DEPARTMENT OF THE ARMY
US ARMY INSTITUTE OF PUBLIC HEALTH
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MCHB-IP-TEP

19 June 2012

MEMORANDUM FOR Environmental Acquisition and Logistics Sustainment Program (AMSRD-FE/Kimberly A. Watts), U.S. Army Research, Development and Engineering Command, 3072 Aberdeen Blvd., Aberdeen Proving Ground, MD 21005

SUBJECT: Toxicology Study No. 87-XE-0DBP-10, Protocol No. 0DBP-38-10-07-01, The subchronic oral toxicity of 2,4-dinitroanisole (DNAN) in rats, September 2010 – March 2011

1. Electronic copy of the subject report is enclosed.

2. Please contact us if this report or any of our services did not meet your expectations.

3. The point of contact is Dr. Emily May Lent, Toxicology Portfolio, Toxicity Evaluation Program, at 410-436-3980, DSN 584-3980, or FAX at 410-436-6710. She may also be reached b electronic mail at usaphctoxinfo@amedd.army.mil.

FOR THE DIRECTOR:

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U.S. ARMY PUBLIC HEALTH COMMAND

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Toxicology Study No. 87-XE-0DBP-10, June 2012
Toxicology Portfolio

The Subchronic Oral Toxicity of 2,4-Dinitroanisole (DNAN) in Rats, September 2010 – March 2011

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General Medical: 500a

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support of Michael Hable of the Laboratory Sciences Portfolio, US Army Public Health Command for his efforts in analyzing the dosing solutions/suspensions used in this study.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
14-06-2012	Technical Report	September 2010 - March 2011

4. TITLE AND SUBTITLE

The subchronic oral toxicity of 2,4-dinitroanisole (DNAN) in rats

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

87-XE-0DBP-10

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

US Army Institute of Public Health
Toxicology Portfolio, ATTN: MCHB-IP-TEP
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8. PERFORMING ORGANIZATION REPORT NUMBER

87-XE-0DBP-10

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

US Army Research Development and Engineering Command (RDECOM)
Environmental Acquisition and Logistics Sustainment Program (EASLP)
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10. SPONSOR/MONITOR'S ACRONYM(S)

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

12. DISTRIBUTION/AVAILABILITY STATEMENT

Distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

To provide information important for protecting the health of military and civilian personnel, three studies were conducted to assess the toxicity of 2,4-dinitroanisole (DNAN). In the subchronic study, male and female Sprague Dawley rats were dosed with DNAN via oral gavage at 0, 1.25, 5, 20, and 80 mg/kg-day. Likely owing to its conversion to 2,4-DNP, an inhibitor of mitochondrial energy homeostasis, DNAN treatment caused an apparent increase in metabolism, leading to reduced feed conversion efficiency and reduced body mass gain in males. Anemia, splenic enlargement, hemosiderosis, and extramedullary hematopoeisis (EMH) indicated that the blood is a target organ for DNAN, with females being more sensitive than males. DNAN was a testicular toxicant, causing decreased mass of the testes and epididymides, as well as degeneration and atrophy of the testicular seminiferous tubules and epididymal aspermia. Stereotypical behavior in males, gait irregularities, and cerebellar glial lesions indicated that DNAN is neurotoxic. A BMDL10 of 2.3 mg/kg-day was derived based on EMH in females.

15. SUBJECT TERMS

oral toxicity, explosives, insensitive munitions, subchronic toxicity, DNAN, 2,4-dinitroanisole

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Emily May Lent
					19b. TELEPHONE NUMBER *(Include area code)*
U	U	U	SAR	251	410-436-7749

Study Title

Toxicology Study No. 87-XE-0DBP-10
Protocol No. 0DBP-38-10-07-01
The subchronic oral toxicity of 2,4-dinitroanisole (DNAN) in rats
September 2010 - March 2011

Data Requirement

Health Effects Testing Guidelines Reference No. OPPTS 870.3100

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Study Completed On

June 2012

Performing Laboratory

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Laboratory Project ID

Protocol No. 0DBP-38-10-07-01

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this report on the basis of its falling within the scope of TSCA § 790.7 (a) – (d).

Company:_____

Company Agent
Typed Name:_____ Date:_____

Title:_____ Signature:_____

Toxicological Study No. 87-XE-0DBP-10, Subchronic toxicity of DNAN, Sept 2010-March 2011

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14 JUN 2012
Date

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

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

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

2. The concentrations of the test article dosing suspensions for the acute portion of the study were not verified analytically in accordance with Good Laboratory Practice Standards. The accuracy of the data reported is considered sufficient for the purposes of the acute study.

3. The statistical analyses of the neurobehavioral evaluation data were conducted by the US Army Public Health Command statisticians. It is not know if these analyses were conducted in accordance with Good Laboratory Practice Standards.

Submitted By:

Study Director:

Emily May Lent — 14 Jun 2012

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Toxicologist
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Date

Approved By:

Shannon M. Wallace — 14 JUN 2012

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1 Summary

1.1 Purpose

This study was conducted to determine the oral LD_{50} resulting from the acute oral administration of DNAN, and to determine if adverse effects occur from a subacute (14 day) and subchronic (90-day) repetitive oral exposure regime of DNAN to male and female rats. 2,4-dinitroanisole (DNAN) is an energetic material being investigated as a less sensitive replacement for 2,4,6-trinitrotoluene (TNT). DNAN is a component of IMX-101, an insensitive munition currently under development. Data from this study will be important for determining appropriate exposure levels and protecting the health of military and civilian personnel potentially exposed to DNAN.

1.2 Conclusions

Mortality occurred in three male rats (days 50, 63, and 77) and one female rat (day 26) all from the 80 mg/kg-day dose group. Rats in the highest dose group (80 mg/kg-day) experienced lethargy, labored/rapid respiration, prostrate and/or recumbent posture, hunched posture, ear twitching, squinting, curled tail, and gait irregularities. A functional observation battery (FOB) and analysis of motor activity at week 13 indicated that rats given 80 mg/kg-day had altered neuromuscular function and decreased activity levels. In the 80 mg/kg-day group, female rats also had reduced sensorimotor responses while male rats had increased excitability responses.

Although food intake was similar among groups for male rats, animals from the 80 mg/kg-day dose group exhibited reduced body mass and a reduced food efficiency ratio. Female rats in the 80 mg/kg-day dose group also had a reduced food efficiency ratio, but had elevated food consumption at several time points during the study. Body mass did not differ among dose groups for female rats. Female rats in the 80 mg/kg-day dose group and male rats in the 20 mg/kg-day group produced higher volumes of urine with lower specific gravity. Despite the increase in volume, urine color was darker in the 20 and 80 mg/kg-day dose groups for both sexes.

Increased mean kidney, liver, and spleen mass were observed in male and female rats given 80 mg/kg-day DNAN. In male rats, increased mean kidney and liver mass were also noted in the 20 mg/kg-day dose group; however, the changes were not associated with treatment related microscopic abnormalities or alterations in clinical chemistry parameters. Decreased mass of the testes and epididymides as well as degeneration and atrophy of the testicular seminiferous tubules and aspermia were also observed in rats from the 80 mg/kg-day group. In females, changes in hematology indicative of anemia, including decreased red blood cell count, hematocrit, and hemoglobin, and increased red cell distribution width were observed in the 80 mg/kg-day group. A dose related increase in extramedullary hematopoeisis was noted in spleens of female rats at 20 and 80 mg/kg-day. Glial lesions within the cerebellum were noted in four rats (1 female/3 males) in the 80 mg/kg-day group.

This study, the first repetitive oral dosing conducted with DNAN, demonstrated a steep dose response curve, with most effects occurring only in the highest doses and occurring at or near lethal doses. Likely owing to its conversion to 2,4-DNP, an inhibitor of mitochondrial energy homeostasis, DNAN treatment resulted in an apparent increase in metabolism leading to reduced feed conversion efficiency and ultimately reduced body mass gain in males. Changes in hematology parameters indicative of anemia, splenic enlargement, hemosiderosis, and extramedullary hematopoeisis indicate that the blood is a target organ for DNAN, with female rats being more sensitive to these effects than males. DNAN demonstrated testicular toxicity that, combined with the documented reproductive/developmental effects of its metabolite, 2,4-DNP, raises concern about the reproductive/developmental toxicity of DNAN. DNAN treatment resulted in progressive development of behavioral neurotoxicity as well as associated brain lesions. Extramedullary hematopoeisis in female rats was identified as the critical endpoint in this study and was used to determine the lower bound of a 95 percent confidence interval on a benchmark dose corresponding to a 10 percent effect level ($BMDL_{10}$). This $BMDL_{10}$ of 2.3 mg/kg-day may be used for development of safe exposure levels.

2 References

See Appendix A for a listing of references.

3 Authority

This toxicology study addresses, in part, the environmental safety and occupational health (ESOH) requirements outlined in Army Regulations (AR) 200-1, AR 40-5, and AR 70-1; Department of Defense Instruction (DoDI) 4715.4; and Army Environmental Requirements and Technology Assessments (AERTA). It was performed as part of an on-going effort by the U.S. Army Environmental Quality Technology (EQT), Ordnance Environmental Program Pollution Prevention Team, to produce safer ordnance. This program is under the direction of the U.S. Army Research, Development, and Engineering Command (USARDECOM) Environmental Acquisition Logistics & Sustainment Program and EQT Pollution Prevention.

4 Background

DNAN is a tan powder with a wax-like consistency that is practically insoluble in water (BAE 2005). It is classified as a flammable solid and is being investigated as a less-sensitive replacement for 2,4,6-Trinitrotoluene (TNT) in melt-cast insensitive munition formulations. DNAN is used industrially in the synthesis of dyes and has been used as an insecticide in the past by the US Military. The use of DNAN as an energetic material in explosive formulations dates back to World War II when it was used as the main ingredient in Amatol 40 for various warheads. At the time, DNAN's use as an ingredient in explosive formulations was based primarily on the scarcity of higher performance materials, such as TNT. Renewed interest in the energetic properties of DNAN has been fueled by the need to develop munitions that are less prone to inadvertent initiation during transport and routine handling. The reduced sensitivity to environmental stimuli and nearly equal performance during testing make DNAN-based formulations desirable replacements for currently fielded munitions (Davies and Provatas 2006). Although DNAN is used industrially in the synthesis of dyes and its use by the US military dates back to World War II when it was used in Amatol 40 and as an ingredient in MYL louse powder (Hayes 1982; Davies and Provatas 2006), information on the toxicity of DNAN is limited.

DNAN is moderately acutely toxic, with an oral LD_{50} of 199 mg/kg in the rat (Dodd et al. 2002). DNAN was reported to cause slight skin and eye irritation with reversibility in 24-48 hours, but did not cause dermal sensitization (Dodd et al. 2002). DNAN was evaluated in the Ames *Salmonella* test (TA98, TA100, TA102, TA1535, and TA1537), with and without metabolic activation (S9), DNAN was mutagenic only in strain TA100 without activation (Dodd et al. 2002). Evaluation using Chinese Hamster Ovary (CHO) cells (AS52/XPRT) indicated no mutagenic induction in the tested cells (Dodd et al. 2002). DNAN was judged to have caused no chromosomal damage and to be non-mutagenic in the *in vivo* mouse bone marrow assay (Dodd et al. 2002). Acute inhalation testing of DNAN aerosolized in acetone resulted in a 4 hour LC_{50} of > 3 g/m^3 in the rat (Hoffman 2000). In a subacute inhalation study using DNAN aerosolized in acetone at 150, 500 and 1500 mg/m^3, all rats in the 1500 mg/m^3 and 8/10 animals in the 500 mg/m^3 group were found dead or euthanized during the exposure period. Clinical signs of toxicity observed prior to euthanasia included decreased food consumption, prostration, irregular gait, lethargy, head bobbing, poor condition, pale, backwards walking, labored breathing, and red nasal discharge. Animals exposed to 500 mg/m^3 gained less weight and consumed less feed during the first week of exposure than the acetone controls. Females in the 150 mg/m^3 had statistically significant decreases, relative to the acetone control group, in mean hemoglobin concentrations, mean corpuscular volume, and mean corpuscular hemoglobin and increases in mean absolute monocytes and liver weight. The urine of both male and female rats exposed to 150 mg/m^3 was darker than acetone controls. The only reported compound related microscopic finding was non-specific minimal metaplasia of laryngeal epithelium in rats exposed to 150 mg/m^3 (Hoffman 2001). No reports of repeated-dose oral testing of DNAN were identified. To ensure its safe use by military personnel and production employees handling the material on a daily basis, the subacute and subchronic oral toxicity of DNAN were investigated. These data will be important in development of safe exposure levels.

The following table identifies the date of critical study events.

Table 1. Critical Study Events

Critical Event	Date of Event
Animal Use Protocol Approved	07/29/10
ALD Animals Received	09/08/10
Study Start	08/03/10
Experimental Start	09/14/10
ALD Necropsies	09/28/10
14-Day Animals Received	09/29/10
14-Day Study Start	10/05/10
14-Day Necropsies	10/19/10 – 10/22/10
90-Day Animals Received	11/10/10, 11/17/10
90-Day Study Start	11/24/10
Ophthalmic Exams	11/18/10, 11/24/10, 2/17/11, 2/23/11
Urinalysis	2/14/11 – 2/16/11; 2/21/11 – 2/24/11
90-Day Necropsies	2/22/11 – 2/23/11; 3/01/11 – 3/02/11
Experimental Completion	03/02/11
Study Completion	06/14/2012

4.1 Methods

4.1.1 Materials

4.1.1.1 Test Substance

Neat DNAN (CAS # 119-27-7) was produced by BAE Systems, Ordnance Systems, 4509 West Stone Drive, Kingsport, TN 37660. The certificate of analysis provided by the supplier indicated that the DNAN (lot#BAE10H281-008) was 100 percent pure. The test article was dried in a vacuum oven at approximately 70 °C for 12-48 hours to remove moisture. Dosing solutions/suspensions were prepared by grinding DNAN using a mortar and pestle to a fine consistency, weighing the required amount of DNAN, and mixing with a measured volume of corn oil. To accommodate the wide range of doses required in the range finding study, six dosing solutions/suspensions were prepared: 5, 10, 25, 50, 100 and 200 mg/ml. Similarly, for the 14-day study seven dosing solutions/suspensions with concentrations of 1, 2, 4, 8, 16, 32 and 64 mg/ml were prepared at the start of the study in sufficient volume for use throughout the study. A one milliliter sample was taken from each dosing solution/suspension for the 14-day study and analyzed using a gas chromatograph equipped with an electron capture detector to verify the concentration. In addition, the homogeneity of the solutions/suspensions was verified by determining the concentration of samples taken from the top, middle, and bottom of the highest concentration (64 mg/ml) suspension. Initial homogeneity samples revealed unacceptable differences between samples taken from the top and bottom of the dosing solution. To correct this problem, larger particles were broken up using a glass rod and the dosing suspension was mixed overnight and re-sampled. Homogeneity results from the second sampling were acceptable; all dosing suspensions were prepared in a similar manner. Samples were collected from a representative suspension (5 mg/ml) at weekly intervals prior to the 14-day study to determine the stability of the dosing suspensions. Results from the stability test indicated that the test compound was stable for at least one month when stored at room temperature. For the 90-day study, four dosing solutions/suspensions were prepared: 0.25, 1, 4 and 16 mg/ml. Dosing solutions/suspensions were prepared in volumes sufficient for approximately two-three weeks of dosing, resulting in preparation of five sets of dosing solutions. A one milliliter sample was taken from each dosing solution/suspension for analytical verification of concentration of each preparation. The dosing solutions/suspensions were mixed for approximately one hour prior to taking analytical samples, prior to dosing, and continued to be mixed throughout the dosing procedure.

4.1.1.2 Animals*

This study was conducted using young adult male and female Sprague Dawley (Crl:CD(SD) CD® IGS) rats obtained from Charles River Laboratories, Wilmington, Massachusetts. All animals were housed in temperature-, relative humidity-, and light controlled rooms. The °F, 30-70 percent humidity, with a 12-hour light/dark cycle. Temperature and humidity conditions were within the target range in the acute and 14-day studies. However, in the 90-day study the temperature was

* Animal use procedures were approved by the United States Army Public Health Command (USAPHC) Institutional Animal Care and Use Committee (IACUC). Animal care and use was conducted in accordance with *The Guide for the Care and Use of Laboratory Animals* and all applicable Federal and DOD regulations. The USAPHC Animal Care and Use Program is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

out of range one day, resulting in a temperature range of 61-79 °F. Relative humidity was out of range three days, giving a range of 11-64 percent. A certified pesticide-free rodent chow (Harlan Teklad®, 8728C Certified Rodent Diet) and drinking quality water were available *ad libitum*. Rats were housed individually in suspended polycarbonate cages with ALPHA-dri® bedding. Each rat was uniquely identified by number via cage card and tail marking. (CD® IGS is a registered trademark of Charles River Laboratories International, Inc.; Teklad® is a registered trademark of Harlan, Teklad; ALPHA-dri® is a registered trademark with Shepard Specialty Papers.)

A total of four male and four female rats not placed on study, but housed in the same room, were used as sentinels and sent to Bioreliance, Rockville, Maryland at the start of the study (two per sex) and at the end of the study (two per sex) to assess the health status of the animals. Serology, bacteriology, pathology, and parasitology were performed. The results of pre-study tests were all negative. The post-study tests were negative in the female rats, whereas *Staphylococcus aureus* and *Klebsiella oxytoca* were isolated from the oral and fecal samples of both male rats.

4.1.1.3 Contract Studies

Tissues from the 90-day study were preserved, packaged and transported to the United States Army Medical Research Institute of Chemical Defense (USAMRICD), Aberdeen Proving Ground, MD, for processing, slide preparation and staining. Slides were returned to the USAPHC for evaluation by an American College of Veterinary Pathology board-certified military veterinary pathologist.

4.1.1.4 Quality Assurance

The USAPHC Quality Systems Office (QSO) audited critical study phases. Appendix B provides the dates of these audits along with the phases audited.

4.1.1.5 Study Personnel

Appendix C lists the names of individuals contributing to study performance.

4.1.2 Acute Study

The approximate lethal dose (ALD) procedure was used to verify the reported LD_{50} in male and female Sprague Dawley (Crl:CD(SD) CD® IGS) rats and to determine dosage levels for the subacute (14-day) study. The general procedures of the acute study followed the Portfolio of Toxicology (PTOX) Standing Operating Procedure (SOP) for the ALD Procedure (USAPHC 2009a) as well as the EPA Health Effects Test Guidelines for Acute Oral Toxicity (OPPTS 870.1100) (USEPA 1998). Twelve male and twelve female Sprague Dawley (Crl:CD(SD) CD® IGS) rats, eight weeks old and weighing 190.0 ± 8.95 and 245.8 ± 7.62 grams, respectively, were administered DNAN suspended in corn oil via oral gavage. Animals were fasted overnight prior to dosing and up to four hours post dosing. One male and one female rat were administered DNAN at each of 12 doses. Doses were selected based on the reported LD_{50} and a dose interval of 1.5x, resulting in doses of 17.6, 26.3, 39.5, 59.3, 88.9, 133.3, 200.0, 300.0, 450.0, 675.0, 1012.50, 1518.8 mg/kg. Animals were observed continuously for 2 hours after dosing and then approximately every half hour for the subsequent seven hours. Animals were held for a subsequent 14-day observation period during which clinical observations were made twice daily and body mass measured daily. Following the 14-day observation period, all animals were euthanized with CO_2 and submitted for gross pathological examination.

4.1.3 14-Day Oral Repeated Dose Toxicity Study

Upon evaluating the results of the range-finding study, a 14-day repeated dose oral toxicity study was conducted in male and female rats in accordance with the PTOX Standing Operating Procedure (SOP) for 14-day Range Finding and 90-Day Oral Toxicity Study in Rats (USAPHC 2009b).

4.1.3.1 Test Substance Administration

Fifty male and female Sprague Dawley (Crl:CD(SD) CD® IGS) rats eight weeks old, weighing 274.1 ± 5.47 and 201.1 ± 7.72 grams, respectively, at the start of dosing were used for this phase of the study. Following a six day acclimatization period, six rats of each sex were randomly distributed, according to body mass, into seven DNAN treatment groups and a vehicle control group (corn oil control). Body mass did not differ among treatment groups prior to initiation of dosing. Dosage levels were set at 1.56, 3.13, 6.25, 12.5, 25, 50, and 100 mg/kg-day. The males and females were each divided into two evenly distributed experimental start dates to facilitate scheduling of necropsies. Vehicle control animals were dosed with corn oil at the same volume per body mass (1.56 ml/kg) as the DNAN exposed animals.

Seven dosing solutions/suspensions, one per dose group, with concentrations of 1, 2, 4, 8, 16, 32, and 64 mg/ml were prepared at the start of the study in sufficient volume for use throughout the study. Dose was determined by the most recent rat mass and volume of solution/suspension administered. The volume of dosing solution/suspension per kilogram of body mass was equivalent across dose groups (1.56 ml/kg). The DNAN solution/suspensions and corn oil control were administered at approximately the same time daily, 7 days per week, for 14 days. Oral dosing was performed using a stainless steel 16 gauge x 2 inch gavage needle.

4.1.3.2 Observations, Body Mass, Food Consumption

Observations for mortality and signs of toxic effects were made at least twice daily, once in the morning and once in the afternoon, except on weekends when observations occurred only in the morning. Additionally, each animal was removed from its cage daily and given a physical/neurobehavioral examination. Examinations included evaluation of skin and fur, eyes and mucous membranes. Respiratory and circulatory effects, autonomic effects such as salivation, central nervous system effects, including tremors and convulsions, changes in the level of activity, gait and posture, reactivity to handling or sensory stimuli, altered strength, and stereotypes or bizarre behavior (e.g., self mutilation, walking backwards) were recorded. Physical examinations were made concurrently with dosing.

Animals were weighed twice pre-study and on study days 0, 1, 3, 7, and 14. Terminal body mass was obtained the morning of necropsy following overnight fasting. Feed was provided *ad libitum* seven days per week in weighed feeder bins. Feeders were reweighed weekly and the mass of the empty feeder was subtracted from the mass of the full feeder to determine the grams of food consumed for each animal.

4.1.3.3 Necropsy

After 14-days of treatment, all surviving rats were anesthetized with carbon dioxide (CO_2), blood was collected by intracardiac puncture, and rats were euthanized using CO_2. Necropsies were scheduled over four days based on the staggered experimental start dates. Necropsy order was randomized across treatment groups. A macroscopic examination was conducted on all terminal animals, noting all lesions and abnormal observations. The adrenals, brain, heart, kidneys, epididymides, liver, ovaries, spleen, testes, thymus, and uterus were removed, trimmed in a uniform manner, and weighed. Paired organs were weighed together.

4.1.3.4 Clinical Chemistry and Hematology

Blood was obtained from CO_2 anesthetized animals via intracardiac puncture at the termination of the study. Blood for clinical chemistry analyses was transferred to collection tubes free of anticoagulant, 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 evaluated included the following: albumin (ALB), alkaline phosphatase (ALKP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), calcium (Ca), cholesterol (CHOL), creatinine (CREA), glucose (fasting) (GLU), globulin (GLOB), lactate dehydrogenase (LDH), inorganic phosphorous (PHOS), total bilirubin (TBIL), total protein (TP), sodium (Na), potassium (K), and chloride (Cl). Results were determined using the VetTest 8008 Chemistry Analyzer and the VetLyte Na, K, Cl Analyzer (IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, ME 04092) on all valid serum samples.

Hematology parameters evaluated included the following: 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). Results were determined using the Cell-Dyn 3700 Hematology Analyzer (Abbott Laboratories, Abbott Park, IL 60064) on all valid samples.

4.1.3.5 Statistical Analyses

For experimental variables measured at the end of the study, dose groups were compared using a one-factor analysis of variance (ANOVA). Organ to brain and organ to body mass ratios were calculated and analyzed similarly to the other parameters measured at the end of the study. If the ANOVA was significant, post hoc tests were used to compare pairs of dose groups, a Tukey's multiple comparison test if the variance of groups were similar and a Dunnett's T3 test if the variances were unequal. Variance equality was determined by a Levene's test. For body mass changes and food consumption over the study days, a repeated measures ANOVA with time as the repeated factor and dose group as between group factor was used to assess changes in times and dose groups. If the ANOVA was significant, post hoc tests were used to compare pairs of dose groups, a Tukey's multiple comparison test if the variance of the groups were similar and a Dunnett's T3 test if the variance were unequal. Variance equality was determined by a Levene's test. SPSS® 16.0 was used to perform all analyses and statistical significance was defined as α=0.05 for all tests. (SPSS® is a registered trademark of IBM Corp.)

4.1.4 90-Day Oral Repeated Dose Toxicity Study

Upon evaluating the results of the 14-day repeated dose study, the 90-day repeated dose oral toxicity study was conducted in male and female rats in accordance with the PTOX Standing Operating Procedure (SOP) for 14-day Range Finding and 90-Day Oral Toxicity Study in Rats (USAPHC 2009b).

4.1.4.1 Test Substance Administration

Fifty male and female Sprague Dawley (Crl:CD(SD) CD® IGS) rats, eight weeks old, weighing 297.1 ± 10.88 and 214.1 ± 9.14 grams, respectively, at the start of dosing, were used for this phase of the study. Following a fourteen day acclimatization period, ten rats of each sex were randomly distributed, according to body mass, into four DNAN treatment groups and a vehicle control group (corn oil control). Body mass did not significantly differ among treatment groups prior to initiation of dosing. Dosage levels were set at 1.25, 5, 20, and 80 mg/kg-day. The males and females were each divided into two evenly distributed experimental start dates to facilitate scheduling of necropsies. Vehicle control animals were dosed with corn oil at the same volume per body mass (5 ml/kg) as the DNAN exposed animals.

Four dosing solutions/suspensions, one per dose group, with concentrations of 0.25, 1, 4, and 16 mg/ml, were prepared in sufficient volume for approximately three weeks of dosing. The concentrations of each of the resulting five batches of dosing solutions/suspensions were verified analytically. Dose was determined by the most recent rat mass and volume of solution/suspension administered. The volume of dosing solution/suspension per kilogram of body mass was equivalent across dose groups (5 ml/kg). The DNAN solution/suspensions and corn oil control were administered at approximately the same time daily, 7 days per week, for 90 days. Oral dosing was performed using a stainless steel 16 gauge x 2 inch gavage needle.

4.1.4.2 Observations, Body Mass, Food Consumption

Observations for mortality and signs of toxic effects were made at least twice daily, once in the morning and once in the afternoon, except on weekends when observations occurred only in the morning. Additionally, each animal was removed from its cage daily and given a physical/neurobehavioral examination. Examinations included evaluation of skin and fur, eyes and mucous membranes. Respiratory and circulatory effects, autonomic effects such as salivation, central nervous system effects, including tremors and convulsions, changes in the level of activity, gait and posture, reactivity to handling or sensory stimuli, altered strength, and stereotypes or bizarre behavior (e.g., self mutilation, walking backwards) were recorded. Physical examinations were made concurrently with dosing.

Animals were weighed twice pre-study and weekly during the study. Terminal body mass was obtained the morning of necropsy following overnight fasting. Feed was provided *ad libitum* seven days per week in weighed feeder bins. Feeders were reweighed weekly and the mass of the empty feeder was subtracted from the mass of the full feeder to determine the grams of food consumed for each animal.

4.1.4.3 Neurobehavioral Evaluations

Potential neurotoxic effects of DNAN were evaluated using the functional observation battery (FOB) and motor activity assessment. The FOB protocol used in this study followed the methods described in McDaniel et al. (1993). Animals were divided into two subsets for each sex, using a stratified random procedure based on dose group. The FOB was conducted on each animal prior

to initiation of dosing and weekly thereafter, with one subset of animals being assessed per day. The order of animals evaluated each day was randomly determined prior to study initiation. The FOB was performed at the same time each morning, prior to dosing. Each rat was removed from its cage and held by the observer to conduct the handheld observation of reactivity and appearance. The rat was then placed on a cart to conduct the open arena observations of gait, arousal, rears, and excretions. Home cage observations were performed weekly on all animals on the same day. During week eleven of dosing, sensorimotor responses were tested after the open arena observations. Observations and FOB were performed by the same evaluator throughout the study; the evaluator was blind to the treatment groups. Motor activity was measured after week eleven of dosing using an open field chamber with automated detection devices.

The home cage observations included signs of agitation, convulsions, tremors, posture, mutilation, and the area mutilated. Each rat was assigned a number corresponding with the observed response. Agitation and mutilation were scored as present (1) or absent (2), area mutilated was only described if present. Convulsions and tremors were scored as absent (1), slight (2), or severe (3). Posture was scored for the following positions: lying down (1), sit/stand (2), rearing (3), flattened (4), lying down with limbs up (5), crouched with head down (6), and/or head bobbing (7), animals demonstrated one or more body postures in one observation.

For handheld observations, each animal was removed from the home cage and the following observations were recorded: ease of removal (ER), reactivity to handling (RH), lacrimation (LAC), salivation (SAL), barbering (BAR), piloerection (PIL), palpebral closure (PC) of left and right eye, exophthalmus (EXO), and pupillary status (PS) of the left and right eye. ER describes the removal of the rat from the home cage and was scored 1-6: very easy, easy, moderately difficult, rat flinches, difficult, and very difficult. RH was scored 1–5: very low, low, moderately low, moderately high and high. Tearing from the eye (LAC), salivation (SAL), eye bulging (EXO), and absence of hair from the forelimbs due to excessive grooming (BAR) were scored as present (1) or absent (2). PC described the eye lid and was scored for left and right eye as normal (1), squinted (2), or closed (3). PS was scored for left and right eye as normal (1), constricted (2), or dilated (3).

Open Arena was conducted following the handheld observations. Each rat was placed on a 36" x 24" cart lined with paper. The rat was allowed to move freely around the arena for three minutes. During this time, observations were scored by an observer blind to the treatment groups. The following observations were recorded: number of rears and grooms, arousal, gait, fecal boli, fecal description, and urine. Rears were defined as the front limbs being lifted from the floor, supported or unsupported. Grooms were defined as any licking, biting, or scratching. Arousal was scored: very low (1), low (some head/body movement and exploration) (2), normal (3), high (slight excitement, sudden darting/freezing) (4), and very high (hyper alert, excited, sudden bouts of running/movement) (5). Gait, the movement/coordination of the rat, was scored: normal (1), too little movement to determine gait (2), ataxia (3), hind limb impairment (4), forelimb impairment (5), walking on toes (6), hunched (7), body drags (8), no movement (9) and unable to move (10). Fecal boli was the absence (1) or presence (2) of fecal matter. If fecal boli was present, fecal description was scored: normal (1), diarrhea (2), soft (3), mucoid (4), and bloody (5). After the three minute assessment the rat was returned to the home cage and the arena sanitized prior to assessment of subsequent rats.

