7.3.3 Organ Mass and Ratios

Organs were weighed during necropsy and were reported as absolute organ mass and as ratios of organ mass to either fasted body mass or brain mass. Similar patterns were observed across both sexes, with statistically significant decreases in absolute and normalized organ masses observed for adrenals, heart, kidneys, and spleen. None of these organs showed an apparent dose response, as effect levels were similar across all dose levels, and statistical significance was observed for nearly all doses regardless of how organs were normalized. Additionally, liver mass was unaffected by DNP administration in males; however, females showed marginal effects, with a significant decrease in absolute liver mass at 28 mg/kg-d and significant increases in absolute liver mass and liver to body mass ratio at 222 mg/kg-d. Males and females showed no effect of DNP administration on the brain or thymus. Additionally, sex-specific organs (i.e., testes, epididymides, ovaries, and uterus), were unaffected by DNP administration. See Appendix J for details of organ mass and organ mass ratios.

7.3.4 Hematology

Rats dosed with DNP showed reductions in numerous blood parameters although these effects tended to be subtle, sporadic, and not dose dependent. In both sexes, white blood cells (WBCs) were reduced at multiple doses, with females showing statistically significant reductions at 14 and 28 mg/kg-d and males showing reductions at 28, 56, and 111 mg/kg-d. In females, this reduction in WBCs was associated with statistically significant decreases in neutrophils, monocytes, eosinophils, and basophils. In males, this reduction was associated with significantly reduced neutrophils, lymphocytes, and eosinophils. Additionally, female rats exhibited statistically significant reductions in red blood cells (RBCs) (28, 56, and 222 mg/kg-d), hemoglobin (28, 56, 111, and 222 mg/kg-d), hematocrit (28, 56, and 222 mg/kg-d), and platelets (28 and 56 mg/kg-d). Males displayed statistically significant effects in multiple RBC indices,

including reduced mean corpuscular volume (111 mg/kg-d) and mean corpuscular hemoglobin (111 and 222 mg/kg-d), and increased red cell distribution width (111 and 222 mg/kg-d). Males also displayed statistically significant increases in mean platelet volume after exposure to lower doses of DNP (14 and 28 mg/kg-d). See Appendix K for details of hematological parameters.

7.3.5 Clinical Chemistry

Potential markers of kidney injury, including blood urea nitrogen (BUN), urea, and creatinine, were significantly elevated in serum at multiple doses in both sexes; however, blood glucose was unaffected. The liver enzyme alanine aminotransferase (ALT) was significantly elevated at multiple doses in both sexes; serum levels in the liver enzymes aspartate aminotransferase (AST) and alkaline phosphatase (ALKP) showed sporadic reductions in females and males, respectively. Total cholesterol was significantly increased in males (111 and 222 mg/kg-d) and females (222 mg/kg-d); triglyceride levels were significantly decreased in males (all doses) and females (14, 28, 56, 111 mg/kg-d). Protein levels, including total protein, albumin, and globulin, were significantly increased in both sexes at nearly all doses; the ratio of albumin/globulin was significantly decreased in both sexes. Numerous electrolytes were significantly altered, with a small but statistically significant increase in sodium and decreases in potassium (females only) and phosphate (both sexes). Calcium and chloride were unchanged. See Appendix L for details of clinical chemistry.

7.3.6 Pathology

Bright yellow urine staining of the fur around the vulva was commonly noted in rats receiving 222 mg/kg-d DNP (8 of 10 females; 1 of 9 males) as well as in 1 of 10 females receiving 111 mg/kg-d. The most common pathological sign observed was purple and/or pink discoloration of the thymus, which was noted at 14 mg/kg-d (4 males; 1 female), 28 mg/kg-d (4 males; 1 female), 56 mg/kg-d (4 males), 111 mg/kg-d (3 males), and 222 mg/kg-d (3 males). Discoloration of the thymus was not noted in any of the control animals. Diffuse lobular yellow discoloration of the liver was occasionally observed in male rats and was evenly distributed across dose groups and vehicle controls (1 at 0 mg/kg-d; 2 at 14 mg/kg-d; 2 at 56 mg/kg-d; 1 at 56 mg/kg-d; 1 at 111 mg/kg-d; 0 at 222 mg/kg-d).

Additional findings that were noted in individual animals were sporadically distributed across dose groups and sexes. These findings included kidney cysts and distended urinary bladder (1 male at 28 mg/kg-d), multifocal discoloration of lungs (female at 0 mg/kg-d), enlarged testis (1 male at 28 mg/kg-d), small testis (1 male at 56 mg/kg-d), yellow-green material adherent to stomach mucosa (1 female at 111 mg/kg-d), white fibrous material attached to stomach mucosa with minimal redness (1 female at 111 mg/kg-d), proliferative nodule on liver (1 male at 222 mg/kg-d), and one male rat containing masses attached to vas deferens and bladder wall, with enlarged kidneys, hydronephrosis and discoloration of the right ventricle (222 mg/kg dose group-d; found dead on day 2 of study). See Appendix M for details of gross pathology.

7.3.7 Histopathology

Treatment-related changes were primarily observed in the stomach of male and female rats. The stomach of each high-dose male and female (222 mg/kg-d) had microscopic findings consisting of squamous epithelial hyperplasia and hyperkeratosis in the non-glandular mucosa, accompanied by submucosal edema, congestion, and neutrophilic and lymphocytic infiltrates. Only the stomach from the highest dose group (222 mg/kg-d group) was microscopically evaluated due to the expected evaluation of the mid-dose groups in the follow-on subchronic (90-day) study. The microscopic changes observed in the stomach were attributed to exposure to the test article and were observed in all of the rats exposed to the highest dose (222 mg/kg-d group, p=0.000).

The incidence of thymic perifollicular congestion and hemorrhage was slightly increased in DNP-exposed rats (2 females at 0 mg/kg-d; 3 males at 222 mg/kg-d; and 4 females at 222 mg/kg-d), but this finding was not statistically significant. This thymic microscopic finding is likely correlated to the gross finding of purple thymic discoloration. Thymic congestion was variably accompanied by increased lymphocyte apoptosis, which is a physiologically normal occurrence that may be increased by stress and is unlikely to be an adverse finding.

In the liver of male and female rats, various abnormalities including lymphocytic infiltrates, hepatocellular necrosis, and vacuolar degeneration distributed in a periportal to centrilobular pattern were observed. These abnormalities were observed equally in controls and dosed animals, and there was no clear evidence of necrosis or hemorrhage. There were minimal to no changes observed in the spleen, bone marrow, and adrenal gland. The kidney had no significant changes in any animals examined with the exception of the kidney of one male rat (18-684) in the 222 mg/kg-d group. This was the rat that died shortly after the onset of dosing and displayed severe kidney pathology consisting of end-stage cortical atrophy and necrosis with hydronephrosis. It also had a mass in the urinary bladder; the renal changes were due to obstruction of urine from the mass and were unrelated to dosing. The histologic changes suggestive of DNP-induced toxicity were equally observed in male and female rats. See Appendix N for the full histopathology report.

7.3.8 Genotoxicity

To determine chromosomal damage, the ratio of micronucleated reticulocytes (MN-RET; immature RBCs) and micronucleated normochromatic erythrocytes (MN-NCE; mature RBCs) were quantified in peripheral blood from male rats in the three highest DNP dose groups and compared to 0 mg/kg-d DNP controls. Additionally, the percent reticulocytes (%RET) among total RBCs was calculated to provide an indication of bone marrow toxicity. Blood from rats collected prior to and after treatment with the genotoxic agent EMS served as additional controls for this assay. The EMS controls showed an increase in MN-RET but not in MN-NCE, indicating acute genotoxicity rather than chronic genotoxicity. Additionally, EMS caused a decrease in %RET in the control rats, indicating bone marrow toxicity.

Treatment with DNP did not cause a statistically significant increase in either MN-RET or MN-NCE, indicating a lack of genotoxicity. DNP treatment caused statistically significant decreases

in %RET at 56 and 111 mg/kg-d, suggesting toxicity to bone marrow; however, this decrease did not follow a dose-dependent pattern and was not significantly decreased in the 222 mg/kg-d dose group. See Appendix O for details of the rat micronucleus assay.

7.4 Determination of Benchmark Dose

Numerous potentially adverse effects were identified at multiple dose levels, including the lowest dose (14 mg/kg-d). The most consistent critical effect was mild kidney injury, as indicated by decreased kidney mass and increased serum levels of BUN, urea, creatinine, total protein, albumin, and globulin. A secondary critical effect was immunological impairment, based on decreased numbers of white blood cells, decreased spleen mass, discoloration of the thymus, and anemia (only females showed anemia). For both critical effects, the severity was considered mild and did not worsen with increasing doses.

None of the critical effects displayed a dose response, with comparable effect sizes exhibited across all doses. According to the U.S. Environmental Protection Agency's (EPA) guidance document on benchmark dose (BMD) modeling, the data should show a graded monotonic response with dose, and the minimum dataset for calculating a BMD should show a biologically or statistically significant dose-related trend in the selected endpoint (reference 16). Since the selected endpoints do not fit the criteria for BMD modeling, no BMD was determined. Instead, the lowest adverse effect level (LOAEL) was established at 14 mg/kg-d for renal and immunological effects.

8. DISCUSSION

DNP, a nitrated derivative of pyrazole, 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 DNP in rats. The current study derived an oral LD_{50} for DNP in female Sprague-Dawley rats at 444 mg/kg using the SADM approach. This LD_{50} is slightly lower than the predicted LD_{50} values derived from QSAR modeling (728.2 mg/kg) and the HCLAT *in vitro* assay (800–900 mg/kg) conducted previously at the Defense Centers for Public Health–Aberdeen (references 6 and 7). Additionally, a study sponsored by the Army Research Development Engineering Center (ARDEC Picatinny Arsenal, New Jersey) showed that acute oral dosing of mice with DNP dissolved in DMSO (N=3 per sex per dose) produced 100% mortality at 1,000 and 2,000 mg/kg, partial mortality at 500 mg/kg, and no mortality at 10, 50, and 100 mg/kg (reference 17). Thus, the current acute data are consistent with previous findings. The oral LD_{50} of pyrazole, the chemical backbone of DNP, was previously estimated at 1,010 mg/kg in the rat and 409 mg/kg in the mouse; the oral LD_{50} of TNT was estimated at 607 mg/kg in rats and 660 mg/kg in mice (reference 18). Thus, the acute toxicity of DNP established in the current study is similar to or slightly more toxic than that of TNT.

Based on the results of acute testing, subacute testing was conducted using 5 doses of DNP (0, 14, 28, 56, 111, 222 mg/kg-d), with the highest dose equal to 50% of the LD_{50}. Daily gavage dosing of DNP in corn oil for 14 days resulted in no compound-related mortality although one rat died during the study due to a large neoplasm in its urinary tract. Clinical signs were generally minor and sporadic, and the only statistically significant impact on body weight over the entire

duration of dosing was for body mass gain (but not absolute body mass) among males dosed at 222 mg/kg-d DNP. Other male dose groups showed transient effects on mass gain, but these effects were often compensated for in other time windows and were not significant over the duration of the study. Additionally, bright yellow perineal staining, which was presumably caused by a urinary metabolite of DNP, was observed on the fur of numerous (primarily female) rats dosed at 222 mg/kg. Thus, minimal DNP-induced clinical signs of toxicity were noted throughout the duration of dosing.

Gross pathological, hematological, and chemical findings tended to be minor in severity, with many of these effects observed at multiple or all doses without an apparent dose-response effect. The most consistent effect of DNP dosing was mild kidney injury, as indicated by a statistically significant 15% decrease in kidney mass, coupled with approximately 70% increases in urea and BUN and an approximate 45% increase in creatinine. These effects, which showed similar magnitudes of effect across sex and dose, are consistent with prerenal azotemia. Prerenal azotemia is a common form of kidney failure where nitrogen waste products accumulate in the blood, which could have multiple underlying etiologies such as dehydration or reduced blood flow or volume. The increase in total protein, albumin, and globulin observed among rats dosed with DNP further support hemoconcentration/dehydration as a mediating factor. Additionally, the modest effect sizes may further support dehydration rather than a pathological condition. Water consumption was not monitored during this study.

Rats dosed with DNP also showed hematological changes that may be indicative of an immunological abnormality. The most consistent hematological effects were statistically significant decreases in white blood cells in both sexes (primarily due to neutrophils and monocytes in females; primarily due to lymphocytes and eosinophils in males) and subtle changes consistent with mild anemia in females. These hematological changes may be further supported by an approximate 23% decrease in spleen mass as well as notations of pink/purple discoloration of the thymus (primarily in males) frequently observed at necropsy among rats dosed with DNP but not among controls.

Other changes in organ masses included an approximate 30% decrease in adrenals and an approximate 18% decrease in heart mass. The mild changes in markers of kidney function, the hematological profiles, and the decreases in multiple organ masses may be considered adverse despite the consistent absence of a dose response for nearly every parameter measured, and the relatively minor magnitudes of effect. Thus, the lowest observed adverse effect level (LOAEL) for this subacute study is 14 mg/kg-d. Studies that include greater durations of dosing are needed to determine whether these effects of DNP are indeed adverse rather than temporary or adaptive responses.

DNP did not cause an increase in chromosomal damage to peripheral blood erythrocytes at doses of 56, 111, and 222 mg/kg-d, based on the MNA. This finding is consistent with a study sponsored by ARDEC in which mice receiving single oral gavage doses of either 125, 250, or 500 mg/kg DNP showed no statistically significant increases in the number of micronucleated polychromatic erythrocytes (reference 17). Both the DCPH-A and ARDEC previously demonstrated in *in vitro* studies that DNP was strongly mutagenic with and without metabolic activation using the Ames assay in tester strains of *S. typhimurium* and *E. coli* (references 19

and 20). Additionally, an ARDEC report demonstrated positive effects of DNP in the chromosomal aberration assay in Chinese Hamster Ovary (CHO) cells at 1,000 micrograms per milliliter (µg/mL) both with and without metabolic activation (reference 21). Thus, despite clear genotoxic effects demonstrated using *in vitro* assays, DNP does not increase micronucleated erythrocytes in rodents. Some regulatory guidelines suggest that two *in vivo* assays are required to follow up on a positive result identified in an *in vitro* assay. Thus, additional indicators of *in vivo* genotoxicity, such as chromosome aberrations, sister chromatid exchanges, nuclear buds, and nucleoplasmic bridges, are still needed in both peripheral cells (e.g., blood and sperm) and target organs to further understand the genotoxic potential of DNP.

Pyrazole, the parent compound of DNP, has exhibited toxicity in rats, mice, and dogs, including centrilobular necrosis of the liver, spleen shrinkage, and atrophic changes in the testes, prostate, and seminal vesicles (reference 22). Pathological effects have also been observed in the thyroid at high doses in the rat, but not in other species, and adrenal necrosis has also been reported (references 22 and 23). Pyrazole was proposed as a potential anti-cancer drug based on preclinical modes; however, in Phase I clinical trials, participants receiving intravenous pyrazole exhibited severe anemia, nausea, vomiting, and marked renal toxicity at 22–24 mg/kg-d for up to 5 days (references 22 and 24). Several of these same tissues were altered in the rats dosed with DNP, including effects to the spleen, adrenals, and kidneys; however, prolonged dosing is required to further understand these potential effects.

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 25). Additionally, the 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 26 and 27). The Occupational Safety and Health Administration (OSHA) has established a maximum allowable occupational exposure of 1.5 mg/m^3 for a 40-hour workweek, and the EPA established an oral reference dose of $5x10^{-4}$ mg/kg-d based on liver effects observed in dogs (LOAEL 0.5 mg/kg-d) (references 26 and 28). Subchronic or chronic dosing with DNP is required to determine how the toxicity of DNP compares to that of TNT.

9. CONCLUSIONS

The acute oral toxicity of DNP was evaluated using the SADM approach in female Sprague-Dawley rats. The LD_{50} was 444 (364-542) mg/kg, with mortality associated with gastric toxicity and enlargement of livers and spleens.

In the 14-day subacute study, no compound-related mortality was observed in either male or female rats after daily oral doses up to 222 mg/kg-d. Clinical signs noted throughout dosing were intermittent, minor in severity, and not dose-dependent. Intermittent effects on body weight gain, but not absolute body weight, were observed among multiple dose groups of males. Males dosed at 222 mg/kg were the only dose group to exhibit a statistically significant decrease in body weight gain over the entire duration of dosing (days 0–13).

Numerous potentially adverse effects were identified at multiple dose levels, including the lowest dose (14 mg/kg). The toxicological effect that was most consistently supported by multiple analyses was mild kidney injury. This was evident based on statistically significant decreases in absolute and relative masses of kidneys and adrenals as well as statistically significant increases in biomarkers of kidney injury (BUN, urea, and creatinine) and blood protein levels (total protein, albumin, and globulin). These effects were observed in both sexes and at nearly all dose levels, including 14 mg/kg. Prerenal azotemia could be a potential cause of these effects.

A secondary critical effect was immunological impairment. This was supported by pathological lesions of the thymus, statistically significant decreases in relative and absolute spleen mass, and decreases in the number of WBCs (both sexes: neutrophils and eosinophils; females only: basophils; males only: lymphocytes). Females also exhibited mild anemia (decreased RBC, HGB, HCT, and PLT). These effects were observed in both sexes and in nearly all dose groups.

Despite the consistent absence of a dose response for nearly every parameter measured, and the relatively minor magnitudes of effect, the rats were determined to have developed mild kidney injury and immunological abnormalities at all doses tested. Thus, the LOAEL for this study is 14 mg/kg. BMD was not determined due to the absence of a monotonic dose response relationship.

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.

Prepared By:

SUSSAN.THOMAS.EDWARD.JR.1537772626 Digitally signed by SUSSAN.THOMAS.EDWARD.JR.1537772626 Date: 2023.08.31 13:23:32 -04'00'

THOMAS E. SUSSAN — Date
Biologist
Health Effects Division (HEF)

Approved By:

QUINN.MICHAEL.J.1282372092 Digitally signed by QUINN.MICHAEL.J.1282372092 Date: 2023.09.01 12:34:29 -04'00'

MICHAEL J. QUINN — Date
Division Chief, Health Effects Division

JOHNSON.MARK.STEVEN.1229380717 Digitally signed by JOHNSON.MARK.STEVEN.1229380717 Date: 2023.09.05 07:53:33 -04'00'

MARK S. JOHNSON — Date
Director, Toxicology

APPENDIX A

REFERENCES

1. Department of the Army (DA). 2007. Regulation 200–1, *Environmental Protection and Enhancement*. https://armypubs.army.mil

2. DA. 2007. Regulation 40–5, *Preventive Medicine*. https://armypubs.army.mil

3. DA. 2003. Regulation 70–1, *Army Acquisition Policy*. https://armypubs.army.mil

4. Department of Defense Directive (DoDD) 4715.1E, *Environment, Safety, and Occupational Health (ESOH),* 2005, Change 1, 2018. https://www.esd.whs.mil/

5. Army Environmental Requirements and Technology Assessments. 2012. Requirement PP-3-02-05, *Compliant Ordnance Lifecycle For Readiness of the Transformation and Objective Forces*. Joint Base San Antonio, Texas.

6. U.S. Army Public Health Center (APHC). 2017. Substance Toxicity Profile- *3,4-Dinitropyrazole [DNP]*. Prepared by W.S. Eck. Aberdeen Proving Ground, Maryland.

7. APHC (Prov). 2015. Toxicology Report No. S.0024589d-15*, Human Cell Line Activation Test of the Novel Energetic 3,4-Dinitropyrazole.* Aberdeen Proving Ground, Maryland.

8. APHC. 2017. Quality Systems and Regulatory Compliance Office (QSARC) 800*, Animal Euthanasia*. Aberdeen Proving Ground, Maryland.

9. U.S. Army Institute of Public Health. 2016. Toxicology Report No. S.0024589d-15, *Direct Peptide Reactivity Assay of the Novel Energetic 3,4-Dinitropyrazole (DNP)*. Aberdeen Proving Ground, Maryland.

10. Accelrys, Inc. 2004. TOPKAT Version 6.2 QSAR.

11. APHC. 2018. Toxicology Report No. S.0002728b-15, *Microtox Toxicity Testing of the Novel Energetics, 1,4-dinitroglycoluril (DNGU), 3,4dinitropyrazole (DNP), 1,4,7-trinitrohexahydro-1H-imidazo[4,5-b]pyrazine-2(3H)-one (HK-56), 2,6-diamino-3,5-dinitro 2,3-dihydropyrazine 1-oxide (LLM-105), and 2,4,6trinitro-3-bromoanisole (TNBA)*. Aberdeen Proving Ground, Maryland.

12. American Society for Testing and Materials (ASTM). 2008. E2552-16, *Standard Guide for Assessing the Environmental and Human Health Impacts of New Compounds for Military Use*. West Conshohocken, Pennsylvania: ASTM International.

13. ASTM. 2011. E1163-10, *Standard Test Method for Estimating Acute Oral Toxicity in Rats, Biological Effects and Environmental Fate.* West Conshohocken, Pennsylvania: ASTM International.

14. Feder PI, CT Olson, DW Hobson, MC Matthews, and RL Joiner. 1991. Stagewise, group sequential experimental designs for quantal responses. one-sample and two-sample comparisons. *Neurosci Biobehav Rev* 15(1):129-33. DOI: 10.1016/s0149-7634(05)80104-6

15. Feder PI, CT Olson, DW Hobson, MC Matthews, and RL Joiner. 1991. Stagewise, adaptive dose allocation for quantal response dose-response studies. *Neurosci Biobehav Rev* 15(1):109-14. DOI: 10-1016/s0149-7634(05)80101-0

16. EPA. 2012. *Benchmark Dose Technical Guidance.* Risk Assessment Forum. Washington, DC.

17. Shore K. 2008. *In Vivo Test for Chemical Induction of Micronucleated Polychromatic Erythrocytes in Mouse Bone Marrow Cells*. Rockville, Maryland: SITEK Research Laboratories.

18. Elfarra AA, I Jakobson, and MW Anders. 1986. Mechanism of S-(1,2-dichlorovinyl)glutathione-induced nephrotoxicity. *Biochem Pharmacol* 35(2):283-8. DOI: 10.1016/0006-2952(86)90527-7

19. APHC. 2018. Toxicology Study No. S.0002728a-15, *Ames Mutagenicity Test of the Novel Energetics: 1,4-dinitroglycoluril (DNGU), 3,4-dinitropyrazole (DNP), 2,6-diamino-3,5-dinitro-2,3-dihydropyrazine 1-oxide (LLM-105), 2,4,6-trinitro-3-bromoanisole (TNBA), and 1,4,7-trinitrohexahydro-1H-imidazo[4,5-b]pyrazine-2(3H)-one (HK-56)*. Aberdeen Proving Ground, Maryland.

20. Kirby P. 2008. *Evaluation of a Test Article in the Salmonella typhimurium/Escherichia coli Plate Incorporation Mutation Assay in the Presence and Absence of Induced Rat Liver S-9*. Rockville, Maryland: SITEK Research Laboratories.

21. Song J. 2008. *Test for Chemical Induction of Chromosome Aberrations in Cultured Chinese Hanster Ovary (CHO) Cells With and Without Metabolic Activation*. Rockville, Maryland: SITEK Research Laboratories.

22. O'Dwyer PJ, SA King, J Plowman, CK Grieshaber, DF Hoth, and B Leyland-Jones. 1988. Pyrazole: preclinical reassessment. *Invest New Drugs* 6(4):305-10. DOI: 10.1007/BF00173649.

23. Ashby J and B Elliott. 1984. Toxicity of Heterocycles. In *Comprehensive Heterocyclic Chemistry,* ed. Alan R. Katritzky and Charles W. Rees. Amsterdam, Netherlands: Elsevier Science Ltd.

24. Wilson WL and NG Bottiglieri. 1962. Phase I Studies with Pyrazole. *Cancer Chemother Rep* 21:137-41. Washington, DC: U.S. Department of Health, Education, and Welfare.

25. Agency for Toxic Substances and Disease Registry. Toxicological Profile for *2,4,6-Trinitrotoluene.* https://www.atsdr.cdc.gov/toxprofiledocs/index.html

26. EPA. 1988. 2,4,6-Trinitrotoluene (TNT); CASRN 118-96-7, in *Integrated Risk Information System (IRIS) Chemical Assessment Summary*. Washington, DC.

27. International Agency for Research on Cancer. 1996. 2,4,6-Trinitrotoluene, in *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 65. Printing Processes and Printing Inks, Carbon Black and Some Nitro Compounds*. https://monographs.iarc.who.int/wp-content/uploads/2018/06/mono65.pdf

28. Occupational Safety and Health Administration. 2018. *2,4,6-TRINITROTOLUENE*. https://www.osha.gov/chemicaldata/chemResult.html?recNo=147

APPENDIX B

QUALITY ASSURANCE STATEMENT

For Protocol No. 30-18-07-01, titled "EFFECTS OF ACUTE AND SUBACUTE ORAL 3,4-DINITROPYRAZOLE (DNP) 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
Study Raw Data Good Laboratory Practice Standard Review	07/21/2023	07/21/2023
Final Report Good Laboratory Practice Standard Review	07/21/2023	07/21/2023

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.*

KEFAUVER.MICHAEL.P.1229209678 Digitally signed by KEFAUVER.MICHAEL.P.1229209678 Date: 2023.08.14 10:33:00 -04'00'

Michael P. Kefauver
GLP Quality Assurance Specialist

08/14/2023
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 the DCPH-A Toxicology Directorate archives 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 the DCPH-A Toxicology Directorate archives.

Wet tissues, histology slides, and paraffin blocks are stored in the Pathology Division archives.

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

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

Protocol No.: 30-18-07-01 **Species:** Rat **Sex:** Female **Route:** Oral Gavage
Test Article: DNP **Diluent:** Corn Oil
Date Dosed: 08/07/2018 (Stage 1), 08/14/2018 (Stage 2), 08/22/2018 (Stage 3)

Stage	Animal ID	Weight (kg)	Nominal Dose (mg/kg)	Stock Concentration (mg/mL)	Time Dosed	Clinical Signs (time noted)	Outcome
1	18-0551	0.1847	200	50	0820	Increased respiration (0838); normal (0929, 1500)	Scheduled necropsy
1	18-0552	0.1717	356	50	0823	Laying on side (0838); prostrate (0929); active and normal (0947, 1500)	Scheduled necropsy
1	18-0553	0.1701	632	100	0830	Panting (0839); prostrate (0929, 1120); active (1130); normal (1500); labored breathing (8/8/18- 0821)	Found dead (8/8/18- 1316)
1	18-0554	0.1835	1125	200	0833	Prostrate (0841)	Found dead (0900)
1	18-0555	0.1650	2000	200	0837	Prostrate (0842)	Found dead (0854)
2	18-0556	0.2109	356	50	0817	Squinting (0841); normal (1117, 1535)	Scheduled necropsy
2	18-0557	0.2141	356	50	0821	Laying on side, labored breathing (0838, 0938); pale (1038); prostrate, labored breathing (1121); slight movement, pale, labored breathing, pale (1535); laying on side, no spontaneous activity (8/15/18- 0745, 1200, 1545)	Found dead (8/16/18- 0625)
2	18-0558	0.2030	474	50	0824	Prostrate (0838); moving about cage (1038); alert, normal (1124, 1535)	Scheduled necropsy
2	18-0559	0.1925	474	50	0826	Wobbly gait (0838); elevated respiratory rate (0841)	Found dead (0850)
2	18-0560	0.1914	632	100	0833	Prostrate (0838); elevated respiratory rate (0841)	Found dead (0918)
2	18-0561	0.1916	632	100	0835	Normal (0932, 1134, 1535); hunched, lethargic, congested breathing, discharge around eyes (8/15/18- 0745, 1200, 1545)	Found dead (8/16/18- 0625)
3	18-0562	0.2316	200	25	0808	Normal (0854, 1109, 1545)	Scheduled necropsy
3	18-0563	0.2398	200	25	0812	Normal (0854, 1112, 1545)	Scheduled necropsy
3	18-0564	0.2364	200	25	0815	Lethargic (0855); did not eat immediately after feed was returned (1120); lethargic (1545); normal (8/23/18- 0740)	Scheduled necropsy
3	18-0565	0.2143	474	63.2	0821	Prostrate (0840); wobbly gait (0913); active (1120); hunched, lethargic, normal breathing (1545); hunched but active (8/23/18- 0740, 1435); active, walks gingerly (8/24/18- 0755)	Scheduled necropsy
3	18-0566	0.2039	474	63.2	0825	Active (0856); hunched (1117); did not immediately eat after feed was returned (1125); hunched, lethargic, normal breathing (1545); hunched, lethargic (8/23/18- 0740); hunched, lethargic, dried brown liquid around mouth, vocalization when handled (8/23/18- 1435); active, walks gingerly (8/24/18- 0755)	Scheduled necropsy
3	18-0567	0.2618	474	63.2	0828	Prostrate (0841); wobbly gait (0911); elevated respiratory rate (0915)	Found dead (0949)
3	18-0568	0.2324	632	63.2	0833	Resting (0907); active (1137); squinting, no spontaneous activity (1545); lethargic, wobbly gait, pale, sunken eyes (8/23/18- 0740); gasping, congested breathing, pale, eyes closed, minimal movement (8/23/18- 1136)	Euthanized (8/23/18- 1207)
3	18-0569	0.2173	632	63.2	0837	Resting (0907); hunched, squinting (1137); eyes closed, discharge around eyes, very lethargic (1545); very lethargic, pale, sunken eyes (8/23/18- 0740)	Found dead (8/23/18- 1020)
3	18-0570	0.2507	632	63.2	0839	Prostrate (0849); labored breathing (0859)	Found dead (0909)

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

Protocol No.: 30-18-07-01 **Species:** Rat **Sex:** Female **Route:** Oral Gavage
Test Article: DNP **Diluent:** Corn Oil
Date Dosed: 08/07/2018 (Stage 1), 08/14/2018 (Stage 2), 08/22/2018 (Stage 3)