Sensorimotor responses were evaluated by testing reactivity to different types of stimuli. Each rat was scored for reaction to the approach of a closed pen, auditory startle response to a loud click, tail pinch response, pinna response and pupillary response to a pen light. Approach was scored: no reaction (1), slow approach (2), approaches energetically (3), jumps/avoids (4), freezes (5), bizarre/attack (6). Auditory/startle was scored: no reaction (1), slight (ear flick) (2),

energetic/vocalize (3), jumps (4), freezes (5) and bizarre/attacks (6). Tail pinch was scored as response (1) or no visible response (2). Pinna response was scored as response (1) or no visible response (2). Pupillary response was scored as eye constricts (1) or does not constrict (2). Righting reflex was measured by placing the rat on its back on a padded surface. The rat was scored on how quickly it turned over onto its feet. Righting reflex was scored: normal (1), impaired (greater than 2 seconds to right) (2), and totally impaired (remains on back or side) (3). To score aerial righting, the rat was held in the air at 20 centimeters with its back horizontal to a padded surface. The rat was released and scored on its ability to turn over to land on its feet. Aerial righting was scored: normal (1), slightly uncoordinated (2), lands on side (3), and lands on back (4). To measure hind limb landing foot splay, the back feet of each rat were moistened with water. The rat was held by the scruff of the neck and the base of the tail and dropped from 20 centimeters onto a cage pad to show foot impressions. Foot splay was measured as the distance between the centers of the foot prints, to the nearest 0.5 centimeter. This was repeated twice and the measures were averaged. Forelimb and hind limb grip strength was assessed following these measurements. Grip strength was measured using Chatillon® Digital Force Meters (Model DFM-10) that were verified using standard weights. The force meters were set to measure the peak force in kilograms, trials were reposted twice and the average was calculated. Forelimb test: the animal was held by the base of the tail and allowed to place forepaws on the grate, the animal was pulled away from the grate at a continuous rate until grip was released and the reading was recorded. For the hind limb test, the animal was held by the base of the tail and allowed to grasp the grate with hind paws, the animal was pulled away from the grate at a continuous rate until grip was released, and the reading was recorded. (Chatillon® is a registered trademark of Ametek Inc.)

Motor activity was assessed using a SmartFrame® Open Field Activity System. The system consisted of four Plexiglas motor activity chambers (41 x 41 x 38 cm) each surrounded by a frame containing 32 evenly spaced (16x and 16y, 2.5 cm apart) infrared photocells. The floor of each chamber was equipped with a hole board containing nine holes equipped with infrared photocells to detect nose poke activity. Activity was measured as basic movement, immobility, x and y ambulation, and nose pokes based on the number of photobeam breaks recorded using the MotorMonitor® sortware (Version 4.14). After acclimation to the test room for at least 30 minutes, animals were removed from the home cage and placed individually into an open field arena for 15 minutes. Data was collected automatically by the system at fifteen equally spaced times while each rat was within the enclosure. After completion of the test, the rat was returned to its home cage and the chamber cleaned prior to testing of subsequent animals. Functioning of the software and chambers were verified prior to each test session by manually disrupting the beams and running a software diagnostic test. (SmartFrame® and MotorMonitor® are registered trademarks of Hamilton Kinder).

4.1.4.4 Ophthalmoscopic Examinations

All study animals were examined pre-study and animals from the control and high dose (80 mg/kg-d) groups were examined within one week of the conclusion of the study. The fundus and anterior chamber of the eye were examined using a Welch Allyn ophthalmoscope after instillation of tropicamide ophthalmic solution (1 percent) (USAPHC 2009c).

4.1.4.5 Urinalysis

During the last two weeks of the study, each animal was placed in a metabolism cage capable of separating urine and feces for one overnight period of approximately 12 hours during which free-catch urine was collected. Animals were fasted during this period, but water was available *ad libitum*. Urine samples were transferred to clear, graduated conical centrifuge tubes and the volume, color, and appearance of each sample were recorded. The color of each sample was determined based on comparison with a urine color chart with nine colors ranging from pale yellow/straw to dark amber. Specific gravity was tested using a refractometer. Multistix 7 Reagent strips were used to conduct chemical analyses including pH, protein, glucose, ketones, bilirubin, blood, urobilinogen, nitrites, and leukocytes (USAPHC 2009d).

4.1.4.6 Necropsy

Rats that died during the course of this study were submitted for gross necropsy. Tissues that were not grossly autolytic were submitted for histopathological evaluation. After 90-days of treatment, all surviving rats were anesthetized with carbon dioxide (CO_2), blood was collected by intracardiac puncture, and rats were euthanized using CO_2. Necropsies were scheduled over four days based on the staggered experimental start dates. Necropsy order was randomized across treatment groups. A macroscopic examination was conducted on all terminal animals, noting all lesions and abnormal observations. The adrenals, brain, heart, kidneys, epididymides, liver, ovaries, spleen, testes, thymus, and uterus were removed, trimmed in a uniform manner, and weighed. Paired organs were weighed together.

4.1.4.7 Sperm Analysis

Cauda epididymal sperm counts were determined using a computer assisted sperm analyzer (TOX IVOS-CASA, Hamilton Thorne Research, Beverly, MA). After removal, trimming, and weighing, one epididymis was further trimmed to select the cauda portion, weighed, placed in 200 µl Roswell Park Memorial Institute-1640 (RPMI-1640; Sigma-Aldrich, St. Louis, MO) medium at 37 °C and minced using fine scissors followed by a gentle grinding with a pestle to release sperm. A chamber of a standard count analysis slide (Leja®) was loaded with the semen suspension and the slide loaded into the semen analyzer. The number of sperm, number of motile sperm, and number of progressive sperm were determined in duplicate for each animal. The data were expressed as millions of sperm per ml of suspension and millions of sperm per gram cauda epididymis. (Leja® is a registered trademark of Leja Products BV.)

4.1.4.8 Clinical Chemistry and Hematology

Blood was obtained from CO_2 anesthetized animals via intracardiac puncture at the termination of the study. Blood for clinical chemistry analyses was transferred to collection tubes free of anticoagulant, 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 evaluated included the following: albumin (ALB), alkaline phosphatase (ALKP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), calcium (Ca), cholesterol (CHOL), creatinine (CREA), glucose (fasting) (GLU), globulin (GLOB), lactate dehydrogenase (LDH), inorganic phosphorous (PHOS), total bilirubin (TBIL), total protein (TP), sodium (Na), potassium (K), and chloride (Cl). Results were determined using the VetTest 8008 Chemistry Analyzer and the VetLyte Na, K, Cl Analyzer (IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, ME 04092) on all valid serum samples.

Hematology parameters evaluated included the following: 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). Results were determined using the Cell-Dyn 3700 Hematology Analyzer (Abbott Laboratories, Abbott Park, IL 60064) on all valid samples.

4.1.4.9 Histopathology

Tissues were appropriately preserved in 10 percent buffered formalin, selectively trimmed and placed in cassettes labeled with protocol number, animal identification number and laboratory assigned accession number. Cassettes were placed in labeled formalin filled bottles and transported to the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) for processing. Tissues were routinely processed and paraffin embedded. All processed and embedded tissues were microtomed at 5 um thick and automatically stained with hematoxylin and eosin and coverslipped. The pathologist examined slides for compound-induced histopathologic changes via light microscopy. The prevalence and severity of findings were graded as compared to controls. Findings were assigned as none, minimal, mild, moderate or severe. The description and criteria of severity grades per particular organs can be reviewed in Appendix W.

4.1.4.10 Statistical Analyses

For experimental variables measured at the end of the study, dose groups were compared using a one-factor analysis of variance (ANOVA). Organ to brain and organ to body mass ratios were calculated and analyzed similarly to the other parameters measured at the end of the study. If the ANOVA was significant, post hoc tests were used to compare pairs of dose groups, a Tukey's multiple comparison test if the variance of groups were similar, and a Dunnett's T3 test if the variances were unequal. Variance equality was determined by a Levene's test. For body mass changes and food consumption over the study days, a repeated measures ANOVA with time as the repeated factor and dose group as between group factor was used to assess changes in times and dose groups. If the ANOVA was significant, post hoc tests were used to compare pairs of dose groups, a Tukey's multiple comparison test if the variance of the groups were similar, and a Dunnett's T3 test if the variance were unequal. Variance equality was determined by a Levene's test. SPSS 16.0 was used to perform all analyses and statistical significance was defined as α=0.05 for all tests.

The neurobehavioral evaluations generated two types of data, continuous/count variables and categorical variables. The continuous/count variables were either measurements or counts of a specified action. The categorical variables were either presence or absence of a response or a severity of occurrence. Due to the low frequency of grooms, this count variable was converted to a categorical (presence/absence) variable for analysis. For the motor activity data, the fifteen interval recordings were averaged to get one single number per rat. The nose poke response was calculated by totaling the nine nose poke recordings per interval and then taking an average over the fifteen minute interval. For continuous data, an ANOVA was used to test for differences between treatment groups, separately for each sex. If the ANOVA revealed significant differences, a Dunnett's C test was used if variances were homogenous and a Dunnett's T3 test if variances differed between treatment groups. Levene's test was used to test the homogeneity of variance among treatment groups. For categorical data, Fisher's exact test was used to test for differences

between treatment groups at each week, for each sex. If significant differences were observed, then a Mann-Whitney test was conducted to compare pairs of treatment groups. SPSS® 16.0 and SAS® version 9.2 were used for all statistical analyses. Statistical significance was defined as $P<0.05$. Details of the statistical analyses can be found in Appendix T. (SPSS® is a registered trademark of IBM Corp.; SAS® is a registered trademark of SAS Institute Inc.)

5 Results

5.1 Analytical Chemistry

The analytical chemistry results are summarized in Tables 2 and 3. All of the dosage levels in Appendices D-W are reported using the nominal concentrations. The following tables provide a summary of the analytical results of each dosing suspension for the 14-day and 90-day studies.

Table 2. 14-day Analytical Results

Nominal Concentration (mg/ml)	Analytical Concentration (mg/ml)
1 (14-day)	1.06
2 (14-day)	2.19
4 (14-day)	4.44
8 (14-day)	8.99
16 (14-day)	17.8
32 (14-day)	36.7
64 (14-day)	62.4
64 top (homogeneity)	45.2
64 middle (homogeneity)	57.7
64 bottom (homogeneity)	66.3
64 top (homogeneity) repeat	66.0
64 middle (homogeneity) repeat	69.5
64 bottom (homogeneity) repeat	73.2
5 (0-day stability)	4.96
5 (7-day stability)	5.10
5 (14-day stability)	5.70
5 (21-day stability)	5.44
5 (28-day stability)	5.92

Table 3. 90-day Analytical Results

Nominal	Analytical Concentration (mg/ml)

Concentration (mg/ml)					
	batch 1	batch 2	batch 3	batch 4	batch 5
0.25	0.30	0.23	0.21	0.22	0.24
1	1.0	0.94	0.94	0.96	0.99
4	4.2	3.7	3.9	3.9	4.2
16	18	17	16	16	NA

5.2 Approximate Lethal Dose Study

The results of the approximate lethal dose (ALD) range-finding study are presented in Appendix D.

Clinical signs including lethargy, rapid respiration/labored breathing, prostrate posture, and salivation were noted in male rats at doses of 88.9 mg/kg and greater and in female rats at doses of 133.3 mg/kg and greater. Female rats additionally exhibited chromodacryorrhea. Clinical signs were apparent approximately fifteen to thirty minutes after dosing and persisted throughout the first day of observation in surviving animals. Mortality occurred in all male rats at doses of 300.0 mg/kg and greater, occurring 3 ± 1.7 hours after administration of the test substance. Mortality occurred in female rats dosed at 300.0 mg/kg and greater with the exception of the rat dosed at 450.0 mg/kg which survived the 14-day observation period. Mortality occurred in female rats 4 ± 1.7 hours after administration of the test substance. Gross pathology observations were unremarkable.

5.3 14-Day Oral Repeated Dose Toxicity Study

5.3.1 Clinical Observations and Mortality

Clinical signs of toxicity, including lethargy, prostrate posture, rapid respiration/labored breathing, dark urine, orange feces, and barbering were observed in the 100 mg/kg-d dose groups. Lethargy, dark urine, congested breathing and barbering were noted in the 50 mg/kg-d groups. Clinical signs in lower dose groups were limited to barbering (see Appendix E).

5.3.2 Body Mass and Food Consumption

Body mass remained constant or decreased slightly (50 and 100 mg/kg-d dose groups) following the first dose of DNAN, but increased with time in all dose groups throughout the remainder of the study. Body mass of male rats given 100 mg/kg-d DNAN was significantly reduced relative to the corn oil control from day 1 through day 13 ($P=0.017$, $P=0.002$, $P<0.001$, $P<0.001$). Male rats in the 50 mg/kg-d day also had significantly reduced body mass at days 7 and 13 ($P=0.037$, $P=0.040$). In female rats, body mass in the 100 mg/kg-d dose group was reduced relative to the corn oil control at day 13; however, body mass did not differ significantly between treated and control groups at any time during the study. Food consumption did not differ significantly between treated and control groups at any time during the study for male or female rats (see Appendices G-I). Feed conversion efficiency was reduced in male rats given 50 or 100 mg/kg-d at day 7 ($P=0.008$, $P<0.001$, respectively) and overall ($P=0.002$, $P<0.001$, respectively). Feed conversion efficiency did not differ among treated and control groups for female rats.

5.3.3 Organ Mass and Ratios

Kidney, liver, and spleen mass to body mass ratios differed significantly between DNAN treated and control groups in both males (P<0.001, P=0.001, P=0.001) and females (P=0.001, P<0.001, P<0.001). Kidney to body mass ratios were increased, relative to the control, in male and female rats given 50 (P=0.011 and P=0.001) and 100 mg/kg-d DNAN (P=0.001 and P=0.001). Liver and spleen to body mass ratios were increased, relative to the control, in males in the 100 mg/kg-d dose group (P=0.002 and P=0.002, respectively) and female rats in the 50 (P=0.010 and P=0.002, respectively) and 100 mg/kg-d groups (P=0.001 and P<0.001, respectively). Spleen to body mass ratios were increased in females in the 25 mg/kg-day group (P=0.028). Spleen mass and spleen to brain mass ratios differed significantly between female rats treated with DNAN and control groups (P<0.001 and P<0.001). Female rats given 50 or 100 mg/kg-d DNAN had increased spleen mass (P=0.002 and P<0.001, respectively) and spleen to brain mass ratios (P=0.003 and P<0.001) (see Appendix N).

5.3.4 Clinical Chemistry

Albumin (ALB) levels were significantly elevated in male rats (P=0.012) given 50 or 100 mg/kg-d, relative to the corn oil control group (P=0.033 and P=0.047). Alanine aminotransferase (ALT) levels were significantly (P<0.001) elevated in female rats in the 50 and 100 mg/kg-d dose groups relative to the control group (P=0.012 and P=0.004, respectively). Female rats in the 100 mg/kg-d DNAN dose group had elevated total bilirubin (TBIL) levels compared to the control group (P=0.003). Cholesterol (CHOL) levels were increased (P=0.005) in female rats in the 50 and 100 mg/kg-d dose groups relative to the control group; however, this difference was only statistically significant for the 50 mg/kg-d group (P=0.002) (see Appendix P).

5.3.5 Hematology

Female rats in the 100 mg/kg-d dose group had significantly reduced red blood cell counts (RBC) (P<0.001), hemoglobin (HGB) (P<0.001), hematocrit (HCT) (P=0.004), and mean cell hemoglobin concentration (MCHC) (P=0.037), compared to corn oil control group. Red cell distribution width (RDW) was increased (P<0.001) in female rats treated with 100 mg/kg-d DNAN. None of the measured hematology parameters differed significantly between treated and control groups in male rats (see Appendix R).

5.3.6 Pathology

Mottled kidney was noted in one male rat from each of the 3.13 and 6.25 mg/kg-d dose groups. Mottled liver was noted in two females (12.5 and 50 mg/kg-d) and three males (control and two 6.25 mg/kg-day). Additional liver findings included two females (one control and one 12.5 mg/kg-d) with focal pale areas. One female in the 50 mg/kg-d group had an hepatic accessory lobe. Dark spleen was noted in five of the six females in the 100 mg/kg-d group. Enlarged mesenteric lymph nodes were appreciated in 18 females: four controls, four 1.56 mg/kg-d, two 3.13 mg/kg-d, two 6.25 mg/kg-d, two 12.5 mg/kg-d, two 50 mg/kg-d, and two 100 mg/kg-d. Raised nodules were found on the mesenteric lymph nodes in eight males (two controls, one 1.56, two 3.13, one 6.25, one 12.5 and one 100 mg/kg-d). Nodules were noted on the jejunum of seven males and seven females: one control, one 1.56, four 3.13, two 6.25, two 12.5, two 50, and two 100 mg/kg-d.

5.4 90-Day Oral Repeated Dose Toxicity Study

5.4.1 Clinical Observations and Mortality

Mortality or morbidity was observed in three males and one female in the 80 mg/kg-d dose group. The female was euthanized on day 19 following observations of lethargy, labored breathing, prostrate posture, ataxia, partial hind limb paralysis, and complete front limb paralysis. The males were found dead on days 51, 64, and 79. Clinical signs of toxicity, including lethargy prostrate or recumbent posture, rapid/labored respiration, ataxia, irregular gait patterns (walking on toes, creeping, dragging hind end, hind end raised, pulling legs up, stiff legs, limping, dragging leg, walking backwards in circles, hind end wobbling, movements jerky, stiff/locked muscle/limb), dark urine, squinting, hunched posture, pulling ears back, twitching ears, twitching tail, body twitching, head shaking, leaning to left, straubbed tail, curled tail, barbering, rough hair coat, piloerection, low arousal, red discharge from nose, chromodacryorrhea were noted in the 80 mg/kg-d dose group. Although the overall signs noted for males and females were similar, there was an apparent gender difference in the pattern of clinical signs. Males were consistently observed in dorsal or lateral recumbency starting approximately three hours after the DNAN was administered and lasting approximately three hours. Males in this posture were alert and responsive, immediately righting themselves when disturbed. Females were observed in this posture relatively infrequently. Males and females both demonstrated gait irregularities, however, males tended to creep with lowered hind quarters while females tended to walk on their toes and pull up their legs and high step while walking; both genders had difficulty moving the hind limbs due to what appeared to be muscle stiffness/tetany. Lethargy, dark urine, dorsal and lateral recumbency, prostrate posture, congested breathing, and labored breathing were noted in males in the 20 mg/kg-d dose group. Clinical signs in females in the 20 mg/kg-d group included dark urine, low arousal, irregular gait (walking on toes), and lateral recumbency. Males in lower dose groups exhibited dark urine, curled tail, dorsal and lateral recumbency, irregular gait (creeping, hind end lowered, stiff muscles), chromodacryorrhea, and barbering. Clinical signs in females in lower dose groups were limited to barbering, alopecia and congested breathing, with the exception of one female in both the 1.25 and 5 mg/kg-d groups with hind limb ataxia. Clinical signs in control animals were limited to barbering, chromodacryorrhea, alopecia, scab, and congested breathing, with the exception of one male which was euthanized on day 68 due to a dosing error (see Appendix F).

5.4.2 Body Mass and Food Consumption

Body mass increased with time for all dose groups throughout the study, with the exception of week 11 when animals were fasted overnight while in metabolism cages. Females in the 80 mg/kg-d dose group additionally lost weight during week 10. Body mass of male rats given 80 mg/kg-d DNAN was significantly reduced relative to the corn oil control from day 7 through day 90 ($P=0.043$, $P=0.031$, $P=0.003$, $P<0.001$, $P<0.001$, $P<0.001$, $P<0.001$, $P<0.001$, $P<0.001$, $P<0.001$, $P<0.001$, $P<0.001$, $P<0.001$). Body mass of female rats did not differ significantly between treated and control groups at any time during the study. Food consumption was increased in males 20 mg/kg-d and females given 20 and 80 mg/kg-d; however this increase was only significant in females in the 80 mg/kg-d group (week 2, $P=0.004$; week 3, $P=0.029$; week 4, $P=0.001$; week 7, $P=0.024$; week 9, $P=0.026$). Males in the 80 mg/kg-d dose group initially exhibited an increase in food consumption similar to that observed in the 20 mg/kg-d group; however, this increase dissipated by week four (see Appendices J-L). Feed conversion efficiency was reduced in male rats given 80 mg/kg-d at weeks one through six ($P<0.001$, $P=0.001$, $P<0.001$, $P=0.024$, $P=0.005$, $P=0.033$, respectively) and overall ($P<0.001$). Feed conversion efficiency did not differ among treated and control groups for female rats.

5.4.3 Neurobehavioral Evaluations

5.4.3.1 Home Cage Observation

There were no differences among treatment groups in any of the home cage parameters: agitation, convulsions, tremors, posture, mutilation, and the area mutilated.

5.4.3.2 Handheld Observation

Males
There were no differences among treatment groups for lacrimation, salivation, piloerection, palpebral closures, exopthalmos and pupillary status. The 80 mg/kg-day dose group had fewer rats that were classified as very easy to remove from the cage at weeks one ($P = 0.0154$), five ($P = 0.0046$), seven ($P = 0.0497$) and eight ($P < 0.001$). To help show the drastic difference in observed responses between the 80 mg/kg-day group and the other four dose groups, an average ease of removal score was calculated for each rat. The 80 mg/kg-day group had the seven highest average scores, pointing towards more difficult ease of removal for this dose group. Reactivity to handling also differed among treatment groups at weeks two ($P = 0.0485$), three ($P = 0.0495$), six ($P = 0.0218$), seven ($P < 0.001$), nine ($P = 0.0422$) and ten ($P = 0.0061$). The 80 mg/kg-day group had fewer low and more moderately high reactivity to handling observations than the other dose groups. The 80 mg/kg-day group had six of the seven highest 11 week average reactivity to handling scores, indicating that reactivity was higher, in general, for rats in this dose group. For both ease of removal and reactivity to handling, responses for rat 102 differed from the remaining rats in the 80 mg/kg-day, with rat 102 appearing less affected by the treatment than the other rats.

Females
There were no differences among treatment groups for ease of removal, reactivity to handling, lacrimation, salivation, piloerection, palpebral closures, exopthalmos and pupillary status. At week 6, the 80 mg/kg-day dose group had more barbering ($P = 0.0289$) observations than the control group. Barbering was, however, present in all dose groups (80 = 5, 20 = 2, 5 = 5, 1.25 = 4, control = 1 rat) during the 11 week study and did not differ between dose groups at any other time point.

5.4.3.3 Open Arena Observation

Males
There were no differences found in grooms, rears, arousal, fecal boli, fecal description, and urine. The 80 mg/kg-day group had fewer normal gait observations than other dose groups at weeks five ($P = 0.002$), nine ($P = 0.011$) and 11 ($P = 0.015$). Generally rats in the 80 mg/kg-day dose group had too little movement to determine gait; however, at week 11 more hunched body position was observed in this group. Additional gait observations included ataxia, hind limb impairment, and walking on toes. If a rat was recorded as either having hind limb impairment, walking on toes or hunched body position, the rat usually displayed all three characteristics.

Females
There were no differences found in grooms, arousal, fecal boli, fecal description, and urine. Females in the 80 mg/kg-day dose group had fewer normal gait observations at weeks nine ($P < 0.001$), ten ($P = 0.050$) and 11 ($P = 0.013$). Similar to the males, females in the 80 mg/kg-day had fewer normal observations and more hunched body position was observed at weeks ten and 11. As with males, hind limb impairment, walking on toes or hunched body position, when observed, typically occurred together. Rats in the 80 mg/kg-day group reared less often than those in the other dose groups at weeks six ($P = 0.004$), seven ($P = 0.020$) and ten ($P = 0.040$).

5.4.3.4 Sensory Motor

Males
There were no differences among treatment groups in any of the sensory motor responses: approach, auditory startle response, tail pinch, pinna response, pupillary response, righting reflex, aerial righting, landing foot splay, forelimb grip strength, and hindlimb grip strength.

Females
There were no differences for auditory startle response, pinna response, pupillary response, righting reflex, aerial righting, landing foot splay, forelimb grip strength, and hindlimb grip strength. Tail pinch and approach differed among the five dose groups for females ($P = 0.020$ and $P = 0.024$, respectively). The 80 mg/kg-day dose group had fewer response observations for tail pinch and fewer slow approach observations for the approach variable. Nine of the ten animals in the 80 mg/kg-day dose group had no reaction responses for the approach variable. There were three, two, three, and four no reaction responses in the control, 1.25, 5, 20 mg/kg-day groups, respectively. Three animals in the 80 mg/kg-day group had no response to the tail pinch whereas all animals in all other dose groups showed a response with the exception of one animal in the 20 mg/kg-day group.

5.4.3.5 Motor Activity

Males
There were no differences among treatment groups in basic movement, immobility, X and Y ambulation. Mean number of nose pokes was lower ($P = 0.009$) in the 80 mg/kg-day group than the other dose groups.

Females
There were no differences among treatment groups in basic movement, immobility, X and Y ambulation. Mean number of nose pokes was lower ($P = 0.014$) in the 80 mg/kg-day group than the other dose groups.

5.4.4 Ophthalmoscopic Examinations

No abnormalities were noted in the terminal ophthalmoscopic examination.

5.4.5 Urinalysis

Urine color, volume, specific gravity, and protein concentration differed significantly between DNAN treated and control groups in both males and females. Urine was significantly darker in the 20 and 80 mg/kg-d dose groups than in the control groups in males ($P=0.003$ and $P<0.001$, respectively) and females ($P<0.001$ and $P<0.001$, respectively). In males, urine color increased from dark yellow in controls to gold and brown in the 20 and 80 mg/kg-d groups, respectively. Urine color in females increased from yellow in controls to dark yellow and gold/amber in the 20 and 80 mg/kg-d groups, respectively. Urine volume increased in a dose dependent manner in females and was significantly higher (3.2 fold) in the 80 mg/kg-d group than the control group ($P<0.001$). Urine volume was also increased (1.7 fold) in the 20 mg/kg-d group, however, this increase was not statistically significant. In males, urine volume was significantly higher (2.0 fold) in the 20 mg/kg-d group than in the control ($P=0.022$). Urine volume was also increased (1.7 fold) in the 80 mg/kg-d group, however, this increase was not statistically significant. Specific gravity decreased in a dose dependent manner in

female rats and was significantly reduced in the 80 mg/kg-d group relative to the control group (P=0.006). In males, specific gravity was significantly lower in the 20 mg/kg-d dose group than in the control group (P=0.039). Protein concentration was significantly lower in females in the 80 mg/kg-d group (P=0.037) and males in the 20 mg/kg/d group (P=0.027) than in the control groups. Bilirubin concentration was increased (2.7 fold) in males in the 80 mg/kg-d group relative to the control group (P=0.009). Urine appearance, glucose, ketones, pH, urobilinogin, and leukocytes did not differ between DNAN treated and control groups (see Appendix U).

5.4.6 Organ Mass and Ratios

Kidney, liver, and spleen mass, organ to body mass ratios, and organ to brain mass ratios differed significantly between DNAN treated and control groups in both males and females. In males, absolute kidney mass did not differ significantly between treated and control groups; however, kidney mass to body mass ratios were increased in the 20 and 80 mg/kg-d groups relative to the control (P=0.001 and P<0.001, respectively). Kidney mass to brain mass ratios were increased in males in the 20 mg/kg-d group only (P=0.036). In females, kidney mass increased in a dose dependent manner and was significantly higher in the 20 and 80 mg/kg-d groups than in the control group (P=0.035 and P=0.035, respectively). Kidney mass to body mass and brain mass ratios were increased, relative to the control, in female rats given 80 mg/kg-d DNAN (P<0.001 and P=0.019). Liver mass to body mass ratios were increased, relative to the control, in males (P<0.001) and females (P=0.001) in the 80 mg/kg-d dose group. Spleen mass to body mass ratios were higher in males and females given 80 mg/kg-d DNAN relative to controls (P<0.001 and P<0.001, respectively). Absolute spleen mass and spleen mass to brain mass ratios were also increased, relative to the control, in females in the 80 mg/kg-d group (P<0.001 and P<0.001, respectively). In males, testes mass, testes to body mass, and testes to brain mass ratios were reduced in the 80 mg/kg-d group (P<0.001, P<0.001 and P<0.001, respectively). Epididymides mass and epididymides to brain mass ratio were also reduced, relative to the control, in males given 80 mg/kg-d DNAN (P<0.001 and P<0.001, respectively). Thymus mass and thymus to brain mass ratio were reduced, relative to the control, in males in the 80 mg/kg-d group (P=0.001 and P<0.001, respectively). Adrenal mass was reduced, relative to the control, in females in the 80 mg/kg-d group (P=0.042) (see Appendix O).

5.4.7 Sperm Analysis

The number of sperm per gram in the cauda epididymis in male rats in the 80 mg/kg-d group was reduced to 4.5 percent of the number of sperm per gram found in controls (P=0.038). No motile sperm were found in any of the animals in the 80 mg/kg-d group. No significant reductions in sperm per gram, percent motile sperm, or percent progressively motile sperm were observed in the 1.25, 5, or 20 mg/kg-d dose groups (see Appendix V).

5.4.8 Clinical Chemistry

Cholesterol levels decreased in a dose dependent manner in DNAN treated males, were significantly lower (1.4 and 1.5 fold, respectively) in the 20 and 80 mg/kg-d groups than in the control group (P=0.007 and P=0.005), and were outside of normal ranges. Blood chloride levels were higher in the 80 mg/kg-d group than in the control group (P=0.007); however, these values were within normal ranges. Blood urea nitrogen (BUN) in males in the 80 mg/kg-d group exceeded the normal range and was elevated relative to the control (1.2 fold); however, the 80 mg/kg-d group did not differ from the control group. Similarly, in females, BUN did not differ significantly between treated and control groups, but was outside of normal ranges and was increased relative to the

control in all groups, particularly the 80 mg/kg-d group (1.4 fold). Alanine aminotransferase (ALT) levels were elevated in both males and females in the 80 mg/kg-d groups relative to the control group; however, ALT levels did not differ significantly between treated and control groups. ALT levels were above reported normal levels in both treated and control groups, up to two-fold in the 80 mg/kg-d group. Alkaline phosphatase (ALKP) levels were slightly above reported normal levels in male rats in the 80 mg/kg-d group. ALKP levels in the 80 mg/kg-d group were not elevated with respect to the control group which also had ALKP levels above reported normal levels. Lactate dehydrogenase levels in males were below the reported normal range in all groups (1.1-1.5 fold). Total bilirubin (TBIL) levels in females were below reported normal ranges (1.56 to 2 fold) in all groups except the 5 mg/kg-d group. Sodium and potassium levels were above reported normal ranges for both males and females in all dose groups (see Appendix Q).

5.4.9 Hematology

White blood cell count (WBC) increased in a dose dependent manner in female rats and was significantly higher (1.5 and 1.7 fold, respectively) in the 20 and 80 mg/kg-d groups relative to the control group (P=0.023 and P=0.002, respectively). WBCs were above normal ranges in female rats in all dose groups of 5 mg/kg-d and greater in all dose groups for male rats. The increase in white blood cell count observed in female rats was due to an increase in lymphocytes in the 80 mg/kg-d group (1.6 fold) (P=0.0074) and monocytes in the 20 and 80 mg/kg-d groups (2.2 and 3.2 fold) (P=0.014 and P<0.001, respectively) relative to the control group. Lymphocyte counts demonstrated a dose dependent increase, with all dose groups of 5 mg/kg-d and greater having counts above reported normal ranges. Lymphocyte counts in male rats exceeded normal ranges in all dose groups; treated and control groups did not differ. Monocyte counts increased in a dose dependent manner in female rats. All monocyte and basophil counts were above normal ranges in both male and female rats.

Red blood cell counts (RBC) in female rats were significantly (P<0.001) reduced (0.8 fold) in the 80 mg/kg-d dose group compared to the corn oil control group. The RBC counts in the 80 mg/kg-d group were also outside of the normal range. In male rats, RBC counts were increased (1.1 fold) in the 5 and 20 mg/kg-d groups relative to the control group (P=0.037 and P<0.001, respectively). RBC counts increased in a dose dependent manner from the control group through the 20 mg/kg-d group, with groups at and above 1.25 mg/kg-d exceeding normal values. RBC counts in the 80 mg/kg-d group, however, approximated those of the control group. Hemoglobin (HGB) and hematocrit (HCT) were significantly reduced (0.88 and 0.91 fold) in female rats in the 80 mg/kg-d group (P<0.001 and P=0.007, respectively) relative to the corn oil control group; both measures were below normal ranges in the 80 mg/kg-d group. In male rats, HCT and HGB did not differ among treated and control groups. Mean cell hemoglobin (MCH) was reduced (0.93) in males in the 20 mg/kg-d group (P=0.002) relative to the control. MCH did not differ among treated and control groups for female rats. MCH values were below normal ranges for both males and females with the exception of the females in the 80 mg/kg-d group. Mean cell hemoglobin concentration (MCHC) was significantly reduced in both males (0.97 fold) and females (0.96 fold) in the 80 mg/kg-d group (P<0.001 and P<0.001, respectively); however, MCHC remained within the normal range. Mean cell volume (MCV) was increased (1.1 fold) in female rats in the 80 mg/kg-d group (P<0.001). In male rats, MCV was reduced (0.93 fold) in the 20 mg/kg-d group (P=0.003). In both males and females, MCV values were below reported normal ranges with the exception of the females in the 80 mg/kg-d group. Red cell distribution width (RDW) was increased in the 20 and 80 mg/kg-d dose groups in both males (1.2 and 1.4 fold; P<0.001 and P<0.001, respectively) and females (1.1 and 1.3 fold; P=0.033 and P<0.001, respectively). Platelet count did not differ between treated and control groups for either male or female rats. Mean platelet volume (MPV) was slightly increased

(1.1 fold) in the 80 mg/kg-d groups; however, this increase was only significant in females (P=0.048) (see Appendix S).

5.4.10 Pathology

The gross pathological examination of the pre-term mortality of the female in the 80 mg/kg-d dose group indicated an enlarged dark spleen, enlarged submandibular lymph nodes, and dark kidneys. Macroscopic findings in the pre-term mortalities in males in the 80 mg/kg-d group included dark spleen (1/3), enlarged kidneys (2/3), dark red focus on kidney (1/3), small testes (1/2), red focal area on jejuna serosa (1/3). The macroscopic findings in the pre-term mortality in the control male, esophageal rupture proximal to thoracic inlet and subcutaneous edema were consistent with a dosing error. Additional macroscopic findings included enlarged heart in two male rats, one control and one in the 5 mg/kg-d group. Pale and/or mottled kidney were noted in nine females, one control, one 1.25 mg/kg-d, three 5 mg/kg-d, one 20 mg/kg-d, and three 80 mg/kg-d animals. One female from the 80 mg/kg-d group was noted as having dark kidneys. In males, kidneys were noted to be mottled and/or pale in three controls, one 1.25 mg/kg-d, and three 5 mg/kg-d animal. Hydronephrosis was noted in one control and one 20 mg/kg-d males. Two males in the 80 mg/kg-d group had enlarged kidneys. Ovarian findings were noted in two females in the 5 mg/kg-d, an adhesion and a hemorrhagic cyst. Three female rats were noted as having pale areas in the liver, one each from the control, 1.25 and 5 mg/kg-d groups. In male rats, liver findings included six rats with diffusely pale livers (three controls, one 1.25 mg/kg-d, and two 5 mg/kg-d), three with mottled livers (one each from control, 1.25 and 5 mg/kg-d), and two 1.25 mg/kg-d rats with enlarged livers. All of the females and eight of the males in the 80 mg/kg-d group were noted as having dark spleens. Small testes were noted in six of the males in the 80 mg/kg-d group. Hydrouterus was noted in five females: one 5 mg/kg-d, two 20 mg/kg-d, and two 80 mg/kg-d. Two control females and four males (one control and three 80 mg/kg-d) had enlarged mesenteric lymph nodes. Six females: one control, one 1.25 mg/kg-d, one 5 mg/kg-d, one 20 mg/kg-d and two 80 mg/kg-d had enlarged submandibular lymph nodes. Additional gross lesions appreciated at the time of necropsy are noted in Appendix W.

5.4.11 Histopathology

Mortality occurred in three males and one female dosed at 80 mg/kg-d. Selected tissues were collected from these animals; no cause of death was identified based on microscopic examination. Test-article related microscopic findings were noted in the testes, epididymides, spleen, liver, brain, and kidney.

Degeneration and atrophy of testicular seminiferous tubules (moderate to severe) was present in nine of nine males examined in the 80 mg/kg-d group. No test article-related changes were noted in the testes in the control and 20 mg/kg-d groups. Seminiferous tubules of the 80 mg/kg-d group retained only Sertoli cells, spermatogonia and early spermatocytes. Absent germ cell layers included all spermatid and late spermatocyte stages resulting in the absence of mature sperm in seminiferous tubules. Testes additionally demonstrated moderate to numerous numbers of atrophic tubules.

Aspermia with eosinophilic cellular tubular debris (moderate to severe) was present in the epididymis of nine of nine males examined in the 80 mg/kg-d group. Few sperm were noted in the cauda of individual animals in the 80 mg/kg-d group.

In the spleen, extramedullary hematopoiesis (minimal to severe) was present in 6/10 males and 0/10 females in the control group, 3/10 males and 3/10 females at 1.25 mg/kg-d, 6/10 males and 3/10 females at 5 mg/kg-d, 6/10 males and 4/10 females at 20 mg/kg-d and 7/10 males and 9/10 females at 80 mg/kg-d. Severity was greater in females than in males and increased with dose in females. Hemosiderin, excess iron deposited in the spleen in normal rats due to the breakdown of old erythrocytes, was present in all rats on study. An increase in hemosiderin deposits can result from hemolytic crisis or hematotoxic insult. A dose related increase in the severity of hemosiderosis was apparent in males. Hemosiderosis of greater severity than observed in the control (minimal) group was present in 3/10 (mild) males at 1.25 mg/kg-d, 4/10 (mild) at 5 mg/kg-d, 7/10 (mild) at 20 mg/kg-d and 10/10 (mild to severe) at 80 mg/kg-d. Hemosiderosis (mild to moderate) was present in 10/10 females at 1.25 mg/kg-d, 7/10 at 5 mg/kg-d, 10/10 at 20 mg/kg-d and 10/10 at 80 mg/kg-d; however the incidence 10/10 and severity (mile to moderate) was similar in control females.

In the liver, lymphohistiocytic infiltrates were observed in treated control groups. Although these aggregates are often considered to be background lesions, the frequency may be increased by treatment. A slight increase in severity with increasing dose was noted in females which may have been treatment related. Lymphohistiocytic infiltrates were present in 10/10 (minimal to mild) control females, 10/10 (mild to moderate) females at 5 mg/kg-d, 9/10 (minimal to moderate) at 20 mg/kg-d, and 9/10 (minimal to severe) at 80 mg/kg-d. Livers from animals in the 1.25 mg/kg-d group were not examined. Focal hepatic biliary hyperplasia (minimal to mild) was present in 1/10 males in the 80 mg/kg-d group and 1/10 females in the 5 mg/kg-d group. Due to the isolated incidence and minimal severity, these lesions are considered incidental and not treatment related.

Cerebellar or brain stem gliosis was noted in 3/10 males and 1/10 females in the 80 mg/kg-d dose group. Two of the males and the female were pre-term mortalities. Microscopically, lesions appeared as spongiotic grey or white matter with increased glial cells and astrocytes occasionally with macrophages (gitter cells). These lesions were considered to be compound related.

Renal mineralization at the corticomedullary junction was present in 3/10 (minimal to mild) control females, 6/10 (minimal to mild) females at 5 mg/kg-d, 10/10 (minimal to moderate) at 20 mg/kg-d, and 7/10 (minimal to mild) at 80 mg/kg-d. Kidneys from the 1.25 mg/kg-d group were not examined. The prevalence of mineralization was higher in DNAN treated females than in the controls; however, a clear dose response was not apparent in either prevalence or severity. Renal mineralization was not noted in males. Other kidney lesions including, basophilic tubules, pelvic dilatation (hydronephrosis), and lymphocytic interstitial infiltrates occurred at similar rates in control and treated animals and were considered background lesions.

Additional lesions noted but determined to be background or incidental due to low frequency of occurrence or comparable occurrence in control and treated groups included: prostatic, epididymal and coagulating gland lymphocytic infiltrates, harderian gland lymphocytic infiltrates, rare lymphoid hyperplasia of submandibular or mesenteric lymph nodes, adrenal gland vacuolation, plasmacytosis of the submandibular lymph node, pancreatic acinar atrophy or degeneration, myocardial necrosis, cardiac lymphocytic infiltrates, and ultimobranchial cysts of the thyroid (see Appendix W).

5.5 Determination of BMDL and $BMDL_{10}$

Extramedullary hematopoeisis (EMH) was identified as the critical endpoint in this study based on the increased incidence in female rats in the DNAN treated groups (Barnes and Dourson 1988, EPA 2002). Benchmark Dose Software (BMDS v.2.1.2) was used to fit mathematical models to the EMH incidence dose response data and calculate a lower-bound confidence limit on a dose corresponding to a 10 percent response rate ($BMDL_{10}$) (EPA 1995, EPA 2000). The Gamma, quantal-linear, and Weibull models were selected based on goodness-of-fit and statistical parameters ($p>0.1$, lowest AIC values and residuals) (Appendix X). A mean BMDL of 4.08 mg/kg-day and a $BMDL_{10}$ of 2.3 mg/kg-day were calculated based on the results of these three models.

5.6 Standing Operating Procedure and Protocol Deviations

The following deviations occurred during the study but were not considered to have compromised the integrity or validity of the study results:

1. As per the protocol and SOP 004, animal room lights are to be set to turn on at 0600 and turn off at 1800. However, on 10/05/10 it was found that the animal room (3203) lights were on at 0515. The light timer for the room was re-set accordingly.

2. The environmental monitoring system (Metasys®) for the animal rooms failed from 11/15/10-11/16/10 and 01/18/11-02/24/11. During these time periods, the system did not have the ability to alarm when/if the temperature and humidity went outside the set ranges. In addition, as per SOP 90, in the event of system failure, Animal Health Technicians are to use a recently calibrated strip chart recorder to monitor temperature/humidity until the system is repaired. However, while the Metasys® was down, a portable thermohydrometer in the animal room was used to document temperature and humidity readings. The thermohydrometer used was past its re-calibration date. (Metasys® is a registered trademark of Johnson Controls)

3. As per SOP 90, when the Johnson Controls system alarms after duty hours, the staff duty officer (SDO) is to notify the Attending Veterinarian (AV) or Animal Health Technicians (AHT) on duty. On 12/7/10 the humidity went out of range and the Johnson Control system alarmed to the SDO; however, the SDO did not contact the AV or the AHT on duty. As a result, the humidity was out of range from 0200-0830. The humidity was 11 percent at 0600 when checked by the AHT.

4. On 02/03/2011, the boilers failed to restart when the generator turned on during a power outage. This resulted in the animal room temperature dropping to 60 degrees before study staff arrived in the morning. Per SOP 004, room temperature was to be maintained between 64 and 79 degrees. Additionally, due to the failure of the Metasys® system (see 2 above), the Johnson Controls system did not alarm after duty hours and no notifications were made as required by SOP 90.

5. As per SOP 73, a complete training file is required before an employee may participate on a study. However, the training file of the pathologist was not complete when necropsies were performed. The required information was added to the training file.

6. Animal #11-0187 was mis-dosed with 1.26 ml of 80 mg/kg on 12/23/10, #11-0171 was mis-dosed with 1.33 ml of 20 mg/kg on 1/19/10 and #11-0179 was not dosed on 1/19/10 due to technician error.

6 Discussion

Clinical signs of toxicity were observed in male rats at doses of 88.9 mg/kg and greater and in female rats at doses of 133.3 mg/kg and greater in the acute phase of the study. Mortality occurred at doses of 300.0 mg/kg and greater, indicating an ALD of 300 mg/kg. These results are in general agreement with the previously reported LD_{50} of 199 mg/kg (Dodd and McDougal 2002).

Both subacute and subchronic exposure to DNAN resulted in reduced body mass in male rats in the highest dose groups (50 and 100 mg/kg-d, and 80 mg/kg-d, respectively), but did not affect body mass in female rats. The reduced body mass was not attributable to a reduction in food intake as food intake did not differ among treatment groups for male rats. The absence of an effect on body mass in female rats may, however, have been due to the increase in food intake observed in female rats. Feed conversion efficiency was reduced in male, but not female rats, in the highest dose groups. These data suggest that the effects of DNAN on body mass may have been due to impacts on metabolism. DNAN is metabolized to 2,4-dinitrophenol (2,4-DNP) (Hayes 1982), a compound which increases basal metabolic rate by uncoupling oxidative phosphorylation (De Felice and Ferreira 2006 and reference therein). Thus, the effects of DNAN on metabolism may be attributable to 2,4-DNP. The disparity in effects of DNAN on body mass between males and females suggests a possible difference in the conversion of DNAN to DNP between the sexes. Preliminary data suggests that although both male and female rats metabolize 2,4-DNAN to 2,4-DNP, males appear to convert a greater proportion of DNAN to DNP (O'Neill and Crouse in prep).

Gender differences in the conversion of DNAN to DNP may also contribute to the differences in effects observed in organ systems. Female rats exhibited enlargement of the spleen, splenic hemosiderosis, and extramedullary hematopoeisis associated with changes in hematology indicative of anemia, including decreased red blood cell count, hematocrit, hemoglobin, and mean corpuscular hemoglobin concentration and increased red cell distribution width, and mean cell volume. Although gross and microscopic changes of the spleen were observed in male rats, the prevalence and severity were lower than that seen in females. Overt anemia was not apparent in male rats; however, a compensated anemia may have been present. As previous studies with rats and dogs demonstrated no hematological abnormalities following subchronic exposure to DNP (ATSDR 1995), the hematological effects observed in the females likely resulted from exposure to the parent compound, DNAN. Again, the differences between the sexes may be attributable to a greater proportion of the dose of DNAN remaining unchanged in female rats, resulting in a higher exposure to DNAN in females than in males.

In contrast, the higher incidence and greater severity of neurological effects in male rats may have resulted from higher DNP exposure. Although both male and female rats developed gait abnormalities and exhibited signs of neurotoxicity in the neurobehavioral assessment, male rats additionally exhibited consistent ventral or lateral recumbency subsequent to DNAN administration and a higher incidence of brain lesions. Ventral or lateral recumbency was noted as a consistent indicator of development of brain lesions in rats exposed to 3-nitroproprionic acid (3-NPA) (Hamilton and Gould 1987). Gait irregularities similar to those noted in the current study, including walking on toes, hunched back and partial disuse of rear legs, have been noted in association with gliovascular lesions in the brain stem of rats exposed to 1,3,5-trinitrobenzene (TNB), 1,3-dinitrobenzene (DNB) and nitrobenzene (NB) (Philbert et al. 2000). Like DNP, 3-NPA and 1,3-DNB disrupt energy metabolism through inhibition of succinate dehydrogenase (SDH) activity (Hamilton and Gould 1987, Phelka et al. 2003). Although both appear to be mediated by SDH inhibition, lesions produced by nitroproprionic acid are characteristically striatal lesions, whereas nitrobenzenes selectively damage the cerebellum (Hamilton and Gould 1987, Phelka et al. 2003).

Recent work has demonstrated that the onset of neuron depleted striatal lesions typically associated with both acute and chronic administration of 3-NPA is preceded by reactive astrocytosis and mild behavioral/gait abnormalities (Cirillo et al. 2010). Neuronal death occurs only after rescue from the disease pathway is no longer possible. The cerebellar gliosis associated with DNAN treatment may similarly represent early stages in a progressive neurodegenerative pathology or maybe a regenerative response to toxic insult (Aschner et al. 1999). The absence of neuronal death may be attributable to low levels of DNP reaching the brain following metabolism of DNAN. As has been hypothesized with 3-NPA, this may lead to less severe metabolic impairment and the build-up of free radicals, nitric oxide and lactic acid which can impair cell functioning. (Newcomb et al. 2005). Progressive nitroproprionic acid induced cell death reportedly is a slow process, taking up to seven days for completion (Koutouzis et al. 1994). Behavioral changes were, however, noted after 3-4 days and overt lesions after five days in rats given high doses of 3-NPA for five days (Blum et al. 2002). In rats given low doses of 3-NPA for two days, behavioral alterations were not apparent until days 5-7, and overt lesions did not manifest (Newcomb et al. 2005).

Delayed onset of lesions has similarly been noted following administration of nitrobenzenes (Chandra et al. 1995, Xu et al. 1999, Philbert et al. 2000). In 1,3,5-TNB exposed rats, 10 days of exposure to 71 mg/kg-day was required to produce brain lesions; rats exposed to 35.5 mg/kg-day for 4 or10 days and 71 mg/kg-day for 4 days did not exhibit brain lesions (Chandra et al.1995). Studies in which 1,3-DNB was given as a single bolus or infused over time demonstrated that a high concentration alone does not induce neurotoxicity; a concentration-time threshold exists (Xu et al. 1999). Chandra et al. (1995) reasoned that the delayed onset of neurotoxicity was attributable to a need for massive doses to accumulate in the brain before damage would ensue. Although this may well be true, the delay may also be the result of progressive neurodegenerative processes that have been noted in the case of 3-NPA to take seven days to result in lesions (Koutouzis et al. 1994).

A delay in onset of neurotoxicity of DNAN or its metabolite(s) was apparent in the onset of gait abnormalities observed during clinical observations as well as during the neurotoxicity evaluation. Significant gait differences were detected in the FOB in males at week five and females at week nine. The appearance of gait abnormalities during clinical observations was variable, with some rats making apparent recovery from one day to the next; however, the mean first day of appearance in the 80 mg/kg-day was day 32±13 in males and day 26±15 in females. This delay in onset may suggest the presence of concentration-time threshold for some aspects of DNAN-induced neurotoxicity as well. Ventral and lateral recumbency, however, a reliable indicator of 3-NPA-induced brain lesions, were present in some rats within the first day of dosing, suggesting either lesions were also present or recumbency is not associated with DNAN-induced brain lesions. The earliest evidence of DNAN-induced brain lesions was in the female euthanized at day 19. That some indications of neurotoxicity were apparent as early as the first day of dosing (recumbency), while the onset of others (gait irregularities) was considerably delayed suggests a progressive neuropathologyy or perhaps separate DNAN and DNP induced neurotoxicities.

Although DNP is neurotoxic at high doses, low doses have been shown to be neuro-protective, maintaining mitochondrial function and reducing oxidative neuronal damage induced by excitotoxic pathways (De Felice and Ferreira 2006 and reference therein). Perhaps the concentrations of DNP present in the brains were such that a neuroprotective effect was established. This might explain the slow onset of gait abnormalities as well as why brain lesions were limited to cerebellar gliosis and did not progress to neuronal death. Although gait irregularities were reported in a subacute inhalation study (Hoffman 2001), this work represents the first documentation of DNAN-induced neurotoxicity associated with brain lesions. Much can be gained by comparison with other

compounds that disrupt mitochondrial energy homeostasis; however, that DNAN-induced neurotoxicity is induced via this mechanism remains speculation at this time.

The mechanism by which DNAN induced testicular toxicity is also not apparent from the current study; however, that it is a testicular toxicant is clear. Males in the 80 mg/kg-day group had reduced testes mass, degeneration and atrophy of testicular seminiferous tubules, severe aspermia with eosinophilic cellular tubular debris of the epididymis, and no detectable sperm in the cauda epididymal sperm analysis. Because germ cells are dependent on the function and processes of other cell types within the testis, disruption of the germ cell supporting environment often results in their death (Creasy 1997). Repetitive and prolonged dosing thus results in progressive germ cell loss, regardless of the mechanism of toxicity. The end result often being seminiferous tubules lined only by Sertoli cells, which, though sensitive to alterations in function, are extremely resistant to cell death (Creasy 1997). The DNAN-induced testicular toxicity is likely attributable to the parent compound rather than its metabolite, DNP. Although 2,4-DNP was toxic to Sertoli-germ cell co-cultures at high concentrations (Takahashi et al. 2003), 2,4-DNP has shown no testicular toxicity in laboratory animals (Matsumoto et al. 2008). Additionally, 2,4-DNP was negative in the rodent Hershberger bioassay, indicating that it is not an anti-androgenic compound (Freyberger and Schladt 2009). A slight increase in the incidence of tailless sperm was noted after 14 days of administration of 30 mg/kg-day 2,4-DNP, suggesting a possible spermatotoxic effect; however, the effect was only observed at a near lethal dose (Takahashi et al. 2004). At the same dose (30 mg/kg-day), 2,4-DNP demonstrated reproductive and developmental toxicity, reducing the number of live births, live birth index, and body weight of pups (Takahashi et al. 2009). Menstrual irregularities have been reported in humans taking 2,4-DNP as a diet aid, indicating possible endocrine activity (ATSDR 1995). Given the testicular toxicity of DNAN and in light of the reproductive and developmental effects of 2,4-DNP, further investigation of the reproductive, developmental and endocrine disrupting effects of DNAN are warranted.

7 Conclusions

Mortality occurred in three male rats (days 50, 63, and 77) and one female rat (day 26) all from the 80 mg/kg-day dose group. Rats in the highest dose group (80 mg/kg-day) experienced lethargy, labored/rapid respiration, prostrate and/or recumbent posture, hunched posture, ear twitching, squinting, curled tail, and gait irregularities. A functional observation battery (FOB) and analysis of motor activity at week 13 indicated that rats given 80 mg/kg-day had altered neuromuscular function and decreased activity levels. In the 80 mg/kg-day group, female rats also had reduced sensorimotor responses while male rats had increased excitability responses.

Although food intake was similar among groups for male rats, animals from the 80 mg/kg-day dose group exhibited reduced body mass and a reduced food efficiency ratio. Female rats in the 80 mg/kg-day dose group also had a reduced food efficiency ratio, but had elevated food consumption at several time points during the study. Body mass did not differ among dose groups for female rats. Female rats in the 80 mg/kg-day dose group and male rats in the 20 mg/kg-day group produced higher volumes of urine with lower specific gravity. Despite the increase in volume, urine color was darker in the 20 and 80 mg/kg-day dose groups for both sexes.

Increased mean kidney, liver, and spleen mass were observed in male and female rats given 80 mg/kg-day DNAN. In male rats, increased mean kidney and liver mass were also noted in the 20 mg/kg-day dose group; however, the changes were not associated with treatment related microscopic abnormalities or alterations in clinical chemistry parameters. Decreased mass of the testes and epididymides as well as degeneration and atrophy of the testicular seminiferous tubules

and aspermia were also observed in rats from the 80 mg/kg-day group. In females, changes in hematology indicative of anemia, including decreased red blood cell count, hematocrit, and hemoglobin, and increased red cell distribution width were observed in the 80 mg/kg-day group. A dose related increase in extramedullary hematopoeisis was noted in spleens of female rats at 20 and 80 mg/kg-day. Glial lesions within the cerebellum were noted in four rats (1 female/3 males) in the 80 mg/kg-day group.

This study, the first repetitive oral dosing conducted with DNAN, demonstrated a steep dose response curve, with most effects occurring only in the highest doses and occurring at or near lethal doses. Likely owing to its conversion to 2,4-DNP, an inhibitor of mitochondrial energy homeostasis, DNAN treatment resulted in an apparent increase in metabolism leading to reduced feed conversion efficiency and ultimately reduced body mass gain in males. Changes in hematology parameters indicative of anemia, splenic enlargement, hemosiderosis, and extramedullary hematopoeisis indicate that the blood is a target organ for DNAN, with female rats being more sensitive to these effects than males. DNAN demonstrated testicular toxicity that, combined with the documented reproductive/developmental effects of its metabolite, 2,4-DNP, raises concern about the reproductive/developmental toxicity of DNAN. DNAN treatment resulted in progressive development of behavioral neurotoxicity as well as associated brain lesions. Extramedullary hematopoeisis in female rats was identified as the critical endpoint in this study and was used to derive a $BMDL_{10}$ of 2.3 mg/kg-day. This $BMDL_{10}$ may be used for development of safe exposure levels.

8 Point of Contact

Questions pertaining to this report should be referred to Emily May Lent at DSN 584-3980, Commercial 410-436-3980, or by e-mail: usaphctoxinfo@amedd.army.mil.

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APPROVED:

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Appendix A

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Appendix B

Quality Assurance Statement

For: Toxicology Study No. 87-XE-0DBP-10, Protocol No. 0DBP-38-10-07-01, The Subchronic Oral Toxicity of 2,4-dinitroanisole (DNAN) in rats, September 2010 - March 2011, the following critical phases were audited/inspected by the Quality Systems Office:

B-1 Pre In-Life Phase of the Study

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Study Protocol GLP Review	06/24/2010	06/24/2010

B-2 In-Life Phase of the Study

Experiment 1 – Acute Test

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Acute Test - Pre-Procedural Provisions	09/20/2010	09/23/2010
Test Article Characterization	09/20/2010	09/23/2010
ALD - Test Article - Receipt and Control	09/20/2010	09/23/2010
ALD - Test System - Observations and Body Weights	09/20/2010	09/23/2010
ALD - Study Endpoint Criteria	09/28/2010	09/30/2010
ALD - Gross Necropsy Procedures	09/28/2010	09/30/2010
ALD - Euthanasia Procedures	09/28/2010	09/30/2010

Experiment 2 – 14-Day Repeated Dose Test

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
14 Day - Test Article Mixing and Administration	10/07/2010	10/15/2010
Verification of Light/Dark Cycle	10/07/2010	10/26/2010
14-Day Test System Identification and Food Supply	10/07/2010	10/15/2010
Purity Confirmation Analysis - Test Article	10/12/2010	10/15/2010
14-day Pre-Procedural Provisions	10/22/2010	10/29/2010
14-day Biosample Collection Procedures	10/22/2010	10/29/2010
14-day Necropsy Procedures	10/22/2010	10/29/2010
Compliance with PTOX SOPs	10/22/2010	11/01/2010

Experiment 3 – 90-Day Subchronic Test

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Animal Room Temperature and Humidity	11/30/2010	12/06/2011
Functional Observation Battery - Baseline Measures	12/02/2010	12/08/2010
SOP/Protocol Compliance - Husbandry Considerations	12/10/2010	12/23/2011
Test Article Dosing and Initial Observations	12/10/2010	12/17/2010
Maintenance and Calibration of Equipment	12/10/2010	12/17/2010
Test System Identification and Observations	12/10/2010	12/17/2010
Control, Storage & Identification of Test Article	01/24/2011	02/10/2011
Study Staff Training Records	01/24/2011	02/10/2011
FOB - Study Animal Room Obs. and Measurements	02/11/2011	02/17/2011
FOB - Motor Activity Measurements	02/15/2011	02/17/2011
Urinalysis - Specimen Collection and Storage	02/24/2011	03/04/2011
Necropsy, Organ & Tissue Collection Procedures	03/02/2011	03/09/2011

B-3 Post In-Life Phase of the Study

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Histopathology Evaluation Procedures	09/14/2011	09/23/2011
Pathology Contributing Scientist Report Review	11/08/2011	11/10/2011
Final Study Report GLP Review	04/24/2010	04/25/2010
Study Raw Data GLP Review	04/24/2010	05/20/2010

Note 1 - All 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 - In addition to the study specific critical phase inspections listed here, general facility and process based inspections not specifically related to this study are done monthly or annually in accordance with QA Standard Procedure.

Note 3 - This report has been audited by the Quality Assurance Unit (QSO), and is considered to be an accurate account of the data generated and of the procedures followed

Michael P. Kefauver
Quality Assurance Specialist, AIPH-QSO

05/10/2012
Date

Appendix C

Archives and Study Personnel

C-1 Archives

All raw data, documentation, records, protocol, and a copy of the final report generated as a result of this study will be archived in room 1026, building E-2100, USAPHC, for a minimum of five (5) 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 Medical Division, Toxicology Portfolio, for a minimum of five (5) years following submission of the final report to the Sponsor.

Some ancillary records pertaining to this study, such as instrument maintenance logs, animal room observation logs, etc., will not be archived until those logbooks have been completed. Once complete they will be archived in room 1026, building E-2100, USAPHC.

Wet tissues, histology slides, and paraffin blocks are stored in building E-5158.

C-2 Personnel

Management: Cindy Landgren, LTC, VC, Portfolio Director, Toxicology (succeeded by Chris E. Hanson, COL, VC, July 2011); Dr. Glenn Leach, Ph.D., Manager, Toxicity Evaluation Program (TEP) (succeeded by Shannon M. Wallace, LTC, VC, March 2012); Dr. Mark S. Johnson, Ph.D., Manager, Health Effects Research Program (HERP).

Study Director: Emily May Lent, Toxicologist, Toxicity Evaluation Program (TEP)

Quality Assurance: Michael P. Kefauver, Quality Assurance Specialist, Quality Systems Office.

Veterinary Support and Animal Care: Anne M. MacLarty, DVM, MAJ, VC; Robert Sunderland, Animal Health Technician; Rebecca Kilby, Animal Health Technician; Jason Williams, Animal Health Technician.

Pathology Lab Coordinator: Patricia Beall, Biologist, TEP

Histopathology: Shannon M. Wallace, DVM, DACVP, LTC, VC, Pathologist, VMD

In-Life Support: Lee C.B. Crouse, Biologist, TEP; Theresa Hanna, Biological Technician, TEP.

Hematology, Clinical Chemistry, Urinalysis: Matthew Bazar, Biologist, TEP; Mark Way, Biologist, TEP.