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	18-0551	200	184.7	193.6	210.0	217.9	217.7	220.8	231.2	237.0
3	18-0562	200	231.6	233.6	239.0	245.0	249.8	254.8	255.2	255.0
3	18-0563	200	239.8	248.8	247.0	247.8	254.1	256.6	258.3	258.1
3	18-0564	200	236.4	220.1	229.5	239.3	246.8	251.4	255.4	258.9
1	18-0552	356	171.7	171.8	181.3	188.7	190.8	194.3	200.4	206.2
2	18-0556	356	210.9	201.7	212.1	224.9	232.5	234.1	234.6	240.5
2	18-0557	356	214.1	200.5	Dead					
2	18-0558	474	203.0	189.4	196.2	214.4	224.8	227.1	226.5	232.4
2	18-0559	474	192.5	Dead						
3	18-0565	474	214.3	197.5	208.8	217	219.7	225.4	228.2	230.5
3	18-0566	474	203.9	192.7	199.1	209.8	211.1	218.5	221	224.8
3	18-0567	474	261.8	Dead						
1	18-0553	632	170.1	157.2	Dead					
2	18-0560	632	191.4	Dead						
2	18-0561	632	191.6	177.6	Dead					
3	18-0568	632	232.4	212.1	Dead					
3	18-0569	632	217.3	204.2	Dead					
3	18-0570	632	250.7	Dead						
1	18-0554	1125	183.5	Dead						
1	18-0555	2000	165.0	Dead						

Table D-3. Acute Gross Pathological Findings

Protocol No.: 30-18-07-01 **Species:** Rat **Sex:** Female **Route:** Oral Gavage
Test Article: DNP **Diluent:** Corn Oil
Date Dosed: 08/07/2018 (Stage 1), 08/14/2018 (Stage 2), 08/22/2018 (Stage 3)

Stage	Animal ID	Nominal Dose (mg/kg)	Outcome (hours after dosing)	Gross Pathological Findings
1	18-0551	200	Scheduled necropsy	Minimal yellow staining around vulva.
3	18-0562	200	Scheduled necropsy	Minimal yellow staining around vulva.
3	18-0563	200	Scheduled necropsy	NGLR
3	18-0564	200	Scheduled necropsy	NGLR
1	18-0552	356	Scheduled necropsy	Minimal yellow staining around tail.
2	18-0556	356	Scheduled necropsy	Minimal yellow staining around vulva.
2	18-0557	356	Found dead (40 h)	Moderate amount of yellow staining around vulva and anus; liver is swollen, patchy tan and dark areas; stomach is markedly swollen with gas; stomach contains ulcerations and specs of dark red material; SI is discolored with dark green contents (post-mortem autolysis and possible ileus); colon is empty; congested and hemorrhaged meninges; hemorrhage within thymus; mild congestion of lungs.
2	18-0558	474	Scheduled necropsy	Minimal yellow staining around anus.
2	18-0559	474	Found dead (0.5 h)	Yellow staining around vulva; large, swollen, dark red, congested liver; white discoloration of the non-glandular stomach mucosa; small intestine is distended with gas and fluid.
3	18-0565	474	Scheduled necropsy	Minimal yellow staining around vulva.
3	18-0566	474	Scheduled necropsy	NGLR
3	18-0567	474	Found dead (1.25 h)	Yellow staining on vulva; liver is enlarged with mottled brown appearance; dark, enlarged spleen; white discoloration of stomach with sloughing; non-glandular and glandular areas have deep red erosion; small amount of clear fluid in esophagus; SI filled with fluid; colon conatins hard formed feces; firm and hard feces stuck in rectum; reddish-brown staining around nostrils; several dark purple patches on external left lung that extends into parenchyma.
1	18-0553	632	Found dead (28 h)	Dark, swollen liver; non-glandular stomach mucosa is white and has areas of sloughing; small intestinal contents have yellow discoloration; congestion of blood vessels throughout body; large amounts of clear fluid in thoracic cavity; yellow staining around vulva.
2	18-0560	632	Found dead (0.75 h)	Yellow staining around vulva; enlarged, dark red, congested liver; white discoloration of stomach mucosa; stomach filled with clear fluid.
2	18-0561	632	Found dead (40 h)	Large amount of yellow staining around vulva, anus, tail mouth and chest; stomach ulceration with hemorrhage; hemorrhage on the dorsal surface of the meninges; cecum and colon are empty.
3	18-0568	632	Euthanized (28 h)	Yellow staining around vulva; distended stomach with fluid and gas, glandular portion has multifocal ulcerations; cecum and colon nearly empty; heart and great vessels distended and congested; congested meningial vessels; lungs are dark red and firm.
3	18-0569	632	Found dead (26 h)	Bright yellow urine; swollen, dark liver; substantial clear fluid in pericardial sac, mediastinum, and pleural cavity; stomach severely distended and contains large amount of fluid and ingesta; glandular portion has multifocal redness, ulceration, and adherent material; SI is slightly distended with fluid and has mild reddish-brown discoloration; nearly empty cecum and colon; meninges are congested and edematous.
3	18-0570	632	Found dead (0.5 h)	Yellow staining on vulva; enlarged liver; slightly enlarged spleen; external hemorrhaging of both stomach regions; 1-3mm portion of liver touching stomach, causing discoloration; errosion and sloughing of distal esophagus; both lungs appear red with blood; congested subcutaneous blood vessels and carotid arteries; clear fluid in proximal intestine.
1	18-0554	1125	Found dead (0.5 h)	Advanced rigor mortis shortly after death; liver and spleen are swollen and dark red; stomach mucosa is white; liver and spleen turned white when stomach contents spilled on them.
1	18-0555	2000	Found dead (0.25 h)	Advanced rigor mortis shortly after death; liver and spleen are swollen and dark red; liver and spleen turned white when stomach contents spilled on them.

Table D-4. LD_{50} Probit Analysis

SEPARATE-SLOPES DOSE-RESPONSE FITS
PERCENTILES WITH CONFIDENCE INTERVALS BASED ON FIELLER'S AND DELTA METHODS

Agent=DNP Treatment Group=female

Agent	Perc-entile	Probit of Percentile	Log(Eff. Dose) for Percentile	Std. Error of Log(Eff. Dose)	Effective Dose for Percentile	Lower Fieller Confidence Bound	Upper Fieller Confidence Bound	Lower Delta Confidence Bound	Upper Delta Confidence Bound
DNP	0.0001	-4.75342	2.16131	0.24286	144.980	0.002	259.76	48.451	433.82
DNP	0.0010	-4.26489	2.21127	0.22017	162.657	0.006	276.40	60.221	439.34
DNP	0.0100	-3.71902	2.26710	0.19492	184.971	0.022	296.41	76.749	445.80
DNP	1.0000	-2.32635	2.40954	0.13141	256.770	0.677	355.86	141.899	464.63
DNP	10.0000	-1.28155	2.51640	0.08601	328.400	8.782	413.01	222.754	484.15
DNP	16.0000	-0.99446	2.54577	0.07441	351.372	17.664	432.53	251.140	491.61
DNP	30.0000	-0.52440	2.59384	0.05732	392.504	54.678	473.23	303.043	508.37
DNP	50.0000	0.00000	2.64748	0.04398	444.098	178.937	563.90	364.140	541.61
DNP	70.0000	0.52440	2.70111	0.04254	502.473	379.577	1036.64	414.692	608.84
DNP	84.0000	0.99446	2.74919	0.05235	561.293	464.830	2866.86	443.189	710.87
DNP	90.0000	1.28155	2.77855	0.06159	600.555	496.705	5651.55	454.825	792.98
DNP	99.0000	2.32635	2.88541	0.10356	768.091	589.106	71713.37	481.319	1225.72

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	18-0723		
	18-0724		
	18-0697		
	18-0698		
	18-0717		
	18-0718		
	18-0719		
	18-0720		
	18-0731		
	18-0732		
14 mg/kg	18-0705	Mistakenly dosed with twice the intended dose (2.8 mg/mL instead of 1.4 mg/mL); treated with control the following day	4,5
	18-0706		
	18-0687		
	18-0688		
	18-0735		
	18-0736		
	18-0743		
	18-0744		
	18-0701		
	18-0702		
28 mg/kg	18-0699		
	18-0700		
	18-0693	Aspirated some compound	11
	18-0694	Alopecia on belly, forearms, neck	4
	18-0713		
	18-0714		
	18-0707		
	18-0708		
	18-0691		
	18-0692		
56 mg/kg	18-0733		
	18-0734		
	18-0715		
	18-0716		
	18-0703		
	18-0704		
	18-0729		
	18-0730		
	18-0745		
	18-0746		
111 mg/kg	18-0689		
	18-0690		
	18-0739		
	18-0740		
	18-0737		
	18-0738		
	18-0741		
	18-0742		
	18-0695		
	18-0696		
222 mg/kg	18-0709		
	18-0710		
	18-0727	Gasping immediately after dosing for <30min due to aspiration	4
		Both rats in cage have yellow fluid (urine or compound)- mostly on its back	8
	18-0728	Both rats in cage have yellow fluid (urine or compound)- mostly on its face and back	8
	18-0711		
	18-0712		
	18-0725		
	18-0726		
	18-0721		
	18-0722		

Note:
Animals with no remarkable observations (i.e., 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	18-0649		
	18-0650		
	18-0685		
	18-0686	Temporary bleeding from mouth (bit tongue)	1
	18-0671		
	18-0672		
	18-0675		
	18-0676		
	18-0657		
	18-0658		
14 mg/kg	18-0637		
	18-0638		
	18-0677		
	18-0678	Chromodacryorrhea in left eye	10
	18-0679	Minor eye injury (right eye)	0
		Dried porphyrin around right eye	1,8,10
	18-0680		
	18-0643		
	18-0644		
	18-0629		
	18-0630		
28 mg/kg	18-0653		
	18-0654		
	18-0641		
	18-0642		
	18-0665		
	18-0666		
	18-0663		
	18-0664	Temporary bleeding from mouth (bit tongue)	8
	18-0623		
	18-0624		
56 mg/kg	18-0661		
	18-0662		
	18-0651		
	18-0652	Aspirated some compound	2,4,7
		Slight bleeding in mouth (bit tongue)	7
	18-0635		
	18-0636		
	18-0633		
	18-0634		
	18-0681		
	18-0682		
111 mg/kg	18-0655	Minor bite wound on side	7
	18-0656		
	18-0645	Slight bleeding in mouth (bit tongue)	12
	18-0646		
	18-0673		
	18-0674		
	18-0669		
	18-0670		
	18-0667		
	18-0668		
222 mg/kg	18-0631	Congested breathing	11
	18-0632		
	18-0647		
	18-0648		
	18-0621		
	18-0622		
	18-0659		
	18-0660		
	18-0683		
	18-0684	Underweight, ruffled fur, hunched	0,1
		Found dead	2

Note:
Animals with no remarkable observations (i.e., 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]	Dose Group	Animal ID	Day 0	Day 3	Day 7	Day 13	Day 14[1]
0 mg/kg	18-0723	184.0	197.7	214.1	226.7	218.3	**56 mg/kg**	18-0733	172.1	185.9	206.7	225.0	216.3
	18-0724	183.4	196.7	216.4	238.8	228.7		18-0734	175.3	196.5	217.6	226.1	220.4
	18-0697	183.5	186.2	201.5	215.1	210.3		18-0715	182.6	191.3	207.9	221.3	211.8
	18-0698	178.4	190.5	199.1	214.1	205.9		18-0716	183.8	196.1	224.2	244.2	230.6
	18-0717	173.0	178.3	190.5	207.9	201.3		18-0703	160.3	178.2	195.0	199.0	196.8
	18-0718	187.3	198.6	214.7	231.8	225.6		18-0704	191.1	203.2	221.0	241.4	228.1
	18-0719	158.7	170.1	182.3	201.2	192.1		18-0729	171.0	169.6	182.3	186.5	186.8
	18-0720	175.2	184.3	196.5	208.0	197.9		18-0730	168.2	180.4	191.2	196.3	194.2
	18-0731	168.3	177.3	187.6	199.7	192.8		18-0745	163.2	182.0	196.7	199.7	196.6
	18-0732	166.7	177.8	188.5	202.5	198.9		18-0746	169.9	181.9	192.2	201.4	194.5
	Mean	**175.9**	**185.8**	**199.1**	**214.6**	**207.2**		**Mean**	**173.8**	**186.5**	**203.5**	**214.1**	**207.6**
	SD	**9.2**	**9.9**	**12.4**	**13.6**	**13.2**		**SD**	**9.6**	**10.2**	**14.2**	**20.1**	**15.7**
14 mg/kg	18-0705	173.2	181.7	198.4	218.8	207.5	**111 mg/kg**	18-0689	202.4	216.0	239.0	260.9	249.7
	18-0706	197.9	209.3	230.1	262.0	245.7		18-0690	180.6	185.5	206.8	220.2	211.3
	18-0687	183.6	191.4	211.2	225.1	214.4		18-0739	184.2	194.4	217.6	232.5	222.0
	18-0688	174.0	179.0	199.2	217.9	204.0		18-0740	173.0	191.7	207.2	221.2	210.9
	18-0735	174.1	193.7	206.9	233.5	222.5		18-0737	178.9	196.2	211.2	233.6	226.0
	18-0736	175.5	185.5	204.1	220.9	209.0		18-0738	179.9	193.3	209.0	210.6	203.5
	18-0743	161.1	172.1	181.6	194.5	191.5		18-0741	175.9	181.2	196.0	210.1	204.2
	18-0744	172.3	195.7	213.2	226.7	216.4		18-0742	173.9	180.2	197.1	201.4	195.4
	18-0701	167.1	175.8	181.1	197.0	188.0		18-0695	144.9	150.4	154.2	170.0	163.7
	18-0702	173.8	198.4	214.8	220.0	216.6		18-0696	168.4	177.4	192.8	212.5	206.5
	Mean	**175.3**	**188.3**	**204.1**	**221.6**	**211.6**		**Mean**	**176.2**	**186.6**	**203.1**	**217.3**	**209.3**
	SD	**9.8**	**11.5**	**15.0**	**18.8**	**16.3**		**SD**	**14.3**	**16.8**	**21.6**	**23.6**	**22.2**
28 mg/kg	18-0699	198.9	213.0	238.7	270.1	256.5	**222 mg/kg**	18-0709	164.9	174.6	192.5	198.4	192.6
	18-0700	172.4	174.6	186.9	212.2	202.0		18-0710	203.7	213.5	217.9	246.5	234.8
	18-0693	163.5	188.0	195.4	201.4	190.1		18-0727	169.8	188.2	193.2	203.9	195.2
	18-0694	183.4	190.2	204.5	229.9	216.8		18-0728	184.2	196.8	214.2	225.9	221.9
	18-0713	168.1	172.3	193.4	201.3	196.2		18-0711	183.7	195.5	204.3	211.2	202.4
	18-0714	180.4	190.2	209.5	210.6	203.0		18-0712	165.2	171.6	191.6	191.4	187.6
	18-0707	169.2	178.7	180.8	194.6	188.1		18-0725	189.2	205.0	218.4	228.1	222.9
	18-0708	184.1	201.2	224.4	242.5	235.0		18-0726	159.2	178.8	190.3	194.9	188.8
	18-0691	167.2	184.3	200.3	202.1	194.7		18-0721	169.0	166.3	188.0	192.6	186.8
	18-0692	157.3	161.9	170.0	193.3	186.1		18-0722	171.8	176.8	192.5	197.3	190.2
	Mean	**174.5**	**185.4**	**200.4**	**215.8**	**206.9**		**Mean**	**176.1**	**186.7**	**200.3**	**209.0**	**202.3**
	SD	**12.2**	**14.7**	**20.3**	**24.6**	**22.9**		**SD**	**13.7**	**15.6**	**12.2**	**18.6**	**17.6**

Note:
[1]Fasted overnight.
Data showed a normal distribution and equal variance. No effects of dose were detected via repeated measures ANOVA.

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	18-0649	350.3	360.5	391.9	431.9	418.0	**56 mg/kg**	18-0661	320.9	328.0	356.5	366.2	356.3
	18-0650	304.9	316.3	336.2	362.5	352.4		18-0662	339.1	350.7	387.2	421.1	408.2
	18-0685	325.8	359.1	385.5	428.3	414.8		18-0651	327.1	341.6	354.7	365.9	355.4
	18-0686	328.8	355.4	382.7	417.2	400.3		18-0652	303.0	313.1	336.4	358.8	348.0
	18-0671	324.2	351.2	379.6	416.8	402.7		18-0635	334.3	352.8	386.9	412.1	399.1
	18-0672	304.5	328.4	348.5	381.6	371.9		18-0636	304.2	319.6	350.4	392.5	381.8
	18-0675	313.8	333.3	353.6	383.9	370.7		18-0633	317.9	313.7	346.9	372.4	360.9
	18-0676	312.5	332.1	337.7	361.2	347.8		18-0634	308.4	303.2	334.6	359.9	354.6
	18-0657	293.2	313.2	334.5	363.6	356.8		18-0681	334.2	353.7	381.8	405.7	395.2
	18-0658	312.1	336.7	364.9	398.9	390.6		18-0682	273.5	289.9	317.2	339.5	327.0
	Mean	**317.0**	**338.6**	**361.5**	**394.6**	**382.6**		**Mean**	**316.3**	**326.6**	**355.3**	**379.4**	**368.7**
	SD	**16.0**	**17.2**	**22.3**	**27.8**	**26.1**		**SD**	**19.8**	**22.4**	**23.7**	**26.8**	**26.1**
14 mg/kg	18-0637	334.6	356.9	391.6	404.9	391.5	**111 mg/kg**	18-0655	353.0	357.8	398.0	428.9	418.9
	18-0638	326.5	346.1	381.9	401.5	386.4		18-0656	316.0	324.9	354.6	375.6	367.9
	18-0677	341.1	360.2	396.8	425.1	410.7		18-0645	322.3	337.7	366.1	381.5	371.5
	18-0678	322.9	341.6	368.8	398.5	388.1		18-0646	324.2	343.6	385.2	404.3	395.0
	18-0679	329.4	356.3	378.3	382.9	373.7		18-0673	310.6	315.7	338.5	358.0	350.6
	18-0680	311.0	340.5	372.0	408.0	389.7		18-0674	325.5	336.6	367.9	387.1	377.7
	18-0643	311.0	327.4	351.8	368.8	355.5		18-0669	334.2	345.9	378.8	402.9	397.0
	18-0644	341.7	362.4	391.0	419.0	399.9		18-0670	293.2	299.3	321.3	330.0	323.3
	18-0629	340.6	363.8	396.4	433.8	420.4		18-0667	308.1	315.6	336.9	350.0	339.6
	18-0630	284.5	302.4	330.4	365.5	354.8		18-0668	313.4	323.0	345.4	371.7	363.8
	Mean	**324.3**	**345.8**	**375.9**	**400.8**	**387.1**		**Mean**	**320.1**	**330.0**	**359.3**	**379.0**	**370.5**
	SD	**18.0**	**19.2**	**21.3**	**22.8**	**21.3**		**SD**	**16.2**	**17.5**	**24.2**	**28.9**	**28.5**
28 mg/kg	18-0653	377.5	397.3	452.6	475.6	460.4	**222 mg/kg**	18-0631	319.9	319.7	349.0	363.5	361.3
	18-0654	338.7	355.2	395.1	426.3	412.3		18-0632	336.2	326.4	363.8	390.3	377.6
	18-0641	316.2	332.5	355.9	378.4	369.2		18-0647	335.6	353.8	385.4	408.7	400.4
	18-0642	323.6	334.1	361.6	376.8	366.5		18-0648	311.7	319.8	347.9	388.1	369.5
	18-0665	303.2	317.0	336.3	350.4	342.7		18-0621	322.7	331.3	363.1	373.7	362.5
	18-0666	346.0	366.0	404.9	434.1	418.8		18-0622	311.6	315.0	339.6	352.3	341.8
	18-0663	292.2	315.8	338.6	361.8	350.0		18-0659	306.8	305.0	335.3	350.9	342.4
	18-0664	326.4	343.1	375.8	381.4	372.0		18-0660	316.3	312.4	334.8	346.7	334.4
	18-0623	311.4	329.4	356.7	365.5	354.9		18-0683	321.6	306.9	333.7	349.5	341.6
	18-0624	275.5	296.5	312.2	315.4	312.5		18-0684					
	Mean	**321.1**	**338.7**	**369.0**	**386.6**	**375.9**		**Mean**	**320.3**	**321.1**	**350.3**	**369.3**	**359.1**
	SD	**28.9**	**28.7**	**40.3**	**46.4**	**43.1**		**SD**	**10.3**	**14.9**	**17.5**	**22.2**	**21.4**

Note:
[1]Fasted overnight.
Data showed a normal distribution and equal variance. No effects of dose were detected via repeated measures ANOVA.

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	18-0723	13.7	16.4	12.6	42.7	**56 mg/kg**	18-0733	13.8	20.8	18.3	52.9
	18-0724	13.3	19.7	22.4	55.4		18-0734	21.2	21.1	8.5	50.8
	18-0697	2.7	15.3	13.6	31.6		18-0715	8.7	16.6	13.4	38.7
	18-0698	12.1	8.6	15.0	35.7		18-0716	12.3	28.1	20.0	60.4
	18-0717	5.3	12.2	17.4	34.9		18-0703	17.9	16.8	4.0	38.7
	18-0718	11.3	16.1	17.1	44.5		18-0704	12.1	17.8	20.4	50.3
	18-0719	11.4	12.2	18.9	42.5		18-0729	-1.4	12.7	4.2	15.5
	18-0720	9.1	12.2	11.5	32.8		18-0730	12.2	10.8	5.1	28.1
	18-0731	9.0	10.3	12.1	31.4		18-0745	18.8	14.7	3.0	36.5
	18-0732	11.1	10.7	14.0	35.8		18-0746	12.0	10.3	9.2	31.5
	Mean	**9.9**	**13.4**	**15.5**	**38.7**		**Mean**	**12.8**	**17.0**	**10.6**	**40.3**
	SD	**3.5**	**3.4**	**3.5**	**7.6**		**SD**	**6.3**	**5.4**	**6.9**	**13.5**
14 mg/kg	18-0705	8.5	16.7	20.4	45.6	**111 mg/kg**	18-0689	13.6	23.0	21.9	58.5
	18-0706	11.4	20.8	31.9	64.1		18-0690	4.9	21.3	13.4	39.6
	18-0687	7.8	19.8	13.9	41.5		18-0739	10.2	23.2	14.9	48.3
	18-0688	5.0	20.2	18.7	43.9		18-0740	18.7	15.5	14.0	48.2
	18-0735	19.6	13.2	26.6	59.4		18-0737	17.3	15.0	22.4	54.7
	18-0736	10.0	18.6	16.8	45.4		18-0738	13.4	15.7	1.6	30.7
	18-0743	11.0	9.5	12.9	33.4		18-0741	5.3	14.8	14.1	34.2
	18-0744	23.4	17.5	13.5	54.4		18-0742	6.3	16.9	4.3	27.5
	18-0701	8.7	5.3	15.9	29.9		18-0695	5.5	3.8	15.8	25.1
	18-0702	24.6	16.4	5.2	46.2		18-0696	9.0	15.4	19.7	44.1
	Mean	**13.0**	**15.8**	**17.6**	**46.4**		**Mean**	**10.4**	**16.5**	**14.2**	**41.1**
	SD	**6.9**	**5.0**	**7.5**	**10.6**		**SD**	**5.1**	**5.6**	**6.8**	**11.5**
28 mg/kg	18-0699	14.1	25.7	31.4	71.2	**222 mg/kg**	18-0709	9.7	17.9	5.9	33.5
	18-0700	2.2	12.3	25.3	39.8		18-0710	9.8	4.4	28.6	42.8
	18-0693	24.5	7.4	6.0	37.9		18-0727	18.4	5.0	10.7	34.1
	18-0694	6.8	14.3	25.4	46.5		18-0728	12.6	17.4	11.7	41.7
	18-0713	4.2	21.1	7.9	33.2		18-0711	11.8	8.8	6.9	27.5
	18-0714	9.8	19.3	1.1	30.2		18-0712	6.4	20.0	-0.2	26.2
	18-0707	9.5	2.1	13.8	25.4		18-0725	15.8	13.4	9.7	38.9
	18-0708	17.1	23.2	18.1	58.4		18-0726	19.6	11.5	4.6	35.7
	18-0691	17.1	16.0	1.8	34.9		18-0721	-2.7	21.7	4.6	23.6
	18-0692	4.6	8.1	23.3	36.0		18-0722	5.0	15.7	4.8	25.5
	Mean	**11.0**	**15.0**	**15.4**	**41.4**		**Mean**	**10.6**	**13.6**	**8.7**	**33.0**
	SD	**7.1**	**7.6**	**10.9**	**13.9**		**SD**	**6.7**	**6.1**	**7.8**	**7.0**

Note:
Data showed a normal distribution; significant error variance was detected via Levene's test for day 7-13 (p=0.044). No effects of dose were detected via repeated measures ANOVA.

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	18-0649	10.2	31.4	40.0	81.6	**56 mg/kg**	18-0661	7.1	28.5	9.7	45.3
	18-0650	11.4	19.9	26.3	57.6		18-0662	11.6	36.5	33.9	82.0
	18-0685	33.3	26.4	42.8	102.5		18-0651	14.5	13.1	11.2	38.8
	18-0686	26.6	27.3	34.5	88.4		18-0652	10.1	23.3	22.4	55.8
	18-0671	27.0	28.4	37.2	92.6		18-0635	18.5	34.1	25.2	77.8
	18-0672	23.9	20.1	33.1	77.1		18-0636	15.4	30.8	42.1	88.3
	18-0675	19.5	20.3	30.3	70.1		18-0633	-4.2	33.2	25.5	54.5
	18-0676	19.6	5.6	23.5	48.7		18-0634	-5.2	31.4	25.3	51.5
	18-0657	20.0	21.3	29.1	70.4		18-0681	19.5	28.1	23.9	71.5
	18-0658	24.6	28.2	34.0	86.8		18-0682	16.4	27.3	22.3	66.0
	Mean	**21.6**	**22.9**	**33.1**	**77.6**		**Mean**	**10.4***	**28.6**	**24.2**	**63.2**
	SD	**7.1**	**7.4**	**6.0**	**16.3**		**SD**	**8.8**	**6.6**	**9.5**	**16.5**
14 mg/kg	18-0637	22.3	34.7	13.3	70.3	**111 mg/kg**	18-0655	4.8	40.2	30.9	75.9
	18-0638	19.6	35.8	19.6	75.0		18-0656	8.9	29.7	21.0	59.6
	18-0677	19.1	36.6	28.3	84.0		18-0645	15.4	28.4	15.4	59.2
	18-0678	18.7	27.2	29.7	75.6		18-0646	19.4	41.6	19.1	80.1
	18-0679	26.9	22.0	4.6	53.5		18-0673	5.1	22.8	19.5	47.4
	18-0680	29.5	31.5	36.0	97.0		18-0674	11.1	31.3	19.2	61.6
	18-0643	16.4	24.4	17.0	57.8		18-0669	11.7	32.9	24.1	68.7
	18-0644	20.7	28.6	28.0	77.3		18-0670	6.1	22.0	8.7	36.8
	18-0629	23.2	32.6	37.4	93.2		18-0667	7.5	21.3	13.1	41.9
	18-0630	17.9	28.0	35.1	81.0		18-0668	9.6	22.4	26.3	58.3
	Mean	**21.4**	**30.1**	**24.9**	**76.5**		**Mean**	**10.0***	**29.3**	**19.7***	**59.0**
	SD	**4.1**	**4.9**	**10.9**	**13.7**		**SD**	**4.7**	**7.4**	**6.4**	**14.0**
28 mg/kg	18-0653	19.8	55.3	23.0	98.1	**222 mg/kg**	18-0631	-0.2	29.3	14.5	43.6
	18-0654	16.5	39.9	31.2	87.6		18-0632	-9.8	37.4	26.5	54.1
	18-0641	16.3	23.4	22.5	62.2		18-0647	18.2	31.6	23.3	73.1
	18-0642	10.5	27.5	15.2	53.2		18-0648	8.1	28.1	40.2	76.4
	18-0665	13.8	19.3	14.1	47.2		18-0621	8.6	31.8	10.6	51.0
	18-0666	20.0	38.9	29.2	88.1		18-0622	3.4	24.6	12.7	40.7
	18-0663	23.6	22.8	23.2	69.6		18-0659	-1.8	30.3	15.6	44.1
	18-0664	16.7	32.7	5.6	55.0		18-0660	-3.9	22.4	11.9	30.4
	18-0623	18.0	27.3	8.8	54.1		18-0683	-14.7	26.8	15.8	27.9
	18-0624	21.0	15.7	3.2	39.9		18-0684				
	Mean	**17.6**	**30.3**	**17.6***	**65.5**		**Mean**	**0.9***	**29.1**	**19.0***	**49.0***
	SD	**3.8**	**11.8**	**9.7**	**19.6**		**SD**	**10.0**	**4.4**	**9.5**	**16.9**

Note:
Data from day 0-3 lacked normality (p=0.042). Rank-transformed data was normally distributed, with equal error variances. *p<0.05 from control group based on one-way ANOVA and Dunnett (2-sided) post hoc test.

APPENDIX H

INDIVIDUAL AND SUMMARY OF FOOD CONSUMPTION

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

Dose Group	Animal Pair ID	Day 0-3	Day 3-7	Day 7-13	Day 0-13
0 mg/kg	18-0723/0724	99.8	134.9	191.7	426.4
	18-0697/0698	83.7	114.5	158.9	357.1
	18-0717/0718	96.0	127.8	159.2	383.0
	18-0719/0720	85.8	108.1	147.7	341.6
	18-0731/0732	78.8	116.1	164.0	358.9
	Mean	88.8	120.3	164.3	373.4
	SD	8.8	10.8	16.4	33.1
14 mg/kg	18-0705/0706	96.0	136.6	180.2	412.8
	18-0687/0688	90.3	129.3	167.5	387.1
	18-0735/0736	95.6	126.2	164.2	386.0
	18-0743/0744	92.5	126.4	147.4	366.3
	18-0701/0702	89.0	120.7	129.3	339.0
	Mean	92.7	127.8	157.7	378.2
	SD	3.1	5.8	19.7	27.5
28 mg/kg	18-0699/0700	104.5	145.6	191.0	441.1
	18-0693/0694	94.1	115.7	146.3	356.1
	18-0713/0714	87.5	128.3	130.1	345.9
	18-0707/0708	87.2	128.9	155.2	371.3
	18-0691/0692	76.7	105.2	121.1	303.0
	Mean	90.0	124.7	148.7	363.5
	SD	10.2	15.2	27.1	50.3
56 mg/kg	18-0733/0734	99.1	136.8	163.7	399.6
	18-0715/0716	104.8	140.5	175.6	420.9
	18-0703/0704	94.6	126.5	150.4	371.5
	18-0729/0730	74.6	109.1	112.0	295.7
	18-0745/0746	94.1	120.3	125.5	339.9
	Mean	93.4	126.6	145.4	365.5
	SD	11.4	12.7	26.4	49.5
111 mg/kg	18-0689/0690	95.3	144.8	175.3	415.4
	18-0739/0740	91.3	126.0	157.5	374.8
	18-0737/0738	93.7	126.0	151.1	370.8
	18-0741/0742	80.7	120.0	128.1	328.8
	18-0695/0696	79.5	109.2	143.0	331.7
	Mean	88.1	125.2	151.0	364.3
	SD	7.5	12.9	17.5	35.7
222 mg/kg	18-0709/0710	90.8	124.5	159.3	374.6
	18-0727/0728	95.0	113.7	156.9	365.6
	18-0711/0712	83.3	121.8	135.7	340.8
	18-0725/0726	85.0	124.4	139.7	349.1
	18-0721/0722	53.8	120.7	138.2	312.7
	Mean	81.6	121.0	146.0	348.6
	SD	16.2	4.4	11.2	24.1

Note:
Data were normally distributed with equal error variances. No significant effects of dose were observed via repeated measures ANOVA.