Archivist: Martha Thompson, Data Acquisition Specialist, TEP

Appendix D

Approximate Lethal Dose Observations

APPENDIX D
APPROXIMATE LETHAL DOSE CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 5 mg/ml[A], 10 mg/ml[B], 25 mg/ml[C], 50 mg/ml[D], 100 mg/ml[E], 200 mg/ml[F] **Date:** 9/14/10
Diluent: corn oil

MALE INDIVIDUAL ANIMAL EFFECTS

Animal No.	Weight	Dose (mg/kg)	Volume (ml)	Time Administered	Clinical Sign	Onset	Recovery
10-1611	257	17.6	0.90[A]	0603	lethargic	0700	0710
10-1612	234	26.3	1.24[A]	0606			
10-1613	237	39.5	0.95[B]	0615	lethargic	0735	0745
10-1614	252	59.3	1.49[B]	0616			
10-1615	245	88.9	0.88[C]	0621	prostrate	0735	0740
10-1615					prostrate	0800	0805
10-1615					lethargic	0830	0600 on 9/16
10-1615					labored/rapid breathing	1230	0600 on 9/15
10-1616	236	133.3	1.25[C]	0623	lethargic	0715	0600 on 9/15
10-1616					diarrhea	1000	
10-1616					prostrate	1230	0600 on 9/15
10-1616					labored/rapid breathing	1230	0600 on 9/15
10-1617	240	200.0	0.96[D]	0631	prostrate	0705	0600 on 9/15
10-1617					labored/rapid breathing	0705	0600 on 9/15
10-1617					salivation	1000	0600 on 9/15
10-1618	245	300.0	1.47[D]	0632	prostrate	0705	
10-1618					labored/rapid breathing	0705	
10-1618					found dead	0850	
10-1619	249	450.0	1.12[E]	0638	prostrate	0658	
10-1619					labored/rapid breathing	0705	
10-1619					salivation	1000	
10-1619					found dead	1230	
10-1620	251	675.0	1.71[E]	0640	prostrate	0658	
10-1620					salivation	0705	
10-1620					labored/rapid breathing	0705	
10-1620					found dead	0920	
10-1621	248	1012.5	1.26[F]	0646	prostrate	0700	
10-1621					labored/rapid breathing	0705	
10-1621					found dead	0925	
10-1622	255	1518.8	1.94[F]	0649	prostrate	0705	
10-1622					labored/rapid breathing	0705	
10-1622					found dead	0807	

APPENDIX D
APPROXIMATE LETHAL DOSE CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Female
Concentration: 5 mg/ml[A], 10 mg/ml[B], 25 mg/ml[C], 50 mg/ml[D], 100 mg/ml[E], 200 mg/ml[F] **Date:** 9/14/10
Diluent: corn oil

FEMALE INDIVIDUAL ANIMAL EFFECTS

Animal No.	Weight	Dose (mg/kg)	Volume (ml)	Time Administered	Clinical Sign	Onset	Recovery
10-1623	196	17.6	0.7[A]	0608			
10-1624	204	26.3	1.08[A]	0611			
10-1625	204	39.5	0.82[B]	0617			
10-1626	191	59.3	1.13[B]	1619			
10-1627	182	88.9	0.66[C]	0624			
10-1628	180	133.3	0.95[C]	0625	lethargic	1230	0600 on 9/15
10-1628					labored/rapid breathing	1230	0600 on 9/15
10-1628					prostrate	1310	0600 on 9/15
10-1629	188	200.0	0.75[D]	0634	lethargic	0743	0800
10-1629					prostrate	0900	0600 on 9/15
10-1629					labored/rapid breathing	1000	0600 on 9/15
10-1629					chromodacryorrhea	0600 on 9/15	
10-1630	184	300.0	1.10[D]	0635	prostrate	0700	
10-1630					labored/rapid breathing	1000	
10-1630					found dead	1025	
10-1631	190	450.0	0.90[E]	0643	salivation	0715	
10-1631					lethargic	0730	
10-1631					prostrate	0742	0800
10-1631					prostrate	0830	
10-1631					labored/rapid breathing	1000	
10-1631					chromodacryorrhea	1545	
10-1632	186	675.0	1.26[E]	0645	salivation	0710	
10-1632					lethargic	0722	
10-1632					prostrate	0735	0800
10-1632					prostrate	0830	
10-1632					labored/rapid breathing	1000	
10-1632					found dead	1230	
10-1633	177	1012.5	0.90[F]	0651	prostrate	0708	
10-1633					labored/rapid breathing	0708	
10-1633					chromodacryorrhea	1000	
10-1633					found dead	1230	
10-1634	198	1518.8	1.50[F]	0652	prostrate	0708	
10-1634					found dead	0900	

Appendix E

14-Day Clinical Observations

APPENDIX E
14-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 1 mg/ml[A], 2 mg/ml[B], 4 mg/ml[C], 8 mg/ml[D], 16 mg/ml[E], 32 mg/ml[F], 32 mg/ml[G]
Diluent: corn oil

MALE INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Clinical Sign	Day of First Appearance	Day of Last Appearance
10-1635	Corn Oil Control	appears normal		
10-1643	Corn Oil Control	appears normal		
10-1645	Corn Oil Control	appears normal		
10-1662	Corn Oil Control	appears normal		
10-1664	Corn Oil Control	appears normal		
10-1675	Corn Oil Control	appears normal		
10-1642	1.56 mg/kg-day[A]	appears normal		
10-1651	1.56 mg/kg-day[A]	appears normal		
10-1656	1.56 mg/kg-day[A]	appears normal		
10-1660	1.56 mg/kg-day[A]	appears normal		
10-1672	1.56 mg/kg-day[A]	appears normal		
10-1678	1.56 mg/kg-day[A]	appears normal		
10-1648	3.13 mg/kg-day[B]	appears normal		
10-1652	3.13 mg/kg-day[B]	appears normal		
10-1657	3.13 mg/kg-day[B]	appears normal		
10-1661	3.13 mg/kg-day[B]	appears normal		
10-1666	3.13 mg/kg-day[B]	appears normal		
10-1683	3.13 mg/kg-day[B]	appears normal		
10-1638	6.25 mg/kg-day[C]	appears normal		
10-1639	6.25 mg/kg-day[C]	appears normal		
10-1641	6.25 mg/kg-day[C]	appears normal		
10-1644	6.25 mg/kg-day[C]	appears normal		
10-1670	6.25 mg/kg-day[C]	appears normal		
10-1680	6.25 mg/kg-day[C]	appears normal		
10-1654	12.5 mg/kg-day[D]	appears normal		
10-1659	12.5 mg/kg-day[D]	appears normal		
10-1663	12.5 mg/kg-day[D]	appears normal		
10-1665	12.5 mg/kg-day[D]	appears normal		
10-1676	12.5 mg/kg-day[D]	appears normal		
10-1681	12.5 mg/kg-day[D]	appears normal		
10-1636	25 mg/kg-day[E]	appears normal		
10-1640	25 mg/kg-day[E]	appears normal		
10-1658	25 mg/kg-day[E]	appears normal		
10-1671	25 mg/kg-day[E]	appears normal		
10-1673	25 mg/kg-day[E]	appears normal		
10-1677	25 mg/kg-day[E]	appears normal		
10-1650	50 mg/kg-day[F]	lethargic	0	0
10-1653	50 mg/kg-day[F]	barbering	5	14
10-1653	50 mg/kg-day[F]	dark urine	12	12
10-1668	50 mg/kg-day[F]	appears normal		
10-1669	50 mg/kg-day[F]	lethargic	0	3
10-1679	50 mg/kg-day[F]	appears normal		
10-1684	50 mg/kg-day[F]	appears normal		
10-1637	100 mg/kg-day[G]	lethargic	2	10
10-1637	100 mg/kg-day[G]	dark urine	5	9
10-1637	100 mg/kg-day[G]	labored/rapid breathing	10	10
10-1646	100 mg/kg-day[G]	lethargic	2	2
10-1646	100 mg/kg-day[G]	dark urine	5	6
10-1647	100 mg/kg-day[G]	lethargic	1	2
10-1647	100 mg/kg-day[G]	prostrate	2	2
10-1647	100 mg/kg-day[G]	labored/rapid breathing	2	2
10-1647	100 mg/kg-day[G]	dark urine	4	5
10-1647	100 mg/kg-day[G]	barbering	13	14
10-1649	100 mg/kg-day[G]	dark urine	5	6
10-1655	100 mg/kg-day[G]	dark urine	4	5
10-1674	100 mg/kg-day[G]	lethargic	2	2
10-1674	100 mg/kg-day[G]	prostrate	2	2
10-1674	100 mg/kg-day[G]	labored/rapid breathing	2	2
10-1674	100 mg/kg-day[G]	dark urine	4	5

APPENDIX E
14-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Female
Concentration: 1 mg/ml[A], 2 mg/ml[B], 4 mg/ml[C], 8 mg/ml[D], 16 mg/ml[E], 32 mg/ml[F], 32 mg/ml[G]
Diluent: corn oil

FEMALE INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Clinical Sign	Day of First Appearance	Day of Last Appearance
10-1693	Corn Oil Control	appears normal		
10-1697	Corn Oil Control	barbering	11	14
10-1703	Corn Oil Control	appears normal		
10-1713	Corn Oil Control	appears normal		
10-1718	Corn Oil Control	appears normal		
10-1722	Corn Oil Control	appears normal		
10-1685	1.56 mg/kg-day[A]	appears normal		
10-1690	1.56 mg/kg-day[A]	barbering	9	14
10-1692	1.56 mg/kg-day[A]	appears normal		
10-1700	1.56 mg/kg-day[A]	appears normal		
10-1701	1.56 mg/kg-day[A]	appears normal		
10-1709	1.56 mg/kg-day[A]	appears normal		
10-1695	3.13 mg/kg-day[B]	appears normal		
10-1699	3.13 mg/kg-day[B]	barbering	1	14
10-1705	3.13 mg/kg-day[B]	appears normal		
10-1714	3.13 mg/kg-day[B]	appears normal		
10-1719	3.13 mg/kg-day[B]	appears normal		
10-1723	3.13 mg/kg-day[B]	appears normal		
10-1702	6.25 mg/kg-day[C]	appears normal		
10-1707	6.25 mg/kg-day[C]	appears normal		
10-1711	6.25 mg/kg-day[C]	appears normal		
10-1715	6.25 mg/kg-day[C]	appears normal		
10-1729	6.25 mg/kg-day[C]	appears normal		
10-1730	6.25 mg/kg-day[C]	appears normal		
10-1687	12.5 mg/kg-day[D]	appears normal		
10-1694	12.5 mg/kg-day[D]	barbering	1	14
10-1725	12.5 mg/kg-day[D]	appears normal		
10-1726	12.5 mg/kg-day[D]	appears normal		
10-1733	12.5 mg/kg-day[D]	appears normal		
10-1734	12.5 mg/kg-day[D]	appears normal		
10-1691	25 mg/kg-day[E]	appears normal		
10-1704	25 mg/kg-day[E]	appears normal		
10-1708	25 mg/kg-day[E]	appears normal		
10-1720	25 mg/kg-day[E]	appears normal		
10-1727	25 mg/kg-day[E]	appears normal		
10-1732	25 mg/kg-day[E]	appears normal		
10-1688	50 mg/kg-day[F]	appears normal		
10-1706	50 mg/kg-day[F]	appears normal		
10-1710	50 mg/kg-day[F]	appears normal		
10-1716	50 mg/kg-day[F]	congested breathing	3	14
10-1717	50 mg/kg-day[F]	barbering	1	14
10-1731	50 mg/kg-day[F]	appears normal		
10-1686	100 mg/kg-day[G]	orange feces	7	7
10-1689	100 mg/kg-day[G]	dark urine	3	3
10-1689	100 mg/kg-day[G]	orange feces	7	7
10-1696	100 mg/kg-day[G]	lethargic	0	0
10-1696	100 mg/kg-day[G]	dark urine	3	4
10-1712	100 mg/kg-day[G]	lethargic	0	8
10-1712	100 mg/kg-day[G]	prostrate	0	0
10-1712	100 mg/kg-day[G]	labored/rapid breathing	0	1
10-1712	100 mg/kg-day[G]	dark urine	3	3
10-1724	100 mg/kg-day[G]	prostrate	0	0
10-1724	100 mg/kg-day[G]	labored/rapid breathing	0	0
10-1724	100 mg/kg-day[G]	dark urine	8	8
10-1728	100 mg/kg-day[G]	lethargic	0	0
10-1728	100 mg/kg-day[G]	prostrate	0	0
10-1728	100 mg/kg-day[G]	labored/rapid breathing	0	0
10-1728	100 mg/kg-day[G]	dark urine	3	3
10-1728	100 mg/kg-day[G]	orange feces	8	10

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0097	Corn Oil Control	Male	Barbering	34	68
11-0097	Corn Oil Control	Male	Red material around nose	68	68
11-0097	Corn Oil Control	Male	Cheeks/neck swollen	68	68
11-0097	Corn Oil Control	Male	Rough hair coat	68	68
11-0097	Corn Oil Control	Male	Congested Breathing	68	68
11-0097	Corn Oil Control	Male	Euthanized	68	68
11-0105	Corn Oil Control	Male	Chromodacryorrhea R eye	6	70
11-0107	Corn Oil Control	Male	Alopecia/staining on rear	18	18
11-0108	Corn Oil Control	Male	Barbering	12	49
11-0108	Corn Oil Control	Male	Scab behind R ear	15	15
11-0108	Corn Oil Control	Male	Barbering	57	90
11-0112	Corn Oil Control	Male	Appears Normal		
11-0116	Corn Oil Control	Male	Barbering	12	90
11-0118	Corn Oil Control	Male	Appears Normal		
11-0140	Corn Oil Control	Male	Appears Normal		
11-0147	Corn Oil Control	Male	Appears Normal		
11-0150	Corn Oil Control	Male	Appears Normal		
11-0095	1.25 mg/kg-day[A]	Male	Appears Normal		
11-0103	1.25 mg/kg-day[A]	Male	Appears Normal		
11-0104	1.25 mg/kg-day[A]	Male	Barbering	5	90
11-0113	1.25 mg/kg-day[A]	Male	Appears Normal		
11-0114	1.25 mg/kg-day[A]	Male	Appears Normal		
11-0122	1.25 mg/kg-day[A]	Male	Appears Normal		
11-0126	1.25 mg/kg-day[A]	Male	Laying on back	22	23
11-0126	1.25 mg/kg-day[A]	Male	Laying on back	28	28
11-0126	1.25 mg/kg-day[A]	Male	Laying on side	29	30
11-0126	1.25 mg/kg-day[A]	Male	Laying on side	35	35
11-0126	1.25 mg/kg-day[A]	Male	Laying on side	38	39
11-0126	1.25 mg/kg-day[A]	Male	Laying on side	47	48
11-0126	1.25 mg/kg-day[A]	Male	Laying on back	48	48
11-0126	1.25 mg/kg-day[A]	Male	Laying on side	57	57
11-0126	1.25 mg/kg-day[A]	Male	Laying on side	61	64
11-0126	1.25 mg/kg-day[A]	Male	Laying on side	65	66
11-0126	1.25 mg/kg-day[A]	Male	Laying on side	68	69
11-0126	1.25 mg/kg-day[A]	Male	Laying on back	77	77
11-0126	1.25 mg/kg-day[A]	Male	Laying on side	78	78
11-0126	1.25 mg/kg-day[A]	Male	Laying on side	81	81
11-0126	1.25 mg/kg-day[A]	Male	Laying on side	83	87
11-0134	1.25 mg/kg-day[A]	Male	Appears Normal		
11-0142	1.25 mg/kg-day[A]	Male	Barbering	4	45
11-0145	1.25 mg/kg-day[A]	Male	Swollen L eyelid	30	31
11-0145	1.25 mg/kg-day[A]	Male	Small scab on L eye lid	31	34
11-0145	1.25 mg/kg-day[A]	Male	Red discahrge from nose	65	65
11-0100	5 mg/kg-day[B]	Male	Barbering	41	44
11-0101	5 mg/kg-day[B]	Male	Chromodacryorrhea R eye	51	55
11-0115	5 mg/kg-day[B]	Male	Dark Urine	42	42
11-0115	5 mg/kg-day[B]	Male	Barbering	56	90
11-0117	5 mg/kg-day[B]	Male	Hind limb ataxia	47	55
11-0117	5 mg/kg-day[B]	Male	Creeping	47	90
11-0117	5 mg/kg-day[B]	Male	Stiff leg muscles	47	47
11-0117	5 mg/kg-day[B]	Male	Tail Curled	47	82
11-0117	5 mg/kg-day[B]	Male	Tail Curled	84	90
11-0117	5 mg/kg-day[B]	Male	Hind end lowered	50	50
11-0117	5 mg/kg-day[B]	Male	Stiff leg muscles	52	90
11-0117	5 mg/kg-day[B]	Male	Hind end lowered	56	90
11-0117	5 mg/kg-day[B]	Male	Laying on side	58	58
11-0117	5 mg/kg-day[B]	Male	Laying on side	84	84
11-0124	5 mg/kg-day[B]	Male	Dark Urine	11	11
11-0124	5 mg/kg-day[B]	Male	Chromodacryorrhea both eyes	60	60
11-0124	5 mg/kg-day[B]	Male	Hind end lowered	64	65
11-0124	5 mg/kg-day[B]	Male	Creeping	64	65
11-0131	5 mg/kg-day[B]	Male	Appears Normal		
11-0135	5 mg/kg-day[B]	Male	Appears Normal		
11-0138	5 mg/kg-day[B]	Male	Appears Normal		
11-0141	5 mg/kg-day[B]	Male	Appears Normal		
11-0146	5 mg/kg-day[B]	Male	Barbering	35	90
11-0146	5 mg/kg-day[B]	Male	Ears twitching	85	85
11-0106	20 mg/kg-day[C]	Male	Laying on side	5	5

Appendix F

90-Day Clinical Observations

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0106	20 mg/kg-day[C]	Male	Laying on side	7	7
11-0106	20 mg/kg-day[C]	Male	Laying on side	11	11
11-0106	20 mg/kg-day[C]	Male	Dark Urine	11	49
11-0120	20 mg/kg-day[C]	Male	Dark Urine	44	75
11-0121	20 mg/kg-day[C]	Male	Dark Urine	4	49
11-0125	20 mg/kg-day[C]	Male	Dark Urine	12	75
11-0125	20 mg/kg-day[C]	Male	Scab behind R ear	15	15
11-0125	20 mg/kg-day[C]	Male	Laying on side	70	70
11-0125	20 mg/kg-day[C]	Male	Laying on side	77	77
11-0125	20 mg/kg-day[C]	Male	Laying on side	84	84
11-0125	20 mg/kg-day[C]	Male	Lethargic	84	84
11-0125	20 mg/kg-day[C]	Male	Labored Breathing	84	84
11-0127	20 mg/kg-day[C]	Male	Dark Urine	12	72
11-0127	20 mg/kg-day[C]	Male	Labored Breathing	27	27
11-0127	20 mg/kg-day[C]	Male	Laying on side	27	27
11-0127	20 mg/kg-day[C]	Male	Laying on side	29	29
11-0127	20 mg/kg-day[C]	Male	Laying on side	34	34
11-0127	20 mg/kg-day[C]	Male	Prostrate	41	41
11-0127	20 mg/kg-day[C]	Male	Laying on side	43	43
11-0127	20 mg/kg-day[C]	Male	Laying on side	49	49
11-0127	20 mg/kg-day[C]	Male	Laying on side	51	51
11-0127	20 mg/kg-day[C]	Male	Laying on side	56	56
11-0127	20 mg/kg-day[C]	Male	Laying on side	58	61
11-0127	20 mg/kg-day[C]	Male	Laying on side	63	63
11-0127	20 mg/kg-day[C]	Male	Hind end lowered	64	66
11-0127	20 mg/kg-day[C]	Male	Creeping	64	66
11-0127	20 mg/kg-day[C]	Male	Laying on side	68	68
11-0127	20 mg/kg-day[C]	Male	Laying on side	70	71
11-0127	20 mg/kg-day[C]	Male	Laying on side	79	79
11-0127	20 mg/kg-day[C]	Male	Prostrate	79	79
11-0127	20 mg/kg-day[C]	Male	Lethargic	79	79
11-0127	20 mg/kg-day[C]	Male	Lethargic	84	84
11-0127	20 mg/kg-day[C]	Male	Laying on side	84	86
11-0127	20 mg/kg-day[C]	Male	Labored Breathing	84	84
11-0127	20 mg/kg-day[C]	Male	Laying on side	88	88
11-0130	20 mg/kg-day[C]	Male	Laying on side	4	4
11-0130	20 mg/kg-day[C]	Male	Dark Urine	11	49
11-0130	20 mg/kg-day[C]	Male	Prostrate	26	26
11-0130	20 mg/kg-day[C]	Male	Laying on side	49	49
11-0133	20 mg/kg-day[C]	Male	Dark Urine	12	75
11-0133	20 mg/kg-day[C]	Male	Abrasion behind R ear	15	18
11-0133	20 mg/kg-day[C]	Male	Scab behind R ear	19	26
11-0133	20 mg/kg-day[C]	Male	Staph infection behind R ear	27	32
11-0133	20 mg/kg-day[C]	Male	Laying on back	29	29
11-0133	20 mg/kg-day[C]	Male	Lethargic	29	29
11-0133	20 mg/kg-day[C]	Male	Alopecia behind right ear	34	37
11-0133	20 mg/kg-day[C]	Male	Barbering	35	76
11-0133	20 mg/kg-day[C]	Male	Laying on back	68	68
11-0133	20 mg/kg-day[C]	Male	Lethargic	68	68
11-0133	20 mg/kg-day[C]	Male	Laying on back	79	76
11-0133	20 mg/kg-day[C]	Male	Lethargic	76	77
11-0133	20 mg/kg-day[C]	Male	Laying on side	77	77
11-0133	20 mg/kg-day[C]	Male	Barbering	78	90
11-0133	20 mg/kg-day[C]	Male	Red material around nose	78	78
11-0133	20 mg/kg-day[C]	Male	Laying on back	79	79
11-0133	20 mg/kg-day[C]	Male	Lethargic	79	79
11-0133	20 mg/kg-day[C]	Male	Laying on side	84	84
11-0133	20 mg/kg-day[C]	Male	Laying on side	89	89
11-0137	20 mg/kg-day[C]	Male	Lethargic	28	28
11-0137	20 mg/kg-day[C]	Male	Prostrate	28	28
11-0137	20 mg/kg-day[C]	Male	Soft Feces	33	33
11-0137	20 mg/kg-day[C]	Male	Dark Urine	43	74
11-0137	20 mg/kg-day[C]	Male	Prostrate	70	70
11-0137	20 mg/kg-day[C]	Male	Lethargic	82	82

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0137	20 mg/kg-day[C]	Male	Prostrate	82	82
11-0139	20 mg/kg-day[C]	Male	Dark Urine	11	71
11-0139	20 mg/kg-day[C]	Male	Laying on back	70	70
11-0139	20 mg/kg-day[C]	Male	Lethargic	70	70
11-0139	20 mg/kg-day[C]	Male	Laying on side	82	82
11-0139	20 mg/kg-day[C]	Male	Lethargic	82	82
11-0148	20 mg/kg-day[C]	Male	Congested Breathing	17	17
11-0148	20 mg/kg-day[C]	Male	Prostrate	29	29
11-0148	20 mg/kg-day[C]	Male	Lethargic	29	29
11-0148	20 mg/kg-day[C]	Male	Dark Urine	44	72
11-0148	20 mg/kg-day[C]	Male	Laying on side	77	77
11-0148	20 mg/kg-day[C]	Male	Lethargic	77	77
11-0148	20 mg/kg-day[C]	Male	Laying on side	83	83
11-0148	20 mg/kg-day[C]	Male	Lethargic	83	83
11-0099	80 mg/kg-day[D]	Male	Dark Urine	1	49
11-0099	80 mg/kg-day[D]	Male	Lethargic	1	1
11-0099	80 mg/kg-day[D]	Male	Prostrate	1	1
11-0099	80 mg/kg-day[D]	Male	Lethargic	4	8
11-0099	80 mg/kg-day[D]	Male	Labored Breathing	4	4
11-0099	80 mg/kg-day[D]	Male	Chromodacryorrhea both eyes	4	4
11-0099	80 mg/kg-day[D]	Male	Prostrate	5	5
11-0099	80 mg/kg-day[D]	Male	Chromodacryorrhea both eyes	6	6
11-0099	80 mg/kg-day[D]	Male	Labored Breathing	7	7
11-0099	80 mg/kg-day[D]	Male	Chromodacryorrhea R eye	7	7
11-0099	80 mg/kg-day[D]	Male	Elevated Respiration Rate	8	8
11-0099	80 mg/kg-day[D]	Male	Prostrate	11	13
11-0099	80 mg/kg-day[D]	Male	Lethargic	11	15
11-0099	80 mg/kg-day[D]	Male	Labored Breathing	11	15
11-0099	80 mg/kg-day[D]	Male	Chromodacryorrhea L eye	12	12
11-0099	80 mg/kg-day[D]	Male	Lethargic	18	28
11-0099	80 mg/kg-day[D]	Male	Laying on side	18	18
11-0099	80 mg/kg-day[D]	Male	Labored Breathing	18	23
11-0099	80 mg/kg-day[D]	Male	Prostrate	19	20
11-0099	80 mg/kg-day[D]	Male	Laying on side	21	23
11-0099	80 mg/kg-day[D]	Male	Head tilt left	21	23
11-0099	80 mg/kg-day[D]	Male	Laying on side	25	25
11-0099	80 mg/kg-day[D]	Male	Labored Breathing	25	28
11-0099	80 mg/kg-day[D]	Male	Irregular Gait	26	26
11-0099	80 mg/kg-day[D]	Male	Prostrate	26	27
11-0099	80 mg/kg-day[D]	Male	Laying on side	28	28
11-0099	80 mg/kg-day[D]	Male	Hind limb ataxia	30	30
11-0099	80 mg/kg-day[D]	Male	Low Arousal	30	32
11-0099	80 mg/kg-day[D]	Male	Prostrate	32	34
11-0099	80 mg/kg-day[D]	Male	Lethargic	32	34
11-0099	80 mg/kg-day[D]	Male	Low Arousal	34	38
11-0099	80 mg/kg-day[D]	Male	Lethargic	39	40
11-0099	80 mg/kg-day[D]	Male	Laying on side	39	39
11-0099	80 mg/kg-day[D]	Male	Labored Breathing	39	40
11-0099	80 mg/kg-day[D]	Male	Prostrate	40	40
11-0099	80 mg/kg-day[D]	Male	Laying on side	43	43
11-0099	80 mg/kg-day[D]	Male	Labored Breathing	43	43
11-0099	80 mg/kg-day[D]	Male	Lethargic	43	43
11-0099	80 mg/kg-day[D]	Male	Labored Breathing	46	46
11-0099	80 mg/kg-day[D]	Male	Prostrate	46	46
11-0099	80 mg/kg-day[D]	Male	Lethargic	46	47
11-0099	80 mg/kg-day[D]	Male	Labored Breathing	49	49
11-0099	80 mg/kg-day[D]	Male	Prostrate	49	49
11-0099	80 mg/kg-day[D]	Male	Lethargic	49	49
11-0099	80 mg/kg-day[D]	Male	Piloerection	50	50
11-0099	80 mg/kg-day[D]	Male	Tail Curled	50	50
11-0099	80 mg/kg-day[D]	Male	Hind end lowered	50	50
11-0099	80 mg/kg-day[D]	Male	Laying on side	50	50
11-0099	80 mg/kg-day[D]	Male	Found Dead	51	51
11-0102	80 mg/kg-day[D]	Male	Lethargic	0	0

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0102	80 mg/kg-day[D]	Male	Dark Urine	2	72
11-0102	80 mg/kg-day[D]	Male	Lethargic	2	2
11-0102	80 mg/kg-day[D]	Male	Prostrate	2	2
11-0102	80 mg/kg-day[D]	Male	Lethargic	5	7
11-0102	80 mg/kg-day[D]	Male	Laying on side	5	5
11-0102	80 mg/kg-day[D]	Male	Laying on side	7	9
11-0102	80 mg/kg-day[D]	Male	Labored Breathing	8	8
11-0102	80 mg/kg-day[D]	Male	Lethargic	12	16
11-0102	80 mg/kg-day[D]	Male	Prostrate	12	12
11-0102	80 mg/kg-day[D]	Male	Labored Breathing	12	16
11-0102	80 mg/kg-day[D]	Male	Laying on side	13	13
11-0102	80 mg/kg-day[D]	Male	Laying on back	14	14
11-0102	80 mg/kg-day[D]	Male	Laying on side	15	16
11-0102	80 mg/kg-day[D]	Male	Lethargic	19	22
11-0102	80 mg/kg-day[D]	Male	Laying on back	19	19
11-0102	80 mg/kg-day[D]	Male	Labored Breathing	19	22
11-0102	80 mg/kg-day[D]	Male	Prostrate	20	23
11-0102	80 mg/kg-day[D]	Male	Irregular Gait	24	36
11-0102	80 mg/kg-day[D]	Male	Lethargic	25	29
11-0102	80 mg/kg-day[D]	Male	Laying on side	27	27
11-0102	80 mg/kg-day[D]	Male	Labored Breathing	27	29
11-0102	80 mg/kg-day[D]	Male	Creeping	28	38
11-0102	80 mg/kg-day[D]	Male	Large volume of urine	30	30
11-0102	80 mg/kg-day[D]	Male	Low Arousal	31	31
11-0102	80 mg/kg-day[D]	Male	Lethargic	33	35
11-0102	80 mg/kg-day[D]	Male	Prostrate	33	33
11-0102	80 mg/kg-day[D]	Male	Labored Breathing	33	33
11-0102	80 mg/kg-day[D]	Male	Lethargic	40	44
11-0102	80 mg/kg-day[D]	Male	Laying on back	40	41
11-0102	80 mg/kg-day[D]	Male	Labored Breathing	40	44
11-0102	80 mg/kg-day[D]	Male	Creeping	40	42
11-0102	80 mg/kg-day[D]	Male	Laying on side	43	43
11-0102	80 mg/kg-day[D]	Male	Creeping	45	45
11-0102	80 mg/kg-day[D]	Male	Prostrate	42	42
11-0102	80 mg/kg-day[D]	Male	Laying on back	44	44
11-0102	80 mg/kg-day[D]	Male	Lethargic	47	50
11-0102	80 mg/kg-day[D]	Male	Laying on back	47	48
11-0102	80 mg/kg-day[D]	Male	Labored Breathing	47	50
11-0102	80 mg/kg-day[D]	Male	Creeping	47	47
11-0102	80 mg/kg-day[D]	Male	Creeping	49	50
11-0102	80 mg/kg-day[D]	Male	Prostrate	49	49
11-0102	80 mg/kg-day[D]	Male	Laying on side	51	51
11-0102	80 mg/kg-day[D]	Male	Irregular Gait	52	53
11-0102	80 mg/kg-day[D]	Male	Hind end lowered	52	53
11-0102	80 mg/kg-day[D]	Male	Labored Breathing	55	56
11-0102	80 mg/kg-day[D]	Male	Lethargic	55	56
11-0102	80 mg/kg-day[D]	Male	Laying on side	55	55
11-0102	80 mg/kg-day[D]	Male	Prostrate	56	56
11-0102	80 mg/kg-day[D]	Male	Laying on side	56	57
11-0102	80 mg/kg-day[D]	Male	Hind end lowered	58	59
11-0102	80 mg/kg-day[D]	Male	Labored Breathing	58	58
11-0102	80 mg/kg-day[D]	Male	Creeping	58	59
11-0102	80 mg/kg-day[D]	Male	Lethargic	58	58
11-0102	80 mg/kg-day[D]	Male	Hind end lowered	61	67
11-0102	80 mg/kg-day[D]	Male	Creeping	61	90
11-0102	80 mg/kg-day[D]	Male	Prostrate	61	61
11-0102	80 mg/kg-day[D]	Male	Labored Breathing	62	62
11-0102	80 mg/kg-day[D]	Male	Lethargic	62	62
11-0102	80 mg/kg-day[D]	Male	Prostrate	64	64
11-0102	80 mg/kg-day[D]	Male	Labored Breathing	64	64
11-0102	80 mg/kg-day[D]	Male	Lethargic	64	64
11-0102	80 mg/kg-day[D]	Male	Red discharge from nose	65	65
11-0102	80 mg/kg-day[D]	Male	Red discharge from nose	67	67
11-0102	80 mg/kg-day[D]	Male	Lethargic	68	69