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

Dose Group	Animal Pair ID	Day 0-3	Day 3-7	Day 7-13	Day 0-13
0 mg/kg	18-0649/0650	130.1	198.9	280.4	609.4
	18-0685/0686	166.5	222.9	311.8	701.2
	18-0671/0672	156.0	201.9	277.8	635.7
	18-0675/0676	130.3	161.2	233.6	525.1
	18-0657/0658	135.8	181.0	258.2	575.0
	Mean	143.7	193.2	272.4	609.3
	SD	16.6	23.3	29.0	66.0
14 mg/kg	18-0637/0638	150.0	205.4	203.7	559.1
	18-0677/0678	135.2	201.9	215.1	552.2
	18-0679/0680	151.9	192.7	204.2	548.8
	18-0643/0644	134.0	186.3	196.8	517.1
	18-0629/0630	139.1	178.1	225.6	542.8
	Mean	142.0	192.9	209.1	544.0
	SD	8.4	11.2	11.3	16.2
28 mg/kg	18-0653/0654	159.6	245.7	252.5	657.8
	18-0641/0642	139.5	172.8	193.3	505.6
	18-0665/0666	138.0	201.8	213.8	553.6
	18-0663/0664	129.9	182.3	189.2	501.4
	18-0623/0624	123.6	157.9	165.8	447.3
	Mean	138.1	192.1	202.9	533.1
	SD	13.6	33.9	32.5	79.2
56 mg/kg	18-0661/0662	116.8	186.7	212.6	516.1
	18-0651/0652	121.4	160.0	181.3	462.7
	18-0635/0636	136.8	194.8	242.0	573.6
	18-0633/0634	96.4	162.3	192.4	451.1
	18-0681/0682	118.8	176.3	202.3	497.4
	Mean	118.0	176.0	206.1	500.2
	SD	14.4	15.1	23.2	48.6
111 mg/kg	18-0655/0656	125.4	208.1	245.0	578.5
	18-0645/0646	140.6	195.9	221.2	557.7
	18-0673/0674	124.8	183.7	208.6	517.1
	18-0669/0670	115.0	174.2	186.0	475.2
	18-0667/0668	108.0	164.5	180.8	453.3
	Mean	122.8	185.3	208.3	516.4
	SD	12.3	17.3	26.3	53.0
222 mg/kg	18-0631/0632	113.5	181.0	228.1	522.6
	18-0647/0648	145.9	198.3	257.3	601.5
	18-0621/0622	114.8	181.9	204.2	500.9
	18-0659/0660	112.1	162.8	211.5	486.4
	†18-0683/0684	81.0	190.6	204.6	476.2
	Mean	113.5	182.9	221.1	517.5
	SD	23.0	13.3	22.4	50.1

Note:
†Rat 18-0683 lost 27.5 g between day -3 and day 0 and was found dead on the morning of day 2. It was assumed that this rat did not contribute to the paired food consumption during day 0-3, and the food consumed in that cage was doubled to reflect the projected consumption by two rats. No significant effect of dose was observed via repeated measures ANOVA, regardless of whether or not these data were included. Data showed normal distribution with equal error variances.

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	18-0723/0724	0.271	0.268	0.183	0.230
	18-0697/0698	0.177	0.209	0.180	0.188
	18-0717/0718	0.173	0.221	0.217	0.207
	18-0719/0720	0.239	0.226	0.206	0.220
	18-0731/0732	0.255	0.181	0.159	0.187
	Mean	0.223	0.221	0.189	0.207
	SD	0.045	0.031	0.023	0.019
14 mg/kg	18-0705/0706	0.207	0.275	0.290	0.266
	18-0687/0688	0.142	0.309	0.195	0.221
	18-0735/0736	0.310	0.252	0.264	0.272
	18-0743/0744	0.372	0.214	0.179	0.240
	18-0701/0702	0.374	0.180	0.163	0.224
	Mean	0.281	0.246	0.218	0.244
	SD	0.103	0.051	0.056	0.023
28 mg/kg	18-0699/0700	0.156	0.261	0.297	0.252
	18-0693/0694	0.333	0.188	0.215	0.237
	18-0713/0714	0.160	0.315	0.069	0.183
	18-0707/0708	0.305	0.196	0.206	0.226
	18-0691/0692	0.283	0.229	0.207	0.234
	Mean	0.247	0.238	0.199	0.226
	SD	0.083	0.052	0.082	0.026
56 mg/kg	18-0733/0734	0.353	0.306	0.164	0.260
	18-0715/0716	0.200	0.318	0.190	0.235
	18-0703/0704	0.317	0.274	0.162	0.240
	18-0729/0730	0.145	0.215	0.083	0.147
	18-0745/0746	0.327	0.208	0.097	0.200
	Mean	0.269	0.264	0.139	0.216
	SD	0.091	0.051	0.047	0.044
111 mg/kg	18-0689/0690	0.194	0.306	0.201	0.236
	18-0739/0740	0.317	0.307	0.183	0.257
	18-0737/0738	0.328	0.244	0.159	0.230
	18-0741/0742	0.144	0.264	0.144	0.188
	18-0695/0696	0.182	0.176	0.248	0.209
	Mean	0.233	0.259	0.187	0.224
	SD	0.084	0.054	0.041	0.027
222 mg/kg	18-0709/0710	0.215	0.179	0.217	0.204
	18-0727/0728	0.326	0.197	0.143	0.207
	18-0711/0712	0.218	0.236	0.049	0.158
	18-0725/0726	0.416	0.200	0.102	0.214
	18-0721/0722	0.043	0.310	0.068	0.157
	Mean	0.244	0.225	0.116	0.188
	SD	0.140	0.052	0.067	0.028

Note: Data were normally distributed with equal variance. No significant effect of dose was observed via repeated measures ANOVA.

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	18-0649/0650	0.166	0.258	0.236	0.228
	18-0685/0686	0.360	0.241	0.248	0.272
	18-0671/0672	0.326	0.240	0.253	0.267
	18-0675/0676	0.300	0.161	0.230	0.226
	18-0657/0658	0.328	0.273	0.244	0.273
	Mean	0.296	0.235	0.242	0.253
	SD	0.076	0.044	0.009	0.024
14 mg/kg	18-0637/0638	0.279	0.343	0.162	0.260
	18-0677/0678	0.280	0.316	0.270	0.289
	18-0679/0680	0.371	0.278	0.199	0.274
	18-0643/0644	0.277	0.284	0.229	0.261
	18-0629/0630	0.295	0.340	0.321	0.321
	Mean	0.301	*0.312	0.236	0.281
	SD	0.040	0.031	0.062	0.025
28 mg/kg	18-0653/0654	0.227	0.387	0.215	0.282
	18-0641/0642	0.192	0.295	0.195	0.228
	18-0665/0666	0.245	0.288	0.203	0.244
	18-0663/0664	0.310	0.304	0.152	0.249
	18-0623/0624	0.316	0.272	0.072	0.210
	Mean	0.258	0.309	*0.167	0.243
	SD	0.054	0.045	0.058	0.027
56 mg/kg	18-0661/0662	0.160	0.348	0.205	0.247
	18-0651/0652	0.203	0.227	0.185	0.204
	18-0635/0636	0.248	0.333	0.278	0.290
	18-0633/0634	-0.098	0.398	0.264	0.235
	18-0681/0682	0.302	0.314	0.228	0.276
	Mean	0.163	*0.324	0.232	0.250
	SD	0.155	0.062	0.039	0.034
111 mg/kg	18-0655/0656	0.109	0.336	0.212	0.234
	18-0645/0646	0.248	0.357	0.156	0.250
	18-0673/0674	0.130	0.295	0.186	0.211
	18-0669/0670	0.155	0.315	0.176	0.222
	18-0667/0668	0.158	0.266	0.218	0.221
	Mean	*0.160	*0.314	0.190	0.228
	SD	0.053	0.036	0.026	0.015
222 mg/kg	18-0631/0632	-0.088	0.369	0.180	0.187
	18-0647/0648	0.180	0.301	0.247	0.249
	18-0621/0622	0.105	0.310	0.114	0.183
	18-0659/0660	-0.051	0.324	0.130	0.153
	†18-0684	-0.363	0.281	0.154	0.117
	Mean	*-0.043	*0.317	*0.165	*0.178
	SD	0.210	0.033	0.052	0.048

Notes:
†Data are based on an individual rat; the paired rat was found dead prior to day 2 and was assumed to have not consumed food prior to death. Data were rank-transformed to obtain a normal distribution.
*p<0.05 compared to control based on repeated measures ANOVA for main effect of dose, followed by one-way ANOVA and Dunnett t (2-sided) post hoc test for each time period.

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	18-0697	210.3	1.860	1.648	0.064	8.182	0.809	0.466	0.689	0.129	0.398
	18-0698	205.9	2.040	1.736	0.065	8.448	0.857	0.510	0.525	0.119	0.404
	18-0723	218.3	1.992	1.739	0.074	8.560	0.930	0.590	0.688	0.114	0.465
	18-0724	228.7	1.952	1.676	0.067	9.235	1.196	0.478	0.743	0.129	0.569
	18-0717	201.3	1.817	1.884	0.067	9.098	1.037	0.504	0.527	0.140	0.494
	18-0718	225.6	1.995	1.723	0.055	8.923	0.891	0.481	0.487	0.141	0.667
	18-0719	192.1	1.862	1.645	0.061	7.862	0.802	0.443	0.673	0.111	0.805
	18-0720	197.9	1.823	1.686	0.081	8.337	1.052	0.427	0.428	0.133	0.620
	18-0731	192.8	1.898	1.749	0.076	7.516	1.018	0.573	0.633	0.117	0.735
	18-0732	198.9	1.878	1.676	0.072	7.259	0.864	0.416	0.479	0.119	0.539
	Mean	207.2	1.912	1.716	0.068	8.342	0.946	0.489	0.587	0.125	0.570
	SD	13.2	0.078	0.070	0.008	0.656	0.127	0.058	0.110	0.011	0.137
14 mg/kg	18-0687	214.4	1.847	1.404	0.049	8.418	0.707	0.293	0.411	0.101	0.936
	18-0688	204.0	1.996	1.386	0.031†	8.016	0.805	0.346	0.506	0.089	1.015
	18-0705	207.5	1.985	1.385	0.043	7.877	0.670	0.394	0.565	0.136	0.602
	18-0706	245.7	1.912	1.673	0.044	9.137	0.738	0.361	0.493	0.120	0.843
	18-0735	222.5	1.891	1.566	0.032	8.666	0.758	0.327	0.579	0.118	0.622
	18-0736	209.0	2.005	1.457	0.039	8.195	0.708	0.349	0.576	0.123	0.559
	18-0743	191.5	2.015	1.424	0.056	7.610	0.655	0.326	0.489	0.129	0.574
	18-0744	216.4	2.006	1.513	0.058	8.648	0.772	0.362	0.583	0.108	0.625
	18-0701	188.0	2.017	1.366	0.059	6.882	0.748	0.344	0.577	0.117	0.611
	18-0702	216.6	2.007	1.576	0.044	8.833	0.793	0.346	0.617	0.133	0.585
	Mean	211.6	1.968	1.475*	0.047*	8.228	0.735*	0.345*	0.540	0.117	0.697
	SD	16.3	0.061	0.103	0.009	0.663	0.050	0.027	0.063	0.015	0.168
28 mg/kg	18-0693	190.1	1.962	1.422	0.044	7.391	0.619	0.339	0.520	0.116	0.455
	18-0694	216.8	1.838	1.489	0.059	8.601	0.703	0.377	0.533	0.099	0.626
	18-0699	256.5	1.968	1.616	0.059	10.289	0.802	0.494	0.793	0.130	0.619
	18-0700	202.0	2.025	1.477	0.037	7.788	0.733	0.406	0.645	0.117	0.661
	18-0713	196.2	2.036	1.374	0.033	7.253	0.727	0.330	0.508	0.115	0.357
	18-0714	203.0	1.986	1.373	0.047	7.917	0.786	0.380	0.565	0.113	0.606
	18-0691	194.7	1.710	1.392	0.027	6.761	0.694	0.263	0.317	0.107	0.479
	18-0692	186.1	1.885	1.541	0.041	7.606	0.796	0.466	0.583	0.106	0.777
	18-0707	188.1	1.814	1.291	0.055	5.914	0.722	0.374	0.398	0.117	0.443
	18-0708	235.0	1.910	1.706	0.043	8.865	0.866	0.414	0.537	0.123	0.629
	Mean	206.9	1.913	1.468*	0.045*	7.839*	0.745*	0.384*	0.540	0.114	0.565
	SD	22.9	0.103	0.125	0.011	1.207	0.069	0.067	0.129	0.009	0.127
56 mg/kg	18-0715	211.8	1.927	1.431	0.043	7.692	0.750	0.345	0.573	0.105	0.796
	18-0716	230.6	1.839	1.739	0.043	9.389	0.766	0.371	0.516	0.192	0.624
	18-0733	216.3	2.038	1.592	0.047	7.925	0.767	0.365	0.564	0.096	0.545
	18-0734	220.4	1.906	1.660	0.041	8.699	0.785	0.362	0.414	0.112	0.598
	18-0703	196.8	1.914	1.371	0.042	7.610	0.883	0.364	0.541	0.125	0.551
	18-0704	228.1	1.928	1.580	0.044	9.254	0.794	0.514	0.463	0.105	0.524
	18-0729	186.8	1.747	1.425	0.042	6.636	0.714	0.301	0.446	0.119	0.432
	18-0730	194.2	1.816	1.314	0.052	8.207	0.713	0.329	0.527	0.091	0.491
	18-0745	196.6	1.825	1.347	0.043	7.732	0.729	0.344	0.723	0.098	0.465
	18-0746	194.5	1.757	1.486	0.042	7.914	0.729	0.400	0.445	0.110	0.776
	Mean	207.6	1.870	1.495*	0.044*	8.106	0.763*	0.370*	0.521	0.115	0.580
	SD	15.7	0.089	0.142	0.003	0.824	0.051	0.057	0.089	0.029	0.123

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
111 mg/kg	18-0689	249.7	1.382	1.562	0.058	10.233	0.843	0.514	0.664	0.126	0.687
	18-0690	211.3	1.961	1.437	0.049	8.582	0.744	0.367	0.419	0.111	0.432
	18-0737	226.0	1.987	1.571	0.035	9.552	0.781	0.373	0.545	0.147	0.861
	18-0738	203.5	1.844	1.297	0.038	7.763	0.773	0.407	0.333	0.135	0.398
	18-0739	222.0	1.976	1.487	0.064	8.979	0.834	0.416	0.521	0.098	0.523
	18-0740	210.9	1.951	1.582	0.060	8.267	0.750	0.475	0.562	0.111	0.648
	18-0695	163.7	1.688	1.088	0.038	7.289	0.706	0.328	0.401	0.103	0.512
	18-0696	206.5	1.945	1.486	0.044	8.298	0.778	0.440	0.500	0.141	0.864
	18-0741	204.2	1.778	1.448	0.054	9.238	0.832	0.408	0.469	0.114	0.450
	18-0742	195.4	1.893	1.270	0.047	8.609	0.802	0.351	0.459	0.100	0.426
	Mean	209.3	1.841	1.423*	0.049*	8.681	0.784*	0.408*	0.487	0.119	0.580
	SD	22.2	0.188	0.159	0.010	0.862	0.044	0.057	0.093	0.018	0.176
222 mg/kg	18-0709	192.6	1.688	1.313	0.048	8.414	0.989	0.336	0.411	0.119	0.453
	18-0710	234.8	1.928	1.657	0.039	11.269	0.952	0.433	0.459	0.132	0.571
	18-0711	202.4	1.896	1.599	0.039	10.345	0.804	0.333	0.489	0.120	0.453
	18-0712	187.6	1.899	1.372	0.031	7.751	0.661	0.425	0.591	0.120	0.477
	18-0727	195.2	1.869	1.538	0.058	8.488	0.760	0.351	0.522	0.141	0.378
	18-0728	221.9	1.944	1.544	0.039	9.364	0.814	0.441	0.573	0.149	0.773
	18-0721	186.8	1.826	1.415	0.037	7.674	0.736	0.382	0.346	0.098	1.011
	18-0722	190.2	1.873	1.274	0.044	7.950	0.717	0.346	0.449	0.120	0.814
	18-0725	222.9	1.921	1.555	0.055	9.454	0.860	0.442	0.664	0.125	0.640
	18-0726	188.8	1.887	1.455	0.046	7.993	0.829	0.309	0.391	0.106	0.482
	Mean	202.3	1.873	1.472*	0.044*	8.870*	0.812*	0.380*	0.490	0.123	0.605
	SD	17.6	0.073	0.127	0.008	1.210	0.102	0.051	0.099	0.015	0.202

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, ovaries, and uterus were log-transformed, and heart and adrenals were rank-transformed to achieve normal distribution of data. After rank-transformation of adrenal masses, error variances significantly differed from equality, based on Levene's test.
†Right adrenal gland partially missing. Considered an outlier due to processing damage and not included in analysis.

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	18-0649	418.0	2.186	3.347	0.073	16.082	1.480	0.740	0.588	3.762	0.882
	18-0650	352.4	2.072	2.519	0.040	11.883	1.387	0.703	0.500	3.168	0.904
	18-0671	402.7	2.043	2.739	0.065	14.027	1.306	0.651	0.556	3.076	0.874
	18-0672	371.9	1.994	2.731	0.066	13.987	1.393	0.772	0.650	3.049	0.894
	18-0685	414.8	2.305	3.041	0.072	14.419	1.469	0.789	0.659	3.433	0.905
	18-0686	400.3	2.021	2.551	0.063	12.050	1.269	0.923	0.833	2.766	0.764
	18-0657	356.8	2.101	2.625	0.069	13.460	1.198	0.703	0.564	3.168	0.879
	18-0658	390.6	2.115	3.188	0.072	14.972	1.521	0.865	0.801	3.661	0.966
	18-0675	370.1	1.995	2.322	0.049	11.479	1.474	0.678	0.581	2.992	0.771
	18-0676	347.8	2.052	2.565	0.058	10.548	1.446	0.666	0.675	3.314	0.982
	Mean	382.5	2.088	2.763	0.063	13.291	1.394	0.749	0.641	3.239	0.882
	SD	26.1	0.096	0.326	0.011	1.741	0.105	0.089	0.107	0.308	0.070
14 mg/kg	18-0637	391.5	2.260	2.236	0.040	12.742	1.051	0.505	0.693	3.432	0.912
	18-0638	386.4	2.128	2.522	0.044	14.961	1.171	0.521	0.801	3.573	0.813
	18-0677	410.7	2.135	2.461	0.045	15.606	1.242	0.567	0.818	3.543	0.864
	18-0678	388.1	2.057	2.203	0.050	13.598	1.165	0.617	0.688	3.571	0.872
	18-0679	373.7	2.114	2.485	0.056	12.809	1.289	0.554	0.400	3.782	1.000
	18-0680	389.7	1.949	2.396	0.066	13.982	1.221	0.606	0.661	2.938	0.851
	18-0629	420.4	2.183	2.375	0.067	13.595	1.073	0.559	0.577	3.444	0.849
	18-0630	354.8	2.179	2.061	0.042	11.765	1.084	0.815	0.569	3.295	1.016
	18-0643	355.5	2.069	2.162	0.051	11.656	1.186	0.624	0.541	2.964	0.813
	18-0644	399.9	2.150	2.548	0.050	14.541	1.138	0.611	0.519	3.073	0.836
	Mean	387.1	2.122	2.345*	0.051*	13.526	1.162*	0.598*	0.627	3.362	0.883
	SD	21.3	0.084	0.168	0.009	1.306	0.077	0.087	0.130	0.286	0.072
28 mg/kg	18-0641	369.2	2.228	2.240	0.044	12.256	1.101	0.558	0.675	3.650	0.918
	18-0642	366.5	2.052	2.284	0.043	12.973	1.202	0.476	0.516	4.012	0.914
	18-0653	460.4	2.255	2.644	0.051	15.535	1.437	0.578	0.626	3.469	0.885
	18-0654	412.3	2.055	2.447	0.052	14.831	1.201	0.485	0.523	3.602	0.915
	18-0665	342.7	1.978	2.034	0.044	10.535	0.995	0.488	0.431	4.844	0.885
	18-0666	418.8	2.230	2.478	0.038	14.927	1.333	0.596	0.752	3.507	0.807
	18-0623	354.9	2.066	2.126	0.044	10.633	0.975	0.405	0.586	3.198	0.842
	18-0624	312.5	1.938	10.170†	0.044	12.631	1.279	0.629	0.453	3.176	0.883
	18-0663	350.0	1.987	2.216	0.046	10.586	1.155	0.443	0.609	3.389	0.852
	18-0664	372.0	2.081	2.179	0.052	12.376	1.113	0.500	0.519	3.085	0.839
	Mean	375.9	2.087	2.294*	0.046*	12.728	1.179*	0.516*	0.569	3.593	0.874
	SD	43.1	0.113	0.193	0.005	1.866	0.144	0.072	0.100	0.516	0.038
56 mg/kg	18-0651	355.4	2.059	2.317	0.044	13.382	1.085	0.614	0.735	3.181	0.822
	18-0652	348.0	1.927	2.291	0.048	12.078	1.021	0.488	0.535	3.041	0.731
	18-0661	356.3	2.032	2.206	0.053	11.881	1.375	0.555	0.517	3.491	0.902
	18-0662	408.2	2.045	2.554	0.049	13.497	1.247	0.650	0.954	3.096	0.772
	18-0635	399.1	2.073	2.352	0.045	14.047	1.119	0.590	0.577	3.484	0.879
	18-0636	381.8	2.195	2.340	0.040	14.447	1.008	0.540	0.759	2.276	0.697
	18-0633	360.9	2.162	2.280	0.050	12.506	1.059	0.577	0.569	3.178	0.795
	18-0634	354.6	2.126	2.442	0.049	13.455	1.176	0.449	0.582	3.372	0.834
	18-0681	395.2	2.168	2.386	0.053	14.400	1.262	0.644	0.583	3.494	1.046
	18-0682	327.0	2.153	2.443	0.042	10.746	1.054	0.460	0.484	3.249	0.827
	Mean	368.7	2.094	2.361*	0.047*	13.044	1.141*	0.557*	0.630	3.186	0.831
	SD	26.1	0.082	0.100	0.004	1.209	0.121	0.072	0.144	0.361	0.098

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
111 mg/kg	18-0645	371.5	1.999	2.436	0.049	12.597	1.197	0.583	0.542	3.614	0.936
	18-0646	395.0	2.014	2.409	0.042	14.066	1.181	0.574	0.659	3.599	0.905
	18-0655	418.9	2.240	2.538	0.018[#]	14.969	1.264	0.717	0.767	3.968	1.011
	18-0656	367.9	1.942	2.418	0.049	12.994	1.086	0.471	0.609	3.013	0.748
	18-0669	397.0	2.092	2.422	0.051	13.608	1.204	0.619	0.522	3.373	0.930
	18-0670	323.3	2.019	2.013	0.046	12.213	0.916	0.491	0.562	2.861	0.779
	18-0673	350.6	2.131	2.790	0.046	13.254	1.158	0.497	0.615	3.597	0.827
	18-0674	377.7	2.051	2.355	0.055	13.544	1.082	0.615	0.715	3.058	0.769
	18-0667	339.6	2.031	2.197	0.024	10.923	1.114	0.546	0.594	2.981	0.776
	18-0668	363.8	2.192	2.360	0.041	12.615	1.105	0.631	0.513	3.248	0.853
	Mean	370.5	2.071	2.394*	0.045*	13.078	1.131*	0.574*	0.610	3.331	0.853
	SD	28.5	0.093	0.202	0.009	1.102	0.096	0.076	0.083	0.358	0.089
222 mg/kg	18-0631	361.3	2.210	2.671	0.035	12.285	1.429	0.495	0.611	3.555	0.795
	18-0632	377.6	2.167	2.428	0.041	14.611	1.179	0.627	0.848	3.189	0.856
	18-0647	400.4	2.152	2.294	0.044	14.659	1.020	0.617	0.642	3.160	0.906
	18-0648	369.5	2.027	2.333	0.040	14.935	1.085	0.625	0.722	3.115	0.667
	18-0621	362.5	2.026	1.964	0.042	14.304	1.072	0.592	0.623	3.231	0.826
	18-0622	341.8	2.028	2.379	0.032	13.401	1.183	0.701	0.759	3.173	0.848
	18-0659	342.4	1.960	2.361	0.044	13.021	1.122	0.728	0.658	3.198	0.832
	18-0660	334.4	2.150	2.288	0.041	11.879	1.089	0.641	0.535	3.376	0.908
	18-0683	341.6	1.894	2.523	0.038	12.608	1.253	0.453	0.775	3.136	0.846
	18-0684[‡]										
	Mean	359.1	2.068	2.360*	0.040*	13.523	1.159*	0.609*	0.686	3.237	0.832
	SD	21.4	0.106	0.192	0.004	1.141	0.123	0.088	0.097	0.141	0.072

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 heart, adrenals, and kidneys were log-transformed to achieve normal distribution of data. For liver mass, error variances significantly differed from equality, based on Levene's test.
[†]Kidneys from 18-0624 were noted to be enlarged and polycystic. This effect was not considered to be compound-related, and this mass was excluded from analyses.
[#]18-0655 adrenal mass was obtained for one gland rather than two. The mass of the one adrenal was reported but not included in analyses.
[‡]Rat 18-0684 died shortly after initiation of dosing, and no organs were collected at necropsy.