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0102	80 mg/kg-day[D]	Male	Laying on side	68	68
11-0102	80 mg/kg-day[D]	Male	Labored Breathing	68	69
11-0102	80 mg/kg-day[D]	Male	Laying on back	69	69
11-0102	80 mg/kg-day[D]	Male	Chromodacryorrhea R eye	69	69
11-0102	80 mg/kg-day[D]	Male	Lethargic	71	72
11-0102	80 mg/kg-day[D]	Male	Prostrate	71	71
11-0102	80 mg/kg-day[D]	Male	Laying on side	72	72
11-0102	80 mg/kg-day[D]	Male	Labored Breathing	72	72
11-0102	80 mg/kg-day[D]	Male	Prostrate	75	75
11-0102	80 mg/kg-day[D]	Male	Lethargic	75	79
11-0102	80 mg/kg-day[D]	Male	Labored Breathing	76	78
11-0102	80 mg/kg-day[D]	Male	Laying on back	76	76
11-0102	80 mg/kg-day[D]	Male	Walks backwards in circles	76	76
11-0102	80 mg/kg-day[D]	Male	Laying on side	77	77
11-0102	80 mg/kg-day[D]	Male	Ears twitching	77	80
11-0102	80 mg/kg-day[D]	Male	Hind legs stiff	77	78
11-0102	80 mg/kg-day[D]	Male	Laying on back	78	78
11-0102	80 mg/kg-day[D]	Male	Hind end dropping	78	78
11-0102	80 mg/kg-day[D]	Male	Prostrate	79	79
11-0102	80 mg/kg-day[D]	Male	Movements jerky	81	81
11-0102	80 mg/kg-day[D]	Male	Prostrate	82	87
11-0102	80 mg/kg-day[D]	Male	Lethargic	82	86
11-0102	80 mg/kg-day[D]	Male	Labored Breathing	82	86
11-0102	80 mg/kg-day[D]	Male	Ears twitching	82	84
11-0102	80 mg/kg-day[D]	Male	Laying on side	83	84
11-0102	80 mg/kg-day[D]	Male	Legs very stiff	86	90
11-0102	80 mg/kg-day[D]	Male	Hind end raised	86	86
11-0102	80 mg/kg-day[D]	Male	Hind end lowered	87	90
11-0102	80 mg/kg-day[D]	Male	Ears twitching	88	90
11-0102	80 mg/kg-day[D]	Male	Lethargic	89	89
11-0102	80 mg/kg-day[D]	Male	Creeping	90	90
11-0109	80 mg/kg-day[D]	Male	Laying on back	0	0
11-0109	80 mg/kg-day[D]	Male	Dark Urine	0	82
11-0109	80 mg/kg-day[D]	Male	Lethargic	2	2
11-0109	80 mg/kg-day[D]	Male	Laying on side	6	6
11-0109	80 mg/kg-day[D]	Male	Prostrate	7	7
11-0109	80 mg/kg-day[D]	Male	Labored Breathing	7	7
11-0109	80 mg/kg-day[D]	Male	Laying on side	8	8
11-0109	80 mg/kg-day[D]	Male	Lethargic	8	9
11-0109	80 mg/kg-day[D]	Male	Prostrate	9	9
11-0109	80 mg/kg-day[D]	Male	Elevated Respiration Rate	9	9
11-0109	80 mg/kg-day[D]	Male	Lethargic	12	16
11-0109	80 mg/kg-day[D]	Male	Labored Breathing	12	12
11-0109	80 mg/kg-day[D]	Male	Prostrate	13	13
11-0109	80 mg/kg-day[D]	Male	Laying on side	14	14
11-0109	80 mg/kg-day[D]	Male	Labored Breathing	14	16
11-0109	80 mg/kg-day[D]	Male	Laying on back	15	15
11-0109	80 mg/kg-day[D]	Male	Prostrate	16	16
11-0109	80 mg/kg-day[D]	Male	Lethargic	19	29
11-0109	80 mg/kg-day[D]	Male	Laying on back	19	19
11-0109	80 mg/kg-day[D]	Male	Labored Breathing	19	22
11-0109	80 mg/kg-day[D]	Male	Prostrate	20	20
11-0109	80 mg/kg-day[D]	Male	Laying on side	20	21
11-0109	80 mg/kg-day[D]	Male	Laying on back	22	22
11-0109	80 mg/kg-day[D]	Male	Prostrate	23	23
11-0109	80 mg/kg-day[D]	Male	Labored Breathing	27	27
11-0109	80 mg/kg-day[D]	Male	Prostrate	27	28
11-0109	80 mg/kg-day[D]	Male	Laying on back	29	29
11-0109	80 mg/kg-day[D]	Male	Labored Breathing	29	29
11-0109	80 mg/kg-day[D]	Male	Barbering	30	58
11-0109	80 mg/kg-day[D]	Male	Straubbed Tail	30	30
11-0109	80 mg/kg-day[D]	Male	Low Arousal	31	31
11-0109	80 mg/kg-day[D]	Male	Labored Breathing	33	33
11-0109	80 mg/kg-day[D]	Male	Prostrate	34	35

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0109	80 mg/kg-day[D]	Male	Lethargic	34	35
11-0109	80 mg/kg-day[D]	Male	Labored Breathing	35	35
11-0109	80 mg/kg-day[D]	Male	Prostrate	40	40
11-0109	80 mg/kg-day[D]	Male	Lethargic	40	44
11-0109	80 mg/kg-day[D]	Male	Laying on side	41	41
11-0109	80 mg/kg-day[D]	Male	Labored Breathing	41	44
11-0109	80 mg/kg-day[D]	Male	Prostrate	42	42
11-0109	80 mg/kg-day[D]	Male	Laying on back	43	43
11-0109	80 mg/kg-day[D]	Male	Prostrate	44	44
11-0109	80 mg/kg-day[D]	Male	Prostrate	47	47
11-0109	80 mg/kg-day[D]	Male	Lethargic	47	47
11-0109	80 mg/kg-day[D]	Male	Labored Breathing	47	47
11-0109	80 mg/kg-day[D]	Male	Creeping	47	47
11-0109	80 mg/kg-day[D]	Male	Tail Curled	47	47
11-0109	80 mg/kg-day[D]	Male	Laying on back	49	49
11-0109	80 mg/kg-day[D]	Male	Labored Breathing	49	49
11-0109	80 mg/kg-day[D]	Male	Lethargic	49	49
11-0109	80 mg/kg-day[D]	Male	Creeping	50	50
11-0109	80 mg/kg-day[D]	Male	Laying on side	51	51
11-0109	80 mg/kg-day[D]	Male	Prostrate	55	56
11-0109	80 mg/kg-day[D]	Male	Lethargic	55	58
11-0109	80 mg/kg-day[D]	Male	Piloerection	55	55
11-0109	80 mg/kg-day[D]	Male	Laying on side	56	58
11-0109	80 mg/kg-day[D]	Male	Labored Breathing	58	58
11-0109	80 mg/kg-day[D]	Male	Hunched posture	61	69
11-0109	80 mg/kg-day[D]	Male	Labored Breathing	62	62
11-0109	80 mg/kg-day[D]	Male	Lethargic	62	62
11-0109	80 mg/kg-day[D]	Male	Laying on side	62	62
11-0109	80 mg/kg-day[D]	Male	Hind end lowered	63	63
11-0109	80 mg/kg-day[D]	Male	Creeping	63	63
11-0109	80 mg/kg-day[D]	Male	Laying on back	64	64
11-0109	80 mg/kg-day[D]	Male	Labored Breathing	64	65
11-0109	80 mg/kg-day[D]	Male	Lethargic	64	65
11-0109	80 mg/kg-day[D]	Male	Laying on side	65	65
11-0109	80 mg/kg-day[D]	Male	Labored Breathing	68	68
11-0109	80 mg/kg-day[D]	Male	Lethargic	68	70
11-0109	80 mg/kg-day[D]	Male	Laying on side	68	68
11-0109	80 mg/kg-day[D]	Male	Prostrate	69	70
11-0109	80 mg/kg-day[D]	Male	Laying on side	71	72
11-0109	80 mg/kg-day[D]	Male	Labored Breathing	71	72
11-0109	80 mg/kg-day[D]	Male	Lethargic	72	72
11-0109	80 mg/kg-day[D]	Male	Creeping	72	72
11-0109	80 mg/kg-day[D]	Male	Hunched posture	74	76
11-0109	80 mg/kg-day[D]	Male	Prostrate	75	75
11-0109	80 mg/kg-day[D]	Male	Lethargic	75	79
11-0109	80 mg/kg-day[D]	Male	Labored Breathing	76	76
11-0109	80 mg/kg-day[D]	Male	Tail Curled	77	77
11-0109	80 mg/kg-day[D]	Male	Prostrate	77	77
11-0109	80 mg/kg-day[D]	Male	Straubbed Tail	78	79
11-0109	80 mg/kg-day[D]	Male	Hunched posture	78	78
11-0109	80 mg/kg-day[D]	Male	Ears twitching	78	83
11-0109	80 mg/kg-day[D]	Male	Laying on back	78	78
11-0109	80 mg/kg-day[D]	Male	Labored Breathing	78	78
11-0109	80 mg/kg-day[D]	Male	Creeping	79	79
11-0109	80 mg/kg-day[D]	Male	Laying on side	79	79
11-0109	80 mg/kg-day[D]	Male	Hunched posture	80	82
11-0109	80 mg/kg-day[D]	Male	Tail Curled	81	81
11-0109	80 mg/kg-day[D]	Male	Leans to the left	82	82
11-0109	80 mg/kg-day[D]	Male	Laying on side	82	83
11-0109	80 mg/kg-day[D]	Male	Labored Breathing	82	86
11-0109	80 mg/kg-day[D]	Male	Lethargic	82	86
11-0109	80 mg/kg-day[D]	Male	Tail Curled	84	84
11-0109	80 mg/kg-day[D]	Male	Laying on back	84	84
11-0109	80 mg/kg-day[D]	Male	Straubbed Tail	85	85

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0109	80 mg/kg-day[D]	Male	Hind legs stiff	85	87
11-0109	80 mg/kg-day[D]	Male	Prostrate	85	87
11-0109	80 mg/kg-day[D]	Male	Creeping	85	87
11-0109	80 mg/kg-day[D]	Male	Ears twitching	87	88
11-0109	80 mg/kg-day[D]	Male	Hunched posture	88	88
11-0109	80 mg/kg-day[D]	Male	Creeping	89	89
11-0109	80 mg/kg-day[D]	Male	Hind end lowered	89	89
11-0109	80 mg/kg-day[D]	Male	Legs stiff	89	89
11-0109	80 mg/kg-day[D]	Male	Lethargic	89	89
11-0109	80 mg/kg-day[D]	Male	Laying on side	89	89
11-0109	80 mg/kg-day[D]	Male	Straubbed Tail	90	90
11-0109	80 mg/kg-day[D]	Male	Ears twitching	90	90
11-0110	80 mg/kg-day[D]	Male	Lethargic	4	5
11-0110	80 mg/kg-day[D]	Male	Laying on side	6	8
11-0110	80 mg/kg-day[D]	Male	Lethargic	7	8
11-0110	80 mg/kg-day[D]	Male	Labored Breathing	7	7
11-0110	80 mg/kg-day[D]	Male	Elevated Respiration Rate	8	8
11-0110	80 mg/kg-day[D]	Male	Laying on side	11	11
11-0110	80 mg/kg-day[D]	Male	Lethargic	11	15
11-0110	80 mg/kg-day[D]	Male	Dark Urine	11	71
11-0110	80 mg/kg-day[D]	Male	Prostrate	12	13
11-0110	80 mg/kg-day[D]	Male	Labored Breathing	13	15
11-0110	80 mg/kg-day[D]	Male	Laying on back	14	15
11-0110	80 mg/kg-day[D]	Male	Lethargic	18	29
11-0110	80 mg/kg-day[D]	Male	Laying on back	18	21
11-0110	80 mg/kg-day[D]	Male	Labored Breathing	18	21
11-0110	80 mg/kg-day[D]	Male	Prostrate	19	19
11-0110	80 mg/kg-day[D]	Male	Laying on side	22	22
11-0110	80 mg/kg-day[D]	Male	Labored Breathing	25	28
11-0110	80 mg/kg-day[D]	Male	Laying on side	25	25
11-0110	80 mg/kg-day[D]	Male	Laying on back	27	28
11-0110	80 mg/kg-day[D]	Male	Lethargic	32	34
11-0110	80 mg/kg-day[D]	Male	Laying on back	32	32
11-0110	80 mg/kg-day[D]	Male	Labored Breathing	32	32
11-0110	80 mg/kg-day[D]	Male	Low Arousal	32	32
11-0110	80 mg/kg-day[D]	Male	Laying on back	34	34
11-0110	80 mg/kg-day[D]	Male	Labored Breathing	34	34
11-0110	80 mg/kg-day[D]	Male	Laying on side	35	35
11-0110	80 mg/kg-day[D]	Male	Lethargic	39	43
11-0110	80 mg/kg-day[D]	Male	Laying on back	39	41
11-0110	80 mg/kg-day[D]	Male	Labored Breathing	39	43
11-0110	80 mg/kg-day[D]	Male	Laying on side	42	42
11-0110	80 mg/kg-day[D]	Male	Laying on back	43	43
11-0110	80 mg/kg-day[D]	Male	Creeping	44	44
11-0110	80 mg/kg-day[D]	Male	Lethargic	46	49
11-0110	80 mg/kg-day[D]	Male	Laying on back	46	47
11-0110	80 mg/kg-day[D]	Male	Labored Breathing	46	47
11-0110	80 mg/kg-day[D]	Male	Laying on back	50	50
11-0110	80 mg/kg-day[D]	Male	Labored Breathing	49	49
11-0110	80 mg/kg-day[D]	Male	Lethargic	55	55
11-0110	80 mg/kg-day[D]	Male	Laying on back	55	55
11-0110	80 mg/kg-day[D]	Male	Labored Breathing	55	55
11-0110	80 mg/kg-day[D]	Male	Laying on side	55	55
11-0110	80 mg/kg-day[D]	Male	Laying on back	57	57
11-0110	80 mg/kg-day[D]	Male	Labored Breathing	57	57
11-0110	80 mg/kg-day[D]	Male	Lethargic	57	57
11-0110	80 mg/kg-day[D]	Male	Hunched posture	59	69
11-0110	80 mg/kg-day[D]	Male	Labored Breathing	60	60
11-0110	80 mg/kg-day[D]	Male	Laying on side	60	60
11-0110	80 mg/kg-day[D]	Male	Lethargic	60	60
11-0110	80 mg/kg-day[D]	Male	Red discharge from nose	61	61
11-0110	80 mg/kg-day[D]	Male	Laying on back	62	62
11-0110	80 mg/kg-day[D]	Male	Labored Breathing	62	64
11-0110	80 mg/kg-day[D]	Male	Lethargic	62	64

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0110	80 mg/kg-day[D]	Male	Creeping	62	62
11-0110	80 mg/kg-day[D]	Male	Prostrate	63	63
11-0110	80 mg/kg-day[D]	Male	Laying on side	64	64
11-0110	80 mg/kg-day[D]	Male	Laying on side	67	68
11-0110	80 mg/kg-day[D]	Male	Lethargic	67	70
11-0110	80 mg/kg-day[D]	Male	Laying on back	69	69
11-0110	80 mg/kg-day[D]	Male	Labored Breathing	69	69
11-0110	80 mg/kg-day[D]	Male	Prostrate	70	70
11-0110	80 mg/kg-day[D]	Male	Squinting	71	72
11-0110	80 mg/kg-day[D]	Male	Ears pulled back	71	72
11-0110	80 mg/kg-day[D]	Male	Hunched posture	71	76
11-0110	80 mg/kg-day[D]	Male	Ears pulled back	74	74
11-0110	80 mg/kg-day[D]	Male	Squinting	74	74
11-0110	80 mg/kg-day[D]	Male	Prostrate	74	74
11-0110	80 mg/kg-day[D]	Male	Lethargic	74	78
11-0110	80 mg/kg-day[D]	Male	Labored Breathing	75	78
11-0110	80 mg/kg-day[D]	Male	Laying on side	75	78
11-0110	80 mg/kg-day[D]	Male	Hunched posture	78	85
11-0110	80 mg/kg-day[D]	Male	Ears pulled back	78	82
11-0110	80 mg/kg-day[D]	Male	Squinting	78	80
11-0110	80 mg/kg-day[D]	Male	Laying on side	81	82
11-0110	80 mg/kg-day[D]	Male	Lethargic	81	85
11-0110	80 mg/kg-day[D]	Male	Laying on back	81	82
11-0110	80 mg/kg-day[D]	Male	Labored Breathing	81	83
11-0110	80 mg/kg-day[D]	Male	Squinting	84	85
11-0110	80 mg/kg-day[D]	Male	Ears twitching	84	84
11-0110	80 mg/kg-day[D]	Male	Laying on side	84	85
11-0110	80 mg/kg-day[D]	Male	Prostrate	84	84
11-0110	80 mg/kg-day[D]	Male	Laying on back	85	85
11-0110	80 mg/kg-day[D]	Male	Ears pulled back	85	85
11-0110	80 mg/kg-day[D]	Male	Labored Breathing	85	85
11-0110	80 mg/kg-day[D]	Male	Creeping	86	86
11-0110	80 mg/kg-day[D]	Male	Hunched posture	87	89
11-0110	80 mg/kg-day[D]	Male	Ears pulled back	87	87
11-0110	80 mg/kg-day[D]	Male	Squinting	87	87
11-0110	80 mg/kg-day[D]	Male	Ears twitching	88	89
11-0110	80 mg/kg-day[D]	Male	Laying on side	88	88
11-0110	80 mg/kg-day[D]	Male	Lethargic	88	89
11-0110	80 mg/kg-day[D]	Male	Labored Breathing	88	88
11-0110	80 mg/kg-day[D]	Male	Squinting	89	89
11-0110	80 mg/kg-day[D]	Male	Prostrate	89	89
11-0110	80 mg/kg-day[D]	Male	Creeping	90	90
11-0110	80 mg/kg-day[D]	Male	Legs stiff	90	90
11-0111	80 mg/kg-day[D]	Male	Lethargic	1	1
11-0111	80 mg/kg-day[D]	Male	Laying on side	1	1
11-0111	80 mg/kg-day[D]	Male	Dark Urine	3	78
11-0111	80 mg/kg-day[D]	Male	Lethargic	4	8
11-0111	80 mg/kg-day[D]	Male	Laying on side	8	8
11-0111	80 mg/kg-day[D]	Male	Elevated Respiration Rate	8	8
11-0111	80 mg/kg-day[D]	Male	Prostrate	11	11
11-0111	80 mg/kg-day[D]	Male	Lethargic	11	15
11-0111	80 mg/kg-day[D]	Male	Labored Breathing	11	11
11-0111	80 mg/kg-day[D]	Male	Laying on side	13	13
11-0111	80 mg/kg-day[D]	Male	Labored Breathing	13	13
11-0111	80 mg/kg-day[D]	Male	Laying on back	15	15
11-0111	80 mg/kg-day[D]	Male	Labored Breathing	15	15
11-0111	80 mg/kg-day[D]	Male	Lethargic	18	28
11-0111	80 mg/kg-day[D]	Male	Laying on side	18	18
11-0111	80 mg/kg-day[D]	Male	Labored Breathing	18	19
11-0111	80 mg/kg-day[D]	Male	Laying on back	19	19
11-0111	80 mg/kg-day[D]	Male	Prostrate	19	19
11-0111	80 mg/kg-day[D]	Male	Labored Breathing	21	21
11-0111	80 mg/kg-day[D]	Male	Shaking Head	21	21
11-0111	80 mg/kg-day[D]	Male	Walking on toes	21	21

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0111	80 mg/kg-day[D]	Male	Prostrate	22	22
11-0111	80 mg/kg-day[D]	Male	Prostrate	25	25
11-0111	80 mg/kg-day[D]	Male	Laying on side	26	28
11-0111	80 mg/kg-day[D]	Male	Labored Breathing	26	28
11-0111	80 mg/kg-day[D]	Male	Low Arousal	30	30
11-0111	80 mg/kg-day[D]	Male	Low Arousal	32	32
11-0111	80 mg/kg-day[D]	Male	Lethargic	32	35
11-0111	80 mg/kg-day[D]	Male	Labored Breathing	33	33
11-0111	80 mg/kg-day[D]	Male	Laying on back	33	33
11-0111	80 mg/kg-day[D]	Male	Laying on side	34	34
11-0111	80 mg/kg-day[D]	Male	Low Arousal	38	38
11-0111	80 mg/kg-day[D]	Male	Lethargic	40	40
11-0111	80 mg/kg-day[D]	Male	Laying on side	42	42
11-0111	80 mg/kg-day[D]	Male	Lethargic	43	43
11-0111	80 mg/kg-day[D]	Male	Lethargic	46	49
11-0111	80 mg/kg-day[D]	Male	Labored Breathing	46	47
11-0111	80 mg/kg-day[D]	Male	Prostrate	47	47
11-0111	80 mg/kg-day[D]	Male	Laying on side	49	49
11-0111	80 mg/kg-day[D]	Male	Labored Breathing	49	49
11-0111	80 mg/kg-day[D]	Male	Lethargic	55	55
11-0111	80 mg/kg-day[D]	Male	Prostrate	55	55
11-0111	80 mg/kg-day[D]	Male	Lethargic	57	57
11-0111	80 mg/kg-day[D]	Male	Prostrate	57	57
11-0111	80 mg/kg-day[D]	Male	Prostrate	61	61
11-0111	80 mg/kg-day[D]	Male	Hunched posture	62	68
11-0111	80 mg/kg-day[D]	Male	Labored Breathing	63	63
11-0111	80 mg/kg-day[D]	Male	Irregular Gait	63	64
11-0111	80 mg/kg-day[D]	Male	Hind end unstable	63	64
11-0111	80 mg/kg-day[D]	Male	Creeping	64	64
11-0111	80 mg/kg-day[D]	Male	Laying on side	64	64
11-0111	80 mg/kg-day[D]	Male	Lethargic	64	64
11-0111	80 mg/kg-day[D]	Male	Lethargic	67	68
11-0111	80 mg/kg-day[D]	Male	Creeping	68	72
11-0111	80 mg/kg-day[D]	Male	Prostrate	68	68
11-0111	80 mg/kg-day[D]	Male	Prostrate	70	70
11-0111	80 mg/kg-day[D]	Male	Lethargic	70	71
11-0111	80 mg/kg-day[D]	Male	Tail Curled	71	71
11-0111	80 mg/kg-day[D]	Male	Laying on side	71	71
11-0111	80 mg/kg-day[D]	Male	Labored Breathing	71	71
11-0111	80 mg/kg-day[D]	Male	Hunched posture	72	76
11-0111	80 mg/kg-day[D]	Male	Squinting	73	74
11-0111	80 mg/kg-day[D]	Male	Ears back/twitching	73	89
11-0111	80 mg/kg-day[D]	Male	Lethargic	74	78
11-0111	80 mg/kg-day[D]	Male	Walking backwards in circles	77	77
11-0111	80 mg/kg-day[D]	Male	Straubbed Tail	77	77
11-0111	80 mg/kg-day[D]	Male	Laying on side	77	77
11-0111	80 mg/kg-day[D]	Male	Labored Breathing	77	78
11-0111	80 mg/kg-day[D]	Male	Laying on back	78	78
11-0111	80 mg/kg-day[D]	Male	Creeping	78	82
11-0111	80 mg/kg-day[D]	Male	Hind legs stiff	78	82
11-0111	80 mg/kg-day[D]	Male	Labored Breathing	81	81
11-0111	80 mg/kg-day[D]	Male	Lethargic	81	85
11-0111	80 mg/kg-day[D]	Male	Prostrate	81	81
11-0111	80 mg/kg-day[D]	Male	Laying on side	82	83
11-0111	80 mg/kg-day[D]	Male	Hunched posture	83	84
11-0111	80 mg/kg-day[D]	Male	Labored Breathing	83	84
11-0111	80 mg/kg-day[D]	Male	Hind legs stiff	85	85
11-0111	80 mg/kg-day[D]	Male	Prostrate	85	85
11-0111	80 mg/kg-day[D]	Male	Hunched posture	86	86
11-0111	80 mg/kg-day[D]	Male	Creeping	87	87
11-0111	80 mg/kg-day[D]	Male	Legs stiff	87	87
11-0111	80 mg/kg-day[D]	Male	Hunched posture	88	88
11-0111	80 mg/kg-day[D]	Male	Lethargic	88	89
11-0111	80 mg/kg-day[D]	Male	Prostrate	88	89

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0111	80 mg/kg-day[D]	Male	Creeping	89	90
11-0111	80 mg/kg-day[D]	Male	Legs stiff	90	90
11-0123	80 mg/kg-day[D]	Male	Labored Breathing	5	5
11-0123	80 mg/kg-day[D]	Male	Dark Urine	6	72
11-0123	80 mg/kg-day[D]	Male	Lethargic	6	9
11-0123	80 mg/kg-day[D]	Male	Laying on side	7	9
11-0123	80 mg/kg-day[D]	Male	Elevated Respiration Rate	9	9
11-0123	80 mg/kg-day[D]	Male	Laying on side	12	12
11-0123	80 mg/kg-day[D]	Male	Lethargic	12	16
11-0123	80 mg/kg-day[D]	Male	Labored Breathing	12	14
11-0123	80 mg/kg-day[D]	Male	Prostrate	13	13
11-0123	80 mg/kg-day[D]	Male	Laying on side	14	14
11-0123	80 mg/kg-day[D]	Male	Prostrate	16	16
11-0123	80 mg/kg-day[D]	Male	Labored Breathing	16	16
11-0123	80 mg/kg-day[D]	Male	Lethargic	19	29
11-0123	80 mg/kg-day[D]	Male	Laying on side	19	19
11-0123	80 mg/kg-day[D]	Male	Labored Breathing	19	19
11-0123	80 mg/kg-day[D]	Male	Prostrate	20	20
11-0123	80 mg/kg-day[D]	Male	Labored Breathing	22	23
11-0123	80 mg/kg-day[D]	Male	Laying on back	22	23
11-0123	80 mg/kg-day[D]	Male	Irregular Gait	24	24
11-0123	80 mg/kg-day[D]	Male	Laying on side	26	26
11-0123	80 mg/kg-day[D]	Male	Labored Breathing	26	29
11-0123	80 mg/kg-day[D]	Male	Laying on side	29	29
11-0123	80 mg/kg-day[D]	Male	Low Arousal	31	31
11-0123	80 mg/kg-day[D]	Male	Lethargic	33	35
11-0123	80 mg/kg-day[D]	Male	Laying on side	33	35
11-0123	80 mg/kg-day[D]	Male	Labored Breathing	40	40
11-0123	80 mg/kg-day[D]	Male	Laying on back	40	40
11-0123	80 mg/kg-day[D]	Male	Lethargic	40	40
11-0123	80 mg/kg-day[D]	Male	Labored Breathing	43	43
11-0123	80 mg/kg-day[D]	Male	Laying on back	43	43
11-0123	80 mg/kg-day[D]	Male	Lethargic	43	44
11-0123	80 mg/kg-day[D]	Male	Labored Breathing	47	49
11-0123	80 mg/kg-day[D]	Male	Laying on back	47	47
11-0123	80 mg/kg-day[D]	Male	Lethargic	47	50
11-0123	80 mg/kg-day[D]	Male	Laying on side	48	49
11-0123	80 mg/kg-day[D]	Male	Prostrate	51	51
11-0123	80 mg/kg-day[D]	Male	Prostrate	55	55
11-0123	80 mg/kg-day[D]	Male	Lethargic	55	56
11-0123	80 mg/kg-day[D]	Male	Laying on side	56	56
11-0123	80 mg/kg-day[D]	Male	Barbering	57	84
11-0123	80 mg/kg-day[D]	Male	Lethargic	58	58
11-0123	80 mg/kg-day[D]	Male	Laying on side	58	58
11-0123	80 mg/kg-day[D]	Male	Labored Breathing	58	58
11-0123	80 mg/kg-day[D]	Male	Lethargic	61	65
11-0123	80 mg/kg-day[D]	Male	Prostrate	61	61
11-0123	80 mg/kg-day[D]	Male	Laying on side	62	65
11-0123	80 mg/kg-day[D]	Male	Labored Breathing	63	65
11-0123	80 mg/kg-day[D]	Male	Hunched posture	64	69
11-0123	80 mg/kg-day[D]	Male	Rough hair coat	64	66
11-0123	80 mg/kg-day[D]	Male	Creeping	65	65
11-0123	80 mg/kg-day[D]	Male	Laying on side	68	69
11-0123	80 mg/kg-day[D]	Male	Labored Breathing	68	69
11-0123	80 mg/kg-day[D]	Male	Lethargic	68	71
11-0123	80 mg/kg-day[D]	Male	Creeping	70	72
11-0123	80 mg/kg-day[D]	Male	Prostrate	70	70
11-0123	80 mg/kg-day[D]	Male	Labored Breathing	71	71
11-0123	80 mg/kg-day[D]	Male	Laying on back	71	71
11-0123	80 mg/kg-day[D]	Male	Hunched posture	73	74
11-0123	80 mg/kg-day[D]	Male	Creeping	74	74
11-0123	80 mg/kg-day[D]	Male	Leaning to the left	75	75
11-0123	80 mg/kg-day[D]	Male	Laying on side	75	76
11-0123	80 mg/kg-day[D]	Male	Lethargic	75	79

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0123	80 mg/kg-day[D]	Male	Hunched posture	76	77
11-0123	80 mg/kg-day[D]	Male	Labored Breathing	76	79
11-0123	80 mg/kg-day[D]	Male	Prostrate	77	77
11-0123	80 mg/kg-day[D]	Male	Leaning to the left	78	78
11-0123	80 mg/kg-day[D]	Male	Laying on side	78	79
11-0123	80 mg/kg-day[D]	Male	Hunched posture	79	79
11-0123	80 mg/kg-day[D]	Male	Creeping	79	81
11-0123	80 mg/kg-day[D]	Male	Hind legs stiff	79	81
11-0123	80 mg/kg-day[D]	Male	Leaning to the left	81	81
11-0123	80 mg/kg-day[D]	Male	Hunched posture	82	87
11-0123	80 mg/kg-day[D]	Male	Ears twitching	82	82
11-0123	80 mg/kg-day[D]	Male	Laying on side	82	82
11-0123	80 mg/kg-day[D]	Male	Labored Breathing	82	84
11-0123	80 mg/kg-day[D]	Male	Lethargic	82	86
11-0123	80 mg/kg-day[D]	Male	Prostrate	83	84
11-0123	80 mg/kg-day[D]	Male	Ears twitching	84	85
11-0123	80 mg/kg-day[D]	Male	Leaning to the left	85	87
11-0123	80 mg/kg-day[D]	Male	Laying on side	86	86
11-0123	80 mg/kg-day[D]	Male	Labored Breathing	86	86
11-0123	80 mg/kg-day[D]	Male	Ears twitching	87	87
11-0123	80 mg/kg-day[D]	Male	Barbering	87	90
11-0123	80 mg/kg-day[D]	Male	Creeping	88	88
11-0123	80 mg/kg-day[D]	Male	Legs stiff	88	88
11-0123	80 mg/kg-day[D]	Male	Body twitching	88	88
11-0123	80 mg/kg-day[D]	Male	Leaning to the left	89	89
11-0123	80 mg/kg-day[D]	Male	Hunched posture	89	89
11-0123	80 mg/kg-day[D]	Male	Ears twitching	89	89
11-0123	80 mg/kg-day[D]	Male	Labored Breathing	89	89
11-0123	80 mg/kg-day[D]	Male	Lethargic	89	89
11-0123	80 mg/kg-day[D]	Male	Prostrate	89	89
11-0123	80 mg/kg-day[D]	Male	Creeping	90	90
11-0129	80 mg/kg-day[D]	Male	Dark Urine	0	72
11-0129	80 mg/kg-day[D]	Male	Lethargic	2	2
11-0129	80 mg/kg-day[D]	Male	Lethargic	5	7
11-0129	80 mg/kg-day[D]	Male	Laying on side	6	7
11-0129	80 mg/kg-day[D]	Male	Laying on back	8	8
11-0129	80 mg/kg-day[D]	Male	Lethargic	9	9
11-0129	80 mg/kg-day[D]	Male	Prostrate	9	9
11-0129	80 mg/kg-day[D]	Male	Scab behind R ear	10	13
11-0129	80 mg/kg-day[D]	Male	Lethargic	12	16
11-0129	80 mg/kg-day[D]	Male	Prostrate	12	12
11-0129	80 mg/kg-day[D]	Male	Laying on side	13	13
11-0129	80 mg/kg-day[D]	Male	Labored Breathing	13	16
11-0129	80 mg/kg-day[D]	Male	Prostrate	14	14
11-0129	80 mg/kg-day[D]	Male	Laying on side	16	16
11-0129	80 mg/kg-day[D]	Male	Lethargic	19	23
11-0129	80 mg/kg-day[D]	Male	Labored Breathing	19	19
11-0129	80 mg/kg-day[D]	Male	Laying on side	19	19
11-0129	80 mg/kg-day[D]	Male	Prostrate	21	23
11-0129	80 mg/kg-day[D]	Male	Labored Breathing	21	23
11-0129	80 mg/kg-day[D]	Male	Lethargic	26	29
11-0129	80 mg/kg-day[D]	Male	Labored Breathing	26	27
11-0129	80 mg/kg-day[D]	Male	Laying on side	26	28
11-0129	80 mg/kg-day[D]	Male	Prostrate	29	29
11-0129	80 mg/kg-day[D]	Male	Labored Breathing	29	29
11-0129	80 mg/kg-day[D]	Male	Irregular Gait	30	30
11-0129	80 mg/kg-day[D]	Male	Walking on toes	30	30
11-0129	80 mg/kg-day[D]	Male	Lethargic	33	35
11-0129	80 mg/kg-day[D]	Male	Labored Breathing	33	35
11-0129	80 mg/kg-day[D]	Male	Laying on side	33	33
11-0129	80 mg/kg-day[D]	Male	Prostrate	34	35
11-0129	80 mg/kg-day[D]	Male	Laying on back	40	42
11-0129	80 mg/kg-day[D]	Male	Lethargic	40	44
11-0129	80 mg/kg-day[D]	Male	Labored Breathing	40	44