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

		DNP in Corn Oil					
	Sex	0 mg/kg	14 mg/kg	28 mg/kg	56 mg/kg	111 mg/kg	222 mg/kg
Fasted Body Weight	Females	207.3±13.2	211.6±16.3	206.9±22.9	207.6±15.7	209.3±22.2	202.3±17.6
	N	10	10	10	10	10	10
	Males	382.5±26.1	387.1±21.3	375.9±43.1	368.7±26.1	370.5±28.5	359.1±21.4
	N	10	10	10	10	10	9
Brain	Females	1.912±0.078	1.968±0.061	1.913±0.103	1.870±0.089	1.841±0.188	1.873±0.073
	N	10	10	10	10	10	10
	Males	2.088±0.096	2.122±0.084	2.087±0.113	2.094±0.082	2.071±0.093	2.068±0.106
	N	10	10	10	10	10	9
Kidneys (2)	Females	1.716±0.070	1.475±0.103*	1.468±0.125*	1.495±0.142*	1.423±0.159*	1.472±0.127*
	N	10	10	10	10	10	10
	Males	2.763±0.326	2.345±0.168*	2.294±0.193*	2.361±0.100*	2.394±0.202*	2.360±0.192*
	N	10	10	9	10	10	9
Adrenals (2)	Females	0.068±0.008	0.047±0.009*	0.045±0.011*	0.044±0.003*	0.049±0.010*	0.044±0.008*
	N	10	9	10	10	10	10
	Males	0.063±0.011	0.051±0.009*	0.046±0.005*	0.047±0.004*	0.045±0.009*	0.040±0.004*
	N	10	10	10	10	9	9
Liver	Females	8.342±0.656	8.228±0.663	7.839±1.207*	8.106±0.824	8.681±0.862	8.870±1.210*
	N	10	10	10	10	10	10
	Males	13.291±1.741	13.526±1.306	12.728±1.866	13.044±1.209	13.078±1.102	13.523±1.141
	N	10	10	10	10	10	9
Heart	Females	0.946±0.127	0.735±0.050*	0.745±0.069*	0.763±0.051*	0.784±0.044*	0.812±0.102*
	N	10	10	10	10	10	10
	Males	1.349±0.105	1.162±0.077*	1.179±0.144*	1.141±0.121*	1.131±0.096*	1.159±0.123*
	N	10	10	10	10	10	9
Spleen	Females	0.489±0.058	0.345±0.027*	0.384±0.067*	0.370±0.057*	0.408±0.057*	0.380±0.051*
	N	10	10	10	10	10	10
	Males	0.749±0.089	0.598±0.087*	0.516±0.072*	0.557±0.072*	0.574±0.076*	0.609±0.088*
	N	10	10	10	10	10	9
Thymus	Females	0.587±0.110	0.540±0.063	0.540±0.129	0.521±0.089	0.487±0.093	0.490±0.099
	N	10	10	10	10	10	10
	Males	0.641±0.107	0.627±0.130	0.569±0.100	0.630±0.144	0.610±0.083	0.686±0.097
	N	10	10	10	10	10	9
Ovaries (2)	Females	0.125±0.011	0.117±0.015	0.114±0.009	0.115±0.029	0.119±0.018	0.123±0.015
	N	10	10	10	10	10	10
Uterus	Females	0.570±0.137	0.697±0.168	0.565±0.127	0.580±0.123	0.580±0.176	0.605±0.202
	N	10	10	10	10	10	10
Testes (2)	Males	3.239±0.308	3.362±0.286	3.593±0.516	3.186±0.361	3.331±0.358	3.237±0.141
	N	10	10	10	10	10	9
Epididymides	Males	0.882±0.070	0.883±0.072	0.874±0.038	0.831±0.098	0.853±0.089	0.832±0.072
	N	10	10	10	10	10	9

Note:
*p<0.05 versus 0 mg/kg 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	18-0697	8.845	7.836	0.304	38.906	3.847	2.216	3.276	0.613	1.893
	18-0698	9.908	8.431	0.316	41.030	4.162	2.477	2.550	0.578	1.962
	18-0723	9.125	7.966	0.339	39.212	4.260	2.703	3.152	0.522	2.130
	18-0724	8.535	7.328	0.293	40.380	5.230	2.090	3.249	0.564	2.488
	18-0717	9.026	9.359	0.333	45.196	5.152	2.504	2.618	0.695	2.454
	18-0718	8.843	7.637	0.244	39.552	3.949	2.132	2.159	0.625	2.957
	18-0719	9.693	8.563	0.318	40.927	4.175	2.306	3.503	0.578	4.191
	18-0720	9.212	8.519	0.409	42.127	5.316	2.158	2.163	0.672	3.133
	18-0731	9.844	9.072	0.394	38.983	5.280	2.972	3.283	0.607	3.812
	18-0732	9.442	8.426	0.362	36.496	4.344	2.092	2.408	0.598	2.710
	Mean	9.247	8.314	0.331	40.281	4.571	2.365	2.836	0.605	2.773
	SD	0.463	0.630	0.049	2.312	0.597	0.296	0.509	0.051	0.766
14 mg/kg	18-0687	8.615	6.549	0.229	39.263	3.298	1.367	1.917	0.471	4.366
	18-0688	9.784	6.794	ND	39.294	3.946	1.696	2.480	0.436	4.975
	18-0705	9.566	6.675	0.207	37.961	3.229	1.899	2.723	0.655	2.901
	18-0706	7.782	6.809	0.179	37.188	3.004	1.469	2.007	0.488	3.431
	18-0735	8.499	7.038	0.144	38.948	3.407	1.470	2.602	0.530	2.796
	18-0736	9.593	6.971	0.187	39.211	3.388	1.670	2.756	0.589	2.675
	18-0743	10.522	7.436	0.292	39.739	3.420	1.702	2.554	0.674	2.997
	18-0744	9.270	6.992	0.268	39.963	3.567	1.673	2.694	0.499	2.888
	18-0701	10.729	7.266	0.314	36.606	3.979	1.830	3.069	0.622	3.250
	18-0702	9.266	7.276	0.203	40.780	3.661	1.597	2.849	0.614	2.701
	Mean	9.363	6.981*	0.225*	38.895	3.490*	1.637*	2.565	0.558	3.298
	SD	0.901	0.284	0.056	1.284	0.307	0.166	0.358	0.083	0.774
28 mg/kg	18-0693	10.321	7.480	0.231	38.880	3.256	1.783	2.735	0.610	2.393
	18-0694	8.478	6.868	0.272	39.673	3.243	1.739	2.458	0.457	2.887
	18-0699	7.673	6.300	0.230	40.113	3.127	1.926	3.092	0.507	2.413
	18-0700	10.025	7.312	0.183	38.554	3.629	2.010	3.193	0.579	3.272
	18-0713	10.377	7.003	0.168	36.967	3.705	1.682	2.589	0.586	1.820
	18-0714	9.783	6.764	0.232	39.000	3.872	1.872	2.783	0.557	2.985
	18-0691	8.783	7.149	0.139	34.725	3.564	1.351	1.628	0.550	2.460
	18-0692	10.129	8.280	0.220	40.870	4.277	2.504	3.133	0.570	4.175
	18-0707	9.644	6.863	0.292	31.441	3.838	1.988	2.116	0.622	2.355
	18-0708	8.128	7.260	0.183	37.723	3.685	1.762	2.285	0.523	2.677
	Mean	9.334	7.128*	0.215*	37.795	3.620*	1.862*	2.601	0.556	2.744
	SD	0.984	0.524	0.047	2.829	0.346	0.294	0.497	0.050	0.644
56 mg/kg	18-0715	9.098	6.756	0.203	36.317	3.541	1.629	2.705	0.496	3.758
	18-0716	7.975	7.541	0.186	40.716	3.322	1.609	2.238	0.833	2.706
	18-0733	9.422	7.360	0.217	36.639	3.546	1.687	2.607	0.444	2.520
	18-0734	8.648	7.532	0.186	39.469	3.562	1.642	1.878	0.508	2.713
	18-0703	9.726	6.966	0.213	38.669	4.487	1.850	2.749	0.635	2.800
	18-0704	8.452	6.927	0.193	40.570	3.481	2.253	2.030	0.460	2.297
	18-0729	9.352	7.628	0.225	35.525	3.822	1.611	2.388	0.637	2.313
	18-0730	9.351	6.766	0.268	42.261	3.671	1.694	2.714	0.469	2.528
	18-0745	9.283	6.851	0.219	39.329	3.708	1.750	3.678	0.498	2.365
	18-0746	9.033	7.640	0.216	40.689	3.748	2.057	2.288	0.566	3.990
	Mean	9.034	7.197*	0.213*	39.018	3.689*	1.778*	2.527	0.555	2.799
	SD	0.528	0.375	0.024	2.215	0.315	0.216	0.503	0.119	0.595

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
111 mg/kg	18-0689	5.535	6.256	0.232	40.981	3.376	2.058	2.659	0.505	2.751
	18-0690	9.281	6.801	0.232	40.615	3.521	1.737	1.983	0.525	2.044
	18-0737	8.792	6.951	0.155	42.265	3.456	1.650	2.412	0.650	3.810
	18-0738	9.061	6.373	0.187	38.147	3.799	2.000	1.636	0.663	1.956
	18-0739	8.901	6.698	0.288	40.446	3.757	1.874	2.347	0.441	2.356
	18-0740	9.251	7.501	0.284	39.199	3.556	2.252	2.665	0.526	3.073
	18-0695	10.312	6.646	0.232	44.527	4.313	2.004	2.450	0.629	3.128
	18-0696	9.419	7.196	0.213	40.184	3.768	2.131	2.421	0.683	4.184
	18-0741	8.707	7.091	0.264	45.240	4.074	1.998	2.297	0.558	2.204
	18-0742	9.688	6.499	0.241	44.058	4.104	1.796	2.349	0.512	2.180
	Mean	8.895	6.801*	0.233*	41.566	3.772*	1.950*	2.322	0.569	2.769
	SD	1.272	0.389	0.041	2.373	0.309	0.185	0.308	0.081	0.770
222 mg/kg	18-0709	8.764	6.817	0.249	43.686	5.135	1.745	2.134	0.618	2.352
	18-0710	8.211	7.057	0.166	47.994	4.055	1.844	1.955	0.562	2.432
	18-0711	9.368	7.900	0.193	51.112	3.972	1.645	2.416	0.593	2.238
	18-0712	10.123	7.313	0.165	41.317	3.523	2.265	3.150	0.640	2.543
	18-0727	9.575	7.879	0.297	43.484	3.893	1.798	2.674	0.722	1.936
	18-0728	8.761	6.958	0.176	42.199	3.668	1.987	2.582	0.671	3.484
	18-0721	9.775	7.575	0.198	41.081	3.940	2.045	1.852	0.525	5.412
	18-0722	9.848	6.698	0.231	41.798	3.770	1.819	2.361	0.631	4.280
	18-0725	8.618	6.976	0.247	42.414	3.858	1.983	2.979	0.561	2.871
	18-0726	9.995	7.707	0.244	42.336	4.391	1.637	2.071	0.561	2.553
	Mean	9.304	7.288*	0.217*	43.742*	4.021	1.877*	2.417	0.608	3.010
	SD	0.666	0.449	0.044	3.254	0.455	0.195	0.432	0.060	1.084

Legend:
ND = No data analyzed
Notes:
*p<0.05 versus 0 mg/kg control group, based on one-way ANOVA and Dunnett (2-sided) post hoc test. Ratios of uterus, adrenals, liver, and kidneys were log-transformed, and heart was rank-transformed 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	18-0649	5.230	8.007	0.175	38.474	3.541	1.770	1.407	9.000	2.110
	18-0650	5.880	7.148	0.114	33.720	3.936	1.995	1.419	8.990	2.565
	18-0671	5.073	6.802	0.161	34.832	3.243	1.617	1.381	7.638	2.170
	18-0672	5.362	7.343	0.177	37.610	3.746	2.076	1.748	8.198	2.404
	18-0685	5.557	7.331	0.174	34.761	3.541	1.902	1.589	8.276	2.182
	18-0686	5.049	6.373	0.157	30.102	3.170	2.306	2.081	6.910	1.909
	18-0657	5.888	7.357	0.193	37.724	3.358	1.970	1.581	8.879	2.464
	18-0658	5.415	8.162	0.184	38.331	3.894	2.215	2.051	9.373	2.473
	18-0675	5.390	6.274	0.132	31.016	3.983	1.832	1.570	8.084	2.083
	18-0676	5.900	7.375	0.167	30.328	4.158	1.915	1.941	9.528	2.823
	Mean	5.474	7.217	0.163	34.690	3.657	1.960	1.677	8.488	2.318
	SD	0.325	0.612	0.024	3.331	0.337	0.204	0.265	0.819	0.274
14 mg/kg	18-0637	5.773	5.711	0.102	32.547	2.685	1.290	1.770	8.766	2.330
	18-0638	5.507	6.527	0.114	38.719	3.031	1.348	2.073	9.247	2.104
	18-0677	5.198	5.992	0.110	37.999	3.024	1.381	1.992	8.627	2.104
	18-0678	5.300	5.676	0.129	35.037	3.002	1.590	1.773	9.201	2.247
	18-0679	5.657	6.650	0.150	34.276	3.449	1.482	1.070	10.120	2.676
	18-0680	5.001	6.148	0.169	35.879	3.133	1.555	1.696	7.539	2.184
	18-0629	5.193	5.649	0.159	32.338	2.552	1.330	1.373	8.192	2.020
	18-0630	6.141	5.809	0.118	33.160	3.055	2.297	1.604	9.287	2.864
	18-0643	5.820	6.082	0.143	32.788	3.336	1.755	1.522	8.338	2.287
	18-0644	5.376	6.372	0.125	36.362	2.846	1.528	1.298	7.684	2.091
	Mean	5.497	6.062*	0.132*	34.910	3.011*	1.556*	1.617	8.700	2.290
	SD	0.350	0.361	0.023	2.292	0.271	0.297	0.311	0.795	0.274
28 mg/kg	18-0641	6.035	6.067	0.119	33.196	2.982	1.511	1.828	9.886	2.486
	18-0642	5.599	6.232	0.117	35.397	3.280	1.299	1.408	10.947	2.494
	18-0653	4.898	5.743	0.111	33.742	3.121	1.255	1.360	7.535	1.922
	18-0654	4.984	5.935	0.126	35.971	2.913	1.176	1.268	8.736	2.219
	18-0665	5.772	5.935	0.128	30.741	2.903	1.424	1.258	14.135	2.582
	18-0666	5.325	5.917	0.091	35.642	3.183	1.423	1.796	8.374	1.927
	18-0623	5.821	5.990	0.124	29.961	2.747	1.141	1.651	9.011	2.372
	18-0624	6.202	ND	0.141	40.419	4.093	2.013	1.450	10.163	2.826
	18-0663	5.677	6.331	0.131	30.246	3.300	1.266	1.740	9.683	2.434
	18-0664	5.594	5.858	0.140	33.269	2.992	1.344	1.395	8.293	2.255
	Mean	5.591	6.001*	0.123*	33.858	3.151*	1.385*	1.515	9.676	2.352
	SD	0.419	0.184	0.015	3.194	0.375	0.249	0.218	1.865	0.282
56 mg/kg	18-0651	5.793	6.519	0.124	37.653	3.053	1.728	2.068	8.950	2.313
	18-0652	5.537	6.583	0.138	34.707	2.934	1.402	1.537	8.739	2.101
	18-0661	5.703	6.191	0.149	33.345	3.859	1.558	1.451	9.798	2.532
	18-0662	5.010	6.257	0.120	33.065	3.055	1.592	2.337	7.585	1.891
	18-0635	5.194	5.893	0.113	35.197	2.804	1.478	1.446	8.730	2.202
	18-0636	5.749	6.129	0.105	37.839	2.640	1.414	1.988	5.961	1.826
	18-0633	5.991	6.318	0.139	34.652	2.934	1.599	1.577	8.806	2.203
	18-0634	5.995	6.887	0.138	37.944	3.316	1.266	1.641	9.509	2.352
	18-0681	5.486	6.037	0.134	36.437	3.193	1.630	1.475	8.841	2.647
	18-0682	6.584	7.471	0.128	32.862	3.223	1.407	1.480	9.936	2.529
	Mean	5.704	6.429*	0.129*	35.370	3.101*	1.507*	1.700	8.685	2.259
	SD	0.443	0.466	0.013	1.993	0.334	0.138	0.315	1.166	0.272

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
111 mg/kg	18-0645	5.381	6.557	0.132	33.908	3.222	1.569	1.459	9.728	2.520
	18-0646	5.099	6.099	0.106	35.610	2.990	1.453	1.668	9.111	2.291
	18-0655	5.347	6.059	ND	35.734	3.017	1.712	1.831	9.472	2.413
	18-0656	5.279	6.572	0.133	35.319	2.952	1.280	1.655	8.190	2.033
	18-0669	5.270	6.101	0.128	34.277	3.033	1.559	1.315	8.496	2.343
	18-0670	6.245	6.226	0.142	37.776	2.833	1.519	1.738	8.849	2.410
	18-0673	6.078	7.958	0.131	37.804	3.303	1.418	1.754	10.260	2.359
	18-0674	5.430	6.235	0.146	35.859	2.865	1.628	1.893	8.096	2.036
	18-0667	5.981	6.469	0.071	32.164	3.280	1.608	1.749	8.778	2.285
	18-0668	6.025	6.487	0.113	34.676	3.037	1.734	1.410	8.928	2.345
	Mean	5.613	6.476*	0.122*	35.313	3.053*	1.548*	1.647	8.991	2.303
	SD	0.418	0.557	0.023	1.706	0.164	0.138	0.191	0.679	0.157
222 mg/kg	18-0631	6.117	7.393	0.097	34.002	3.955	1.370	1.691	9.839	2.200
	18-0632	5.739	6.430	0.109	38.694	3.122	1.660	2.246	8.445	2.267
	18-0647	5.375	5.729	0.110	36.611	2.547	1.541	1.603	7.892	2.263
	18-0648	5.486	6.314	0.108	40.419	2.936	1.691	1.954	8.430	1.805
	18-0621	5.589	5.418	0.116	39.459	2.957	1.633	1.719	8.913	2.279
	18-0622	5.933	6.960	0.094	39.207	3.461	2.051	2.221	9.283	2.481
	18-0659	5.724	6.895	0.129	38.029	3.277	2.126	1.922	9.340	2.430
	18-0660	6.429	6.842	0.123	35.523	3.257	1.917	1.600	10.096	2.715
	18-0683	5.544	7.386	0.111	36.909	3.668	1.326	2.269	9.180	2.477
	18-0684‡	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	5.771	6.596*	0.111*	37.650	3.242*	1.702	1.914	9.047	2.324
	SD	0.336	0.688	0.011	2.063	0.420	0.281	0.277	0.705	0.251

Legend:
ND = No data analyzed
Notes:
*p<0.05 versus 0 mg/kg control group, based on one-way ANOVA and Dunnett (2-sided) post hoc test.
Ratios of heart, kidney, and spleen masses were rank-transformed to achieve normal distribution of data.
‡Rat 18-0684 died shortly after initiation of dosing, and no organs were collected at necropsy.

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

		DNP in Corn Oil					
	Sex	**0 mg/kg**	**14 mg/kg**	**28 mg/kg**	**56 mg/kg**	**111 mg/kg**	**222 mg/kg**
Brain	Females	9.247±0.463	9.363±0.901	9.334±0.984	9.034±0.528	8.895±1.272	9.304±03666
	N	10	10	10	10	10	10
	Males	5.474±0.325	5.497±0.350	5.592±0.441	5.709±0.500	5.622±0.522	5.771±0.336
	N	10	10	10	10	10	9
Kidneys (2)	Females	8.314±0.630	6.981±0.284*	7.128±0.524*	7.197±0.375*	6.801±0.389*	7.288±0.449*
	N	10	10	10	10	10	10
	Males	7.217±0.612	6.062±0.361*	6.031±0.656*	6.440±0.613*	6.479±0.592*	6.596±0.688*
	N	10	10	9	10	10	9
Adrenals (2)	Females	0.331±0.049	0.225±0.056*	0.215±0.047*	0.213±0.024*	0.233±0.041*	0.217±0.044*
	N	10	9	10	10	10	10
	Males	0.163±0.024	0.132±0.023*	0.124±0.020*	0.129±0.014*	0.121±0.022*	0.111±0.011*
	N	10	10	10	10	9	9
Liver	Females	40.281±2.302	38.895±1.284	37.795±2.829	39.018±2.215	41.566±2.373	43.742±3.254*
	N	10	10	10	10	10	10
	Males	34.690±3.331	34.910±2.292	34.091±5.306	35.429±2.873	35.405±3.171	37.650±2.063
	N	10	10	10	10	10	9
Heart	Females	4.571±0.597	3.490±0.307*	3.620±0.346*	3.689±0.315*	3.772±0.309*	4.021±0.455
	N	10	10	10	10	10	10
	Males	3.657±0.337	3.011±0.271*	3.169±0.511*	3.111±0.419*	3.057±0.230*	3.242±0.420*
	N	10	10	10	10	10	9
Spleen	Females	2.365±0.296	1.637±0.166*	1.862±0.294*	1.778±0.216*	1.950±0.185*	1.877±0.195*
	N	10	10	10	10	10	10
	Males	1.960±0.204	1.556±0.297*	1.387±0.256*	1.514±0.205*	1.554±0.200*	1.702±0.281
	N	10	10	10	10	10	9
Thymus	Females	2.836±0.509	2.565±0.358	2.601±0.497	2.527±0.503	2.322±0.308	2.417±0.432
	N	10	10	10	10	10	10
	Males	1.677±0.265	1.617±0.311	1.513±0.196	1.718±0.433	1.655±0.256	1.914±0.277
	N	10	10	10	10	10	9
Ovaries (2)	Females	0.605±0.051	0.558±0.083	0.556±0.050	0.555±0.119	0.569±0.081	0.608±0.060
	N	10	10	10	10	10	10
Uterus	Females	2.773±0.766	3.298±0.774	2.744±0.644	2.799±0.595	2.769±0.770	3.010±1.084
	N	10	10	10	10	10	10
Testes (2)	Males	8.488±0.819	8.700±0.795	9.654±1.742	8.688±1.185	9.013±0.954	9.047±0.705
	N	10	10	10	10	10	9
Epididymides	Males	2.318±0.274	2.290±0.274	2.350±0.267	2.261±0.284	2.309±0.226	2.324±0.251
	N	10	10	10	10	10	9

Note:
*p<0.05 versus 0 mg/kg 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	18-0697	0.886	0.034	4.399	0.435	0.251	0.370	0.069	0.214
	18-0698	0.851	0.032	4.141	0.420	0.250	0.257	0.058	0.198
	18-0723	0.873	0.037	4.297	0.467	0.296	0.345	0.057	0.233
	18-0724	0.859	0.034	4.731	0.613	0.245	0.381	0.066	0.291
	18-0717	1.037	0.037	5.002	0.571	0.277	0.290	0.077	0.272
	18-0718	0.864	0.028	4.473	0.447	0.241	0.244	0.071	0.334
	18-0719	0.883	0.033	4.222	0.431	0.238	0.361	0.060	0.432
	18-0720	0.925	0.044	4.573	0.577	0.234	0.235	0.073	0.340
	18-0731	0.921	0.040	3.960	0.536	0.302	0.334	0.062	0.387
	18-0732	0.892	0.038	3.865	0.460	0.222	0.255	0.063	0.287
	Mean	0.899	0.036	4.366	0.496	0.256	0.307	0.066	0.299
	SD	0.054	0.005	0.347	0.071	0.027	0.057	0.007	0.075
14 mg/kg	18-0687	0.760	0.027	4.558	0.383	0.159	0.223	0.055	0.507
	18-0688	0.694	ND	4.016	0.403	0.173	0.254	0.045	0.509
	18-0705	0.698	0.022	3.968	0.338	0.198	0.285	0.069	0.303
	18-0706	0.874	0.023	4.779	0.386	0.189	0.258	0.063	0.441
	18-0735	0.828	0.017	4.583	0.401	0.173	0.306	0.062	0.329
	18-0736	0.727	0.019	4.087	0.353	0.174	0.287	0.061	0.279
	18-0743	0.707	0.028	3.777	0.325	0.162	0.243	0.064	0.285
	18-0744	0.754	0.029	4.311	0.385	0.180	0.291	0.054	0.312
	18-0701	0.677	0.029	3.412	0.371	0.171	0.286	0.058	0.303
	18-0702	0.785	0.022	4.401	0.395	0.172	0.307	0.066	0.291
	Mean	0.750*	0.024*	4.189	0.374*	0.175*	0.274	0.060	0.356
	SD	0.064	0.004	0.417	0.027	0.012	0.028	0.007	0.092
28 mg/kg	18-0693	0.725	0.022	3.767	0.315	0.173	0.265	0.059	0.232
	18-0694	0.810	0.032	4.680	0.382	0.205	0.290	0.054	0.341
	18-0699	0.821	0.030	5.228	0.408	0.251	0.403	0.066	0.315
	18-0700	0.729	0.018	3.846	0.362	0.200	0.319	0.058	0.326
	18-0713	0.675	0.016	3.562	0.357	0.162	0.250	0.056	0.175
	18-0714	0.691	0.024	3.986	0.396	0.191	0.284	0.057	0.305
	18-0691	0.814	0.016	3.954	0.406	0.154	0.185	0.063	0.280
	18-0692	0.818	0.022	4.035	0.422	0.247	0.309	0.056	0.412
	18-0707	0.712	0.030	3.260	0.398	0.206	0.219	0.064	0.244
	18-0708	0.893	0.023	4.641	0.453	0.217	0.281	0.064	0.329
	Mean	0.769*	0.023*	4.096	0.390*	0.201*	0.281	0.060	0.296
	SD	0.071	0.006	0.588	0.038	0.033	0.059	0.004	0.066
56 mg/kg	18-0715	0.743	0.022	3.992	0.389	0.179	0.297	0.054	0.413
	18-0716	0.946	0.023	5.105	0.417	0.202	0.281	0.104	0.339
	18-0733	0.781	0.023	3.889	0.376	0.179	0.277	0.047	0.267
	18-0734	0.871	0.022	4.564	0.412	0.190	0.217	0.059	0.314
	18-0703	0.716	0.022	3.976	0.461	0.190	0.283	0.065	0.288
	18-0704	0.820	0.023	4.800	0.412	0.267	0.240	0.054	0.272
	18-0729	0.816	0.024	3.799	0.409	0.172	0.255	0.068	0.247
	18-0730	0.724	0.029	4.519	0.393	0.181	0.290	0.050	0.270
	18-0745	0.738	0.024	4.237	0.399	0.188	0.396	0.054	0.255
	18-0746	0.846	0.024	4.504	0.415	0.228	0.253	0.063	0.442
	Mean	0.800*	0.024*	4.338	0.408*	0.198*	0.279	0.062	0.311
	SD	0.074	0.002	0.431	0.023	0.029	0.048	0.016	0.068

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
111 mg/kg	18-0689	1.130	0.042	7.404	0.610	0.372	0.480	0.091	0.497
	18-0690	0.733	0.025	4.376	0.379	0.187	0.214	0.057	0.220
	18-0737	0.791	0.018	4.807	0.393	0.188	0.274	0.074	0.433
	18-0738	0.703	0.021	4.210	0.419	0.221	0.181	0.073	0.216
	18-0739	0.753	0.032	4.544	0.422	0.211	0.264	0.050	0.265
	18-0740	0.811	0.031	4.237	0.384	0.243	0.288	0.057	0.332
	18-0695	0.645	0.023	4.318	0.418	0.194	0.238	0.061	0.303
	18-0696	0.764	0.023	4.266	0.400	0.226	0.257	0.072	0.444
	18-0741	0.814	0.030	5.196	0.468	0.229	0.264	0.064	0.253
	18-0742	0.671	0.025	4.548	0.424	0.185	0.242	0.053	0.225
	Mean	0.781*	0.027	4.791	0.432	0.226*	0.270	0.065	0.319
	SD	0.135	0.007	0.968	0.068	0.055	0.080	0.013	0.104
222 mg/kg	18-0709	0.778	0.028	4.985	0.586	0.199	0.243	0.070	0.268
	18-0710	0.859	0.020	5.845	0.494	0.225	0.238	0.068	0.296
	18-0711	0.843	0.021	5.456	0.424	0.176	0.258	0.063	0.239
	18-0712	0.722	0.016	4.082	0.348	0.224	0.311	0.063	0.251
	18-0727	0.823	0.031	4.541	0.407	0.188	0.279	0.075	0.202
	18-0728	0.794	0.020	4.817	0.419	0.227	0.295	0.077	0.398
	18-0721	0.775	0.020	4.203	0.403	0.209	0.189	0.054	0.554
	18-0722	0.680	0.023	4.245	0.383	0.185	0.240	0.064	0.435
	18-0725	0.809	0.029	4.921	0.448	0.230	0.346	0.065	0.333
	18-0726	0.771	0.024	4.236	0.439	0.164	0.207	0.056	0.255
	Mean	0.786*	0.023*	4.733	0.435	0.203*	0.261	0.066	0.323
	SD	0.054	0.005	0.586	0.066	0.024	0.048	0.007	0.109

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

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	18-0649	1.531	0.033	7.357	0.677	0.339	0.269	1.721	0.403
	18-0650	1.216	0.019	5.735	0.669	0.339	0.241	1.529	0.436
	18-0671	1.341	0.032	6.866	0.639	0.319	0.272	1.506	0.428
	18-0672	1.370	0.033	7.015	0.699	0.387	0.326	1.529	0.448
	18-0685	1.319	0.031	6.256	0.637	0.342	0.286	1.489	0.393
	18-0686	1.262	0.031	5.962	0.628	0.457	0.412	1.369	0.378
	18-0657	1.249	0.033	6.406	0.570	0.335	0.268	1.508	0.418
	18-0658	1.507	0.034	7.079	0.719	0.409	0.379	1.731	0.457
	18-0675	1.164	0.025	5.754	0.739	0.340	0.291	1.500	0.386
	18-0676	1.250	0.028	5.140	0.705	0.325	0.329	1.615	0.479
	Mean	1.321	0.030	6.357	0.668	0.359	0.307	1.550	0.423
	SD	0.121	0.005	0.716	0.050	0.044	0.054	0.111	0.033
14 mg/kg	18-0637	0.989	0.018	5.638	0.465	0.223	0.307	1.519	0.404
	18-0638	1.185	0.021	7.031	0.550	0.245	0.376	1.679	0.382
	18-0677	1.153	0.021	7.310	0.582	0.266	0.383	1.659	0.405
	18-0678	1.071	0.024	6.611	0.566	0.300	0.334	1.736	0.424
	18-0679	1.175	0.026	6.059	0.610	0.262	0.189	1.789	0.473
	18-0680	1.229	0.034	7.174	0.626	0.311	0.339	1.507	0.437
	18-0629	1.088	0.031	6.228	0.492	0.256	0.264	1.578	0.389
	18-0630	0.946	0.019	5.399	0.497	0.374	0.261	1.512	0.466
	18-0643	1.045	0.025	5.634	0.573	0.302	0.261	1.433	0.393
	18-0644	1.185	0.023	6.763	0.529	0.284	0.241	1.429	0.389
	Mean	1.107*	0.024*	6.385	0.549*	0.282*	0.296	1.584	0.416
	SD	0.094	0.005	0.692	0.053	0.042	0.063	0.126	0.033
28 mg/kg	18-0641	1.005	0.020	5.501	0.494	0.250	0.303	1.638	0.412
	18-0642	1.113	0.021	6.322	0.586	0.232	0.251	1.955	0.445
	18-0653	1.173	0.023	6.889	0.637	0.256	0.278	1.538	0.392
	18-0654	1.191	0.025	7.217	0.584	0.236	0.255	1.753	0.445
	18-0665	1.028	0.022	5.326	0.503	0.247	0.218	2.449	0.447
	18-0666	1.111	0.017	6.694	0.598	0.267	0.337	1.573	0.362
	18-0623	1.029	0.021	5.147	0.472	0.196	0.284	1.548	0.408
	18-0624	ND	0.023	6.518	0.660	0.325	0.234	1.639	0.456
	18-0663	1.115	0.023	5.328	0.581	0.223	0.306	1.706	0.429
	18-0664	1.047	0.025	5.947	0.535	0.240	0.249	1.482	0.403
	Mean	1.090*	0.022*	6.089	0.565*	0.247*	0.271	1.728	0.420
	SD	0.066	0.002	0.740	0.062	0.033	0.037	0.287	0.030
56 mg/kg	18-0651	1.125	0.021	6.499	0.527	0.298	0.357	1.545	0.399
	18-0652	1.189	0.025	6.268	0.530	0.253	0.278	1.578	0.379
	18-0661	1.086	0.026	5.847	0.677	0.273	0.254	1.718	0.444
	18-0662	1.249	0.024	6.600	0.610	0.318	0.467	1.514	0.378
	18-0635	1.135	0.022	6.776	0.540	0.285	0.278	1.681	0.424
	18-0636	1.066	0.018	6.582	0.459	0.246	0.346	1.037	0.318
	18-0633	1.055	0.023	5.784	0.490	0.267	0.263	1.470	0.368
	18-0634	1.149	0.023	6.329	0.553	0.211	0.274	1.586	0.392
	18-0681	1.101	0.024	6.642	0.582	0.297	0.269	1.612	0.482
	18-0682	1.135	0.020	4.991	0.490	0.214	0.225	1.509	0.384
	Mean	1.129*	0.023*	6.232	0.546*	0.266*	0.301	1.525	0.397
	SD	0.058	0.002	0.547	0.064	0.036	0.071	0.188	0.045

Table J-8 (Continued). 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
111 mg/kg	18-0645	1.219	0.025	6.302	0.599	0.292	0.271	1.808	0.468
	18-0646	1.196	0.021	6.984	0.586	0.285	0.327	1.787	0.449
	18-0655	1.133	ND	6.683	0.564	0.320	0.342	1.771	0.451
	18-0656	1.245	0.025	6.691	0.559	0.243	0.314	1.551	0.385
	18-0669	1.158	0.024	6.505	0.576	0.296	0.250	1.612	0.445
	18-0670	0.997	0.023	6.049	0.454	0.243	0.278	1.417	0.386
	18-0673	1.309	0.022	6.220	0.543	0.233	0.289	1.688	0.388
	18-0674	1.148	0.027	6.604	0.528	0.300	0.349	1.491	0.375
	18-0667	1.082	0.012	5.378	0.548	0.269	0.292	1.468	0.382
	18-0668	1.077	0.019	5.755	0.504	0.288	0.234	1.482	0.389
	Mean	1.156*	0.022*	6.317	0.546*	0.277*	0.295	1.608	0.412
	SD	0.091	0.005	0.485	0.043	0.029	0.038	0.147	0.036
222 mg/kg	18-0631	1.209	0.016	5.559	0.647	0.224	0.276	1.609	0.360
	18-0632	1.120	0.019	6.743	0.544	0.289	0.391	1.472	0.395
	18-0647	1.066	0.020	6.812	0.474	0.287	0.298	1.468	0.421
	18-0648	1.151	0.020	7.368	0.535	0.308	0.356	1.537	0.329
	18-0621	0.969	0.021	7.060	0.529	0.292	0.308	1.595	0.408
	18-0622	1.173	0.016	6.608	0.583	0.346	0.374	1.565	0.418
	18-0659	1.205	0.022	6.643	0.572	0.371	0.336	1.632	0.424
	18-0660	1.064	0.019	5.525	0.507	0.298	0.249	1.570	0.422
	18-0683	1.332	0.020	6.657	0.662	0.239	0.409	1.656	0.447
	18-0684‡	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	1.143*	0.019*	6.553	0.561*	0.295*	0.333	1.567	0.403
	SD	0.105	0.002	0.622	0.062	0.046	0.054	0.065	0.037

Legend:
ND = No data analyzed
Notes:
*p<0.05 versus 0 mg/kg control group, based on one-way ANOVA and Dunnett (2-sided) post hoc test. Ratios of masses adrenals, and thymus were rank-transformed and liver and testes were log-transformed in order to achieve normal distribution of data.
‡Rat 18-0684 died shortly after initiation of dosing, and no organs were collected at necropsy.