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0129	80 mg/kg-day[D]	Male	Laying on side	43	43
11-0129	80 mg/kg-day[D]	Male	Prostrate	44	44
11-0129	80 mg/kg-day[D]	Male	Laying on back	47	47
11-0129	80 mg/kg-day[D]	Male	Lethargic	47	50
11-0129	80 mg/kg-day[D]	Male	Labored Breathing	47	47
11-0129	80 mg/kg-day[D]	Male	Laying on side	48	48
11-0129	80 mg/kg-day[D]	Male	Prostrate	49	49
11-0129	80 mg/kg-day[D]	Male	Laying on back	50	50
11-0129	80 mg/kg-day[D]	Male	Labored Breathing	50	50
11-0129	80 mg/kg-day[D]	Male	Laying on side	51	51
11-0129	80 mg/kg-day[D]	Male	Lethargic	55	58
11-0129	80 mg/kg-day[D]	Male	Labored Breathing	55	55
11-0129	80 mg/kg-day[D]	Male	Laying on side	55	55
11-0129	80 mg/kg-day[D]	Male	Laying on back	56	56
11-0129	80 mg/kg-day[D]	Male	Right hind limb stiff/locked/dragging	57	57
11-0129	80 mg/kg-day[D]	Male	Laying on side	57	57
11-0129	80 mg/kg-day[D]	Male	Labored Breathing	57	58
11-0129	80 mg/kg-day[D]	Male	Laying on back	58	58
11-0129	80 mg/kg-day[D]	Male	Limping on R hind limb	58	58
11-0129	80 mg/kg-day[D]	Male	Laying on back	61	61
11-0129	80 mg/kg-day[D]	Male	Lethargic	61	64
11-0129	80 mg/kg-day[D]	Male	Labored Breathing	61	62
11-0129	80 mg/kg-day[D]	Male	Prostrate	62	63
11-0129	80 mg/kg-day[D]	Male	Laying on side	64	64
11-0129	80 mg/kg-day[D]	Male	Labored Breathing	64	64
11-0129	80 mg/kg-day[D]	Male	Irregular Gait	64	65
11-0129	80 mg/kg-day[D]	Male	Hind end raised	64	66
11-0129	80 mg/kg-day[D]	Male	Laying on back	68	68
11-0129	80 mg/kg-day[D]	Male	Lethargic	68	69
11-0129	80 mg/kg-day[D]	Male	Labored Breathing	68	69
11-0129	80 mg/kg-day[D]	Male	Laying on side	69	69
11-0129	80 mg/kg-day[D]	Male	Laying on back	71	71
11-0129	80 mg/kg-day[D]	Male	Lethargic	71	72
11-0129	80 mg/kg-day[D]	Male	Labored Breathing	71	72
11-0129	80 mg/kg-day[D]	Male	Laying upside down	72	72
11-0129	80 mg/kg-day[D]	Male	Laying on back	75	75
11-0129	80 mg/kg-day[D]	Male	Lethargic	75	79
11-0129	80 mg/kg-day[D]	Male	Labored Breathing	75	76
11-0129	80 mg/kg-day[D]	Male	Prostrate	76	77
11-0129	80 mg/kg-day[D]	Male	Labored Breathing	78	79
11-0129	80 mg/kg-day[D]	Male	Laying on back	79	79
11-0129	80 mg/kg-day[D]	Male	Laying on side	79	80
11-0129	80 mg/kg-day[D]	Male	Laying on back	82	86
11-0129	80 mg/kg-day[D]	Male	Lethargic	82	86
11-0129	80 mg/kg-day[D]	Male	Labored Breathing	82	86
11-0129	80 mg/kg-day[D]	Male	Tail Curled	83	83
11-0129	80 mg/kg-day[D]	Male	Hind end raised	84	84
11-0129	80 mg/kg-day[D]	Male	Walking on toes	84	84
11-0129	80 mg/kg-day[D]	Male	Left hind limb ataxia	85	90
11-0129	80 mg/kg-day[D]	Male	Limping	85	85
11-0129	80 mg/kg-day[D]	Male	Prostrate	85	86
11-0129	80 mg/kg-day[D]	Male	Pulling up legs when walking	86	86
11-0129	80 mg/kg-day[D]	Male	Hind end raised	86	86
11-0129	80 mg/kg-day[D]	Male	Ears twitching	87	87
11-0129	80 mg/kg-day[D]	Male	Laying on side	87	87
11-0129	80 mg/kg-day[D]	Male	Pulling up legs when walking	88	90
11-0129	80 mg/kg-day[D]	Male	Tail Curled	88	88
11-0129	80 mg/kg-day[D]	Male	Lethargic	89	89
11-0129	80 mg/kg-day[D]	Male	Labored Breathing	89	89
11-0129	80 mg/kg-day[D]	Male	Laying on side	89	89
11-0132	80 mg/kg-day[D]	Male	Laying on side	1	1
11-0132	80 mg/kg-day[D]	Male	Lethargic	1	1
11-0132	80 mg/kg-day[D]	Male	Lethargic	4	4
11-0132	80 mg/kg-day[D]	Male	Laying on side	5	5

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0132	80 mg/kg-day[D]	Male	Laying on back	6	8
11-0132	80 mg/kg-day[D]	Male	Dark Urine	7	78
11-0132	80 mg/kg-day[D]	Male	Labored Breathing	7	7
11-0132	80 mg/kg-day[D]	Male	Lethargic	8	8
11-0132	80 mg/kg-day[D]	Male	Elevated Respiration Rate	8	8
11-0132	80 mg/kg-day[D]	Male	Lethargic	11	15
11-0132	80 mg/kg-day[D]	Male	Laying on back	11	12
11-0132	80 mg/kg-day[D]	Male	Labored Breathing	11	15
11-0132	80 mg/kg-day[D]	Male	Laying on side	13	13
11-0132	80 mg/kg-day[D]	Male	Laying on back	14	15
11-0132	80 mg/kg-day[D]	Male	Lethargic	18	21
11-0132	80 mg/kg-day[D]	Male	Labored Breathing	18	21
11-0132	80 mg/kg-day[D]	Male	Laying on back	18	18
11-0132	80 mg/kg-day[D]	Male	Prostrate	18	18
11-0132	80 mg/kg-day[D]	Male	Laying on side	19	20
11-0132	80 mg/kg-day[D]	Male	Laying on back	21	21
11-0132	80 mg/kg-day[D]	Male	Prostrate	22	22
11-0132	80 mg/kg-day[D]	Male	Soft Feces	22	23
11-0132	80 mg/kg-day[D]	Male	Lethargic	25	28
11-0132	80 mg/kg-day[D]	Male	Labored Breathing	25	28
11-0132	80 mg/kg-day[D]	Male	Laying on back	25	25
11-0132	80 mg/kg-day[D]	Male	Irregular Gait	25	25
11-0132	80 mg/kg-day[D]	Male	Walking on toes	26	28
11-0132	80 mg/kg-day[D]	Male	Straubbed Tail	26	28
11-0132	80 mg/kg-day[D]	Male	Laying on side	26	26
11-0132	80 mg/kg-day[D]	Male	Laying on back	27	28
11-0132	80 mg/kg-day[D]	Male	Irregular Gait	30	32
11-0132	80 mg/kg-day[D]	Male	Walking on toes	30	32
11-0132	80 mg/kg-day[D]	Male	Lethargic	32	33
11-0132	80 mg/kg-day[D]	Male	Labored Breathing	32	33
11-0132	80 mg/kg-day[D]	Male	Laying on back	33	33
11-0132	80 mg/kg-day[D]	Male	Soft Feces	37	37
11-0132	80 mg/kg-day[D]	Male	Prostrate	39	39
11-0132	80 mg/kg-day[D]	Male	Lethargic	39	43
11-0132	80 mg/kg-day[D]	Male	Labored Breathing	39	41
11-0132	80 mg/kg-day[D]	Male	Laying on back	40	41
11-0132	80 mg/kg-day[D]	Male	Prostrate	42	42
11-0132	80 mg/kg-day[D]	Male	Labored Breathing	43	43
11-0132	80 mg/kg-day[D]	Male	Laying on back	43	43
11-0132	80 mg/kg-day[D]	Male	Lethargic	46	49
11-0132	80 mg/kg-day[D]	Male	Labored Breathing	46	49
11-0132	80 mg/kg-day[D]	Male	Laying on side	46	46
11-0132	80 mg/kg-day[D]	Male	Laying on back	47	47
11-0132	80 mg/kg-day[D]	Male	Laying on side	48	48
11-0132	80 mg/kg-day[D]	Male	Laying on back	49	49
11-0132	80 mg/kg-day[D]	Male	Lethargic	54	55
11-0132	80 mg/kg-day[D]	Male	Labored Breathing	54	55
11-0132	80 mg/kg-day[D]	Male	Laying on side	54	54
11-0132	80 mg/kg-day[D]	Male	Prostrate	55	55
11-0132	80 mg/kg-day[D]	Male	Prostrate	57	57
11-0132	80 mg/kg-day[D]	Male	Labored Breathing	57	57
11-0132	80 mg/kg-day[D]	Male	Lethargic	57	57
11-0132	80 mg/kg-day[D]	Male	Laying on back	61	61
11-0132	80 mg/kg-day[D]	Male	Lethargic	61	64
11-0132	80 mg/kg-day[D]	Male	Labored Breathing	61	64
11-0132	80 mg/kg-day[D]	Male	Prostrate	62	62
11-0132	80 mg/kg-day[D]	Male	Laying on side	63	63
11-0132	80 mg/kg-day[D]	Male	Prostrate	64	64
11-0132	80 mg/kg-day[D]	Male	Laying on back	67	67
11-0132	80 mg/kg-day[D]	Male	Lethargic	67	70
11-0132	80 mg/kg-day[D]	Male	Labored Breathing	67	69
11-0132	80 mg/kg-day[D]	Male	Laying on side	68	68
11-0132	80 mg/kg-day[D]	Male	Prostrate	70	70
11-0132	80 mg/kg-day[D]	Male	Prostrate	74	74

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0132	80 mg/kg-day[D]	Male	Lethargic	74	78
11-0132	80 mg/kg-day[D]	Male	Labored Breathing	75	78
11-0132	80 mg/kg-day[D]	Male	Laying on back	75	75
11-0132	80 mg/kg-day[D]	Male	Laying on side	76	76
11-0132	80 mg/kg-day[D]	Male	Prostrate	77	77
11-0132	80 mg/kg-day[D]	Male	Squinting	78	80
11-0132	80 mg/kg-day[D]	Male	Ears back/twitching	78	86
11-0132	80 mg/kg-day[D]	Male	Hunched posture	78	83
11-0132	80 mg/kg-day[D]	Male	Laying on back	78	78
11-0132	80 mg/kg-day[D]	Male	Prostrate	81	81
11-0132	80 mg/kg-day[D]	Male	Lethargic	81	85
11-0132	80 mg/kg-day[D]	Male	Labored Breathing	81	85
11-0132	80 mg/kg-day[D]	Male	Laying on side	82	82
11-0132	80 mg/kg-day[D]	Male	Squinting	83	83
11-0132	80 mg/kg-day[D]	Male	Prostrate	83	85
11-0132	80 mg/kg-day[D]	Male	Leaning to the left	83	83
11-0132	80 mg/kg-day[D]	Male	Laying on back	84	85
11-0132	80 mg/kg-day[D]	Male	Leaning to the left	85	85
11-0132	80 mg/kg-day[D]	Male	Prostrate	88	89
11-0132	80 mg/kg-day[D]	Male	Lethargic	88	89
11-0132	80 mg/kg-day[D]	Male	Labored Breathing	88	89
11-0132	80 mg/kg-day[D]	Male	Ears twitching	88	90
11-0132	80 mg/kg-day[D]	Male	Hunched posture	89	89
11-0132	80 mg/kg-day[D]	Male	Creeping	90	90
11-0132	80 mg/kg-day[D]	Male	Legs stiff	90	90
11-0144	80 mg/kg-day[D]	Male	Lethargic	33	2
11-0144	80 mg/kg-day[D]	Male	Laying on back	2	2
11-0144	80 mg/kg-day[D]	Male	Dark Urine	2	48
11-0144	80 mg/kg-day[D]	Male	Laying on back	5	9
11-0144	80 mg/kg-day[D]	Male	Lethargic	6	6
11-0144	80 mg/kg-day[D]	Male	Labored Breathing	6	6
11-0144	80 mg/kg-day[D]	Male	Laying on side	7	7
11-0144	80 mg/kg-day[D]	Male	Lethargic	8	9
11-0144	80 mg/kg-day[D]	Male	Lethargic	12	12
11-0144	80 mg/kg-day[D]	Male	Laying on back	12	12
11-0144	80 mg/kg-day[D]	Male	Labored Breathing	12	12
11-0144	80 mg/kg-day[D]	Male	Lethargic	14	16
11-0144	80 mg/kg-day[D]	Male	Laying on side	14	16
11-0144	80 mg/kg-day[D]	Male	Labored Breathing	14	16
11-0144	80 mg/kg-day[D]	Male	Lethargic	19	29
11-0144	80 mg/kg-day[D]	Male	Labored Breathing	19	23
11-0144	80 mg/kg-day[D]	Male	Laying on back	19	20
11-0144	80 mg/kg-day[D]	Male	Laying on side	21	21
11-0144	80 mg/kg-day[D]	Male	Laying on side	23	23
11-0144	80 mg/kg-day[D]	Male	Prostrate	26	26
11-0144	80 mg/kg-day[D]	Male	Laying on side	27	27
11-0144	80 mg/kg-day[D]	Male	Labored Breathing	27	29
11-0144	80 mg/kg-day[D]	Male	Laying on back	28	29
11-0144	80 mg/kg-day[D]	Male	Laying on side	33	33
11-0144	80 mg/kg-day[D]	Male	Labored Breathing	33	35
11-0144	80 mg/kg-day[D]	Male	Lethargic	33	35
11-0144	80 mg/kg-day[D]	Male	Laying on back	34	35
11-0144	80 mg/kg-day[D]	Male	Labored Breathing	40	44
11-0144	80 mg/kg-day[D]	Male	Lethargic	40	44
11-0144	80 mg/kg-day[D]	Male	Laying on back	40	44
11-0144	80 mg/kg-day[D]	Male	Labored Breathing	47	50
11-0144	80 mg/kg-day[D]	Male	Lethargic	47	50
11-0144	80 mg/kg-day[D]	Male	Prostrate	47	47
11-0144	80 mg/kg-day[D]	Male	Laying on back	47	48
11-0144	80 mg/kg-day[D]	Male	Laying on side	49	49
11-0144	80 mg/kg-day[D]	Male	Prostrate	50	50
11-0144	80 mg/kg-day[D]	Male	Laying on back	51	51
11-0144	80 mg/kg-day[D]	Male	Labored Breathing	55	57
11-0144	80 mg/kg-day[D]	Male	Lethargic	55	58

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0144	80 mg/kg-day[D]	Male	Prostrate	55	56
11-0144	80 mg/kg-day[D]	Male	Laying on back	57	57
11-0144	80 mg/kg-day[D]	Male	Prostrate	58	58
11-0144	80 mg/kg-day[D]	Male	Laying on back	61	61
11-0144	80 mg/kg-day[D]	Male	Labored Breathing	61	61
11-0144	80 mg/kg-day[D]	Male	Lethargic	61	63
11-0144	80 mg/kg-day[D]	Male	Prostrate	62	63
11-0144	80 mg/kg-day[D]	Male	Laying on side	62	62
11-0144	80 mg/kg-day[D]	Male	Labored Breathing	63	63
11-0144	80 mg/kg-day[D]	Male	Found dead	64	64
11-0149	80 mg/kg-day[D]	Male	Dark Urine	1	71
11-0149	80 mg/kg-day[D]	Male	Lethargic	1	1
11-0149	80 mg/kg-day[D]	Male	Lethargic	4	6
11-0149	80 mg/kg-day[D]	Male	Laying on side	5	5
11-0149	80 mg/kg-day[D]	Male	Laying on back	7	7
11-0149	80 mg/kg-day[D]	Male	Laying on side	7	8
11-0149	80 mg/kg-day[D]	Male	Lethargic	8	8
11-0149	80 mg/kg-day[D]	Male	Elevated Respiration Rate	8	8
11-0149	80 mg/kg-day[D]	Male	Lethargic	11	11
11-0149	80 mg/kg-day[D]	Male	Lethargic	13	15
11-0149	80 mg/kg-day[D]	Male	Laying on side	13	13
11-0149	80 mg/kg-day[D]	Male	Labored Breathing	13	13
11-0149	80 mg/kg-day[D]	Male	Labored Breathing	15	15
11-0149	80 mg/kg-day[D]	Male	Lethargic	18	23
11-0149	80 mg/kg-day[D]	Male	Laying on side	18	19
11-0149	80 mg/kg-day[D]	Male	Labored Breathing	18	21
11-0149	80 mg/kg-day[D]	Male	Prostrate	19	19
11-0149	80 mg/kg-day[D]	Male	Laying on back	20	21
11-0149	80 mg/kg-day[D]	Male	Prostrate	22	22
11-0149	80 mg/kg-day[D]	Male	Lethargic	25	28
11-0149	80 mg/kg-day[D]	Male	Laying on side	25	25
11-0149	80 mg/kg-day[D]	Male	Laying on back	26	26
11-0149	80 mg/kg-day[D]	Male	Labored Breathing	26	26
11-0149	80 mg/kg-day[D]	Male	Walking on toes	27	30
11-0149	80 mg/kg-day[D]	Male	Laying on side	28	28
11-0149	80 mg/kg-day[D]	Male	Labored Breathing	28	28
11-0149	80 mg/kg-day[D]	Male	Irregular Gait	29	30
11-0149	80 mg/kg-day[D]	Male	Lethargic	32	32
11-0149	80 mg/kg-day[D]	Male	Laying on side	32	32
11-0149	80 mg/kg-day[D]	Male	Labored Breathing	32	32
11-0149	80 mg/kg-day[D]	Male	Lethargic	33	34
11-0149	80 mg/kg-day[D]	Male	Laying on side	33	34
11-0149	80 mg/kg-day[D]	Male	Labored Breathing	39	39
11-0149	80 mg/kg-day[D]	Male	Lethargic	39	43
11-0149	80 mg/kg-day[D]	Male	Laying on back	39	39
11-0149	80 mg/kg-day[D]	Male	Prostrate	40	40
11-0149	80 mg/kg-day[D]	Male	Prostrate	42	42
11-0149	80 mg/kg-day[D]	Male	Lethargic	46	46
11-0149	80 mg/kg-day[D]	Male	Labored Breathing	48	49
11-0149	80 mg/kg-day[D]	Male	Lethargic	48	49
11-0149	80 mg/kg-day[D]	Male	Laying on side	48	49
11-0149	80 mg/kg-day[D]	Male	Prostrate	50	50
11-0149	80 mg/kg-day[D]	Male	Lethargic	55	55
11-0149	80 mg/kg-day[D]	Male	Laying on side	56	56
11-0149	80 mg/kg-day[D]	Male	Lethargic	57	57
11-0149	80 mg/kg-day[D]	Male	Prostrate	61	61
11-0149	80 mg/kg-day[D]	Male	Lethargic	61	61
11-0149	80 mg/kg-day[D]	Male	Laying on side	63	63
11-0149	80 mg/kg-day[D]	Male	Prostrate	67	68
11-0149	80 mg/kg-day[D]	Male	Lethargic	67	71
11-0149	80 mg/kg-day[D]	Male	Labored Breathing	67	67
11-0149	80 mg/kg-day[D]	Male	Laying on side	69	69
11-0149	80 mg/kg-day[D]	Male	Labored Breathing	69	69
11-0149	80 mg/kg-day[D]	Male	Prostrate	70	70

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0149	80 mg/kg-day[D]	Male	Labored Breathing	71	71
11-0149	80 mg/kg-day[C]	Male	Laying on side	71	71
11-0149	80 mg/kg-day[D]	Male	Hunched posture	72	72
11-0149	80 mg/kg-day[D]	Male	Squinting	72	72
11-0149	80 mg/kg-day[D]	Male	Lethargic	74	78
11-0149	80 mg/kg-day[D]	Male	Laying on side	74	74
11-0149	80 mg/kg-day[D]	Male	Prostrate	75	75
11-0149	80 mg/kg-day[D]	Male	Creeping	75	75
11-0149	80 mg/kg-day[D]	Male	Squinting	76	76
11-0149	80 mg/kg-day[D]	Male	Ears back/twitching	76	76
11-0149	80 mg/kg-day[D]	Male	Hunched posture	76	76
11-0149	80 mg/kg-day[D]	Male	Laying on side	76	78
11-0149	80 mg/kg-day[D]	Male	Labored Breathing	77	78
11-0149	80 mg/kg-day[D]	Male	Creeping	77	78
11-0149	80 mg/kg-day[D]	Male	Prostrate	78	78
11-0149	80 mg/kg-day[D]	Male	Found dead	79	79

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0154	Corn Oil Control	Female	Appears Normal		
11-0162	Corn Oil Control	Female	Barbering	0	2
11-0162	Corn Oil Control	Female	Feces full of bedding	40	41
11-0162	Corn Oil Control	Female	Feces full of bedding	57	57
11-0162	Corn Oil Control	Female	Feces full of bedding	89	89
11-0168	Corn Oil Control	Female	Congested Breathing	66	66
11-0171	Corn Oil Control	Female	Appears Normal		
11-0173	Corn Oil Control	Female	Small Scab behind right ear	20	21
11-0175	Corn Oil Control	Female	Appears Normal		
11-0190	Corn Oil Control	Female	Appears Normal		
11-0191	Corn Oil Control	Female	Appears Normal		
11-0197	Corn Oil Control	Female	Barbering	0	74
11-0206	Corn Oil Control	Female	Barbering	2	2
11-0152	1.25 mg/kg-day[A]	Female	Small scab on center of neck	14	14
11-0159	1.25 mg/kg-day[A]	Female	Barbering	20	55
11-0165	1.25 mg/kg-day[A]	Female	Barbering	0	90
11-0165	1.25 mg/kg-day[A]	Female	Scab behind right ear	19	29
11-0165	1.25 mg/kg-day[A]	Female	Alopecia behind right ear	23	29
11-0170	1.25 mg/kg-day[A]	Female	Barbering	0	90
11-0172	1.25 mg/kg-day[A]	Female	Congested Breathing	17	20
11-0172	1.25 mg/kg-day[A]	Female	Scab on lower right lip	40	41
11-0172	1.25 mg/kg-day[A]	Female	Slight right hind limb ataxia	52	52
11-0172	1.25 mg/kg-day[A]	Female	Hind limb ataxia	79	83
11-0172	1.25 mg/kg-day[A]	Female	Legs sprawled	79	83
11-0172	1.25 mg/kg-day[A]	Female	Jerky movements	80	80
11-0172	1.25 mg/kg-day[A]	Female	Barbering	83	90
11-0176	1.25 mg/kg-day[A]	Female	Appears Normal		
11-0178	1.25 mg/kg-day[A]	Female	Congested Breathing	2	90
11-0181	1.25 mg/kg-day[A]	Female	Barbering	1	90
11-0192	1.25 mg/kg-day[A]	Female	Bedding in feces	42	42
11-0192	1.25 mg/kg-day[A]	Female	Bedding in feces	90	90
11-0196	1.25 mg/kg-day[A]	Female	Congested Breathing	47	47
11-0156	5 mg/kg-day[B]	Female	Congested Breathing	59	60
11-0157	5 mg/kg-day[B]	Female	Barbering	4	41
11-0157	5 mg/kg-day[B]	Female	Scant Feces/small,dry	42	42
11-0157	5 mg/kg-day[B]	Female	Barbering	43	90
11-0157	5 mg/kg-day[B]	Female	Hind end raised	68	69
11-0157	5 mg/kg-day[B]	Female	Unusual Gait	68	69
11-0157	5 mg/kg-day[B]	Female	Hind limb ataxia	68	69
11-0157	5 mg/kg-day[B]	Female	Left hind limb ataxia	70	90
11-0157	5 mg/kg-day[B]	Female	Left hind limb Stiff/Limping	70	85
11-0157	5 mg/kg-day[B]	Female	Not bearing weight on left hind leg	72	73
11-0157	5 mg/kg-day[B]	Female	Not bearing weight on left hind leg	76	76
11-0157	5 mg/kg-day[B]	Female	Left Hind Foot Clenched	78	81
11-0157	5 mg/kg-day[B]	Female	Bearing some weight on left hind leg	82	84
11-0157	5 mg/kg-day[B]	Female	Left Hind Foot Clenched	83	85
11-0157	5 mg/kg-day[B]	Female	Slightly favoring Left hind leg	85	86
11-0157	5 mg/kg-day[B]	Female	Limping	87	90
11-0166	5 mg/kg-day[B]	Female	Appears Normal		
11-0174	5 mg/kg-day[B]	Female	Barbering	16	50
11-0187	5 mg/kg-day[B]	Female	Laying on side	21	21
11-0189	5 mg/kg-day[B]	Female	Mucousy diarrhea in cage	74	74
11-0193	5 mg/kg-day[B]	Female	Appears Normal		
11-0202	5 mg/kg-day[B]	Female	Appears Normal		
11-0204	5 mg/kg-day[B]	Female	Appears Normal		
11-0153	20 mg/kg-day[C]	Female	Barbering	7	41
11-0153	20 mg/kg-day[C]	Female	Low Arousal	24	24
11-0153	20 mg/kg-day[C]	Female	Dark Urine	36	68
11-0153	20 mg/kg-day[C]	Female	Barbering	43	90
11-0155	20 mg/kg-day[C]	Female	Dark Urine	39	47
11-0160	20 mg/kg-day[C]	Female	Barbering	23	90
11-0160	20 mg/kg-day[C]	Female	Dark Urine	42	42
11-0164	20 mg/kg-day[C]	Female	Dark Urine	64	67
11-0167	20 mg/kg-day[C]	Female	Dark Urine	43	68

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0179	20 mg/kg-day[C]	Female	Dark Urine	43	68
11-0183	20 mg/kg-day[C]	Female	Walking on Toes	23	24
11-0183	20 mg/kg-day[C]	Female	Irregular Gait	24	24
11-0183	20 mg/kg-day[C]	Female	Walking on Toes	26	34
11-0183	20 mg/kg-day[C]	Female	Irregular Gait	27	28
11-0183	20 mg/kg-day[C]	Female	Walking on Toes	43	43
11-0183	20 mg/kg-day[C]	Female	Dark Urine	43	47
11-0183	20 mg/kg-day[C]	Female	Hind end raised	42	42
11-0183	20 mg/kg-day[C]	Female	Scant Feces/small,dry	42	42
11-0183	20 mg/kg-day[C]	Female	Hind end slightly raised	45	90
11-0183	20 mg/kg-day[C]	Female	Walking on Toes	46	68
11-0183	20 mg/kg-day[C]	Female	Walking on Toes	70	83
11-0183	20 mg/kg-day[C]	Female	Laying on side	82	82
11-0183	20 mg/kg-day[C]	Female	Walking on Toes	85	90
11-0184	20 mg/kg-day[C]	Female	Laying on side	33	33
11-0184	20 mg/kg-day[C]	Female	Dark Urine	39	39
11-0194	20 mg/kg-day[C]	Female	Appears Normal		
11-0198	20 mg/kg-day[C]	Female	Dark Urine	40	68
11-0151	80 mg/kg-day[D]	Female	Laying on back	1	1
11-0151	80 mg/kg-day[D]	Female	Labored Breathing	1	1
11-0151	80 mg/kg-day[D]	Female	Dark Urine	5	19
11-0151	80 mg/kg-day[D]	Female	Laying on back	8	8
11-0151	80 mg/kg-day[D]	Female	Labored Breathing	8	9
11-0151	80 mg/kg-day[D]	Female	Lethargic	8	9
11-0151	80 mg/kg-day[D]	Female	Lethargic	13	13
11-0151	80 mg/kg-day[D]	Female	Laying on side	14	15
11-0151	80 mg/kg-day[D]	Female	Ataxia	15	19
11-0151	80 mg/kg-day[D]	Female	Dragging hind end	15	17
11-0151	80 mg/kg-day[D]	Female	Labored Breathing	16	16
11-0151	80 mg/kg-day[D]	Female	Lethargic	16	16
11-0151	80 mg/kg-day[D]	Female	Prostrate	16	16
11-0151	80 mg/kg-day[D]	Female	Labored Breathing	19	19
11-0151	80 mg/kg-day[D]	Female	Straubbed Tail	19	19
11-0151	80 mg/kg-day[D]	Female	Partial back limb paralysis	19	19
11-0151	80 mg/kg-day[D]	Female	Complete front limb paralysis	19	19
11-0151	80 mg/kg-day[D]	Female	Euthanized	19	19
11-0158	80 mg/kg-day[D]	Female	Dark Urine	4	64
11-0158	80 mg/kg-day[D]	Female	Lethargic	7	9
11-0158	80 mg/kg-day[D]	Female	Lethargic	14	15
11-0158	80 mg/kg-day[D]	Female	Laying on side	14	15
11-0158	80 mg/kg-day[D]	Female	Labored Breathing	15	15
11-0158	80 mg/kg-day[D]	Female	Lethargic	17	18
11-0158	80 mg/kg-day[D]	Female	Irregular Gait	18	18
11-0158	80 mg/kg-day[D]	Female	Laying on side	19	19
11-0158	80 mg/kg-day[D]	Female	Labored Breathing	20	20
11-0158	80 mg/kg-day[D]	Female	Laying on side	21	21
11-0158	80 mg/kg-day[D]	Female	Laying on side	33	33
11-0158	80 mg/kg-day[D]	Female	Lethargic	35	36
11-0158	80 mg/kg-day[D]	Female	Prostrate	35	36
11-0158	80 mg/kg-day[D]	Female	Laying on side	40	41
11-0158	80 mg/kg-day[D]	Female	Lots of dark urine	42	42
11-0158	80 mg/kg-day[D]	Female	Lethargic	47	47
11-0158	80 mg/kg-day[D]	Female	Lethargic	49	49
11-0158	80 mg/kg-day[D]	Female	Lethargic	53	53
11-0158	80 mg/kg-day[D]	Female	Prostrate	53	53
11-0158	80 mg/kg-day[D]	Female	Laying on side	54	55
11-0158	80 mg/kg-day[D]	Female	Lethargic	55	56
11-0158	80 mg/kg-day[D]	Female	Prostrate	56	57
11-0158	80 mg/kg-day[D]	Female	Lethargic	57	57
11-0158	80 mg/kg-day[D]	Female	Squinting	57	57
11-0158	80 mg/kg-day[D]	Female	Ears twitching	57	57
11-0158	80 mg/kg-day[D]	Female	Lethargic	60	60
11-0158	80 mg/kg-day[D]	Female	Squinting	60	61
11-0158	80 mg/kg-day[D]	Female	Ears twitching	60	61