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

		DNP in Corn Oil					
	Sex	**0 mg/kg**	**14 mg/kg**	**28 mg/kg**	**56 mg/kg**	**111 mg/kg**	**222 mg/kg**
Kidneys (2)	Females	0.899±0.054	0.750±0.064*	0.769±0.071*	0.800±0.074*	0.871±0.135*	0.786±0.054*
	N	10	10	10	10	10	10
	Males	1.321±0.121	1.107±0.094*	1.090±0.066*	1.129±0.058*	1.156±0.091*	1.143±0.105*
	N	10	10	9	10	10	9
Adrenals (2)	Females	0.036±0.005	0.024±0.004*	0.023±0.006*	0.024±0.002*	0.027±0.007	0.023±0.005*
	N	10	9	10	10	10	10
	Males	0.030±0.005	0.024±0.005*	0.022±0.002*	0.023±0.002*	0.022±0.005*	0.019±0.002*
	N	10	10	10	10	9	9
Liver	Females	4.366±0.347	4.189±0.417	4.096±0.588	4.338±0.431	4.791±0.968	4.733±0.586
	N	10	10	10	10	10	10
	Males	6.357±0.716	6.385±0.692	6.089±0.740	6.232±0.547	6.317±0.485	6.553±0.622
	N	10	10	10	10	10	9
Heart	Females	0.496±0.071	0.374±0.027*	0.390±0.038*	0.408±0.023*	0.432±0.068	0.435±0.066
	N	10	10	10	10	10	10
	Males	0.668±0.050	0.549±0.053*	0.565±0.062*	0.546±0.064*	0.546±0.043*	0.561±0.062*
	N	10	10	10	10	10	9
Spleen	Females	0.256±0.027	0.175±0.012*	0.201±0.033*	0.198±0.029*	0.226±0.055*	0.203±0.024*
	N	10	10	10	10	10	10
	Males	0.359±0.044	0.282±0.042*	0.247±0.033*	0.266±0.036*	0.277±0.029*	0.295±0.046*
	N	10	10	10	10	10	9
Thymus	Females	0.307±0.057	0.274±0.028	0.281±0.059	0.279±0.048	0.270±0.080	0.261±0.048
	N	10	10	10	10	10	10
	Males	0.307±0.054	0.296±0.063	0.271±0.037	0.301±0.071	0.295±0.038	0.333±0.054
	N	10	10	10	10	10	9
Ovaries (2)	Females	0.066±0.007	0.060±0.007	0.060±0.004	0.062±0.016	0.065±0.013	0.066±0.007
	N	10	10	10	10	10	10
Uterus	Females	0.299±0.075	0.356±0.092	0.296±0.066	0.311±0.068	0.319±0.104	0.323±0.109
	N	10	10	10	10	10	10
Testes (2)	Males	1.550±0.111	1.584±0.126	1.728±0.287	1.525±0.188	1.608±0.147	1.567±0.065
	N	10	10	10	10	10	9
Epididymides	Males	0.423±0.033	0.416±0.033	0.420±0.030	0.397±0.045	0.412±0.036	0.403±0.037
	N	10	10	10	10	10	9

Note:
*p<0.05 versus 0 mg/kg control group, based on one-way ANOVA and Dunnett (2-sided) 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	18-0697	10.20	0.894	8.780	7.880	77.400	0.667	6.550	0.070	0.688	0.674	6.620	8.95	18.5	52.7	58.8	20.7	35.2	16.5	1185.0	5.06
	18-0698	12.50	1.870	14.900	9.120	72.700	0.753	6.000	0.042	0.336	0.757	6.040	8.72	17.3	49.4	56.7	19.8	35.0	16.6	1148.0	5.09
	18-0723	11.00	0.800	7.300	9.240	84.300	0.524	4.780	0.031	0.282	0.368	3.360	7.68	16.8	46.6	60.7	21.9	36.1	16.1	995.0	5.46
	18-0724	12.50	1.570	12.600	9.690	77.800	0.540	4.330	0.117	0.938	0.548	4.390	8.09	17.3	48.8	60.3	21.4	35.4	16.8	1195.0	5.04
	18-0717	12.10	0.806	6.640	10.600	87.400	0.302	2.490	0.044	0.359	0.378	3.110	8.47	17.2	48.6	57.3	20.4	35.5	18.0	1252.0	4.38
	18-0718	7.54	1.340	17.700	5.380	71.400	0.521	6.900	0.042	0.551	0.260	3.450	8.77	17.8	52.2	59.6	20.4	34.2	17.4	1002.0	4.97
	18-0719	13.00	2.150	16.500	9.620	73.800	0.737	5.650	0.068	0.523	0.464	3.560	9.12	18.5	53.1	58.2	20.2	34.8	16.4	1183.0	4.47
	18-0720	3.11	0.716	23.000	1.720	55.400	0.381	12.300	0.058	1.880	0.228	7.350	9.28	17.9	51.0	54.9	19.2	35.0	16.9	1026.0	4.65
	18-0731	16.00	1.610	10.100	13.100	81.800	0.635	3.960	0.092	0.573	0.575	3.590	7.93	17.1	47.7	60.2	21.6	35.8	15.9	1192.0	5.14
	18-0732	15.40	2.270	14.800	11.500	74.700	0.702	4.570	0.132	0.859	0.785	5.110	8.47	17.1	47.8	56.5	20.1	35.7	16.2	1467.0	6.09
	Mean	11.34	1.403	13.232	8.785	75.670	0.576	5.753	0.070	0.699	0.504	4.658	8.55	17.6	49.8	58.3	20.6	35.3	16.7	1164.5	5.04
	SD	3.77	0.582	5.164	3.222	8.816	0.151	2.648	0.034	0.468	0.197	1.534	0.52	0.6	2.3	1.9	0.8	0.6	0.6	139.9	0.50
14 mg/kg	18-0687	5.32	0.366	6.890	4.110	77.300	0.338	6.350	0.028	0.533	0.475	8.930	8.16	16.6	46.3	56.8	20.4	35.8	17.4	1210.0	6.03
	18-0688	4.71	0.489	10.400	3.690	78.200	0.198	4.210	0.076	1.610	0.263	5.580	7.71	15.5	43.8	56.9	20.1	35.3	15.8	1075.0	5.17
	18-0705	11.20	0.240	2.140	10.700	95.300	0.027	0.244	0.015	0.132	0.241	2.150	8.68	17.9	50.3	57.9	20.6	35.5	16.6	1178.0	5.22
	18-0706	6.64	0.637	9.600	5.090	76.700	0.503	7.570	0.050	0.759	0.355	5.350	7.69	15.6	44.2	57.4	20.3	35.3	16.8	1191.0	5.42
	18-0735	9.41	0.912	9.700	7.360	78.200	0.307	3.260	0.083	0.881	0.744	7.910	8.14	17.0	47.3	58.1	20.8	35.9	17.2	1097.0	5.65
	18-0736	6.73	0.540	8.020	5.620	83.500	0.409	6.070	0.019	0.281	0.143	2.130	8.47	16.7	47.2	55.8	19.7	35.3	16.1	1151.0	4.63
	18-0743	11.80	0.560	4.740	10.300	87.200	0.411	3.480	0.058	0.490	0.480	4.060	8.93	18.9	52.2	58.4	21.2	36.3	15.8	1129.0	5.42
	18-0744	9.88	0.510	5.170	8.620	87.200	0.273	2.770	0.053	0.540	0.425	4.300	7.73	16.0	45.1	58.4	20.7	35.5	16.8	912.0	6.28
	18-0701	6.29	0.197	3.140	5.480	87.100	0.242	3.850	0.038	0.606	0.335	5.330	8.12	16.1	45.8	56.4	19.9	35.3	15.8	1138.0	5.15
	18-0702	7.08	0.150	2.120	6.200	87.600	0.243	3.440	0.022	0.314	0.463	6.550	8.08	16.3	46.4	57.4	20.1	35.1	15.3	1019.0	4.99
	Mean	7.91*	0.460*	6.192	6.717	83.830	0.295*	4.124	0.044	0.615	0.392	5.229	8.17	16.7	46.9*	57.4	20.4	35.5	16.4	1110.0	5.40
	SD	2.48	0.230	3.178	2.457	6.112	0.133	2.087	0.024	0.414	0.167	2.213	0.42	1.1	2.6	0.9	0.5	0.4	0.7	90.0	0.49
28 mg/kg	18-0693	5.77	0.163	2.830	4.970	86.000	0.330	5.720	0.026	0.443	0.287	4.970	8.41	16.9	47.6	56.6	20.1	35.5	16.6	1143.0	5.27
	18-0694	3.09	0.130	4.200	2.800	90.600	0.067	2.180	0.015	0.487	0.077	2.510	7.43	15.9	45.2	60.8	21.4	35.2	15.8	592.0	5.21
	18-0699	11.10	0.387	3.480	10.000	89.800	0.262	2.360	0.043	0.386	0.440	3.950	7.22	15.9	45.2	62.7	22.1	35.2	15.4	1080.0	5.24
	18-0700	7.68	0.256	3.330	6.800	88.600	0.272	3.540	0.017	0.217	0.332	4.320	7.69	15.1	43.6	56.7	19.7	34.8	14.9	1238.0	4.68
	18-0713	10.90	0.316	2.910	9.820	90.400	0.298	2.750	0.048	0.446	0.380	3.490	7.67	15.1	42.6	55.5	19.7	35.5	15.6	1092.0	4.77
	18-0714	7.14	0.231	3.240	6.350	89.000	0.297	4.170	0.029	0.411	0.225	3.160	8.02	16.8	48.3	60.3	21.0	34.8	16.2	979.0	5.99
	18-0691	9.50	0.261	2.750	8.680	91.400	0.258	2.720	0.024	0.257	0.276	2.900	8.66	17.8	50.7	58.5	20.5	35.0	15.5	1004.0	4.35
	18-0692	6.96	0.314	4.510	5.750	82.700	0.455	6.530	0.083	1.190	0.354	5.090	7.91	16.0	45.3	57.3	20.2	35.2	16.2	801.0	5.00
	18-0707	4.53	0.174	3.830	4.020	88.700	0.165	3.640	0.023	0.516	0.148	3.270	8.50	17.0	48.7	57.3	20.0	34.9	16.3	968.0	4.91
	18-0708	7.45	0.268	3.600	6.740	90.400	0.177	2.380	0.059	0.793	0.210	2.820	7.80	15.2	42.9	55.0	19.4	35.4	15.9	979.0	4.73
	Mean	7.41*	0.250*	3.468*	6.593	88.760*	0.258*	3.599	0.037*	0.515	0.273*	3.648	7.93*	16.2*	46.0*	58.1	20.4	35.2	15.8	987.6*	5.02
	SD	2.59	0.079	0.585	2.377	2.609	0.105	1.489	0.022	0.284	0.111	0.902	0.47	0.9	2.7	2.5	0.9	0.3	0.5	182.1	0.45
56 mg/kg	18-0715	6.55	0.325	4.960	5.420	82.700	0.429	6.550	0.034	0.513	0.344	5.250	7.66	16.1	45.7	59.6	21.0	35.3	15.9	998.0	6.34
	18-0716	8.46	0.474	5.600	7.400	87.500	0.233	2.760	0.030	0.358	0.318	3.760	7.74	16.3	46.7	60.4	21.1	34.9	15.0	668.0	5.17
	18-0733	13.00	0.371	2.840	11.500	88.500	0.665	5.110	0.082	0.627	0.387	2.970	8.09	16.5	45.8	56.7	20.4	35.9	17.0	936.0	5.33
	18-0734	8.99	0.439	4.890	7.610	84.700	0.493	5.490	0.055	0.615	0.384	4.270	7.70	15.5	44.4	57.7	20.2	34.9	15.1	1033.0	5.06
	18-0703	10.20	0.293	2.890	9.310	91.600	0.184	1.810	0.056	0.547	0.318	3.130	7.66	16.3	46.1	60.1	21.3	35.5	16.8	1073.0	5.02
	18-0704	12.00	0.973	8.110	10.100	83.900	0.409	3.400	0.040	0.335	0.512	4.270	9.09	18.2	51.7	56.9	20.1	35.3	15.7	1087.0	4.42
	18-0729	9.94	0.368	3.700	8.900	89.600	0.322	3.240	0.021	0.215	0.325	3.270	8.03	16.2	46.3	57.6	20.1	34.9	15.2	697.0	5.30
	18-0730	8.92	0.236	2.650	8.020	89.800	0.268	3.010	0.059	0.661	0.343	3.840	8.05	16.6	45.7	56.7	20.6	36.3	16.4	1052.0	5.43
	18-0745	10.70	0.403	3.780	9.410	88.400	0.429	4.030	0.042	0.392	0.362	3.400	7.93	16.8	47.2	59.5	21.2	35.6	16.2	1049.0	5.67
	18-0746	9.39	0.301	3.210	8.390	89.300	0.341	3.640	0.036	0.379	0.327	3.480	7.59	15.7	44.8	59.0	20.7	35.0	15.7	521.0	5.12
	Mean	9.82	0.418*	4.263*	8.606	87.600*	0.377	3.904	0.046	0.464	0.362	3.764	7.95*	16.4*	46.4*	58.4	20.7	35.4	15.9	911.4*	5.29
	SD	1.83	0.208	1.688	1.661	2.893	0.140	1.424	0.018	0.149	0.058	0.684	0.44	0.7	2.0	1.5	0.5	0.5	0.7	204.4	0.49

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)
111 mg/kg	18-0689	10.50	0.618	5.900	8.830	84.300	0.356	3.400	0.033	0.314	0.635	6.070	7.36	15.8	45.0	61.1	21.4	35.0	16.2	1030.0	6.30
	18-0690	9.26	0.321	3.470	8.360	90.300	0.272	2.940	0.030	0.330	0.273	2.950	7.91	16.2	45.9	58.0	20.4	35.2	15.2	1145.0	5.03
	18-0737	10.80	0.353	3.270	9.300	86.300	0.645	5.980	0.094	0.868	0.380	0.353	8.51	16.6	47.1	55.3	19.5	35.3	17.6	1166.0	4.54
	18-0738	8.79	0.612	9.960	7.360	83.600	0.460	5.230	0.034	0.388	0.332	3.780	8.78	17.4	49.8	56.7	19.8	35.0	19.3	930.0	6.33
	18-0739	10.60	1.010	9.500	8.030	75.800	0.928	8.760	0.069	0.656	0.559	5.280	8.44	16.9	48.8	57.9	20.0	34.6	15.8	1037.0	5.74
	18-0740	6.55	0.307	4.680	5.680	86.700	0.371	5.670	0.031	0.476	0.159	2.430	8.00	16.5	46.1	57.7	20.6	35.7	15.5	1148.0	4.93
	18-0695	4.60	0.227	4.930	3.240	70.400	0.551	12.000	0.045	0.972	0.540	11.800	8.67	17.4	50.1	57.8	20.1	34.7	17.0	1190.0	4.24
	18-0696	10.30	0.602	5.850	8.930	86.800	0.339	3.290	0.102	0.990	0.314	3.050	7.89	15.4	46.3	58.7	19.6	33.3	15.4	1173.0	5.22
	18-0741	9.70	0.400	4.130	8.840	91.100	0.113	1.170	0.022	0.231	0.325	3.350	7.98	16.4	45.9	57.5	20.6	35.7	16.4	923.0	4.91
	18-0742	7.09	0.317	4.470	6.110	86.200	0.370	5.220	0.025	0.351	0.266	3.750	8.30	16.6	46.7	56.2	20.0	35.5	17.0	1179.0	4.83
	Mean	8.82	0.477*	5.616*	7.468	84.150	0.441	5.366	0.049	0.558	0.378	4.281	8.18	16.5*	47.2	57.7	20.2	35.0	16.5	1092.1	5.21
	SD	2.08	0.236	2.335	1.923	6.391	0.225	3.122	0.029	0.291	0.151	3.060	0.43	0.6	1.8	1.6	0.6	0.7	1.2	103.7	0.70
222 mg/kg	18-0709	10.60	0.452	4.260	9.030	85.100	0.392	3.700	0.110	1.040	0.621	5.860	7.82	14.9	43.3	55.3	19.1	34.5	17.0	975.0	4.50
	18-0710	6.52	0.516	7.920	4.560	69.900	0.646	9.910	0.107	1.640	0.692	10.600	7.55	14.8	41.6	55.1	19.6	35.5	17.0	1192.0	4.73
	18-0711	8.52	0.381	4.470	7.370	86.500	0.220	2.580	0.070	0.825	0.481	5.640	8.05	15.5	43.9	54.5	19.3	35.4	17.1	1223.0	5.15
	18-0712	13.40	0.917	6.850	11.000	87.500	0.347	2.590	0.084	0.624	0.330	2.460	8.22	15.8	45.2	55.0	19.3	35.0	15.6	911.0	4.78
	18-0727	10.80	1.120	10.300	8.640	79.800	0.534	4.930	0.043	0.395	0.493	4.550	7.54	16.2	46.1	61.1	21.5	35.1	16.5	1294.0	5.10
	18-0728	11.90	0.553	4.660	8.610	72.600	1.490	12.500	0.115	0.969	1.100	9.260	7.89	16.3	47.3	59.9	20.6	34.4	14.6	1137.0	5.37
	18-0721	6.41	0.300	4.680	5.540	86.400	0.245	3.820	0.040	0.631	0.289	4.500	8.19	16.1	45.6	55.7	19.6	35.2	16.6	1014.0	4.71
	18-0722	9.28	0.450	4.850	7.980	86.000	0.408	4.390	0.107	1.160	0.338	3.640	8.00	15.8	45.4	56.7	19.8	34.9	18.3	1165.0	4.51
	18-0725	9.19	0.229	2.490	8.550	93.100	0.150	1.630	0.053	0.578	0.201	2.190	7.45	15.1	43.3	58.1	20.3	35.0	15.3	598.0	5.45
	18-0726	6.10	0.570	9.350	5.030	82.400	0.241	3.950	0.050	0.826	0.209	3.430	7.81	15.5	44.1	56.5	19.9	35.2	17.2	1086.0	4.92
	Mean	9.27	0.549*	5.983*	7.631	82.930	0.467	5.000	0.078	0.869	0.475	5.213	7.85*	15.6*	44.6*	56.8	19.9	35.0	16.5	1059.5	4.92
	SD	2.46	0.274	2.509	2.024	7.070	0.390	3.463	0.030	0.357	0.275	2.775	0.27	0.5	1.7	2.2	0.7	0.4	1.1	200.4	0.34

Note:
*p<0.05 versus 0 mg/kg control group, based on one-way ANOVA and Dunnett (2-sided) post hoc test. Data for MONO%, EOS, EOS%, NEU, BASO, and HCT were log-transformed, and data for BASO%, NEU%, LYM%, MONO, and PLT were rank-transformed 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	18-0649	13.60	1.120	8.220	10.900	79.500	0.870	6.380	0.100	0.735	0.702	5.150	8.29	16.3	46.8	56.4	19.7	34.9	18.8	1011.0	4.92
	18-0650	15.50	1.440	9.270	12.400	79.900	0.776	5.010	0.116	0.751	0.789	5.090	9.70	18.7	53.6	55.3	19.2	34.8	18.0	1036.0	4.74
	18-0657	22.70	2.210	9.760	19.400	85.700	0.489	2.150	0.082	0.362	0.469	2.070	7.85	16.2	46.3	58.9	20.6	35.0	15.7	1057.0	4.68
	18-0658	19.40	2.670	13.800	14.500	74.800	1.280	6.590	0.168	0.868	0.775	4.000	8.25	15.5	45.0	54.5	18.8	34.5	17.6	1093.0	5.29
	18-0671	20.20	2.300	11.300	15.500	76.600	0.919	4.540	0.045	0.223	1.470	7.270	8.24	16.5	46.8	56.8	20.1	35.4	17.1	1040.0	5.80
	18-0672	16.60	2.460	14.800	12.600	75.500	0.776	4.670	0.050	0.299	0.795	4.780	8.23	16.7	47.9	58.2	20.3	34.8	15.8	1237.0	5.75
	18-0675	14.90	1.180	7.960	12.400	83.200	0.680	4.580	0.120	0.808	0.511	3.440	8.09	16.5	47.5	58.6	20.3	34.7	16.7	1043.0	5.25
	18-0676	21.10	1.080	5.110	18.800	89.100	0.398	1.890	0.165	0.781	0.665	3.150	7.86	16.8	46.1	58.7	21.3	36.3	15.4	939.0	5.15
	18-0685	21.30	1.350	6.340	17.100	80.300	1.410	6.620	0.098	0.458	1.350	6.310	8.57	17.4	49.1	57.3	20.3	35.4	18.0	1126.0	4.79
	18-0686	17.90	1.210	6.750	14.300	79.900	0.984	5.490	0.051	0.287	1.350	7.550	8.25	16.8	48.0	58.2	20.4	35.0	15.8	967.0	5.31
	Mean	18.32	1.702	9.331	14.790	80.450	0.858	4.792	0.100	0.557	0.888	4.881	8.33	16.7	47.7	57.3	20.1	35.1	16.9	1054.9	5.17
	SD	3.08	0.629	3.171	2.884	4.506	0.315	1.672	0.044	0.253	0.365	1.787	0.52	0.8	2.4	1.5	0.7	0.5	1.2	84.0	0.40
14 mg/kg	18-0629	19.40	1.280	6.580	16.500	85.200	0.412	2.120	0.035	0.182	1.140	5.860	9.15	18.9	52.3	57.1	20.7	36.2	16.4	1150.0	5.07
	18-0630	17.70	1.840	10.400	14.500	82.100	0.483	2.730	0.053	0.297	0.798	4.510	8.63	16.9	47.6	55.1	19.5	35.5	16.8	1318.0	5.40
	18-0637	16.90	1.690	10.000	13.900	82.100	0.470	2.790	0.046	0.271	0.810	4.790	7.92	16.4	46.1	58.2	20.8	35.7	17.5	988.0	7.74
	18-0638	13.70	1.230	9.020	11.500	84.100	0.401	2.930	0.045	0.327	0.498	3.640	8.21	16.3	47.1	57.3	19.9	34.7	16.6	968.0	5.79
	18-0643	15.80	1.120	7.090	12.100	76.600	0.786	4.970	0.069	0.437	1.720	10.900	9.01	17.3	49.0	54.4	19.2	35.3	17.8	1415.0	5.89
	18-0644	12.50	0.945	7.570	10.500	84.100	0.430	3.450	0.049	0.395	0.557	4.460	9.18	18.1	50.7	55.2	19.8	35.8	20.6	1053.0	6.20
	18-0677	20.20	1.880	9.300	15.800	78.400	1.450	7.210	0.033	0.163	1.000	4.960	8.47	17.5	49.5	58.4	20.6	35.3	18.3	1093.0	5.18
	18-0678	12.60	1.140	9.030	10.200	81.100	0.538	4.260	0.030	0.235	0.684	5.410	7.38	15.8	43.6	59.0	21.5	36.4	16.1	1246.0	6.62
	18-0679	14.90	1.230	8.220	12.800	85.700	0.429	2.880	0.029	0.193	0.448	3.010	8.90	18.0	50.2	56.4	20.2	35.8	17.5	1086.0	6.94
	18-0680	16.80	1.410	8.370	13.900	82.500	0.704	4.190	0.029	0.171	0.794	4.720	8.31	16.9	48.0	57.7	20.4	35.3	17.1	871.0	5.39
	Mean	16.05	1.377	8.558	13.170	82.190	0.610	3.753	0.042*	0.267*	0.845	5.226	8.52	17.2	48.4	56.9	20.3	35.6	17.5	1118.8	6.02*
	SD	2.66	0.321	1.232	2.131	2.898	0.322	1.493	0.013	0.096	0.376	2.152	0.58	0.9	2.5	1.6	0.7	0.5	1.3	167.3	0.86
28 mg/kg	18-0623	11.50	0.687	5.990	10.100	88.200	0.356	3.100	0.031	0.268	0.280	2.440	8.78	17.1	49.1	55.9	19.5	34.9	18.1	988.0	5.78
	18-0624	20.20	1.370	6.800	16.900	83.900	0.768	3.800	0.134	0.663	0.969	4.800	7.14	14.9	41.4	58.0	20.8	35.9	16.1	1222.0	5.95
	18-0641	13.10	1.060	8.120	10.700	82.100	0.535	4.090	0.033	0.255	0.714	5.460	8.34	16.3	46.8	56.1	19.6	34.9	16.9	1262.0	4.58
	18-0642	12.70	1.370	10.800	9.690	76.300	0.829	6.530	0.091	0.720	0.718	5.650	9.89	18.8	53.8	54.4	19.0	34.9	18.0	1172.0	5.05
	18-0653	15.00	1.240	8.290	11.400	76.400	1.300	8.680	0.079	0.529	0.914	6.110	8.93	16.7	47.7	53.4	18.7	35.1	21.1	1056.0	5.96
	18-0654	8.55	1.150	13.400	5.880	68.800	0.625	7.310	0.015	0.173	0.886	10.400	7.95	15.7	44.9	56.5	19.7	34.9	18.7	1359.0	6.45
	18-0663	14.90	1.810	12.200	11.400	76.600	0.889	5.970	0.014	0.093	0.763	5.130	8.83	18.5	52.8	59.8	20.9	35.0	17.6	991.0	5.81
	18-0664	10.50	1.100	10.400	8.050	76.700	0.572	5.450	0.040	0.379	0.740	7.050	8.29	16.9	47.3	57.0	20.4	35.8	17.9	1279.0	6.81
	18-0665	10.70	0.619	5.800	9.190	86.100	0.258	2.420	0.021	0.201	0.580	0.544	9.06	18.0	50.2	55.4	19.9	35.9	19.4	1021.0	5.86
	18-0666	13.10	0.845	6.450	10.800	82.400	0.620	4.740	0.046	0.353	0.794	6.060	8.26	16.3	46.1	55.8	19.7	35.3	17.9	1071.0	6.49
	Mean	13.03*	1.125†	8.825	10.411*	79.750	0.675	5.209	0.050*	0.363	0.736	5.364	8.55	16.9	48.0	56.2	19.8	35.3	18.2	1142.1	5.87*
	SD	3.22	0.355	2.717	2.838	5.809	0.294	1.959	0.039	0.211	0.196	2.614	0.74	1.2	3.7	1.8	0.7	0.4	1.4	133.8	0.66
56 mg/kg	18-0633	16.30	1.160	7.110	14.100	86.600	0.502	3.090	0.052	0.320	0.473	2.910	8.16	16.0	45.6	55.9	19.6	35.1	18.9	1369.0	5.46
	18-0634	9.41	1.550	16.500	7.030	74.700	0.421	4.470	0.017	0.182	0.395	4.200	9.17	18.5	51.5	56.2	20.2	36.0	18.6	1177.0	5.48
	18-0635	6.77	0.783	11.600	4.250	62.800	0.651	9.620	0.035	0.519	1.050	15.500	8.79	18.2	51.3	58.4	20.7	35.4	19.6	1080.0	5.97
	18-0636	12.80	1.410	11.100	10.500	82.200	0.372	2.910	0.041	0.323	0.455	3.550	7.68	16.0	45.3	59.0	20.9	35.4	19.3	1071.0	4.90
	18-0651	15.00	1.030	6.850	13.300	88.400	0.368	2.450	0.092	0.611	0.259	1.720	8.40	16.3	46.4	55.3	19.4	35.2	17.7	1015.0	4.87
	18-0652	12.70	1.540	12.200	10.000	79.100	0.591	4.670	0.019	0.152	0.487	3.840	8.83	16.6	46.9	53.1	18.8	35.3	19.3	1274.0	4.97
	18-0661	12.60	2.240	17.800	8.870	70.400	0.745	5.910	0.058	0.456	0.683	5.420	8.50	16.6	46.8	55.0	19.5	35.4	17.4	1151.0	5.05
	18-0662	17.10	0.946	5.520	14.500	84.500	0.924	5.420	0.053	0.309	0.723	4.220	8.05	16.4	47.9	59.5	20.4	34.2	16.8	692.0	5.42
	18-0681	12.70	0.841	6.630	10.900	85.700	0.580	4.570	0.021	0.164	0.369	2.910	8.40	16.3	46.7	55.6	19.4	34.9	16.0	936.0	4.63
	18-0682	18.10	2.320	12.800	13.800	76.100	1.170	6.430	0.041	0.229	0.811	4.470	8.96	17.3	48.9	54.6	19.3	35.3	18.6	1156.0	5.61
	Mean	13.35*	1.382	10.811	10.725*	79.050	0.632	4.954	0.043*	0.327	0.571	4.874	8.49	16.8	47.7	56.3	19.8	35.2	18.2	1092.1	5.24
	SD	3.47	0.546	4.251	3.356	8.129	0.256	2.094	0.023	0.157	0.241	3.869	0.46	0.9	2.2	2.1	0.7	0.5	1.2	187.2	0.41