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0158	80 mg/kg-day[D]	Female	Hunched posture	61	61
11-0158	80 mg/kg-day[D]	Female	Squinting	63	67
11-0158	80 mg/kg-day[D]	Female	Ears twitching	63	82
11-0158	80 mg/kg-day[D]	Female	Lethargic	63	64
11-0158	80 mg/kg-day[D]	Female	Labored Breathing	63	63
11-0158	80 mg/kg-day[D]	Female	Laying on side	64	64
11-0158	80 mg/kg-day[D]	Female	Hunched posture	66	69
11-0158	80 mg/kg-day[D]	Female	Prostrate	67	67
11-0158	80 mg/kg-day[D]	Female	Lethargic	67	67
11-0158	80 mg/kg-day[D]	Female	Squinting	69	73
11-0158	80 mg/kg-day[D]	Female	Prostrate	69	69
11-0158	80 mg/kg-day[D]	Female	Labored Breathing	69	71
11-0158	80 mg/kg-day[D]	Female	Laying on back	70	70
11-0158	80 mg/kg-day[D]	Female	Hunched posture	71	73
11-0158	80 mg/kg-day[D]	Female	Body twitching	71	72
11-0158	80 mg/kg-day[D]	Female	Laying on side	71	71
11-0158	80 mg/kg-day[D]	Female	Lethargic	71	71
11-0158	80 mg/kg-day[D]	Female	Blinking repeatedly	74	74
11-0158	80 mg/kg-day[D]	Female	Prostrate	75	76
11-0158	80 mg/kg-day[D]	Female	Lethargic	75	76
11-0158	80 mg/kg-day[D]	Female	Squinting	76	76
11-0158	80 mg/kg-day[D]	Female	Squinting	78	78
11-0158	80 mg/kg-day[D]	Female	Tail twitching	78	78
11-0158	80 mg/kg-day[D]	Female	Prostrate	78	78
11-0158	80 mg/kg-day[D]	Female	Lethargic	78	78
11-0158	80 mg/kg-day[D]	Female	Squinting	79	81
11-0158	80 mg/kg-day[D]	Female	Laying on side	83	83
11-0158	80 mg/kg-day[D]	Female	Lethargic	83	83
11-0158	80 mg/kg-day[D]	Female	Head Shaking	84	84
11-0158	80 mg/kg-day[D]	Female	Straubbed tail	84	84
11-0158	80 mg/kg-day[D]	Female	Creeping	84	84
11-0158	80 mg/kg-day[D]	Female	Ears twitching	84	84
11-0158	80 mg/kg-day[D]	Female	Laying on side	85	85
11-0158	80 mg/kg-day[D]	Female	Squinting	86	89
11-0158	80 mg/kg-day[D]	Female	Ears twitching	86	87
11-0158	80 mg/kg-day[D]	Female	Creeping	87	87
11-0158	80 mg/kg-day[D]	Female	Legs stiff	87	87
11-0158	80 mg/kg-day[D]	Female	Ears pulled back	88	88
11-0158	80 mg/kg-day[D]	Female	Ears twitching	89	90
11-0158	80 mg/kg-day[D]	Female	Creeping	89	90
11-0177	80 mg/kg-day[D]	Female	Dark Urine	0	71
11-0177	80 mg/kg-day[D]	Female	Laying on side	13	13
11-0177	80 mg/kg-day[D]	Female	Lethargic	14	15
11-0177	80 mg/kg-day[D]	Female	Laying on side	15	15
11-0177	80 mg/kg-day[D]	Female	Lethargic	21	21
11-0177	80 mg/kg-day[D]	Female	Barbering	21	90
11-0177	80 mg/kg-day[D]	Female	Irregular Gait	25	25
11-0177	80 mg/kg-day[D]	Female	Walking on Toes	25	25
11-0177	80 mg/kg-day[D]	Female	Laying on side	25	25
11-0177	80 mg/kg-day[D]	Female	Back legs knock kneed	24	25
11-0177	80 mg/kg-day[D]	Female	Hopping	25	26
11-0177	80 mg/kg-day[D]	Female	Walking high on back legs	26	26
11-0177	80 mg/kg-day[D]	Female	Laying on side	27	27
11-0177	80 mg/kg-day[D]	Female	Laying on side	34	35
11-0177	80 mg/kg-day[D]	Female	Lethargic	35	35
11-0177	80 mg/kg-day[D]	Female	Laying on side	39	39
11-0177	80 mg/kg-day[D]	Female	Walking on Toes	39	39
11-0177	80 mg/kg-day[D]	Female	Hind limb ataxia	39	39
11-0177	80 mg/kg-day[D]	Female	Hopping on toes	39	39
11-0177	80 mg/kg-day[D]	Female	Right hind limb ataxia	40	40
11-0177	80 mg/kg-day[D]	Female	Hind end slightly raised	46	50
11-0177	80 mg/kg-day[D]	Female	Laying on side	47	47
11-0177	80 mg/kg-day[D]	Female	Hind end slightly raised	53	53
11-0177	80 mg/kg-day[D]	Female	Walking high on back legs	57	57

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0177	80 mg/kg-day[D]	Female	Dropping hind end when walking	57	58
11-0177	80 mg/kg-day[D]	Female	Unusual Gait	58	65
11-0177	80 mg/kg-day[D]	Female	Hind end raised	58	68
11-0177	80 mg/kg-day[D]	Female	Walking on Toes	58	68
11-0177	80 mg/kg-day[D]	Female	Legs stiff	58	63
11-0177	80 mg/kg-day[D]	Female	Dropping hind end when walking	60	61
11-0177	80 mg/kg-day[D]	Female	Dropping hind end when walking	69	71
11-0177	80 mg/kg-day[D]	Female	Hind end raised	72	75
11-0177	80 mg/kg-day[D]	Female	Laying on side	76	76
11-0177	80 mg/kg-day[D]	Female	Dropping hind end when walking	77	77
11-0177	80 mg/kg-day[D]	Female	Hind end raised	78	83
11-0177	80 mg/kg-day[D]	Female	Walking on Toes	78	79
11-0177	80 mg/kg-day[D]	Female	Straubbed tail	79	79
11-0177	80 mg/kg-day[D]	Female	Dropping hind end when walking	80	80
11-0177	80 mg/kg-day[D]	Female	Tail curled	84	84
11-0177	80 mg/kg-day[D]	Female	Feet splayed	84	85
11-0177	80 mg/kg-day[D]	Female	Dropping hind end when walking	86	89
11-0177	80 mg/kg-day[D]	Female	Prostrate	88	89
11-0177	80 mg/kg-day[D]	Female	Lethargic	88	89
11-0180	80 mg/kg-day[D]	Female	Lethargic	0	1
11-0180	80 mg/kg-day[D]	Female	Dark Urine	0	71
11-0180	80 mg/kg-day[D]	Female	Elevated Respiration Rate	1	1
11-0180	80 mg/kg-day[D]	Female	Lethargic	12	12
11-0180	80 mg/kg-day[D]	Female	Laying on side	13	13
11-0180	80 mg/kg-day[D]	Female	Prostrate	18	18
11-0180	80 mg/kg-day[D]	Female	Laying on side	20	20
11-0180	80 mg/kg-day[D]	Female	Labored Breathing	20	20
11-0180	80 mg/kg-day[D]	Female	Irregular Gait	22	25
11-0180	80 mg/kg-day[D]	Female	Walking on Toes	22	25
11-0180	80 mg/kg-day[D]	Female	Back legs knock kneed	25	25
11-0180	80 mg/kg-day[D]	Female	Barbering	25	26
11-0180	80 mg/kg-day[D]	Female	High stepping	25	25
11-0180	80 mg/kg-day[D]	Female	Walking high on back legs	26	26
11-0180	80 mg/kg-day[D]	Female	Laying on side	32	32
11-0180	80 mg/kg-day[D]	Female	Laying on back	33	33
11-0180	80 mg/kg-day[D]	Female	Laying on side	34	34
11-0180	80 mg/kg-day[D]	Female	Prostrate	41	41
11-0180	80 mg/kg-day[D]	Female	Laying on side	54	54
11-0180	80 mg/kg-day[D]	Female	Prostrate	57	57
11-0180	80 mg/kg-day[D]	Female	Lethargic	57	57
11-0180	80 mg/kg-day[D]	Female	Hind end raised	68	68
11-0180	80 mg/kg-day[D]	Female	Lethargic	68	68
11-0180	80 mg/kg-day[D]	Female	Dropping hind end when walking	73	73
11-0180	80 mg/kg-day[D]	Female	Lethargic	76	76
11-0180	80 mg/kg-day[D]	Female	Dropping hind end when walking	77	80
11-0180	80 mg/kg-day[D]	Female	Prostrate	78	78
11-0180	80 mg/kg-day[D]	Female	Lethargic	78	78
11-0180	80 mg/kg-day[D]	Female	Laying on side	81	81
11-0180	80 mg/kg-day[D]	Female	Dropping hind end when walking	82	83
11-0180	80 mg/kg-day[D]	Female	Laying on side	83	83
11-0180	80 mg/kg-day[D]	Female	Dropping hind end when walking	86	87
11-0180	80 mg/kg-day[D]	Female	Creeping	87	87
11-0180	80 mg/kg-day[D]	Female	Hind legs stiff	87	87
11-0180	80 mg/kg-day[D]	Female	Dropping hind end when walking	89	89
11-0180	80 mg/kg-day[D]	Female	Creeping	89	90
11-0180	80 mg/kg-day[D]	Female	Hind legs stiff	89	90
11-0182	80 mg/kg-day[D]	Female	Lethargic	0	1
11-0182	80 mg/kg-day[D]	Female	Prostrate	0	0
11-0182	80 mg/kg-day[D]	Female	Dark Urine	1	71
11-0182	80 mg/kg-day[D]	Female	Feces full of bedding	1	1
11-0182	80 mg/kg-day[D]	Female	Laying on side	1	1
11-0182	80 mg/kg-day[D]	Female	Labored Breathing	7	9
11-0182	80 mg/kg-day[D]	Female	Lethargic	9	9
11-0182	80 mg/kg-day[D]	Female	Prostrate	9	9

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-36-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0182	80 mg/kg-day[D]	Female	Labored Breathing	12	12
11-0182	80 mg/kg-day[D]	Female	Lethargic	12	14
11-0182	80 mg/kg-day[D]	Female	Laying on side	12	12
11-0182	80 mg/kg-day[D]	Female	Irregular Gait	14	16
11-0182	80 mg/kg-day[D]	Female	Laying on side	15	15
11-0182	80 mg/kg-day[D]	Female	Lethargic	16	17
11-0182	80 mg/kg-day[D]	Female	Prostrate	18	18
11-0182	80 mg/kg-day[D]	Female	Labored Breathing	19	19
11-0182	80 mg/kg-day[D]	Female	Laying on side	19	19
11-0182	80 mg/kg-day[D]	Female	Barbering	21	90
11-0182	80 mg/kg-day[D]	Female	Labored Breathing	25	25
11-0182	80 mg/kg-day[D]	Female	Lethargic	25	25
11-0182	80 mg/kg-day[D]	Female	Right hind limb ataxia	31	33
11-0182	80 mg/kg-day[D]	Female	Lethargic	32	32
11-0182	80 mg/kg-day[D]	Female	Prostrate	32	32
11-0182	80 mg/kg-day[D]	Female	Walking on Toes	32	32
11-0182	80 mg/kg-day[D]	Female	Laying on side	35	36
11-0182	80 mg/kg-day[D]	Female	Labored Breathing	36	36
11-0182	80 mg/kg-day[D]	Female	Lethargic	36	36
11-0182	80 mg/kg-day[D]	Female	Laying on side	41	41
11-0182	80 mg/kg-day[D]	Female	Lethargic	42	42
11-0182	80 mg/kg-day[D]	Female	Prostrate	42	42
11-0182	80 mg/kg-day[D]	Female	Dropping hind end when walking	47	47
11-0182	80 mg/kg-day[D]	Female	Laying on side	47	47
11-0182	80 mg/kg-day[D]	Female	Lethargic	50	51
11-0182	80 mg/kg-day[D]	Female	Prostrate	50	51
11-0182	80 mg/kg-day[D]	Female	Labored Breathing	50	51
11-0182	80 mg/kg-day[D]	Female	Laying on back	53	53
11-0182	80 mg/kg-day[D]	Female	Lethargic	54	55
11-0182	80 mg/kg-day[D]	Female	Prostrate	54	54
11-0182	80 mg/kg-day[D]	Female	Labored Breathing	54	55
11-0182	80 mg/kg-day[D]	Female	Laying on side	55	55
11-0182	80 mg/kg-day[D]	Female	Laying on back	56	57
11-0182	80 mg/kg-day[D]	Female	Dropping hind end when walking	56	57
11-0182	80 mg/kg-day[D]	Female	Labored Breathing	57	57
11-0182	80 mg/kg-day[D]	Female	Lethargic	57	57
11-0182	80 mg/kg-day[D]	Female	Hind end raised	58	58
11-0182	80 mg/kg-day[D]	Female	Legs stiff/ataxia	58	61
11-0182	80 mg/kg-day[D]	Female	Eyes protruding	58	76
11-0182	80 mg/kg-day[D]	Female	Tail curled	58	60
11-0182	80 mg/kg-day[D]	Female	Dropping hind end when walking	60	63
11-0182	80 mg/kg-day[D]	Female	Lethargic	60	60
11-0182	80 mg/kg-day[D]	Female	Laying on side	60	60
11-0182	80 mg/kg-day[D]	Female	Part of dose on chin	62	62
11-0182	80 mg/kg-day[D]	Female	Lethargic	63	64
11-0182	80 mg/kg-day[D]	Female	Laying on side	64	64
11-0182	80 mg/kg-day[D]	Female	Dropping hind end when walking	66	67
11-0182	80 mg/kg-day[D]	Female	Labored Breathing	67	67
11-0182	80 mg/kg-day[D]	Female	Laying on back	67	67
11-0182	80 mg/kg-day[D]	Female	Lethargic	67	68
11-0182	80 mg/kg-day[D]	Female	Laying on side	68	69
11-0182	80 mg/kg-day[D]	Female	Laying on back	70	71
11-0182	80 mg/kg-day[D]	Female	Lethargic	71	71
11-0182	80 mg/kg-day[D]	Female	Dropping hind end when walking	72	73
11-0182	80 mg/kg-day[D]	Female	Straubbed tail	73	74
11-0182	80 mg/kg-day[D]	Female	Laying on side	74	74
11-0182	80 mg/kg-day[D]	Female	Dropping hind end when walking	76	77
11-0182	80 mg/kg-day[D]	Female	Laying on side	76	76
11-0182	80 mg/kg-day[D]	Female	Tail Curled	77	77
11-0182	80 mg/kg-day[D]	Female	Creeping	78	78
11-0182	80 mg/kg-day[D]	Female	Eyes protruding	78	90
11-0182	80 mg/kg-day[D]	Female	Dropping hind end when walking	79	80
11-0182	80 mg/kg-day[D]	Female	Ears twitching	79	79
11-0182	80 mg/kg-day[D]	Female	Straubbed tail	81	81

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0182	80 mg/kg-day[D]	Female	Ears twitching	81	84
11-0182	80 mg/kg-day[D]	Female	Lethargic	81	84
11-0182	80 mg/kg-day[D]	Female	Labored Breathing	81	85
11-0182	80 mg/kg-day[D]	Female	Laying on side	81	81
11-0182	80 mg/kg-day[D]	Female	Dropping hind end when walking	82	83
11-0182	80 mg/kg-day[D]	Female	Laying on back	82	83
11-0182	80 mg/kg-day[D]	Female	Creeping	84	90
11-0182	80 mg/kg-day[D]	Female	Prostrate	84	84
11-0182	80 mg/kg-day[D]	Female	Hind end lowered	85	89
11-0182	80 mg/kg-day[D]	Female	Legs stiff	85	90
11-0182	80 mg/kg-day[D]	Female	Laying on side	85	85
11-0182	80 mg/kg-day[D]	Female	Straubbed tail	86	86
11-0182	80 mg/kg-day[D]	Female	Dropping hind end when walking	87	87
11-0182	80 mg/kg-day[D]	Female	Hind leg ataxia	88	89
11-0182	80 mg/kg-day[D]	Female	Bouncing while walking	88	90
11-0182	80 mg/kg-day[D]	Female	Prostrate	88	89
11-0182	80 mg/kg-day[D]	Female	Lethargic	88	89
11-0182	80 mg/kg-day[D]	Female	Labored Breathing	89	89
11-0186	80 mg/kg-day[D]	Female	Eating bedding	0	0
11-0186	80 mg/kg-day[D]	Female	Laying on back with legs out	1	1
11-0186	80 mg/kg-day[D]	Female	Dark Urine	1	72
11-0186	80 mg/kg-day[D]	Female	Laying on side	9	9
11-0186	80 mg/kg-day[D]	Female	Lethargic	9	9
11-0186	80 mg/kg-day[D]	Female	Labored Breathing	9	9
11-0186	80 mg/kg-day[D]	Female	Barbering	12	14
11-0186	80 mg/kg-day[D]	Female	Laying on side with legs out	12	13
11-0186	80 mg/kg-day[D]	Female	Lethargic	12	13
11-0186	80 mg/kg-day[D]	Female	Labored Breathing	12	13
11-0186	80 mg/kg-day[D]	Female	Laying on side	15	16
11-0186	80 mg/kg-day[D]	Female	Lethargic	16	16
11-0186	80 mg/kg-day[D]	Female	Labored Breathing	16	16
11-0186	80 mg/kg-day[D]	Female	Irregular Gait	17	17
11-0186	80 mg/kg-day[D]	Female	Laying on side	19	19
11-0186	80 mg/kg-day[D]	Female	Laying on side	21	21
11-0186	80 mg/kg-day[D]	Female	Labored Breathing	22	22
11-0186	80 mg/kg-day[D]	Female	Barbering	23	69
11-0186	80 mg/kg-day[D]	Female	Laying on side	27	27
11-0186	80 mg/kg-day[D]	Female	Walking on Toes	27	27
11-0186	80 mg/kg-day[D]	Female	Pulling legs up when walking	27	27
11-0186	80 mg/kg-day[D]	Female	Knock kneed	27	27
11-0186	80 mg/kg-day[D]	Female	Right hind limb ataxia	29	34
11-0186	80 mg/kg-day[D]	Female	Slight Hopping	29	30
11-0186	80 mg/kg-day[D]	Female	Hopping	31	31
11-0186	80 mg/kg-day[D]	Female	Walking on Toes	31	33
11-0186	80 mg/kg-day[D]	Female	Hind limbs stiff/locked	31	31
11-0186	80 mg/kg-day[D]	Female	Laying on side	34	34
11-0186	80 mg/kg-day[D]	Female	Laying on side	36	36
11-0186	80 mg/kg-day[D]	Female	Walking on Toes	37	90
11-0186	80 mg/kg-day[D]	Female	Pulling legs up when walking	37	42
11-0186	80 mg/kg-day[D]	Female	Hind limb ataxia	37	44
11-0186	80 mg/kg-day[D]	Female	Hind limbs stiff/locked	37	42
11-0186	80 mg/kg-day[D]	Female	Tail curled	41	58
11-0186	80 mg/kg-day[D]	Female	Hind end raised	43	75
11-0186	80 mg/kg-day[D]	Female	Unusual Gait	46	66
11-0186	80 mg/kg-day[D]	Female	Legs stiff	46	65
11-0186	80 mg/kg-day[D]	Female	Laying on side	56	57
11-0186	80 mg/kg-day[D]	Female	Dropping hind end when walking	64	64
11-0186	80 mg/kg-day[D]	Female	Laying on side	65	65
11-0186	80 mg/kg-day[D]	Female	Lethargic	65	65
11-0186	80 mg/kg-day[D]	Female	Laying on side	69	69
11-0186	80 mg/kg-day[D]	Female	Labored Breathing	69	69
11-0186	80 mg/kg-day[D]	Female	Legs stiff	69	75
11-0186	80 mg/kg-day[D]	Female	Dropping hind end when walking	70	70
11-0186	80 mg/kg-day[D]	Female	Kicking hind legs out while walking	75	75

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0186	80 mg/kg-day[D]	Female	Hind end raised	77	90
11-0186	80 mg/kg-day[D]	Female	Dropping hind end when walking	76	82
11-0186	80 mg/kg-day[D]	Female	Lethargic	79	80
11-0186	80 mg/kg-day[D]	Female	Labored Breathing	79	80
11-0186	80 mg/kg-day[D]	Female	Legs stiff	80	85
11-0186	80 mg/kg-day[D]	Female	Ears twitching	80	80
11-0186	80 mg/kg-day[D]	Female	Laying on side	82	83
11-0186	80 mg/kg-day[D]	Female	Creeping	83	83
11-0186	80 mg/kg-day[D]	Female	Lethargic	83	83
11-0186	80 mg/kg-day[D]	Female	Labored Breathing	83	83
11-0186	80 mg/kg-day[D]	Female	Kicking hind legs out while walking	84	90
11-0186	80 mg/kg-day[D]	Female	Tail curled	85	90
11-0186	80 mg/kg-day[D]	Female	Barbering	86	86
11-0186	80 mg/kg-day[D]	Female	Eyes protruding	86	86
11-0186	80 mg/kg-day[D]	Female	Laying on side	86	86
11-0186	80 mg/kg-day[D]	Female	Labored Breathing	86	86
11-0186	80 mg/kg-day[D]	Female	Dropping hind end when walking	87	90
11-0186	80 mg/kg-day[D]	Female	Legs stiff	87	90
11-0186	80 mg/kg-day[D]	Female	Labored Breathing	89	89
11-0188	80 mg/kg-day[D]	Female	Dark Urine	0	71
11-0188	80 mg/kg-day[D]	Female	Lethargic	0	1
11-0188	80 mg/kg-day[D]	Female	Laying on side	1	1
11-0188	80 mg/kg-day[D]	Female	Elevated Respiration Rate	1	1
11-0188	80 mg/kg-day[D]	Female	Lethargic	6	6
11-0188	80 mg/kg-day[D]	Female	Prostrate	6	6
11-0188	80 mg/kg-day[D]	Female	Lethargic	8	8
11-0188	80 mg/kg-day[D]	Female	Laying on side	8	8
11-0188	80 mg/kg-day[D]	Female	Labored Breathing	8	8
11-0188	80 mg/kg-day[D]	Female	Lethargic	11	12
11-0188	80 mg/kg-day[D]	Female	Prostrate	11	12
11-0188	80 mg/kg-day[D]	Female	Laying on side	12	12
11-0188	80 mg/kg-day[D]	Female	Labored Breathing	12	12
11-0188	80 mg/kg-day[D]	Female	Lethargic	14	14
11-0188	80 mg/kg-day[D]	Female	Prostrate	14	14
11-0188	80 mg/kg-day[D]	Female	Labored Breathing	19	19
11-0188	80 mg/kg-day[D]	Female	Prostrate	19	19
11-0188	80 mg/kg-day[D]	Female	Irregular Gait	23	25
11-0188	80 mg/kg-day[D]	Female	Walking on Toes	23	25
11-0188	80 mg/kg-day[D]	Female	Right hind limb ataxia	24	26
11-0188	80 mg/kg-day[D]	Female	Legs knock kneed	25	25
11-0188	80 mg/kg-day[D]	Female	High stepping	25	25
11-0188	80 mg/kg-day[D]	Female	Creeping	27	28
11-0188	80 mg/kg-day[D]	Female	Laying on side	33	33
11-0188	80 mg/kg-day[D]	Female	Laying on side	35	35
11-0188	80 mg/kg-day[D]	Female	Lethargic	36	36
11-0188	80 mg/kg-day[D]	Female	Lethargic	39	39
11-0188	80 mg/kg-day[D]	Female	Barbering	40	79
11-0188	80 mg/kg-day[D]	Female	Laying on side	40	40
11-0188	80 mg/kg-day[D]	Female	Creeping	41	41
11-0188	80 mg/kg-day[D]	Female	Prostrate	42	42
11-0188	80 mg/kg-day[D]	Female	Lethargic	42	42
11-0188	80 mg/kg-day[D]	Female	Dropping hind end when walking	43	43
11-0188	80 mg/kg-day[D]	Female	Low arousal	46	46
11-0188	80 mg/kg-day[D]	Female	Laying on side	48	48
11-0188	80 mg/kg-day[D]	Female	Unusual Gait	52	53
11-0188	80 mg/kg-day[D]	Female	Hind end raised	52	53
11-0188	80 mg/kg-day[D]	Female	Walking on Toes	52	53
11-0188	80 mg/kg-day[D]	Female	Laying on side	53	53
11-0188	80 mg/kg-day[D]	Female	Laying on side	55	56
11-0188	80 mg/kg-day[D]	Female	Hunched posture	58	59
11-0188	80 mg/kg-day[D]	Female	Squinting	58	59
11-0188	80 mg/kg-day[D]	Female	Ears back/twitching	58	79
11-0188	80 mg/kg-day[D]	Female	Laying on side	60	60
11-0188	80 mg/kg-day[D]	Female	Squinting	61	61

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0188	80 mg/kg-day[D]	Female	Tail twitching	61	61
11-0188	80 mg/kg-day[D]	Female	Hunched posture	62	64
11-0188	80 mg/kg-day[D]	Female	Lethargic	62	62
11-0188	80 mg/kg-day[D]	Female	Tail twitching	64	65
11-0188	80 mg/kg-day[D]	Female	Laying on side	64	64
11-0188	80 mg/kg-day[D]	Female	Lethargic	64	64
11-0188	80 mg/kg-day[D]	Female	Hunched posture	66	66
11-0188	80 mg/kg-day[D]	Female	Lethargic	67	67
11-0188	80 mg/kg-day[D]	Female	Tail twitching	67	69
11-0188	80 mg/kg-day[D]	Female	Squinting	68	68
11-0188	80 mg/kg-day[D]	Female	Hunched posture	68	70
11-0188	80 mg/kg-day[D]	Female	Labored Breathing	70	70
11-0188	80 mg/kg-day[D]	Female	Lethargic	70	71
11-0188	80 mg/kg-day[D]	Female	Prostrate	70	71
11-0188	80 mg/kg-day[D]	Female	Squinting	71	73
11-0188	80 mg/kg-day[D]	Female	Tail twitching	71	71
11-0188	80 mg/kg-day[D]	Female	Hunched posture	72	75
11-0188	80 mg/kg-day[D]	Female	Straubbed tail	73	74
11-0188	80 mg/kg-day[D]	Female	Squinting	76	76
11-0188	80 mg/kg-day[D]	Female	Hunched posture	77	77
11-0188	80 mg/kg-day[D]	Female	Squinting	78	78
11-0188	80 mg/kg-day[D]	Female	Leaning to the left	79	79
11-0188	80 mg/kg-day[D]	Female	Dropping hind end when walking	80	80
11-0188	80 mg/kg-day[D]	Female	Tail curled	80	80
11-0188	80 mg/kg-day[D]	Female	Hunched posture	81	81
11-0188	80 mg/kg-day[D]	Female	Ears twitching	81	84
11-0188	80 mg/kg-day[D]	Female	Tail twitching	81	81
11-0188	80 mg/kg-day[D]	Female	Lethargic	81	83
11-0188	80 mg/kg-day[D]	Female	Prostrate	81	81
11-0188	80 mg/kg-day[D]	Female	Labored Breathing	81	81
11-0188	80 mg/kg-day[D]	Female	Feet Splayed	82	82
11-0188	80 mg/kg-day[D]	Female	Laying on side	82	85
11-0188	80 mg/kg-day[D]	Female	Labored Breathing	83	83
11-0188	80 mg/kg-day[D]	Female	Straubbed tail	83	83
11-0188	80 mg/kg-day[D]	Female	Walking on Toes	83	83
11-0188	80 mg/kg-day[D]	Female	Kicking legs out while walking	83	83
11-0188	80 mg/kg-day[D]	Female	Creeping	84	85
11-0188	80 mg/kg-day[D]	Female	Hind legs stiff	84	85
11-0188	80 mg/kg-day[D]	Female	Tail curled	84	86
11-0188	80 mg/kg-day[D]	Female	Barbering	84	85
11-0188	80 mg/kg-day[D]	Female	Eyes protruding	86	88
11-0188	80 mg/kg-day[D]	Female	Dropping hind end when walking	86	87
11-0188	80 mg/kg-day[D]	Female	Barbering	87	90
11-0188	80 mg/kg-day[D]	Female	Ears twitching	87	90
11-0188	80 mg/kg-day[D]	Female	Hunched posture	88	89
11-0188	80 mg/kg-day[D]	Female	Squinting	88	89
11-0188	80 mg/kg-day[D]	Female	Creeping	90	90
11-0188	80 mg/kg-day[D]	Female	Hind legs stiff	90	90
11-0195	80 mg/kg-day[D]	Female	Lethargic	1	2
11-0195	80 mg/kg-day[D]	Female	Prostrate	2	2
11-0195	80 mg/kg-day[D]	Female	Dark Urine	5	72
11-0195	80 mg/kg-day[D]	Female	Lethargic	8	8
11-0195	80 mg/kg-day[D]	Female	Lethargic	13	13
11-0195	80 mg/kg-day[D]	Female	Lethargic	21	21
11-0195	80 mg/kg-day[D]	Female	Prostrate	21	21
11-0195	80 mg/kg-day[D]	Female	Prostrate	35	36
11-0195	80 mg/kg-day[D]	Female	Lethargic	36	36
11-0195	80 mg/kg-day[D]	Female	Laying on side	49	49
11-0195	80 mg/kg-day[D]	Female	Right hind leg ataxia	63	65
11-0195	80 mg/kg-day[D]	Female	Straubbed tail	63	63
11-0195	80 mg/kg-day[D]	Female	Hind end raised	71	72
11-0195	80 mg/kg-day[D]	Female	Lethargic	71	71
11-0195	80 mg/kg-day[D]	Female	Dropping hind end when walking	75	76
11-0195	80 mg/kg-day[D]	Female	Dropping hind end when walking	81	84