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)
111 mg/kg	18-0645	11.40	1.130	9.920	8.890	78.100	0.630	5.540	0.054	0.477	0.675	5.940	8.44	16.6	47.3	56.1	19.7	35.1	19.4	1022.0	5.67
	18-0646	7.92	0.596	7.530	6.810	86.100	0.223	2.820	0.024	0.299	0.260	3.290	8.18	16.6	47.5	58.0	20.3	34.9	16.8	1078.0	5.56
	18-0655	13.10	1.070	8.140	10.400	79.200	0.880	6.710	0.077	0.586	0.070	5.320	8.39	16.3	46.8	55.8	19.4	34.8	19.1	1103.0	4.32
	18-0656	12.30	0.861	7.020	10.500	85.400	0.297	2.420	0.096	0.784	0.535	4.360	8.49	16.3	46.8	55.1	19.2	34.8	19.0	1093.0	6.15
	18-0667	16.40	1.020	6.210	14.300	87.100	0.503	3.060	0.106	0.647	0.484	2.940	9.07	17.4	48.6	53.6	19.2	35.8	19.6	1079.0	4.72
	18-0668	16.60	1.230	7.430	13.900	83.900	0.656	3.960	0.053	0.317	0.731	4.410	8.47	16.3	46.1	54.4	19.3	35.4	19.0	1366.0	5.33
	18-0669	13.60	2.140	15.700	9.830	72.100	0.651	4.770	0.082	0.604	0.993	6.840	8.70	16.5	47.0	54.0	19.0	35.1	18.7	1532.0	5.52
	18-0670	11.90	0.849	7.150	10.500	88.000	0.272	2.290	0.028	0.239	0.276	2.330	8.92	16.7	46.2	51.8	18.7	36.0	19.7	1204.0	5.37
	18-0673	15.50	1.020	6.560	12.600	81.200	0.729	4.690	0.062	0.398	1.110	7.120	9.49	18.4	52.2	55.0	19.4	35.4	19.9	1267.0	5.78
	18-0674	12.20	0.629	5.160	10.500	86.300	0.482	3.960	0.046	0.377	0.518	4.250	9.00	17.2	49.5	54.9	19.1	34.8	18.8	1072.0	5.01
	Mean	13.09*	1.055*	8.082	10.823*	82.740	0.532	4.022	0.063	0.473	0.565	4.680	8.72	16.8	47.8	54.9*	19.3*	35.2	19.0*	1181.6	5.34
	SD	2.62	0.433	2.953	2.262	5.041	0.216	1.433	0.027	0.177	0.325	1.614	0.40	0.7	1.9	1.6	0.4	0.4	0.9	162.4	0.53
222 mg/kg	18-0621	14.80	2.720	18.400	10.500	71.000	0.608	4.110	0.088	0.596	0.881	5.950	7.98	16.1	47.0	58.9	20.2	34.2	18.4	955.0	6.70
	18-0622	19.00	1.000	5.270	16.200	85.500	0.876	4.620	0.180	0.951	0.700	3.690	8.42	16.1	45.7	54.3	19.1	35.2	18.1	1214.0	5.03
	18-0631	15.60	0.956	6.130	13.300	85.300	0.385	2.470	0.073	0.469	0.882	5.650	8.18	15.8	45.3	55.4	19.4	35.0	20.5	1346.0	6.33
	18-0632	16.70	1.370	8.210	14.000	83.900	0.514	3.080	0.092	0.553	0.711	4.260	8.45	16.4	47.5	56.2	19.4	34.5	19.4	1135.0	5.81
	18-0647	11.40	1.690	14.800	7.150	62.400	1.030	9.020	0.212	1.860	1.360	11.900	8.10	16.1	46.2	57.1	19.9	34.8	17.7	1256.0	5.45
	18-0648	13.30	1.930	14.500	9.800	73.600	0.639	4.800	0.047	0.354	0.899	6.750	8.20	16.0	45.7	55.7	19.5	35.0	20.1	1103.0	5.03
	18-0659	12.00	0.817	6.810	9.610	80.100	0.706	5.880	0.087	0.722	0.776	6.470	8.07	15.7	45.3	56.2	19.4	34.6	21.1	1508.0	5.26
	18-0660	13.80	0.961	6.970	11.500	83.200	0.632	4.580	0.190	1.380	0.528	3.830	8.49	16.3	46.3	54.6	19.2	35.2	19.8	1070.0	4.79
	18-0683	18.60	1.280	6.880	15.700	84.600	0.832	4.480	0.079	0.423	0.673	3.620	8.56	16.6	46.8	54.7	19.4	35.5	18.7	1225.0	5.08
	18-0684	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	15.02	1.414	9.774	11.973	78.844	0.691	4.782	0.116	0.812	0.823	5.791	8.27	16.1	46.2	55.9	19.5*	34.9	19.3*	1201.3	5.50
	SD	2.70	0.613	4.783	3.033	8.098	0.196	1.871	0.060	0.505	0.235	2.600	0.21	0.3	0.8	1.4	0.3	0.4	1.2	162.3	0.65

Legend:
ND = No data analyzed
Note:
*p<0.05, and †p=0.05 versus 0 mg/kg control group, based on one-way ANOVA and Dunnett (2-sided) post hoc test. Data for EOS, EOS%, NEU, BASO%, and PLT were log-transformed, and data for NEU%, LYM%, BASO, HCT, and HGB were rank-transformed to achieve normal distribution of data. Data for MCH were not normally distributed after transformation, so ranked data were analyzed by Kruskal-Wallis followed by the Mann-Whitney U post hoc test.

Table K-3. Hematology Summary in Females and Males

				DNP in Corn Oil					
		Sex		0 mg/kg	14 mg/kg	28 mg/kg	56 mg/kg	111 mg/kg	222 mg/kg
WBC	(K/uL)	Females		11.34±3.77	7.91±2.48*	7.41±2.59*	9.82±1.83	8.82±2.08	9.27±2.46
			N	10	10	10	10	10	10
		Males		18.32±3.08	16.05±2.66	13.03±3.22*	13.35±3.47*	13.09±2.62*	15.02±2.70
			N	10	10	10	10	10	9
NEU	(K/uL)	Females		1.403±0.582	0.460±0.230*	0.250±0.079*	0.418±0.208*	0.477±0.236*	0.549±0.274*
			N	10	10	10	10	10	10
		Males		1.702±0.629	1.377±0.321	1.125±0.355†	1.382±0.546	1.055±0.433*	1.414±0.613
			N	10	10	10	10	10	9
	(%N)	Females		13.232±5.164	6.192±3.178	3.468±0.585*	4.263±1.688*	5.616±2.335*	5.983±0.274*
			N	10	10	10	10	10	10
		Males		9.331±3.171	8.558±1.232	8.825±2.717	10.811±4.251	8.082±2.953	9.774±4.783
			N	10	10	10	10	10	9
LYM	(K/uL)	Females		8.785±3.222	6.717±2.457	6.593±2.377	8.606±1.661	7.468±1.923	7.631±2.024
			N	10	10	10	10	10	10
		Males		14.790±2.884	13.170±2.131	10.411±2.838*	10.725±3.356*	10.823±2.262*	11.973±3.033
			N	10	10	10	10	10	9
	(%L)	Females		75.670±8.816	83.830±6.112	88.760±2.609*	87.600±2.893*	84.150±6.391	82.930±7.070
			N	10	10	10	10	10	10
		Males		80.450±4.506	82.190±2.898	79.750±5.809	79.050±8.129	82.740±5.041	78.844±8.098
			N	10	10	10	10	10	9
MONO	(K/uL)	Females		0.576±0.151	0.295±0.133*	0.258±0.105*	0.377±0.140	0.441±0.225	0.467±0.390
			N	10	10	10	10	10	10
		Males		0.858±0.315	0.610±0.322	0.675±0.294	0.632±0.256	0.532±0.216	0.691±0.196
			N	10	10	10	10	10	9
	(%M)	Females		5.753±2.648	4.124±2.087	3.599±1.489	3.904±1.424	5.366±3.122	5.000±3.463
			N	10	10	10	10	10	10
		Males		4.792±1.672	3.753±1.493	5.209±1.959	4.954±2.094	4.022±1.433	4.782±1.871
			N	10	10	10	10	10	9
EOS	(K/uL)	Females		0.070±0.034	0.044±0.024	0.037±0.022*	0.046±0.018	0.049±0.029	0.078±0.030
			N	10	10	10	10	10	10
		Males		0.100±0.044	0.042±0.013*	0.050±0.039*	0.043±0.023*	0.063±0.027	0.116±0.060
			N	10	10	10	10	10	9
	(%E)	Females		0.699±0.468	0.615±0.414	0.515±0.284	0.464±0.149	0.558±0.291	0.869±0.357
			N	10	10	10	10	10	10
		Males		0.557±0.253	0.267±0.096*	0.363±0.211	0.327±0.157	0.473±0.177	0.812±0.505
			N	10	10	10	10	10	9
BASO	(K/uL)	Females		0.504±0.197	0.392±0.167	0.273±0.111*	0.362±0.058	0.378±0.151	0.475±0.275
			N	10	10	10	10	10	10
		Males		0.888±0.365	0.845±0.376	0.736±0.196	0.571±0.241	0.565±0.325	0.823±0.235
			N	10	10	10	10	10	9
	(%B)	Females		4.658±1.534	5.229±2.213	3.648±0.902	3.764±0.684	4.281±3.060	5.213±2.775
			N	10	10	10	10	10	10
		Males		4.881±1.787	5.226±2.152	5.364±2.614	4.874±3.869	4.680±1.614	5.791±2.600
			N	10	10	10	10	10	9
RBC	(M/uL)	Females		8.55±0.522	8.17±0.42	7.93±0.47*	7.95±0.44*	8.18±0.43	7.85±0.27*
			N	10	10	10	10	10	10
		Males		8.33±0.52	8.52±0.58	8.55±0.74	8.49±0.46	8.72±0.40	8.27±0.21
			N	10	10	10	10	10	9
HGB	(g/dL)	Females		17.6±0.6	16.7±1.1	16.2±0.9*	16.4±0.7*	16.5±0.6*	15.6±0.5*
			N	10	10	10	10	10	10
		Males		16.7±0.8	17.2±0.9	16.9±1.2	16.8±0.9	16.8±0.7	16.1±0.3
			N	10	10	10	10	10	9

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

			DNP in Corn Oil					
		Sex	0 mg/kg	14 mg/kg	28 mg/kg	56 mg/kg	111 mg/kg	222 mg/kg
HCT	(%)	Females	49.8±2.3	46.9±2.6*	46.0±2.7*	46.4±2.0*	47.2±1.8	44.6±1.7*
		N	10	10	10	10	10	10
		Males	47.7±2.4	48.4±2.5	48.0±3.7	47.7±2.2	47.8±1.9	46.2±0.8
		N	10	10	10	10	10	9
MCV	(fL)	Females	58.3±1.9	57.4±0.9	58.1±2.5	58.4±1.5	57.7±1.6	56.8±2.2
		N	10	10	10	10	10	10
		Males	57.3±1.5	56.9±1.6	56.2±1.8	56.3±2.1	54.9±1.6*	55.9±1.4
		N	10	10	10	10	10	9
MCH	(pg)	Females	20.6±0.8	20.4±0.5	20.4±0.9	20.7±0.5	20.2±0.6	19.9±0.7
		N	10	10	10	10	10	10
		Males	20.1±0.7	20.3±0.7	19.8±0.7	19.8±0.7	19.3±0.4*	19.5±0.3*
		N	10	10	10	10	10	9
MCHC	(g/dL)	Females	35.3±0.6	35.5±0.4	35.2±0.3	35.4±0.5	35.0±0.7	35.0±0.4
		N	10	10	10	10	10	10
		Males	35.1±0.5	35.6±0.5	35.3±0.4	35.2±0.5	35.2±0.4	34.9±0.4
		N	10	10	10	10	10	9
RDW	(%)	Females	16.7±0.6	16.4±0.7	15.8±0.5	15.9±0.7	16.5±1.2	16.5±1.1
		N	10	10	10	10	10	10
		Males	16.9±1.2	17.5±1.3	18.2±1.4	18.2±1.2	19.0±0.9*	19.3±1.2*
		N	10	10	10	10	10	9
PLT	(K/uL)	Females	1164.5±139.9	1110.0±90.0	987.6±182.1*	911.4±204.4*	1092.1±103.7	1059.5±200.4
		N	10	10	10	10	10	10
		Males	1054.9±84.0	1118.8±167.3	1142.1±133.8	1092.1±187.2	1181.6±162.4	1201.3±162.3
		N	10	10	10	10	10	9
MPV	(fL)	Females	5.04±0.50	5.40±0.49	5.02±0.45	5.29±0.49	5.21±0.70	4.92±0.34
		N	10	10	10	10	10	10
		Males	5.17±0.40	6.02±0.86*	5.87±0.66*	5.24±0.41	5.34±0.53	5.50±0.65
		N	10	10	10	10	10	9

Note:
*p<0.05, and †p=0.05 versus 0 mg/kg control group, based on one-way ANOVA and Dunnett (2-sided) 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	18-0697	3.95	1.34	50.1	156.8	84.8	18.79	56.93	12.33	75	0.33	2.94	87.99	19.90	6.89	47	40.2	147	11.8	107
	18-0698	4.26	1.46	32.7	99.3	120.1	18.18	55.08	12.63	83	0.33	2.92	102.11	19.60	7.18	55	38.9	150	9.9	107
	18-0723	4.11	1.32	42.0	132.8	76.8	14.02	50.07	13.59	95	0.28	3.12	100.64	19.27	7.23	75	30.0	145	11.5	107
	18-0724	4.01	1.36	36.3	172.8	83.2	14.21	36.43	13.27	120	0.39	2.94	102.37	20.09	6.95	79	30.4	144	13.8	108
	18-0717	4.03	1.33	41.0	171.1	75.5	15.47	49.90	12.90	80	0.31	3.03	131.76	17.08	7.06	46	33.1	145	11.7	107
	18-0718	3.94	1.50	29.7	149.4	86.7	14.30	44.69	11.83	77	0.32	2.62	72.61	16.52	6.56	36	30.6	149	11.2	107
	18-0719	3.91	1.37	63.3	207.0	83.2	12.38	49.53	13.00	89	0.25	2.85	330.17	17.09	6.76	51	26.5	146	12.5	105
	18-0720	4.38	1.33	31.9	188.8	66.2	12.85	42.84	12.86	110	0.30	3.30	161.64	22.15	7.68	68	27.5	146	ND	107
	18-0731	3.74	1.19	35.8	178.3	80.7	13.69	85.57	13.53	78	0.16	3.15	299.59	22.97	6.89	42	29.3	143	13.8	105
	18-0732	3.74	1.47	42.1	202.4	98.1	10.28	34.27	14.01	65	0.30	2.54	417.45	18.69	6.28	56	22.0	145	13.3	110
	Mean	4.01	1.37	40.5	165.9	85.5	14.42	50.5	13.00	87	0.30	2.94	181	19.34	6.95	56	30.9	146	12.2	107
	SD	0.20	0.09	10.0	32.8	14.7	2.56	14.3	0.64	17	0.06	0.23	122	2.12	0.38	14	5.5	2	1.3	1
14 mg/kg	18-0687	4.21	1.34	62.9	159.6	71.1	24.63	63.15	11.56	116	0.39	3.15	129.20	12.40	7.36	35	52.7	152	7.6	107
	18-0688	4.01	1.23	65.4	97.6	66.5	24.86	51.79	12.36	128	0.48	3.25	145.25	12.55	7.26	33	53.2	149	8.0	107
	18-0705	4.59	1.29	72.8	116.6	125.1	32.24	65.80	13.07	106	0.49	3.56	148.40	14.23	8.15	37	69.0	150	10.4	106
	18-0706	4.22	1.04	51.0	99.8	79.2	25.47	32.24	12.98	126	0.79	4.07	233.76	13.58	8.29	45	54.5	152	8.1	107
	18-0735	4.42	1.18	41.6	141.9	68.6	32.90	62.07	13.08	123	0.53	3.76	113.64	11.58	8.18	28	70.4	151	7.9	105
	18-0736	4.95	1.14	58.8	184.2	82.8	23.37	70.80	14.19	110	0.33	4.35	270.32	13.78	9.30	32	50.0	152	8.3	106
	18-0743	4.29	1.10	74.9	131.1	121.1	25.19	62.97	13.14	128	0.40	3.91	65.38	14.70	8.20	28	53.9	150	9.0	108
	18-0744	4.45	1.09	61.7	90.6	74.0	29.11	72.78	14.27	102	0.40	4.10	359.71	12.97	8.55	35	62.3	149	8.3	107
	18-0701	4.50	1.27	60.3	110.5	64.2	20.84	52.10	13.04	75	0.40	3.55	223.03	12.05	8.05	21	44.6	150	8.8	107
	18-0702	4.44	1.33	43.4	129.2	72.7	16.96	47.12	12.70	73	0.36	3.35	148.88	13.25	7.79	23	36.3	149	8.9	109
	Mean	4.41*	1.20*	59.3*	126.1	82.5	25.56*	58.1	13.04	109	0.46*	3.71*	184	13.11*	8.11*	32*	54.7*	150*	8.5*	107
	SD	0.25	0.11	11.1	29.6	22.1	4.87	12.3	0.79	20	0.13	0.40	87	0.99	0.58	7	10.4	1	0.8	1
28 mg/kg	18-0693	4.33	1.23	41.4	81.4	67.6	19.95	46.40	12.50	95	0.43	3.51	111.69	12.43	7.84	22	42.7	152	8.7	104
	18-0694	4.40	1.16	71.1	97.4	105.4	28.74	82.11	13.63	131	0.35	3.79	309.16	12.43	8.19	48	61.5	152	7.5	106
	18-0699	4.41	1.11	50.6	109.6	60.1	25.14	53.49	13.40	132	0.47	3.99	243.33	13.35	8.40	51	53.8	151	8.3	102
	18-0700	4.08	1.23	57.3	128.9	66.3	29.16	59.51	11.90	109	0.49	3.32	141.98	12.92	7.40	24	62.4	152	8.3	107
	18-0713	4.45	1.26	52.2	130.4	70.1	26.50	67.94	13.25	100	0.39	3.53	202.35	11.41	7.98	33	56.7	151	7.8	107
	18-0714	4.70	1.09	40.9	83.9	76.6	26.17	51.31	13.44	79	0.51	4.30	216.77	14.33	9.00	17	56.0	152	8.6	108
	18-0691	4.33	1.16	49.0	117.4	121.5	17.57	43.93	12.95	101	0.40	3.72	142.42	15.39	8.05	30	37.6	152	9.5	109
	18-0692	4.34	1.49	79.4	186.4	155.0	16.87	48.20	14.14	123	0.35	2.92	293.98	16.93	7.26	47	36.1	148	11.6	106
	18-0707	4.34	1.29	52.0	191.3	80.9	28.97	37.63	12.21	121	0.77	3.37	102.69	12.37	7.71	19	62.0	151	6.9	106
	18-0708	4.45	1.11	69.0	97.2	73.1	27.43	42.20	13.70	115	0.65	4.02	235.04	12.67	8.47	36	58.7	151	6.7	105
	Mean	4.38*	1.21*	56.3*	122.4	87.7	24.65*	53.3	13.11	111	0.48*	3.65*	200	13.42*	8.03*	33*	52.8*	151*	8.4*	106
	SD	0.15	0.12	12.9	38.8	30.4	4.74	13.4	0.71	17	0.14	0.40	73	1.66	0.52	13	10.1	1	1.4	2
56 mg/kg	18-0715	4.89	1.17	37.3	62.2	64.4	21.59	58.35	13.31	101	0.37	4.18	265.97	11.65	9.07	33	46.2	154	8.8	107
	18-0716	4.48	1.23	40.7	84.2	65.3	30.14	65.62	13.57	130	0.46	3.65	200.26	14.15	8.13	50	64.5	148	9.5	103
	18-0733	4.42	1.03	46.9	114.4	80.6	34.39	70.19	13.54	141	0.49	4.31	175.34	13.67	8.73	30	73.6	147	9.1	104
	18-0734	4.38	1.15	46.6	85.7	61.0	18.08	47.59	13.60	134	0.38	3.82	203.53	12.91	8.20	38	38.7	149	8.1	104
	18-0703	4.09	1.22	53.8	135.7	87.7	22.62	48.12	12.10	70	0.47	3.36	104.83	12.93	7.45	69	48.4	149	7.7	106
	18-0704	4.38	1.09	82.7	137.2	66.1	24.63	70.36	13.25	132	0.35	4.01	177.51	14.28	8.39	29	52.7	148	9.7	105
	18-0729	4.41	1.15	57.0	95.5	88.5	28.79	61.25	13.64	88	0.47	3.82	235.11	13.60	8.23	29	61.6	150	8.3	106
	18-0730	4.22	1.17	51.6	162.1	140.2	30.42	66.13	12.86	101	0.46	3.62	84.07	14.08	7.84	25	65.1	150	10.6	109
	18-0745	4.43	1.02	42.6	142.3	61.1	26.78	68.66	12.51	93	0.39	4.33	173.91	13.15	8.76	25	57.3	148	12.2	105
	18-0746	4.05	0.97	34.3	63.3	98.9	27.66	53.20	12.23	98	0.52	4.19	151.81	14.49	8.24	32	59.2	155	8.1	108
	Mean	4.38*	1.12*	49.4	108.3*	81.4	26.51*	60.9	13.06	109	0.44*	3.93*	177	13.49*	8.30*	36*	56.7*	150*	9.2	106
	SD	0.23	0.09	13.7	35.1	24.6	4.84	8.8	0.59	24	0.06	0.33	55	0.86	0.47	14	10.4	3	1.4	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)
111 mg/kg	18-0689	4.24	1.08	50.6	81.6	62.1	21.17	52.92	13.43	168	0.40	3.94	231.49	12.17	8.18	52	45.3	152	8.0	106
	18-0690	4.39	1.09	33.6	94.3	67.0	24.07	60.16	12.44	91	0.40	4.04	132.03	13.04	8.43	29	51.5	152	8.9	107
	18-0737	4.23	1.26	81.8	347.8	76.0	26.40	64.40	12.60	117	0.41	3.37	155.65	13.10	7.60	20	56.5	150	9.0	106
	18-0738	3.97	0.95	40.5	77.5	84.7	24.86	50.74	12.71	96	0.49	4.16	97.31	13.10	8.13	26	53.2	152	8.7	108
	18-0739	4.31	0.99	42.7	113.8	67.2	30.65	56.77	13.19	156	0.54	4.36	201.20	15.20	8.67	40	65.6	152	8.4	107
	18-0740	4.30	1.11	51.0	110.8	73.6	21.68	54.21	12.61	147	0.40	3.88	195.71	11.17	8.18	40	46.4	149	9.0	109
	18-0695	4.33	1.31	74.6	277.9	79.7	20.47	40.94	12.39	103	0.50	3.30	94.28	13.37	7.63	25	43.8	153	7.5	108
	18-0696	4.13	1.10	66.7	122.0	61.8	18.18	51.94	13.13	115	0.35	3.74	204.75	12.47	7.87	24	38.9	152	8.5	107
	18-0741	4.42	1.12	46.5	123.9	95.6	29.39	54.43	12.54	115	0.54	3.93	112.56	14.04	8.35	27	62.9	151	7.4	105
	18-0742	4.36	1.07	65.3	117.6	94.2	21.54	43.96	12.22	146	0.49	4.09	85.73	14.83	8.45	31	46.1	154	8.3	107
	Mean	4.27*	1.11*	55.3*	146.7	76.2	23.84*	53.0	12.73	125*	0.45*	3.88*	151	13.25*	8.15*	31*	51.0*	152*	8.3*	107
	SD	0.13	0.11	15.9	90.5	12.3	4.02	6.9	0.39	27	0.07	0.33	54	1.21	0.35	10	8.6	1	0.6	1
222 mg/kg	18-0709	3.90	0.99	67.4	150.6	93.9	26.87	68.90	13.06	103	0.39	3.95	192.91	12.30	7.85	27	57.5	150	7.9	105
	18-0710	4.14	1.02	52.4	197.6	66.4	12.99	41.91	13.28	157	0.31	4.05	223.38	12.23	8.19	45	27.8	150	7.2	105
	18-0711	4.02	0.99	44.3	108.2	72.1	22.43	49.85	12.71	123	0.45	4.05	164.96	11.94	8.07	38	48.0	153	7.2	109
	18-0712	4.31	1.11	64.4	127.4	77.8	30.56	61.12	12.81	92	0.50	3.90	274.10	12.06	8.21	31	65.4	151	7.9	109
	18-0727	4.49	0.98	58.4	114.9	71.6	28.83	90.10	13.31	128	0.32	4.59	213.68	13.41	9.08	44	61.7	152	8.6	107
	18-0728	3.99	0.98	67.9	193.7	96.5	26.73	78.62	12.95	163	0.34	4.07	157.66	13.25	8.06	34	57.2	152	7.6	107
	18-0721	4.10	1.15	80.0	309.8	79.9	22.38	47.62	12.20	119	0.47	3.57	110.26	12.65	7.67	20	47.9	150	9.2	108
	18-0722	4.27	1.12	60.4	139.5	85.3	19.30	41.96	13.26	137	0.46	3.80	178.76	12.43	8.07	36	41.3	153	7.9	106
	18-0725	4.38	1.13	59.8	121.1	63.0	20.19	59.37	14.03	118	0.34	3.86	306.59	10.69	8.24	49	43.2	151	7.4	107
	18-0726	4.19	1.01	44.5	256.1	73.0	17.99	43.88	13.35	110	0.41	4.13	248.33	12.47	8.32	31	38.5	152	8.0	106
	Mean	4.18	1.05*	60.0*	171.9	78.0	22.83*	58.3	13.10	125*	0.40*	4.00*	207	12.34*	8.18*	36*	48.9*	151*	7.9*	107
	SD	0.19	0.07	11.0	67.2	11.1	5.45	16.6	0.48	22	0.07	0.26	59	0.75	0.37	9	11.7	1	0.6	1

Note:

*p<0.05 versus 0 mg/kg control group, based on one-way ANOVA and Dunnett (2-sided) post hoc test for normally distributed data and Kruskal-Wallis and Mann-Whitney U post hoc test for data that are not normally distributed. Data for AST, ALKP, CREA, and TRIG were log-transformed, and data for K, PHOS, and TP were rank-transformed to achieve normal distribution of data. Data for Na and Cl were not normally distributed after transformation, so ranked data were analyzed by Kruskal-Wallis followed by the 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	18-0649	3.74	1.18	49.1	250.1	73.3	8.13	33.88	12.71	90	0.24	3.17	288.88	15.83	6.91	60	17.4	146	10.3	105
mg/kg	18-0650	3.61	1.20	49.7	215.9	123.9	8.79	28.34	13.41	86	0.31	3.02	197.76	19.21	6.63	89	18.8	149	10.9	108
	18-0657	3.59	1.25	90.8	359.6	77.8	14.58	48.60	13.05	97	0.30	2.87	370.89	13.52	6.46	102	31.2	148	7.9	104
	18-0658	3.51	1.31	50.4	268.7	91.4	8.08	32.34	12.58	67	0.25	2.67	198.84	15.06	6.18	105	17.3	148	8.7	104
	18-0671	3.58	1.44	54.2	186.5	88.0	10.70	31.47	13.35	90	0.34	2.48	304.97	16.44	6.06	166	22.9	147	10.0	105
	18-0672	3.55	1.38	38.9	267.4	78.3	8.97	33.23	12.21	77	0.27	2.58	246.97	16.90	6.13	83	19.2	149	11.4	107
	18-0675	3.71	1.18	44.2	263.3	71.1	11.31	43.49	13.42	65	0.26	3.15	264.68	13.86	6.86	110	24.2	148	7.2	106
	18-0676	3.46	1.41	56.1	238.5	81.2	13.04	40.74	12.14	92	0.32	2.46	175.64	14.17	5.92	86	27.9	144	9.7	106
	18-0685	3.56	1.36	57.7	189.1	73.7	11.36	39.16	12.95	101	0.29	2.62	229.37	16.11	6.18	138	24.3	145	9.9	104
	18-0686	3.43	1.28	39.0	166.5	89.3	10.23	42.64	12.35	97	0.24	2.68	152.90	13.09	6.11	79	21.9	149	7.2	107
	Mean	3.57	1.30	53.0	240.6	84.8	10.52	37.4	12.82	86	0.28	2.77	243	15.42	6.34	102	22.5	147	9.3	106
	SD	0.10	0.10	14.8	55.7	15.5	2.14	6.5	0.49	13	0.04	0.27	66	1.87	0.35	31	4.6	2	1.5	1
14	18-0629	4.11	1.10	64.0	234.3	76.1	27.34	63.57	13.07	118	0.43	3.75	263.52	14.19	7.86	53	58.5	147	9.7	104
mg/kg	18-0630	3.83	1.06	57.4	220.8	66.7	17.62	56.83	13.30	136	0.31	3.62	199.01	14.10	7.45	44	37.7	150	9.1	106
	18-0637	3.70	1.09	68.8	230.2	82.9	9.53	27.24	13.56	77	0.35	3.40	356.65	12.90	7.10	41	20.4	150	7.7	107
	18-0638	3.99	1.05	50.6	123.5	70.3	12.62	28.68	13.20	101	0.44	3.80	256.90	11.35	7.79	46	27.0	150	8.5	107
	18-0643	3.93	1.16	85.9	374.1	70.4	20.70	36.97	12.56	83	0.56	3.38	213.50	13.52	7.31	51	44.3	147	11.2	104
	18-0644	4.02	1.05	40.6	143.7	80.6	14.95	33.99	12.93	109	0.44	3.82	159.12	13.74	7.84	55	32.0	149	9.7	107
	18-0677	3.84	1.17	61.7	175.8	65.4	18.46	52.74	13.65	134	0.35	3.29	188.87	13.53	7.13	93	39.5	148	9.6	106
	18-0678	3.72	1.14	53.0	342.2	62.1	17.06	38.76	12.98	115	0.44	3.26	222.23	11.09	6.98	55	36.5	148	8.1	104
	18-0679	4.09	1.17	54.7	130.6	73.3	17.15	43.97	12.96	94	0.39	3.50	121.17	13.71	7.59	30	36.7	151	8.2	106
	18-0680	4.00	1.17	52.4	274.0	85.0	21.73	58.73	12.85	105	0.37	3.41	179.71	14.12	7.41	62	46.5	148	7.8	111
	Mean	3.92*	1.12*	58.9	224.9	73.3*	17.72*	44.1	13.11	107	0.41*	3.52*	216	13.23	7.45*	53*	37.9*	149	9.0	106
	SD	0.14	0.05	12.3	86.1	7.7	4.94	13.1	0.33	20	0.07	0.21	65	1.12	0.32	17	10.6	1	1.1	2
28	18-0623	3.87	1.05	60.1	309.4	67.0	21.64	47.03	12.17	84	0.46	3.69	149.50	11.10	7.56	26	46.3	150	7.5	106
mg/kg	18-0624	3.70	1.04	49.4	198.9	66.9	41.59	56.97	12.84	115	0.73	3.56	184.63	12.42	7.26	65	89.0	151	7.2	108
	18-0641	3.69	1.16	66.6	395.5	75.4	14.67	31.22	13.56	133	0.47	3.19	309.72	13.74	6.88	53	31.4	149	8.4	108
	18-0642	3.85	0.99	68.5	265.8	72.7	17.52	39.83	12.77	97	0.44	3.89	124.15	13.85	7.74	49	37.5	150	9.2	107
	18-0653	4.08	1.07	44.5	92.9	66.3	17.06	43.73	13.88	102	0.39	3.83	275.52	13.90	7.91	62	36.5	149	8.5	105
	18-0654	3.90	1.10	57.1	170.3	70.1	23.79	46.64	13.87	100	0.51	3.54	377.28	13.09	7.44	57	50.9	149	8.3	106
	18-0663	3.98	1.10	76.3	242.7	77.8	19.21	64.02	13.29	83	0.30	3.63	272.88	12.80	7.61	37	41.1	148	9.8	106
	18-0664	3.53	1.10	58.8	174.0	65.7	17.94	39.88	12.39	86	0.45	3.21	169.03	12.40	6.74	45	38.4	147	9.2	109
	18-0665	3.88	1.01	65.0	166.2	78.7	18.74	43.58	12.99	107	0.43	3.84	194.42	13.17	7.72	31	40.1	151	9.7	108
	18-0666	4.01	0.90	51.4	140.6	68.6	17.99	40.89	13.72	121	0.44	4.46	285.44	12.73	8.47	45	38.5	149	8.8	105
	Mean	3.85*	1.05*	59.8	215.6	70.9*	21.02*	45.4	13.15	103	0.46*	3.68*	234	12.92*	7.53*	47*	45.0*	149*	8.7	107
	SD	0.17	0.07	9.7	89.2	4.9	7.65	9.3	0.61	17	0.11	0.36	81	0.85	0.50	13	16.4	1	0.9	1
56	18-0633	3.95	1.37	90.1	292.6	69.9	13.69	34.23	12.54	145	0.40	2.89	273.55	12.38	6.84	51	29.3	151	7.1	105
mg/kg	18-0634	3.80	1.16	68.6	270.6	80.7	16.96	44.64	12.52	106	0.38	3.28	129.10	14.31	7.08	50	36.3	148	10.5	108
	18-0635	3.81	1.04	62.5	115.9	68.0	14.30	36.67	13.32	91	0.39	3.65	243.22	13.16	7.46	39	30.6	152	8.7	108
	18-0636	3.99	1.11	71.2	184.9	70.8	15.89	45.39	13.93	141	0.35	3.59	396.47	14.12	7.58	74	34.0	151	8.6	106
	18-0651	3.92	1.01	87.1	238.5	81.1	16.26	56.08	12.97	81	0.29	3.90	152.21	11.61	7.82	42	34.8	148	8.4	105
	18-0652	3.82	1.09	56.8	173.5	95.1	13.46	42.06	14.11	131	0.32	3.49	280.57	14.70	7.31	60	28.8	147	10.7	108
	18-0661	3.91	1.33	66.4	274.3	79.8	14.58	39.40	13.03	93	0.37	2.95	182.44	12.67	6.86	49	31.2	151	7.6	110
	18-0662	3.89	1.04	64.5	195.7	75.8	14.86	53.07	13.37	82	0.28	3.74	208.92	11.95	7.63	96	31.8	149	7.7	106
	18-0681	3.77	1.01	49.0	156.7	89.1	16.96	51.40	12.46	88	0.33	3.72	115.31	11.11	7.49	55	36.3	148	8.0	107
	18-0682	3.66	1.09	70.8	189.7	78.6	14.77	42.19	13.20	110	0.35	3.37	144.72	12.89	7.03	46	31.6	149	9.6	108
	Mean	3.85*	1.13*	68.7	209.2	78.9	15.17*	44.5	13.15	107	0.35	3.46*	213	12.89*	7.31*	56*	32.5*	149*	8.7	107
	SD	0.10	0.13	12.5	57.5	8.5	1.27	7.1	0.57	24	0.04	0.34	87	1.20	0.34	17	2.7	2	1.2	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)
111	18-0645	3.81	0.98	63.5	219.6	81.9	16.03	33.39	13.80	93	0.48	3.89	360.54	13.48	7.70	52	34.3	150	8.7	105
mg/kg	18-0646	3.93	1.10	45.8	200.2	66.8	14.86	32.30	12.64	117	0.46	3.58	141.70	13.05	7.51	40	31.8	153	7.7	108
	18-0655	3.97	1.15	86.3	291.5	85.4	17.52	44.93	13.52	93	0.39	3.44	280.16	15.41	7.41	68	37.5	151	8.6	105
	18-0656	3.89	1.11	63.5	316.8	70.7	15.47	41.80	13.88	115	0.37	3.51	370.29	14.11	7.40	65	33.1	149	8.9	104
	18-0667	3.79	1.14	93.5	297.8	75.5	18.51	47.45	12.07	100	0.39	3.33	129.61	12.64	7.12	41	39.6	147	9.9	106
	18-0668	3.96	1.06	96.3	239.4	68.1	20.14	45.77	13.52	120	0.44	3.73	291.24	11.96	7.69	55	43.1	146	8.2	107
	18-0669	3.85	0.98	59.5	155.3	82.7	18.65	45.48	12.93	95	0.41	3.92	161.48	12.73	7.77	61	39.9	151	8.0	106
	18-0670	3.73	1.04	95.5	292.5	76.7	14.53	37.26	12.03	98	0.39	3.58	144.93	11.80	7.31	42	31.1	149	8.8	107
	18-0673	3.71	1.09	68.8	195.3	83.0	16.50	51.55	12.17	117	0.32	3.40	85.64	14.08	7.11	46	35.3	149	11.0	105
	18-0674	4.05	1.07	76.0	293.0	65.1	22.57	62.70	13.42	107	0.36	3.77	189.34	12.62	7.82	49	48.3	148	9.8	106
	Mean	3.87*	1.07*	74.9*	250.1	75.6	17.48*	44.3	13.00	106	0.40*	3.62*	215	13.19*	7.48*	52*	37.4*	149*	9.0	106
	SD	0.11	0.06	17.5	55.4	7.5	2.54	9.0	0.73	11	0.05	0.20	102	1.10	0.26	10	5.4	2	1.0	1
222	18-0621	3.47	0.87	59.8	322.4	70.3	20.47	55.32	13.36	168	0.37	3.99	244.94	10.30	7.46	105	43.8	150	6.0	107
mg/kg	18-0622	3.50	0.94	87.8	284.8	79.2	15.65	29.54	13.69	114	0.53	3.73	265.67	13.14	7.23	60	33.5	146	8.3	107
	18-0631	3.51	0.97	71.4	375.5	74.8	22.90	63.60	12.85	115	0.36	3.63	206.11	13.42	7.14	55	49.0	146	8.6	105
	18-0632	3.49	0.88	51.9	359.2	80.1	16.08	51.86	13.82	94	0.31	3.95	258.34	14.28	7.44	93	34.4	147	7.6	106
	18-0647	3.95	0.99	95.7	313.4	78.9	19.44	72.00	14.02	112	0.27	3.98	340.80	11.99	7.93	98	41.6	149	7.8	106
	18-0648	3.95	0.97	85.3	214.2	73.7	15.23	52.53	14.36	132	0.29	4.09	288.31	13.09	8.04	121	32.6	151	7.7	105
	18-0659	3.59	0.89	73.9	329.5	86.1	17.34	40.32	13.00	135	0.43	4.03	177.84	13.66	7.62	65	37.1	146	8.9	107
	18-0660	3.47	1.02	71.2	245.2	90.7	15.14	37.85	13.07	102	0.40	3.39	269.49	14.03	6.86	51	32.4	147	8.2	112
	18-0683	3.54	1.15	60.5	202.3	74.5	16.17	55.75	12.38	113	0.29	3.08	238.99	12.30	6.62	35	34.6	147	9.3	105
	18-0684	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	3.61	0.96*	73.1*	294.1	78.7	17.60*	51.0	13.39	121*	0.36	3.76*	255	12.91*	7.37*	76	37.7*	148	8.0	107
	SD	0.20	0.09	14.4	61.8	6.4	2.73	13.2	0.63	22	0.08	0.34	47	1.23	0.47	29	5.8	2	1.0	2

Legend:
ND = No data analyzed
Note:
*p<0.05 versus 0 mg/kg control group, based on one-way ANOVA and Dunnett (2-sided) post hoc test for normally distributed data and Kruskal-Wallis and Mann-Whitney U post hoc test for data that are not normally distributed. Data for ALB/GLOB were log-transformed, and data for PHOS, TRIG, TP, Urea, and BUN were rank-transformed to achieve normal distribution of data. Rank-transformed data for BUN, TP, and Urea contained unequal error variances and were analyzed via Dunnett T3 post hoc tests. Data for Na and Cl were not normally distributed after transformation, so ranked data were analyzed by Kruskal-Wallis followed by the Mann-Whitney U post hoc test.

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

		Sex	DNP in Corn Oil 0 mg/kg	14 mg/kg	28 mg/kg	56 mg/kg	111 mg/kg	222 mg/kg
Albumin (ALB)	**(g/dL)**	Females	4.01±0.20	4.41±0.25*	4.38±0.15*	4.38±0.23*	4.27±0.13*	4.18±0.19
		N	10	10	10	10	10	10
		Males	3.57±0.10	3.92±0.14*	3.85±0.17*	3.85±0.10*	3.87±0.11*	3.61±0.20
		N	10	10	10	10	10	9
ALB / GLOB		Females	1.37±0.09	1.20±0.11*	1.21±0.12*	1.12±0.09*	1.11±0.11*	1.05±0.07*
		N	10	10	10	10	10	10
		Males	1.30±0.10	1.12±0.05*	1.05±0.07*	1.13±0.13*	1.07±0.06*	0.96±0.09*
		N	10	10	10	10	10	9
Alanine aminotrans-ferase (ALT)	**(U/L)**	Females	40.5±10.0	59.3±11.1*	56.3±12.9*	49.4±13.7	55.3±15.9*	60.0±11.0*
		N	10	10	10	10	10	10
		Males	53.0±14.8	58.9±12.3	59.8±9.7	68.7±12.5	74.9±17.5*	73.1±14.4*
		N	10	10	10	10	10	9
Alkaline phosphatase (ALKP)	**(U/L)**	Females	165.9±32.8	126.1±29.6	122.4±38.8	108.3±35.1*	146.7±90.5	171.9±67.2
		N	10	10	10	10	10	10
		Males	240.6±55.7	224.9±86.1	215.6±89.2	209.2±57.5	250.1±55.4	294.1±61.8
		N	10	10	10	10	10	9
Aspartate aminotrans-ferase (AST)	**(U/L)**	Females	85.5±14.7	82.5±22.1	87.7±30.4	81.4±24.6	76.2±12.3	78.0±11.1
		N	10	10	10	10	10	10
		Males	84.8±15.5	73.3±7.7*	70.9±4.9*	78.9±8.5	75.6±7.5	78.7±6.4
		N	10	10	10	10	10	9
Blood urea nitrogen (BUN)	**(md/dL)**	Females	14.42±2.56	25.56±4.87*	24.65±4.74*	26.51±4.84*	23.84±4.02*	22.83±5.45*
		N	10	10	10	10	10	10
		Males	10.52±2.14	17.72±4.94*	21.02±7.65*	15.17±1.27*	17.48±2.54*	17.60±2.73*
		N	10	10	10	10	10	9
BUN / CREA		Females	50.5±14.3	58.1±12.3	53.3±13.4	61.0±8.8	53.1±6.9	58.3±16.6
		N	10	10	10	10	10	10
		Males	37.4±6.5	44.2±13.1	45.4±9.3	44.5±7.2	44.3±9.0	51.0±13.2
		N	10	10	10	10	10	9
Calcium (Ca)	**(mg/dL)**	Females	13.00±0.64	13.04±0.79	13.11±0.71	13.06±0.59	12.73±0.39	13.10±0.48
		N	10	10	10	10	10	10
		Males	12.82±0.49	13.11±0.33	13.15±061	13.15±0.57	13.00±0.73	13.39±0.63
		N	10	10	10	10	10	9
Cholesterol (CHOL)	**(mg/dL)**	Females	87±17	109±20	111±17	109±24	125±27*	125±22*
		N	10	10	10	10	10	10
		Males	86±13	107±20	103±17	107±24	106±11	121±22*
		N	10	10	10	10	10	9
Creatinine (CREA)	**(mg/dL)**	Females	0.30±0.06	0.46±0.13*	0.48±0.14*	0.44±0.06*	0.45±0.07*	0.40±0.07*
		N	10	10	10	10	10	10
		Males	0.28±0.04	0.41±0.07*	0.46±0.11*	0.35±0.04	0.40±0.05*	0.36±0.08
		N	10	10	10	10	10	9
Globulin (GLOB)	**(g/dL)**	Females	2.94±0.23	3.71±0.40*	3.65±0.40*	3.93±0.33*	3.88±0.33*	4.00±0.26*
		N	10	10	10	10	10	10
		Males	2.77±0.27	3.52±0.21*	3.68±0.36*	3.46±0.34*	3.62±0.20*	3.76±0.34*
		N	10	10	10	10	10	9
Glucose (GLU)	**(mg/dL)**	Females	181±122	184±87	200±73	177±55	151±54	207±59
		N	10	10	10	10	10	10
		Males	243±66	216±65	234±81	213±87	215±102	255±47
		N	10	10	10	10	10	9
Phosphate (PHOS)	**(mg/dL)**	Females	19.34±2.12	13.11±0.99*	13.42±1.66*	13.49±0.86*	13.25±1.21*	12.34±0.75*
		N	10	10	10	10	10	10
		Males	15.42±1.87	13.23±1.12	12.92±0.85*	12.89±1.20*	13.19±1.10*	12.91±1.23*
		N	10	10	10	10	10	9
Total Protein (TP)	**(g/dL)**	Females	6.95±0.38	8.11±0.58*	8.03±0.52*	8.30±0.47*	8.15±0.35*	8.18±0.37*
		N	10	10	10	10	10	10
		Males	6.34±0.35	7.45±0.32*	7.53±0.50*	7.31±0.34*	7.48±0.26*	7.37±0.47*
		N	10	10	10	10	10	9

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

		Sex		DNP in Corn Oil					
				0 mg/kg	14 mg/kg	28 mg/kg	56 mg/kg	111 mg/kg	222 mg/kg
Triglycer-ides (TRIG)	(g/dL)	Females		56±14	32±7*	33±13*	36±14*	31±10*	36±9*
			N	10	10	10	10	10	10
		Males		102±31	53±17*	47±13*	56±17*	52±10*	76±29
			N	10	10	10	10	10	9
UREA	(mg/dL)	Females		30.9±5.5	54.7±10.4*	52.8±10.1*	56.7±10.4	51.0±8.6*	48.9±11.7*
			N	10	10	10	10	10	10
		Males		22.5±4.6	37.9±10.6*	45.0±16.4*	32.5±2.7*	37.4±5.4*	37.7±5.8*
			N	10	10	10	10	10	9
Sodium (Na)	(mmol/L)	Females		146±2	150±1*	151±1*	150±3*	152±1*	151±1*
			N	10	10	10	10	10	10
		Males		147±2	149±1	149±1*	149±2*	149±2*	148±2
			N	10	10	10	10	10	9
Potassium (K)	(mmol/L)	Females		12.2±1.3	8.5±0.8*	8.4±1.4*	9.2±1.4	8.3±0.6*	7.9±0.6*
			N	10	10	10	10	10	10
		Males		9.3±1.5	9.0±1.1	8.7±0.9	8.7±1.2	9.0±1.0	8.0±1.0
			N	10	10	10	10	10	9
Chloride (Cl)	(mmol/L)	Females		107±1	107±1	106±2	106±2	107±1	107±1
			N	10	10	10	10	10	10
		Males		106±1	106±2	107±1	107±2	106±1	107±2
			N	10	10	10	10	10	9

Note:
*p<0.05 versus 0 mg/kg 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 are not normally distributed.

APPENDIX M

GROSS PATHOLOGY DATA

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

DOSE	SEX	ANIMAL ID	GROSS OBSERVATIONS
0 mg/kg	Female	18-0697	NGLR
		18-0698	NGLR
		18-0723	NGLR
		18-0724	NGLR
		18-0717	Several multifocal 2-3mm areas of discoloration on the lungs
		18-0718	NGLR
		18-0719	NGLR
		18-0720	NGLR
		18-0731	NGLR
		18-0732	NGLR
	Male	18-0649	NGLR
		18-0650	NGLR
		18-0671	NGLR
		18-0672	NGLR
		18-0685	NGLR
		18-0686	Mild diffuse yellow discoloration of the liver in a lobular pattern
		18-0657	NGLR
		18-0658	NGLR
		18-0675	NGLR
		18-0676	NGLR
DOSE	SEX	ANIMAL ID	GROSS OBSERVATIONS
14 mg/kg	Female	18-0687	NGLR
		18-0688	NGLR
		18-0705	NGLR
		18-0706	NGLR
		18-0735	NGLR
		18-0736	Purple discoloration of thymus
		18-0743	NGLR
		18-0744	NGLR
		18-0701	NGLR
		18-0702	NGLR
	Male	18-0637	Diffuse tan/yellow discoloration of the liver in a lobular pattern
		18-0638	Diffuse tan/yellow discoloration of the liver in a lobular pattern; Purple discoloration of the thymus
		18-0677	NGLR
		18-0678	NGLR
		18-0679	NGLR
		18-0680	NGLR
		18-0629	Purple discoloration of thymus
		18-0630	NGLR
		18-0643	Mild purple discoloration of thymus
		18-0644	Purple discoloration of half of thymus

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

DOSE	SEX	ANIMAL ID	GROSS OBSERVATIONS
28 mg/kg	Female	18-0693	NGLR
		18-0694	NGLR
		18-0699	Purple discoloration of thymus
		18-0700	NGLR
		18-0713	NGLR
		18-0714	NGLR
		18-0691	NGLR
		18-0692	NGLR
		18-0707	NGLR
		18-0708	NGLR
	Male	18-0641	Purple discoloration of 1/4 of thymus
		18-0642	NGLR
		18-0653	Mild multi-focal tan discoloration of 2/3 of liver
		18-0654	NGLR
		18-0665	One testis is enlarged by ~15%; Purple discoloration on half of thymus
		18-0666	Light pink discoloration of thymus
		18-0623	Purple discoloration of 90% of thymus
		18-0624	Diffuse severe yellow discooration of liver in a lobular pattern; Kidneys enlarged 2-3 times normal with pale discoloration and cysts that are <1mm in diameter; Urinary bladder is distended, contains mucus blob
		18-0663	NGLR
		18-0664	Mild yellow discoloration of the liver in a lobular pattern
DOSE	SEX	ANIMAL ID	GROSS OBSERVATIONS
56 mg/kg	Female	18-0715	NGLR
		18-0716	NGLR
		18-0733	NGLR
		18-0734	NGLR
		18-0703	NGLR
		18-0704	NGLR
		18-0729	NGLR
		18-0730	NGLR
		18-0745	NGLR
		18-0746	NGLR
	Male	18-0651	Mild purple discoloration of half of thymus
		18-0652	NGLR
		18-0661	NGLR
		18-0662	NGLR
		18-0635	Diffuse yellow discoloration of the liver in a lobular pattern; Purple discoloration of 1/4 of thymus
		18-0636	One testis is 1/4 size of opposite/normal testis; Purple discoloration of thymus
		18-0633	NGLR
		18-0634	NGLR
		18-0681	NGLR
		18-0682	Purple discoloration of 1/4 of thymus

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

DOSE	SEX	ANIMAL ID	GROSS OBSERVATIONS
111 mg/kg	Female	18-0689	Yellow-greenish material loosely adhered to stomach mucosa
		18-0690	NGLR
		18-0737	NGLR
		18-0738	NGLR
		18-0739	NGLR
		18-0740	NGLR
		18-0695	White fibrous material attached to mucosa of stomach with minimal redness
		18-0696	NGLR
		18-0741	NGLR
		18-0742	Minimal yellow staining of fur of ventral abdomen, chest, and mandible
	Male	18-0645	NGLR
		18-0646	NGLR
		18-0655	NGLR
		18-0656	Minimal diffuse yellow discoloration of the liver in a lobular pattern
		18-0669	Purple/pink discoloration of thymus
		18-0670	NGLR
		18-0673	NGLR
		18-0674	NGLR
		18-0667	NGLR
		18-0668	Purple discoloration of 1/3 of thymus
DOSE	SEX	ANIMAL ID	GROSS OBSERVATIONS
222 mg/kg	Female	18-0709	Yellow staining of fur on ventral surface
		18-0710	Yellow staining on fur of vulva
		18-0711	Yellow staining of fur on vulva and abdomen
		18-0712	Mild yellow staining of fur on abdomen
		18-0727	Yellow staining of fur around vulva and abdomen
		18-0728	Yellow staining of fur around vulva and abdomen
		18-0721	Yellow staining of fur around vuluva and abdomen; Uterus is dilated up to 4mm in diameter with fluid throughout
		18-0722	Yellow staining of fur around vulva and abdomen
		18-0725	NGLR
		18-0726	NGLR
	Male	18-0631	NGLR
		18-0632	NGLR
		18-0647	NGLR
		18-0648	Diffuse purple discoloration of thymus
		18-0621	Purple discoloration of thymus
		18-0622	NGLR
		18-0659	Mild yellow staining of hair around penis
		18-0660	Thymus is diffusely light pink in color
		18-0683	Liver has 1 0.25cm nodule, same consistency as surrounding liver (proliferative nodule)
		18-0684*	1x2x1 cm dark red mass attached to vas deferens; 1x2x3 mm mass attached to bladder wall; Kidneys are markedly enlarged; Hydronephrosis; Right ventricular wall has several multifocal tan/white areas of discoloration

Legend:
NGLR=No gross lesion reported
Note: *Pathological observations not considered to be compound-dependent

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

	0 mg/kg		14 mg/kg		28 mg/kg		56 mg/kg		111 mg/kg		222 mg/kg	
	M	F	M	F	M	F	M	F	M	F	M	F
NGLR	9	9	5	9	3	9	6	10	7	7	4	2
Skin, yellow staining, perineum (vulva, abdomen, or prepuce)										1	1	8
Thymus, diffuse, light pink					1				1		1	
Thymus, purple discoloration			4	1	3	1	4		2		2	
Hydronephrosis											1	
Kidney, enlarged					1						1	
Kidney, cyst					1							
Uterus, enlarged and/or fluid filled												1
Bladder, mass											1	
Urinary bladder, distended with mucous					1							
Liver, diffuse yellow discoloration	1		2		2		1		1			
Liver, lobular pattern	1		2		2		1		1			
Liver, nodule											1	
liver, tan multi-focal					1							
Lungs, multi-focal, discolration		1										
Heart, multi-focal discoloration											1	
Stomach, white fibrinous material										1		
Stomach, yellow-greenish material adhered to mucosa										1		
Vas deferens, red mass											1	
Testis, small							1					
Testis, enlarged					1							

Legend:
NGLR=No gross lesion reported

APPENDIX N

HISTOPATHOLOGY REPORT

PATHOLOGY REPORT
FOR

Protocol No. 30-18-07-01

Study Title
EFFECTS OF SUBACUTE ORAL 3,4-DINITROPYRAZOLE (DNP) EXPOSURE TO RATS (*RATTUS NORVEGICUS*).

Study Director:
Thomas E. Sussan, PhD

Prepared by:
LTC Keith Koistinen, DVM, Diplomate, ACVP

Performing Laboratory
U.S. Army Public Health Center
Toxicology (MCHB-PH-TEV)
5158 Blackhawk Road
Aberdeen Proving Ground, MD 21010-5403

Table of Contents

1 Objective

This study/experiment was conducted in order to assess the toxicity of a new experimental explosive DNP, via oral dosing/gavage with DNP daily for 14 consecutive days. This experiment was followed by a subchronic 90 day study.

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 animal exposures by group.

Table 1. Animal Exposure Summary

Group #	Chemical	Route	Exposure Length (days)	Target Concentration (mg/kg)	# of animals exposed (males and females)
0	Vehicle Control	Oral gavage	14	0	20
1	DNP	Oral gavage	14	14	20
2	DNP	Oral gavage	14	28	20
3	DNP	Oral gavage	14	56	20
4	DNP	Oral gavage	14	111	20
5	DNP	Oral gavage	14	222	20

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; epididymides; 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 tissues/slides from only the high dose group (222 mg/kg-d), and vehicle control were processed and analyzed. Limited tissue evaluation from the presumed target organs and from only the high dose and control groups were evaluated because this 14 day study was conducted prior to a subchronic (90 day) exposure study, and effects on other tissues and dosages will be further refined from the subchronic study. The tissues microscopically evaluated included the liver, spleen, kidney, adrenal gland, stomach, thymus, and bone marrow aspirate. 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.

3 Results

3.1 Gross Findings

Bright yellow urine staining of the hair around the vulva was commonly noted in rats in the high dose group (222 mg/kg-d). This finding was most frequently noted in the female rats in the high dose group (**8 of 10 females**). Slight purple discoloration of the thymus and slight yellow discoloration of the liver was observed in a few animals from multiple groups (1 to 4 animals per group). Despite consistent microscopic changes in the stomach observed in the high dose group (222 mg/kg-d), only two animals in the study had gross changes in the stomach, which consisted of white fibrinous to yellow-greenish material adhered to the gastric mucosa.

Additional findings were noted in individual animals that were sporadically distributed across dose groups and sexes. These findings included kidney cysts and distended urinary bladder (1 male at 28 mg/kg), multifocal discoloration of lungs (female at 0 mg/kg), enlarged testis (1 male at 28 mg/kg), small testis (1 male at 56 mg/kg), yellow-green material adherent to stomach mucosa (1 female at 111 mg/kg), white fibrinous material attached to stomach mucosa (1 female at 111 mg/kg), proliferative nodule on liver (1 male at 222 mg/kg), and one rat containing a mass attached to the bladder wall with enlarged kidneys, hydronephrosis and discoloration of the right ventricle (222 mg/kg-d dose group).

3.2 Histopathology

Treatment-related changes were observed in the stomach of male and female rats. The stomach of all of the male and female rats in the high dose group (222 mg/kg-d) had 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. Thymic perifollicular congestion and hemorrhage was occasionally observed and had a slightly increased incidence in DNP exposed rats (2 females at 0 mg/kg; 3 males at 222 mg/kg; and 4 females at 222 mg/kg), but this finding was not statistically significant. This thymic microscopic finding is likely correlated to the gross finding of purple thymic discoloration. Thymic congestion was variably accompanied by increased lymphocyte apoptosis, and lymphocyte apoptosis is a physiologically normal occurrence that may be increased by stress, and is unlikely to be an adverse finding. Only the stomach from highest dose group (222 mg/kg-d group) was microscopically evaluated due to the expected evaluation of the mid-dose groups in the follow-on sub-chronic (90 day) study. The stomach microscopic changes is attributed to exposure to the test article and was observed in all of the rats exposed to the highest dose (222 mg/kg-d group, p=0.000).

In the liver of male and female rats, various abnormalities including lymphocytic infiltrates, hepatocellular necrosis, and vacuolar degeneration distributed in a periportal to centrilobular pattern were observed. These abnormalities were seen in nearly equally in controls and dosed animals, and there was no clear evidence of necrosis, hemorrhage. There were no or minimal changes observed in the spleen, bone marrow, and adrenal gland. The kidney had no significant changes in any animals examined, except the kidney of one male rat (18-684) in the 222 mg/kg-d group displayed severe kidney pathology consisting of end-stage cortical atrophy and necrosis with hydronephrosis. This rat also had a mass in the urinary bladder, and the renal changes are due to obstruction of urine from the mass, and are unrelated to dosing. The histologic changes suggestive of DNP-induced toxicity was equally observed in male and female rats. See Appendix G for details.

4 Discussion

Despite consistent microscopic changes in the stomach observed in the high dose group (222 mg/kg-d), these microscopic findings were not severe enough to be noted macroscopically. Only two animals in the study had gross changes in the stomach, which consisted of white fibrinous to yellow-greenish material adhered to the gastric mucosa.

Thymic congestion was variably accompanied by increased lymphocyte apoptosis. Lymphocyte apoptosis is a physiologically normal process that can be increased by stress, and it is difficult to separate potential causes.

The microscopic changes in the stomach are likely attributed to a direct irritant effect. These lesions were only observed in the non-glandular mucosa 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 unlikely or questionable.

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.

Prepared By:

Digitally signed by
KOISTINEN.KEITH.AARON.1246838085
Date: 2022.01.25 17:11:46 -05'00'

KEITH A. KOISTINEN
Chief, Office of Toxicologic Pathology

Date

References

1. Hailey JR, Walker NJ, Sells DM, Brix AE, Jokinen MP, Nyska A. Classification of proliferative hepatocellular lesions in harlan sprague-dawley rats chronically exposed to dioxin-like compounds. *Toxicol Pathol,* 2005, 33: 165–174

2. Karbe E, Kerlin RL Cystic degeneration/Spongiosis hepatis in rats. *Toxicol Pathol*, 2002, 30: 216–227

3. Kittel B, Ruehl-Fehlert C, Morawietz G, Klapwijk J, Elwell MR, Lenz B, et al.: Revised guides for organ sampling and trimming in rats and mice--Part 2. A joint publication of the RITA and NACAD groups. *Exp Toxicol Pathol* 2004:55(6):413-431.

4. Morawietz G, Ruehl-Fehlert C, Kittel B, Bube A, Keane K, Halm S, et al.: Revised guides for organ sampling and trimming in rats and mice--Part 3. A joint publication of the RITA and NACAD groups. *Exp Toxicol Pathol* 2004:55(6):433-449.

5. QSARC SOP 800, *Animal Euthanasia*, 2017, Army Public Health Center, Aberdeen Proving Ground, MD.

Table 2: Incidence of Histopathologic Finding by Group

		Lesion Incidence			
	Sex	M	F	M	F
	Dose Group (mg/kg)	0	0	222	222
	Number of Animals	10	10	10	10
Liver	# Examined	10	10	8	10
	Essentially normal tissue	2	1	5	4
	Vacuolar degeneration, lipid-type	7	8	2	6
	Vacuolar degeneration, lipid-type, diffuse	2		2	
	Vacuolar degeneration, lipid-type, multifocal	1			
	Vacuolar degeneration, lipid-type, periportal	2	8		6
	Vacuolar degeneration, lipid-type, centrilobular	1			
	Vacuolar degeneration, glycogen-type (rarefaction)	4	1	1	
	Cytoplasmic Inclusions, hepatocyte (plasma influx)		1		2
	Hepatocellular hypertrophy			1	
	Necrosis, hepatocellular, single cell	2	1		
	Infiltrates, lymphocytic	2	2	4	3
Spleen	# Examined	10	10	9	10
	Essentially normal tissue	10	10	9	10
Kidney	# Examined	10	10	10	10
	Essentially normal tissue	8	10	9	9
	Infiltrate, interstitial, lymphocytic	2			1
	Tubular epithelial cell degeneration	1			
	Inflammation, neutrophilic, intratubular, multifocal	1			
	End stage kidney, cortical atrophy and necrosis			1	
	Infarct, wedge segmental necrosis			1	
	Tubulitis, neutrophilic inflammation			1	
	Hydronephrosis, pelvic dilatation			1	
Adrenal gland	# Examined	10	10	10	10
	Essentially normal tissue	10	9	9	9
	Cortical epithelial cells, vacuolation, increased		1	1	
	Nodular Hyperplasia, cortex				1
Stomach	# Examined	10	10	9	10
	Essentially normal tissue	9	7		
	Squamous epithelial hyperplasia, Non-glandular stomach			9	9
	Hyperkeratosis, non-glandular mucosa			9	10
	Submucosal edema		1	10	9
	Cellular infiltrates, neutrophilic and lymphocytic		1	9	9
	Congestion, submucosa			9	7
	Intravascular thrombi			1	
	Degeneration, epithelium, glandular crypts	1	1	2	1
	Ectasia, glandular	1	2	2	1
	Intraglandular debris, crypt abscesses	1	2	2	1
	Granulation tissue, submucosal, nonglandular region			1	
Thymus	Absent			2	
	# Examined	10	10	8	10
	Essentially normal tissue	9	7	5	3
	Abscess, focal	1			
	Perifollicular congestion/hemorrhage		2	3	4
	Lymphocyte apoptosis, increased		2	2	6
Bone marrow, aspirate	Absent			1	
	# Examined	10	7	8	8
	Essentially normal tissue	10	7	8	8

Table 3: Statistical Analysis Results

		Female						Male					
DNP Dose Group (mg/kg)		0	14	28	56	111	222	0	14	28	56	111	222
Stomach	n	10					10	10					9
Essentially normal tissue	incidence	7**					0	9**					0
Squamous epithelial hyperplasia, Non-glandular stomach	incidence	0					9**	0					9**
Hyperkeratosis, non-glandular mucosa	incidence	0					10**	0					9**
Submucosal edema	incidence	1					9**	0					10**
Cellular infiltrates, neutrophilic and lymphocytic	incidence	1					9**	0					9**
Congestion, submucosa	incidence	0					7**	0					9**
Thymus	n	10					10	10					8
Perifollicular congestion/hemorrhage	incidence	2					4	0					3
Gross Findings	n	10	10	10	10	10	10	10	10	10	10	10	10
No Gross Lesion Recognized	incidence	9	9	9	10	7	2*	9	5	3*	6	7	4
Thymus, purple discoloration	incidence	0	1	1	0	0	0	0	4	3	4	2	2
Skin, yellow staining, perineum (vulva, abdomen, or prepuce)	incidence	0	0	0	0	1	8**	0	0	0	0	0	1

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

Table 4A: Male High dose and Control Individual Animal Histopathologic Findings

	Chemical	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP
	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	FD
	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	FD
	Date of necropsy	10/9	10/9	10/11	10/11	10/10	10/10	10/11	10/11	10/10	10/10	10/10	10/10	10/9	10/9	10/9	10/9	10/9	10/10	10/10	10/11	10/1
	Sex	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
	Dose Group	0	0	0	0	0	0	0	0	0	0	222	222	222	222	222	222	222	222	222	222	222
	Animal Number 18-0XXX	649	650	657	658	671	672	675	676	685	686	621	622	631	632	647	648	659	660	683	684	
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		
	Essentially normal tissue			0				0				0		0			0		0	0		
	Vacuolar degeneration, lipid-type	2	1		1		1		1	2	2				3	1						
	Vacuolar degeneration, lipid-type, diffuse	2	1												3	1						
	Vacuolar degeneration, lipid-type, multifocal		1																			
	Vacuolar degeneration, lipid-type, periportal								1		2											
	Vacuolar degeneration, lipid-type, centrilobular									2												
	Vacuolar degeneration, glycogen-type (rarefaction)				2	1	1		1									1				
	Cytoplasmic inclusions, hepatocyte (plasma influx/congestion droplets)																					
	Hepatocellular hypertrophy														1							
	Necrosis, hepatocellular, single cell	2									1											
	Infiltrates, lymphocytic	1							1				1		1	1		2				
Spleen	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	0	0	0	0	0	0	0	0	0	0	0	0		
	slide missing																				1	
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		
	Infiltrate, interstitial, lymphocytic			1	2																	
	Tubular epithelial cell degeneration				1																	
	Inflammation, neutrophilic, intratubular, multifocal				1																	
	End stage kidney, cortical atrophy and necrosis																				4	
	Infarct, wedge segmental necrosis																				4	
	Tubulitis, neutrophilic inflammation																				5	
	Hydronephrosis, pelvic dilatation																				3	
Adrenal gland	Autolysis (5=most severe, precludes analysis)																				4	
	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	1	0	0	0	0	
	Cortical epithelial cells, vacuolation, increased																					
	Nodular Hyperplasia, cortex																					
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											
	Squamous epithelial hyperplasia, Non-glandular stomach											2	3	3	3	3	3	3	3	3		
	Hyperkeratosis, non-glandular mucosa											2	3	3	3	3	3	1	2	2		
	Submucosal edema											1	2	3	2	1	1	1	2	1	1	
	Cellular infiltrates, neutrophilic and lymphocytic												2	2	3	2	2	1	2	1	2	
	Congestion, submucosa											1	2	1	1	3	1	1	1	2		
	Intravascular thrombi																				2	
	Degeneration, epithelium, glandular crypts								1					2	1							
	Ectasia, glandular								1					2	2							
	Intraglandular debris, crypt abscesses								1					2	1							
	Granulation tissue, submucosal, nonglandular region															2						
Thymus	Absent (1=true, blank not true)																			1	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			
	Essentially normal tissue	0	0	0	0		0	0	0	0	0		0	0		0		0	0			
	Abscess, focal					1																
	Perifollicular congestion/hemorrhage											1			1		2					
	Lymphocyte apoptosis, increased														2		1					
Bone marrow, aspirate	Absent (1=true, blank not true)																				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		
	Essentially normal tissue	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0		
	Sample of adequate diagnostic quality (1- yes, 0-non-diagnostic	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1		

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).

Table 4B: Female High dose and Control Individual Animal Histopathologic Findings

Chemical		DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP	DNP
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		10/2	10/2	10/3	10/3	10/3	10/3	10/2	10/2	10/4	10/4	10/2	10/2	10/3	10/3	10/4	10/4	10/4	10/4	10/3	10/3
Sex		F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
Dose Group		0	0	0	0	0	0	0	0	0	0	222	222	222	222	222	222	222	222	222	222
Animal Number 18-0XXX		697	698	717	718	719	720	723	724	731	732	709	710	711	712	721	722	725	726	727	728
	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		
	Vacuolar degeneration, lipid-type			1	1	1	1	1	1	1	1	1	1			1	2			1	1
	Vacuolar degeneration, lipid-type, diffuse																				
	Vacuolar degeneration, lipid-type, multifocal																				
	Vacuolar degeneration, lipid-type, periportal			1	1	1	1	1	1	1	1	1	1			1	2			1	1
	Vacuolar degeneration, lipid-type, centrilobular																				
Liver	Vacuolar degeneration, glycogen-type (rarefaction)	1																			
	Cytoplasmic inclusions, hepatocyte (plasma influx/congestion droplets)				1								1			1					
	Hepatocellular hypertrophy																				
	Necrosis, hepatocellular, single cell			1																	
	Infiltrates, lymphocytic							1			1						1			1	1
Spleen	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
	slide missing																				
	Autolysis (5=most severe, precludes analysis)																				
Kidney	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
	Infiltrate, interstitial, lymphocytic												1								
	Tubular epithelial cell degeneration																				
	Inflammation, neutrophilic, intratubular, multifocal																				
	End stage kidney, cortical atrophy and necrosis																				
	Infarct, wedge segmental necrosis																				
	Tubulitis, neutrophilic inflammation																				
	Hydronephrosis, pelvic dilatation																				
	Autolysis (5=most severe, precludes analysis)																				
Adrenal gland	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
	Cortical epithelial cells, vacuolation, increased								1												
	Nodular Hyperplasia, cortex																	1			
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										
	Squamous epithelial hyperplasia, Non-glandular stomach											2	2	2	2	1	3	2	2		2
	Hyperkeratosis, non-glandular mucosa											2	2	2	1	1	2	3	2	1	3
	Submucosal edema			1								2	1	2	2	1	1	3	1		1
	Cellular infiltrates, neutrophilic and lymphocytic					1						1	1	2	2	1	2	2	2		2
	Congestion, submucosa											1	1	2	1			2		1	1
	Intravascular thrombi																				
	Degeneration, epithelium, glandular crypts	1																		1	
	Ectasia, glandular	1		1																1	
	Intraglandular debris, crypt abscesses	1		1																1	
	Granulation tissue, submucosal, nonglandular region																				
	Absent (1=true, blank not true)																				
Thymus	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		
	Abscess, focal																				
	Perifollicular congestion/hemorrhage			1					2							1		2		1	1
	Lymphocyte apoptosis, increased					1			1				2	1	1	1		2		1	
	Absent (1=true, blank not true)																				
Bone marrow, aspirate	Tissue Examined (1=true, blank=not examined)			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
	Sample of adequate diagnostic quality (1- yes, 0-non-diagnostic)	0	0	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1

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).

APPENDIX O

GENOTOXICITY DATA

Table O-1. Individual and Summary Data for Micronucleus Assay

Dose Group	Animal ID	%RET	MN-RET per 10^6 RET	MN-NCE per 10^6 NCE
0 mg/kg	18-0649	1.89	700	108
	18-0650	1.32	450	88
	18-0685	2.18	1300	78
	18-0686	1.01	1000	139
	18-0671	1.62	1300	141
	18-0672	1.25	1250	158
	18-0675	1.27	4600	104
	18-0676	1.14	850	95
	18-0657	1.36	1550	134
	18-0658	1.52	1600	235
	Mean	1.46	1460	128
	SD	0.36	1163	46
56 mg/kg	18-0661	0.45	1550	186
	18-0662	0.84	1200	72
	18-0651	0.39	950	99
	18-0652	1.06	1500	66
	18-0635	0.58	1350	87
	18-0636	1.85	1050	118
	18-0633	0.56	1400	93
	18-0634	0.40	650	72
	18-0681	0.40	800	109
	18-0682	0.77	1550	100
	Mean	0.73*	1200	100
	SD	0.45	324	35
111 mg/kg	18-0655	0.97	750	91
	18-0656	0.83	1050	116
	18-0645	0.77	1950	115
	18-0646	1.12	1550	110
	18-0673	1.05	750	94
	18-0674	0.49	900	104
	18-0669	0.64	800	125
	18-0670	0.23	600	60
	18-0667	0.46	1050	108
	18-0668	0.56	1000	85
	Mean	0.71*	1040	101
	SD	0.29	412	19
222 mg/kg	18-0631	1.01	1250	134
	18-0632	1.05	1750	246
	18-0647	1.30	1450	176
	18-0648	1.44	1250	174
	18-0621	0.62	1050	173
	18-0622	0.73	950	79
	18-0659	2.36	650	108
	18-0660	0.91	650	198
	18-0683	0.68	1100	186
	18-0684	ND	ND	ND
	Mean	1.12	1122	164
	SD	0.54	356	50
(-) Control	18-0625	2.44	850	70
	18-0626	3.13	1600	50
	18-0627	3.09	2400	115
	18-0628	3.15	1500	213
	18-0639	3.25	850	57
	18-0640	3.95	350	109
	Mean	3.17	1258	102
	SD	0.48	726	61
(+) Control	18-0625	0.31	5900	61
	18-0626	1.03	8300	81
	18-0627	0.41	21500	78
	18-0628	0.78	13750	101
	18-0639	0.73	11600	72
	18-0640	1.98	7800	39
	Mean	0.87	11475	72
	SD	0.60	5663	21

Legend:
MN = Micronucleated; RET = Reticulocytes; NCE = Normochromatic erythrocytes
Note:
*p<0.05 versus 0 mg/kg control group, based on one-way ANOVA and Dunnett t (2-sided) post hoc test.
MN-RET and MN-NCE data were log-transformed to achieve a normal distribution.

APPENDIX P

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 3,4-Dinitropyrazole (DNP) Exposure to Rats (*Rattus norvegicus*)

PROTOCOL NUMBER: 30-18-07-01

DATE OF APPROVAL: 11 JULY 2018

STUDY DIRECTOR/PRINCIPAL INVESTIGATOR (SD/PI):

Thomas E. Sussan
Ph.D. /Biologist
Toxicology Directorate, Health Effects Division
(410) 436-6590
thomas.e.sussan2.civ@mail.mil

PRIMARY CO-INVESTIGATOR(S):

Allison M. Jackovitz
Biologist
Toxicology Directorate, Toxicity Evaluation Division
(410)436-8772
allison.m.jackovitz.civ@mail.mil

CO-INVESTIGATOR(S):

Valerie H Adams
Ph.D., D.A.B.T. / Biologist
Toxicology Directorate, Health Effects Division
(410)436-5063
valerie.h.adams.civ@mail.mil

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
DNP: 2,4-Dinitropyrazole
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
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

Animal Use Protocol: Effects of Acute and Subacute Oral 3,4-Dinitropyrazole (DNP) Exposure to Rats (*Rattus norvegicus*)

U.S. EPA: United States Environmental Protection Agency

I. NON-TECHNICAL SYNOPSIS:

The purpose of this study is to assess the toxicity of a new experimental explosive DNP, 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 DNP 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 DNP daily for 14 consecutive days. At the end of each experiment, all animals will be euthanized and their tissues will be examined for DNP 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. DNP has progressed through this program as a potential replacement for TNT. Currently, *in vivo* toxicity data for DNP is unavailable.

This protocol will assess the acute oral toxicity of DNP 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 DNP 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: (3,4-dinitro-1H-pyrazole or dinitropyrazole* or DNP or 38858-92-3) 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 DNP toxicity was made by searching the above databases. A total of 12 references were identified from the literature searches. Only one of these references pertained to DNP, while the other 14 references pertained to unrelated explosive compounds. The reference pertaining to DNP is an in vitro study that is discussed in section V.1.1 [4] and is not a duplication of the current protocol.

Additionally, PubMed, DTIC, Web of Science, Scopus, FedRIP, TOXNET, EBSCO-Host's CINAHL Plus, Yale University, and the Cochrane Library, were also searched for this duplication of effort and all collectively identified no *in vivo* studies of DNP.

III. OBJECTIVE/HYPOTHESIS:

The objectives of this research are to (1) determine the oral LD50 for DNP 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 DNP 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. This data will also be used to support a potential follow-up 90-day oral toxicity study.

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, 3,4-dinitropyrazole, 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 APHC LS Directorate-Client Services Division- Method Development Section. Method of analysis will be validated prior to the start of the study.

Table 1. DNP Chemical/Physical Properties

Name	3,4-Dinitropyrazole
Synonym	3,4-Dinitro-1H-pyrazole
CASRN	38858-92-3
Molecular Formula	$C_3H_2N_4O_4$
Molecular Weight (g/mol)	158.073
Aqueous Solubility @ 25°C (mg/L)	5.193×10^4 (EPI Suite 4.1 estimate)

V.1. Experimental Design and General Procedures: The acute toxicity of DNP will be determined in female rats. In Experiment 1, DNP 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 DNP 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 DNP 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 DNP includes a predicted oral LD50 of 728.2 mg/kg based on TOPKAT modeling [6] and an estimated LD50 of 800-900 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 ¼ log intervals (e.g., 200, 356, 632, 1125, 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 DNP 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 DNP 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 DNP. 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 6 M 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 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 DNP 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 DNP in the rat. The collected LD50 values (CI and slope) and the derived repeated dose LOAEL and NOAEL values for DNP will be compared to that of TNT and alternative explosives. There are no non-animal alternatives that would provide the necessary toxicological information on DNP 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 DNP 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 DNP 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 DNP, 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 DNP 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 DNP was conducted, but retrieved 0 hits after searching PubMed, Web of Science, 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: DNP 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 DNP. 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

DNP 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 anticoagulant 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 DNP, 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 (> 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 in the event of 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 nonduty 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.

Animal Use Protocol: Effects of Acute and Subacute Oral 3,4-Dinitropyrazole (DNP) Exposure to Rats (*Rattus norvegicus*)

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)	
	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);	

Animal Use Protocol: Effects of Acute and Subacute Oral 3,4-Dinitropyrazole (DNP) Exposure to Rats (*Rattus norvegicus*)

		Rat Peripheral Bleeding (7/2009; 6/24/14; 8/11/14; 9/17/14);	
	CO_2 anesthesia/ euthanasia	Rat techniques: euthanasia (7/19/07); CO2 euthanasia (7/9/09; 5/13/15)	
	Cardiac blood collection	Cardiac Bleed (5/13/15)	
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)	
	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 (4/30-6/4/2018)	
Emily Lent	Handling & observations	Rat handling (7/19/07)	Ph.D., Natural Resources and Environmental Studies, M.S.,
	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)	Wildlife Biology 18+ Yrs animal research experience
	CO_2 anesthesia/ euthanasia	Rat euthanasia via CO2 (7/19/07; 11/18/10; 3/16/12; 8/2014)	
	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 3,4-Dinitropyrazole (DNP) 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)

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		7/11/18
(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)

1. ASTM, *E1163-10: Standard Test Method for Estimating Acute Oral Toxicity in Rats, Biological Effects and Environmental Fate*. 2011, ASTM International: Conshohocken, PA.
2. OECD, *Test Guideline 423: Acute Oral Toxicity - Acute Toxic Class Method*. 2001.
3. APHC, *IACUC SOP 1.3. Standing Operating Procedures for Animal Use Protocol Development, Submission, Review, and Consideration by the IACUC*. 2015, USAPHC.
4. APHC (Prov), *Toxicology Study No. S.0024589d-15, Human Cell Line Activation Test of the Novel Energetic 3,4-Dinitropyrazole*, E.N. Reinke, Editor. 2016, U.S. Army Public Health Center (Provisional): Aberdeen Proving Ground, MD.
5. USEPA, *Health Effects Test Guidelines OPPTS 870.1100 Acute Oral Toxicity* 2002.
6. APHC, *Substance Toxicity Profile, 3,4-Dinitropyrazole [DNP]*. 2017, APHC, Toxicology Directorate: Aberdeen Proving Ground, MD.
7. APHC, *QSARC 800, Animal Euthanasia*. 2017, Quality Systems and Regulatory Compliance Office (QSARC) Animal Program (AP): Aberdeen Proving Ground, MD.
8. APHC, *TOX SOP 011 Clinical Chemistry Analysis of Blood Specimens*. 2018: Aberdeen Proving Ground, MD.
9. APHC, *TOX SOP 013 Cell-Dyn Hematology Analyzer*. 2018: Aberdeen Proving Ground, MD.
10. APHC, TOX SOP 079 *Prothrombin Time and Activated Partial Thromboplastin Time*. 2016, Toxicology Directorate: Aberdeen Proving Ground.
11. USEPA, *Good Laboratory Practices Standards Compliance Monitoring Program, 40 CFR part 160 and part 792*. 2011.
12. NRC, *Guide for the Care and Use of Laboratory Animals*. 2011, National Research Council: Washington, DC.
13. Feder, P.I., et al., *Stagewise, group sequential experimental designs for quantal responses. one-sample and two-sample comparisons.* Neurosci Biobehav Rev, 1991. **15**(1): p. 129-33.
14. Feder, P.I., et al., *Stagewise, adaptive dose allocation for quantal response dose-response studies.* Neurosci Biobehav Rev, 1991. **15**(1): p. 109-14.
15. USEPA, *Health Effects Test Guidelines, OPPTS 870.3050 Repeated Dose 28–Day Oral Toxicity Study in Rodents, EPA 712–C–00–366*. 2000.
16. APHC, *TOX SOP 005, Handling and Storage of Test Records, Data, and Specimens*. 2017, Toxicology Directorate: Aberdeen Proving Ground, MD.
17. Gad, S. and C. Chengelis, *Acute Toxicology Testing: Perspectives and Horizons*. 1988, Caldwell, NJ: Taylor & Francis.
18. *Sex difference in the micronucleus test. The Collaborative Study Group for the Micronucleus Test*. Mutat Res, 1986. **172**(2): p. 151-63.
19. Erkekoglu, P., B. Giray, and N. Basaran, *3R principle and alternative toxicity testing Methods/3R ilkesi ve alternatif toksisite inceleme yöntemleri.* FABAD Journal of Pharmaceutical Sciences, 2011. **36**(2): p. 101-117.
20. Moore, S.C. and E.R. Desantis, *Treatment of complications associated with systemic sclerosis.* Am J Health Syst Pharm, 2008. **65**(4): p. 315-21.

21. Beilin, B., et al., *Narcotic-induced suppression of natural killer cell activity in ventilated and nonventilated rats.* Clin Immunol Immunopathol, 1992. **64**(2): p. 173-6.
22. Piersma, F.E., et al., *Interference of pain control employing opioids in in vivo immunological experiments.* Lab Anim, 1999. **33**(4): p. 328-33.
23. Rouveix, B., *Opiates and immune function. Consequences on infectious diseases with special reference to AIDS.* Therapie, 1992. **47**(6): p. 503-12.
24. Thomas, P.T., H.N. Bhargava, and R.V. House, *Immunomodulatory effects of in vitro exposure to morphine and its metabolites.* Pharmacology, 1995. **50**(1): p. 51-62.
25. Butelman, E.R., et al., *Butorphanol: characterization of agonist and antagonist effects in rhesus monkeys.* J Pharmacol Exp Ther, 1995. **272**(2): p. 845-53.
26. Gerak, L.R., et al., *Antinociceptive and respiratory effects of nalbuphine in rhesus monkeys.* J Pharmacol Exp Ther, 1994. **271**(2): p. 993-9.
27. Soma, L.R., *Anesthetic and analgesic considerations in the experimental animal.* Ann N Y Acad Sci, 1983. **406**: p. 32-47.
28. Negishi, C., et al., *Alfentanil reduces the febrile response to interleukin-2 in humans.* Crit Care Med, 2000. **28**(5): p. 1295-300.
29. Malov, V.A., et al., *[The effect of cyclooxygenase inhibition on the indices of the thrombocyte-vascular link in hemostasis and on the free-radical processes of lipid oxidation in experimental Salmonella intoxication].* Nauchnye Doki Vyss Shkoly Biol Nauki, 1991(7): p. 58-63.
30. APHC, *QSARC 806, Test System Observations*. 2017, Quality Systems and Regulatory Compliance Office (QSARC) Animal Program (AP): Aberdeen Proving Ground.
31. APHC, *QSARC 805, Animal Bleeding Technique*. 2017, Quality Systems and Regulatory Compliance Office (QSARC) Animal Program (AP): Aberdeen Proving Ground, MD.
32. APHC, *QSARC 804, Individual Animal Identification*. 2017, Quality Systems and Regulatory Compliance Office (QSARC) Animal Program (AP): Aberdeen Proving Ground, MD.
33. APHC, *QSARC 707, Animal Health Technician Animal Husbandry and Vivarium Duties*. 2017, Quality Systems and Regulatory Compliance Office (QSARC) Veterinary Medicine Office (VMO): Aberdeen Proving Ground, MD.
34. APHC, *QSARC 802, Animal Environmental Enrichment*. 2017, Quality Systems and Regulatory Compliance Office (QSARC) Animal Program (AP): Aberdeen Proving Ground, MD.
35. APHC, *Regulation 385-1: Safety and Occupational Health Program, US Army Public Health Command*. 2010: Aberdeen Proving Ground, MD.
36. APHC, *Regulation 385-5: Occupational Health and Safety of Animal Users, US Army Public Health Command*. 2006: Aberdeen Proving Ground, MD.
37. APHC, *QSARC 807 Health and Safety of Laboratory Personnel*. 2017, Quality Systems and Regulatory Compliance Office (QSARC) Animal Program (AP): Aberdeen Proving Ground, MD.
38. APHC, *TOX SOP 051 Safety Precautions and Procedures for Handling Energetic Materials*. 2018, Toxicology Directorate: Aberdeen Proving Ground, MD.

ARMY PUBLIC HEALTH CENTER
PROTOCOL REVIEW AND APPROVAL FORM

Test Type: **30** IACUC protocol number: **18-07-01**

TITLE: Effects of Acute and Subacute Oral 3,4-Dinitropyrazole (DNP) 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 — *Date*

SUSSAN.THOMAS.EDWARD.1537772626 Digitally signed by SUSSAN.THOMAS.EDWARD.1537772626 Date: 2018.05.08 11:23:27 -04'00' — *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 — *Date*

BAZAR.MATTHEW.A.1241429322 Digitally signed by BAZAR.MATTHEW.A.1241429322 Date: 2018.05.07 07:39:26 -04'00' — *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 — *Date*

LEE.ROBYN.BELLEK.1267390289 Digitally signed by LEE.ROBYN.BELLEK.1267390289 Date: 2018.05.07 08:55:26 -04'00' — *Signature*

4. ATTENDING VETERINARIAN REVIEW

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

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

2018-05-07 — *Date*

BONHAGE.MICHAEL.ROBERT.1014029881 Digitally signed by BONHAGE.MICHAEL.ROBERT.1014029881 Date: 2018.05.07 11:17:25 -04'00' — *Signature*

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

Printed Name (First, MI, Last): Kristin T. Newkirk

Signature: NEWKIRK.KRISTIN.TORELL.1014786895 Digitally signed by NEWKIRK.KRISTIN.TORELL.1014786895 Date: 2018.07.11 16:48:43 -04'00'

6. INSTITUTIONAL OFFICIAL REVIEW

Printed Name (First, MI, Last): John J. Resta

Signature: RESTA.JOHN.J.1229129305 Digitally signed by RESTA.JOHN.J.1229129305 Date: 2018.07.12 10:37:17 -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 #: **30-18-07-01** *Protocol Approval Date:* 11 July 2018

PROTOCOL TITLE: Effects of Acute and Subacute Oral 3,4-Dinitropyrazole (DNP) Exposure to Rats (Rattus norvegicus)

Modification Request #: **1** *Submission Date:* 12 July 2018

Study Director/ Principal Investigator Signature: SUSSAN.THOMAS.EDWARD.1537772626 Digitally signed by SUSSAN.THOMAS.EDWARD.1537772626 Date: 2018.07.12 13:14:14 -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 12:24:06 -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
Date

DECK.ADAM.T.1267159900 Digitally signed by DECK.ADAM.T.1267159900 Date: 2018.07.24 08:07:40 -04'00'

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

ID 002 REV 0 JUNE 2017 Page 1 of 1

APHC IACUC Animal Use Protocol Modification

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

PROTOCOL #: **30-18-07-01** Protocol Approval Date: 11 July 2018

PROTOCOL TITLE: **Effects of Acute and Subacute Oral 3,4-Dinitropyrazole (DNP) 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/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	**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 #: **30-18-07-01** *Protocol Approval Date:* 11 July 2018

PROTOCOL TITLE: Effects of Acute and Subacute Oral 3,4-Dinitropyrazole (DNP) 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 15:33:15 -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 14:00:50 -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 14:01:18 -04'00'

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

Aug 1, 2018 (Date)

MOCCIA.KRINON.DODEKA.1167935563 Digitally signed by MOCCIA.KRINON.DODEKA.1167935563 Date: 2018.08.01 15:54:56 -04'00'

Safety Review Signature (*if applicable*):

Date

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

Date

IACUC Chair Review Signature:

Aug 2, 2018 (Date)

NEWKIRK.KRISTIN.TORELL.1014786895 Digitally signed by NEWKIRK.KRISTIN.TORELL.1014786895 Date: 2018.08.02 07:53:29 -04'00'

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 #: **30-18-07-01** Protocol Approval Date: 11 July 2018

PROTOCOL TITLE: **Effects of Acute and Subacute Oral 3,4-Dinitropyrazole (DNP) 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.

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	**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 *(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	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):

N/A

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