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0195	80 mg/kg-day[D]	Female	Laying on side	83	83
11-0195	80 mg/kg-day[D]	Female	Lethargic	83	83
11-0195	80 mg/kg-day[D]	Female	Laying on side	85	85
11-0195	80 mg/kg-day[D]	Female	Tail Curled	86	86
11-0195	80 mg/kg-day[D]	Female	Labored Breathing	86	86
11-0195	80 mg/kg-day[D]	Female	Dropping hind end when walking	88	90
11-0200	80 mg/kg-day[D]	Female	Labored Breathing	0	0
11-0200	80 mg/kg-day[D]	Female	Prostrate	0	0
11-0200	80 mg/kg-day[D]	Female	Dark Urine	1	72
11-0200	80 mg/kg-day[D]	Female	Lethargic	1	2
11-0200	80 mg/kg-day[D]	Female	Barbering	1	90
11-0200	80 mg/kg-day[D]	Female	Prostrate	2	2
11-0200	80 mg/kg-day[D]	Female	Lethargic	7	8
11-0200	80 mg/kg-day[D]	Female	Lethargic	13	14
11-0200	80 mg/kg-day[D]	Female	Walking on Toes	29	29
11-0200	80 mg/kg-day[D]	Female	Left hind limb ataxia/nearly dragging	29	29
11-0200	80 mg/kg-day[D]	Female	Tail curled	29	29
11-0200	80 mg/kg-day[D]	Female	Both hind limbs ataxia	30	41
11-0200	80 mg/kg-day[D]	Female	Hopping	30	30
11-0200	80 mg/kg-day[D]	Female	Pulling legs up when walking	30	41
11-0200	80 mg/kg-day[D]	Female	Hopping on toes	31	32
11-0200	80 mg/kg-day[D]	Female	Hind limbs stiff/locked	31	41
11-0200	80 mg/kg-day[D]	Female	Tail curled	33	45
11-0200	80 mg/kg-day[D]	Female	Walking on Toes	33	48
11-0200	80 mg/kg-day[D]	Female	Lethargic	36	37
11-0200	80 mg/kg-day[D]	Female	Hind end raised	43	47
11-0200	80 mg/kg-day[D]	Female	Dropping hind end when walking	46	46
11-0200	80 mg/kg-day[D]	Female	Unusual Gait	46	47
11-0200	80 mg/kg-day[D]	Female	Tail curled	49	75
11-0200	80 mg/kg-day[D]	Female	Hind end raised	50	52
11-0200	80 mg/kg-day[D]	Female	Hind end raised	55	58
11-0200	80 mg/kg-day[D]	Female	Walking on Toes	55	56
11-0200	80 mg/kg-day[D]	Female	Hind end raised	61	61
11-0200	80 mg/kg-day[D]	Female	Walking on Toes	63	63
11-0200	80 mg/kg-day[D]	Female	Hind end raised	63	63
11-0200	80 mg/kg-day[D]	Female	Hind end raised	69	70
11-0200	80 mg/kg-day[D]	Female	Ears twitching	73	73
11-0200	80 mg/kg-day[D]	Female	Tail twitching	75	75
11-0200	80 mg/kg-day[D]	Female	Ears twitching	75	75
11-0200	80 mg/kg-day[D]	Female	Laying on side	76	76
11-0200	80 mg/kg-day[D]	Female	Lethargic	76	76
11-0200	80 mg/kg-day[D]	Female	Tail curled	77	78
11-0200	80 mg/kg-day[D]	Female	Hind end raised	78	78
11-0200	80 mg/kg-day[D]	Female	Ears twitching	79	83
11-0200	80 mg/kg-day[D]	Female	Hunched posture	80	81
11-0200	80 mg/kg-day[D]	Female	Squinting	81	81
11-0200	80 mg/kg-day[D]	Female	Tail curled	82	82
11-0200	80 mg/kg-day[D]	Female	Hunched posture	83	83
11-0200	80 mg/kg-day[D]	Female	Walking on Toes	84	84
11-0200	80 mg/kg-day[D]	Female	Tail curled	84	87
11-0200	80 mg/kg-day[D]	Female	Hind end raised	84	84
11-0200	80 mg/kg-day[D]	Female	Squinting	88	88
11-0200	80 mg/kg-day[D]	Female	Ears pulled back	88	88
11-0200	80 mg/kg-day[D]	Female	Hind end raised	89	90
11-0200	80 mg/kg-day[D]	Female	Walking on Toes	89	90
11-0200	80 mg/kg-day[D]	Female	Straubbed tail	89	90
11-0201	80 mg/kg-day[D]	Female	Lethargic	1	1
11-0201	80 mg/kg-day[D]	Female	Dark Urine	1	73
11-0201	80 mg/kg-day[D]	Female	Lethargic	7	8
11-0201	80 mg/kg-day[D]	Female	Lethargic	12	14
11-0201	80 mg/kg-day[D]	Female	Lethargic	26	26
11-0201	80 mg/kg-day[D]	Female	Right hind limb ataxia	38	40
11-0201	80 mg/kg-day[D]	Female	Right hind limb stiff/dragging	38	40
11-0201	80 mg/kg-day[D]	Female	Right hind limb muscle locked	38	40

APPENDIX F
90-DAY CLINICAL OBSERVATIONS

Study No.: 85-XE-0DBP-11 **Protocol No.:** 0DBP-38-10-07-01
Chemical Substance: 2,4-Dinitroanisole
Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male
Concentration: 0.25 mg/ml[A], 1 mg/ml[B], 4 mg/ml[C], 16 mg/ml[D]
Diluent: corn oil

INDIVIDUAL ANIMAL EFFECTS

Animal No.	Dose Group	Sex	Clinical Sign	Day of First Appearance	Day of Last Appearance
11-0201	80 mg/kg-day[D]	Female	Hind limb ataxia	41	43
11-0201	80 mg/kg-day[D]	Female	Hind limbs stiff/locked	41	43
11-0201	80 mg/kg-day[D]	Female	Pulling legs up when walking	41	43
11-0201	80 mg/kg-day[D]	Female	Walking on Toes	41	43
11-0201	80 mg/kg-day[D]	Female	Prostrate	65	65
11-0201	80 mg/kg-day[D]	Female	Lethargic	65	65
11-0201	80 mg/kg-day[D]	Female	Prostrate	72	73
11-0201	80 mg/kg-day[D]	Female	Lethargic	72	73
11-0201	80 mg/kg-day[D]	Female	Hunched posture	76	76
11-0201	80 mg/kg-day[D]	Female	Hunched posture	80	80
11-0201	80 mg/kg-day[D]	Female	Ears twitching	81	84
11-0201	80 mg/kg-day[D]	Female	Hunched posture	82	82
11-0201	80 mg/kg-day[D]	Female	Laying on side	83	83
11-0201	80 mg/kg-day[D]	Female	Lethargic	83	84
11-0201	80 mg/kg-day[D]	Female	Hind end raised	87	87
11-0201	80 mg/kg-day[D]	Female	Walking on Toes	87	87
11-0201	80 mg/kg-day[D]	Female	Hunched posture	88	89

Appendix G

Individual and Summary of 14-Day Body Mass Data

Table G-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

14-Day Individual Body Mass (grams)
Male Rats

Group	Animal ID	Day 0	Day 1	Day 3	Day 7	Day 13
Corn Oil Control	10-1635	268	277	291	321	363
	10-1643	278	285	307	336	372
	10-1645	277	282	302	334	372
	10-1662	276	279	295	335	375
	10-1664	282	288	297	328	359
	10-1675	280	281	296	328	363
	Mean	**276.8**	**282.0**	**298.0**	**330.3**	**367.3**
	SD	**4.83**	**4.00**	**5.66**	**5.75**	**6.47**
1.56 mg/kg	10-1642	276	281	293	333	370
	10-1651	278	282	297	324	358
	10-1656	270	274	286	324	341
	10-1660	278	284	303	332	374
	10-1672	278	283	304	323	352
	10-1678	277	280	298	341	380
	Mean	**276.2**	**280.7**	**296.8**	**329.5**	**362.5**
	SD	**3.13**	**3.56**	**6.68**	**7.12**	**14.75**
3.13 mg/kg	10-1648	271	279	292	331	360
	10-1652	277	281	297	324	360
	10-1657	275	282	295	326	357
	10-1661	272	279	300	339	358
	10-1666	265	273	293	322	361
	10-1683	281	285	298	324	360
	Mean	**273.5**	**279.8**	**295.8**	**327.7**	**359.3**
	SD	**5.50**	**4.02**	**3.06**	**6.35**	**1.51**
6.25 mg/kg	10-1638	274	281	309	340	381
	10-1639	268	270	288	318	364
	10-1641	271	281	294	329	367
	10-1644	272	277	298	318	351
	10-1670	260	270	286	307	342
	10-1680	263	267	275	311	334
	Mean	**268.0**	**274.3**	**291.7**	**320.5**	**356.5**
	SD	**5.48**	**6.12**	**11.57**	**12.14**	**17.40**
12.5 mg/kg	10-1654	277	281	289	322	365
	10-1659	273	275	293	323	366
	10-1663	281	287	304	342	387
	10-1665	279	284	302	337	368
	10-1676	279	281	301	335	375
	10-1681	274	285	297	330	374
	Mean	**277.2**	**282.2**	**297.7**	**331.5**	**372.5**
	SD	**3.13**	**4.22**	**5.79**	**7.97**	**8.22**
25 mg/kg	10-1636	264	267	289	315	348
	10-1640	267	269	287	319	351
	10-1658	273	275	286	313	344
	10-1671	276	279	300	336	361
	10-1673	278	291	309	344	377
	10-1677	271	267	276	308	349
	Mean	**271.5**	**274.7**	**291.2**	**322.5**	**355.0**
	SD	**5.32**	**9.33**	**11.62**	**14.24**	**12.18**
50 mg/kg	10-1650	284	281	296	321	353
	10-1653	269	269	280	307	328
	10-1668	274	264	280	303	339
	10-1669	278	269	280	312	349
	10-1679	272	277	291	312	344
	10-1684	285	284	297	324	364
	Mean	**277.0**	**274.0**	**287.3**	**313.2**	**346.2**
	SD	**6.51**	**7.85**	**8.29**	**8.04**	**12.32**
100 mg/kg	10-1637	267	266	275	309	332
	10-1646	278	278	286	303	332
	10-1647	275	273	284	305	342
	10-1649	272	268	273	298	322
	10-1655	270	257	260	295	337
	10-1674	272	273	284	311	344
	Mean	**272.3**	**269.2**	**277.0**	**303.5**	**334.8**
	SD	**3.83**	**7.31**	**9.88**	**6.19**	**8.01**

Table G-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

14-Day Individual Body Mass (grams)
Female Rats

Group	Animal ID	Day 0	Day 1	Day 3	Day 7	Day 13
	10-1693	178	183	190	199	206
Corn Oil	10-1697	194	194	208	225	239
Control	10-1703	209	213	222	231	233
	10-1713	192	199	210	213	224
	10-1718	197	202	216	225	235
	10-1722	214	212	222	241	255
	Mean	**197.3**	**200.5**	**211.3**	**222.3**	**232.0**
	SD	**12.86**	**11.33**	**11.98**	**14.62**	**16.30**
	10-1685	202	196	206	224	226
1.56 mg/kg	10-1690	209	207	215	221	240
	10-1692	207	212	221	225	244
	10-1700	205	210	219	228	248
	10-1701	207	210	223	235	241
	10-1709	197	201	213	228	228
	Mean	**204.5**	**206.0**	**216.2**	**226.8**	**237.8**
	SD	**4.37**	**6.23**	**6.21**	**4.79**	**8.86**
	10-1695	205	204	219	230	240
3.13 mg/kg	10-1699	201	206	207	222	236
	10-1705	204	217	223	235	243
	10-1714	204	210	217	220	232
	10-1719	203	201	213	220	238
	10-1723	197	197	210	216	231
	Mean	**202.3**	**205.8**	**214.8**	**223.8**	**236.7**
	SD	**2.94**	**7.03**	**5.95**	**7.17**	**4.63**
	10-1702	200	202	209	218	235
6.25 mg/kg	10-1707	219	213	230	240	255
	10-1711	197	205	212	221	228
	10-1715	202	203	216	223	242
	10-1729	197	195	211	227	243
	10-1730	208	214	216	222	244
	Mean	**203.8**	**205.3**	**215.7**	**225.2**	**241.2**
	SD	**8.47**	**7.17**	**7.55**	**7.83**	**9.11**
	10-1687	194	194	201	210	227
12.5 mg/kg	10-1694	195	200	208	221	232
	10-1725	196	206	209	214	223
	10-1726	207	207	217	220	247
	10-1733	199	199	214	228	230
	10-1734	203	207	213	232	246
	Mean	**199.0**	**202.2**	**210.3**	**220.8**	**234.2**
	SD	**5.10**	**5.34**	**5.65**	**8.26**	**10.03**
	10-1691	219	211	217	224	234
25 mg/kg	10-1704	205	205	201	219	237
	10-1708	189	195	196	205	214
	10-1720	198	191	199	206	228
	10-1727	204	204	210	223	238
	10-1732	202	197	214	215	238
	Mean	**202.8**	**200.5**	**206.2**	**215.3**	**231.5**
	SD	**9.83**	**7.42**	**8.66**	**8.26**	**9.38**
	10-1688	193	193	207	227	243
50 mg/kg	10-1706	201	195	206	223	241
	10-1710	203	195	216	227	239
	10-1716	197	189	187	200	219
	10-1717	189	193	209	215	224
	10-1731	216	209	217	228	249
	Mean	**199.8**	**195.7**	**207.0**	**220.0**	**235.8**
	SD	**9.43**	**6.89**	**10.83**	**10.92**	**11.70**
	10-1686	197	188	195	200	224
100 mg/kg	10-1689	196	198	204	214	225
	10-1696	193	198	201	215	219
	10-1712	200	192	197	205	233
	10-1724	204	199	203	212	224
	10-1728	207	215	236	236	238
	Mean	**199.5**	**198.3**	**206.0**	**213.7**	**227.2**
	SD	**5.24**	**9.22**	**15.10**	**12.37**	**6.97**

Table G-2
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of Body Mass (grams)
Male Rats

Period		Corn Oil Control	2,4-dinitroanisole (DNAN) 1.56 mg/kg	3.13 mg/kg	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Day 0	Mean	276.8	276.2	273.5	268.0	277.2	271.5	277.0	272.3
	SD	4.83	3.13	5.50	5.48	3.13	5.32	6.51	3.83
	N	6	6	6	6	6	6	6	6
Day 1	Mean	282.0	280.7	279.8	274.3	282.2	274.7	274.0	269.2*
	SD	4.00	3.56	4.02	6.12	4.22	9.33	7.85	7.31
	N	6	6	6	6	6	6	6	6
Day 3	Mean	298.0	296.8	295.8	291.7	297.7	291.2	287.3	277.0*
	SD	5.66	6.68	3.06	11.57	5.79	11.62	8.29	9.88
	N	6	6	6	6	6	6	6	6
Day 7	Mean	330.3	329.5	327.7	320.5	331.5	322.5	313.2*	303.5*
	SD	5.75	7.12	6.35	12.14	7.97	14.24	8.04	6.19
	N	6	6	6	6	6	6	6	6
Day 13	Mean	367.3	362.5	359.3	356.5	372.5	355.0	346.2*	334.8*
	SD	6.47	14.75	1.51	17.40	8.22	12.18	12.32	8.01
	N	6	6	6	6	6	6	6	6

*Significantly different from corn oil control

Table G-2
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of Body Mass (grams)
Female Rats

Period		Corn Oil Control	2,4-dinitroanisole (DNAN) 1.56 mg/kg	3.13 mg/kg	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Day 0	Mean	197.3	204.5	202.3	203.8	199.0	202.8	199.8	199.5
	SD	12.86	4.37	2.94	8.47	5.10	9.83	9.43	5.24
	N	6	6	6	6	6	6	6	6
Day 1	Mean	200.5	206.0	205.8	205.3	202.2	200.5	195.7	198.3
	SD	11.33	6.23	7.03	7.17	5.34	7.42	6.89	9.22
	N	6	6	6	6	6	6	6	6
Day 3	Mean	211.3	216.2	214.8	215.7	210.3	206.2	207.0	206.0
	SD	11.98	6.21	5.95	7.55	5.65	8.66	10.83	15.10
	N	6	6	6	6	6	6	6	6
Day 7	Mean	222.3	226.8	223.8	225.2	220.8	215.3	220.0	213.7
	SD	14.62	4.79	7.17	7.83	8.26	8.26	10.92	12.37
	N	6	6	6	6	6	6	6	6
Day 13	Mean	232.0	237.8	236.7	241.2	234.2	231.5	235.8	227.2
	SD	16.30	8.86	4.63	9.11	10.03	9.38	11.70	6.97
	N	6	6	6	6	6	6	6	6

*Significantly different from corn oil control

Appendix H

Individual and Summary of 14-Day Body Mass Gain Data

Table H-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

14-Day Individual Body Mass Gain (grams)
Male Rats

Group	Animal ID	Day 0-1	Day 1-3	Day 3-7	Day 7-13	Net
	10-1635	9	14	30	42	95
Corn Oil	10-1643	7	22	29	36	94
Control	10-1645	5	20	32	38	95
	10-1662	3	16	40	40	99
	10-1664	6	9	31	31	77
	10-1675	1	15	32	35	83
	Mean	**5.2**	**16.0**	**32.3**	**37.0**	**90.5**
	SD	**2.86**	**4.60**	**3.93**	**3.90**	**8.53**
	10-1642	5	12	40	37	94
1.56 mg/kg	10-1651	4	15	27	34	80
	10-1656	4	12	38	17	71
	10-1660	6	19	29	42	96
	10-1672	5	21	19	29	74
	10-1678	3	18	43	39	103
	Mean	**4.5**	**16.2**	**32.7**	**33.0**	**86.3**
	SD	**1.05**	**3.76**	**9.18**	**9.01**	**13.09**
	10-1648	8	13	39	29	89
3.13 mg/kg	10-1652	4	16	27	36	83
	10-1657	7	13	31	31	82
	10-1661	7	21	39	19	86
	10-1666	8	20	29	39	96
	10-1683	4	13	26	36	79
	Mean	**6.3**	**16.0**	**31.8**	**31.7**	**85.8**
	SD	**1.86**	**3.69**	**5.81**	**7.20**	**6.05**
	10-1638	7	28	31	41	107
6.25 mg/kg	10-1639	2	18	30	46	96
	10-1641	10	13	35	38	96
	10-1644	5	21	20	33	79
	10-1670	10	16	21	35	82
	10-1680	4	8	36	23	71
	Mean	**6.3**	**17.3**	**28.8**	**36.0**	**88.5**
	SD	**3.27**	**6.86**	**6.85**	**7.85**	**13.37**
	10-1654	4	8	33	43	88
12.5 mg/kg	10-1659	2	18	30	43	93
	10-1663	6	17	38	45	106
	10-1665	5	18	35	31	89
	10-1676	2	20	34	40	96
	10-1681	11	12	33	44	100
	Mean	**5.0**	**15.5**	**33.8**	**41.0**	**95.3**
	SD	**3.35**	**4.55**	**2.64**	**5.18**	**6.86**
	10-1636	3	22	26	33	84
25 mg/kg	10-1640	2	18	32	32	84
	10-1658	2	11	27	31	71
	10-1671	3	21	36	25	85
	10-1673	13	18	35	33	99
	10-1677	-4	9	32	41	78
	Mean	**3.2**	**16.5**	**31.3**	**32.5**	**83.5**
	SD	**5.49**	**5.32**	**4.08**	**5.13**	**9.27**
	10-1650	-3	15	25	32	69
50 mg/kg	10-1653	0	11	27	21	59
	10-1668	-10	16	23	36	65
	10-1669	-9	11	32	37	71
	10-1679	5	14	21	32	72
	10-1684	-1	13	27	40	79
	Mean	**-3.0**	**13.3**	**25.8**	**33.0**	**69.2**
	SD	**5.69**	**2.07**	**3.82**	**6.63**	**6.77**
	10-1637	-1	9	34	23	65
100 mg/kg	10-1646	0	8	17	29	54
	10-1647	-2	11	21	37	67
	10-1649	-4	5	25	24	50
	10-1655	-13	3	35	42	67
	10-1674	1	11	27	33	72
	Mean	**-3.2**	**7.8**	**26.5**	**31.3**	**62.5**
	SD	**5.12**	**3.25**	**7.09**	**7.45**	**8.55**

Table H-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

14-Day Individual Body Mass Gain (grams)
Female Rats

Group	Animal ID	Day 0-1	Day 1-3	Day 3-7	Day 7-13	Net
	10-1693	5	7	9	7	28
Corn Oil	10-1697	0	14	17	14	45
Control	10-1703	4	9	9	2	24
	10-1713	7	11	3	11	32
	10-1718	5	14	9	10	38
	10-1722	-2	10	19	14	41
	Mean	3.2	10.8	11.0	9.7	34.7
	SD	3.43	2.79	5.93	4.59	8.04
	10-1685	-6	10	18	2	24
1.56 mg/kg	10-1690	-2	8	6	19	31
	10-1692	5	9	4	19	37
	10-1700	5	9	9	20	43
	10-1701	3	13	12	6	34
	10-1709	4	12	15	0	31
	Mean	1.5	10.2	10.7	11.0	33.3
	SD	4.51	1.94	5.35	9.34	6.41
	10-1695	-1	15	11	10	35
3.13 mg/kg	10-1699	5	1	15	14	35
	10-1705	13	6	12	8	39
	10-1714	6	7	3	12	28
	10-1719	-2	12	7	18	35
	10-1723	0	13	6	15	34
	Mean	3.5	9.0	9.0	12.8	34.3
	SD	5.68	5.25	4.43	3.60	3.56
	10-1702	2	7	9	17	35
6.25 mg/kg	10-1707	-6	17	10	15	36
	10-1711	8	7	9	7	31
	10-1715	1	13	7	19	40
	10-1729	-2	16	16	16	46
	10-1730	6	2	6	22	36
	Mean	1.5	10.3	9.5	16.0	37.3
	SD	5.13	5.92	3.51	5.06	5.13
	10-1687	0	7	9	17	33
12.5 mg/kg	10-1694	5	8	13	11	37
	10-1725	10	3	5	9	27
	10-1726	0	10	3	27	40
	10-1733	0	15	14	2	31
	10-1734	4	6	19	14	43
	Mean	3.2	8.2	10.5	13.3	35.2
	SD	4.02	4.07	5.99	8.41	5.95
	10-1691	-8	6	7	10	15
25 mg/kg	10-1704	0	-4	18	18	32
	10-1708	6	1	9	9	25
	10-1720	-7	8	7	22	30
	10-1727	0	6	13	15	34
	10-1732	-5	17	1	23	36
	Mean	-2.3	5.7	9.2	16.2	28.7
	SD	5.32	7.06	5.81	5.91	7.69
	10-1688	0	14	20	16	50
50 mg/kg	10-1706	-6	11	17	18	40
	10-1710	-8	21	11	12	36
	10-1716	-8	-2	13	19	22
	10-1717	4	16	6	9	35
	10-1731	-7	8	11	21	33
	Mean	-4.2	11.3	13.0	15.8	36.0
	SD	5.00	7.89	4.94	4.54	9.14
	10-1686	-9	7	5	24	27
100 mg/kg	10-1689	2	6	10	11	29
	10-1696	5	3	14	4	26
	10-1712	-8	5	8	28	33
	10-1724	-5	4	9	12	20
	10-1728	8	21	0	2	31
	Mean	-1.2	7.7	7.7	13.5	27.7
	SD	7.14	6.68	4.76	10.50	4.55

Appendix I

Individual and Summary of 14-Day Food Consumption Data

Table I-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

14-Day Individual Food Consumption (grams)
Male Rats

Group	Animal ID	Days 0-7	Days 7-13	Total
Corn Oil Control	10-1635	179.1	167.0	346.1
	10-1643	196.4	166.3	362.7
	10-1645	188.0	170.0	358.0
	10-1662	199.2	188.6	387.8
	10-1664	184.3	165.0	349.3
	10-1675	181.4	171.8	353.2
	Mean	**188.1**	**171.5**	**359.5**
	SD	**8.15**	**8.77**	**15.08**
1.56 mg/kg	10-1642	194.9	171.0	365.9
	10-1651	182.7	161.7	344.4
	10-1656	179.0	156.7	335.7
	10-1660	212.6	195.8	408.4
	10-1672	191.4	165.5	356.9
	10-1678	188.5	176.9	365.4
	Mean	**191.5**	**171.3**	**362.8**
	SD	**11.83**	**13.93**	**25.31**
3.13 mg/kg	10-1648	191.4	160.1	351.5
	10-1652	200.0	166.5	366.5
	10-1657	213.2	182.2	395.4
	10-1661	199.5	173.4	372.9
	10-1666	177.7	154.5	332.2
	10-1683	194.4	178.8	373.2
	Mean	**196.0**	**169.3**	**365.3**
	SD	**11.68**	**10.82**	**21.51**
6.25 mg/kg	10-1638	209.0	193.3	402.3
	10-1639	185.2	168.2	353.4
	10-1641	196.1	175.4	371.5
	10-1644	175.2	159.6	334.8
	10-1670	186.5	159.6	346.1
	10-1680	171.4	156.0	327.4
	Mean	**187.2**	**168.7**	**355.9**
	SD	**13.80**	**13.98**	**27.41**
12.5 mg/kg	10-1654	171.2	166.9	338.1
	10-1659	199.4	185.8	385.2
	10-1663	197.9	195.6	393.5
	10-1665	206.6	190.4	397.0
	10-1676	188.3	176.8	365.1
	10-1681	186.3	171.1	357.4
	Mean	**191.6**	**181.1**	**372.7**
	SD	**12.50**	**11.30**	**23.10**
25 mg/kg	10-1636	181.8	160.8	342.6
	10-1640	194.1	184.4	378.5
	10-1658	184.4	172.2	356.6
	10-1671	195.6	181.1	376.7
	10-1673	212.0	187.9	399.9
	10-1677	165.3	180.3	345.6
	Mean	**188.9**	**177.8**	**366.7**
	SD	**15.70**	**9.83**	**22.22**
50 mg/kg	10-1650	172.5	170.1	342.6
	10-1653	178.0	170.3	348.3
	10-1668	ND	175.3	ND
	10-1669	171.2	189.7	360.9
	10-1679	184.6	176.0	360.6
	10-1684	199.9	206.7	406.6
	Mean	**181.2**	**181.4**	**363.8**
	SD	**11.70**	**14.33**	**25.20**
100 mg/kg	10-1637	189.9	181.5	371.4
	10-1646	169.5	193.9	363.4
	10-1647	175.7	189.8	365.5
	10-1649	158.9	173.2	332.1
	10-1655	158.1	191.4	349.5
	10-1674	188.4	197.0	385.4
	Mean	**170.1**	**189.1**	**359.2**
	SD	**13.87**	**8.85**	**18.42**

Table I-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

14-Day Individual Food Consumption (grams)
Female Rats

Group	Animal ID	Days 0-7	Days 7-13	Total
Corn Oil Control	10-1693	119.8	100.6	220.4
	10-1697	136.8	120.3	257.1
	10-1703	141.7	114.3	256.0
	10-1713	142.1	112.5	254.6
	10-1718	139.3	109.8	249.1
	10-1722	137.6	117.5	255.1
	Mean	**136.2**	**112.5**	**248.7**
	SD	**8.32**	**6.90**	**14.15**
1.56 mg/kg	10-1685	136.3	103.2	239.5
	10-1690	131.0	118.1	249.1
	10-1692	136.7	122.0	258.7
	10-1700	132.3	124.9	257.2
	10-1701	145.1	111.1	256.2
	10-1709	142.8	111.1	253.9
	Mean	**137.4**	**115.1**	**252.4**
	SD	**5.61**	**8.08**	**7.17**
3.13 mg/kg	10-1695	136.2	117.5	253.7
	10-1699	136.9	114.5	251.4
	10-1705	155.1	116.0	271.1
	10-1714	139.6	113.0	252.6
	10-1719	133.5	112.5	246.0
	10-1723	137.2	117.2	254.4
	Mean	**139.8**	**115.1**	**254.9**
	SD	**7.77**	**2.12**	**8.50**
6.25 mg/kg	10-1702	133.4	108.3	241.7
	10-1707	133.2	122.5	255.7
	10-1711	133.4	113.9	247.3
	10-1715	139.6	121.1	260.7
	10-1729	148.0	122.2	270.2
	10-1730	137.8	125.8	263.6
	Mean	**137.6**	**119.0**	**256.5**
	SD	**5.78**	**6.54**	**10.58**
12.5 mg/kg	10-1687	122.2	115.1	237.3
	10-1694	146.4	120.4	266.8
	10-1725	119.8	112.2	232.0
	10-1726	152.0	136.4	288.4
	10-1733	135.3	112.4	247.7
	10-1734	150.1	124.0	274.1
	Mean	**137.6**	**120.1**	**257.7**
	SD	**14.14**	**9.25**	**22.23**
25 mg/kg	10-1691	124.2	112.1	236.3
	10-1704	129.4	115.7	245.1
	10-1708	123.3	109.3	232.6
	10-1720	127.1	118.2	245.3
	10-1727	129.3	116.1	245.4
	10-1732	136.4	120.9	257.3
	Mean	**128.3**	**115.4**	**243.7**
	SD	**4.71**	**4.17**	**8.61**
50 mg/kg	10-1688	136.2	122.3	258.5
	10-1706	128.8	108.6	237.4
	10-1710	133.9	118.8	252.7
	10-1716	107.7	107.1	214.8
	10-1717	126.1	110.3	236.4
	10-1731	134.5	120.1	254.6
	Mean	**127.9**	**114.5**	**242.4**
	SD	**10.59**	**6.60**	**16.35**
100 mg/kg	10-1686	107.0	108.5	215.5
	10-1689	113.0	113.1	226.1
	10-1696	123.4	110.9	234.3
	10-1712	119.3	129.5	248.8
	10-1724	ND	131.1	ND
	10-1728	157.3	119.9	277.2
	Mean	**124.0**	**118.8**	**240.4**
	SD	**19.63**	**9.67**	**23.91**

Table I-2
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of Food Consumption (grams)
Male Rats

Period		Corn Oil Control	2,4-dinitroanisole (DNAN) 1.56 mg/kg	3.13 mg/kg	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Days 0-7	Mean	188.1	191.5	196.0	187.2	191.6	188.9	181.2	170.1
	SD	8.15	11.83	11.68	13.80	12.50	15.70	11.70	13.87
	N	6	6	6	6	6	6	5	6
Days 7-14	Mean	171.5	171.3	169.3	168.7	181.1	177.8	181.4	189.1
	SD	8.77	13.93	10.82	13.98	11.30	9.83	14.33	8.85
	N	6	6	6	6	6	6	6	6
Net	Mean	359.5	362.8	365.3	355.9	372.7	366.7	363.8	359.2
	SD	15.08	25.31	21.51	27.41	23.10	22.22	25.20	18.42
	N	6	6	6	6	6	6	5	6

*Significantly different from corn oil control

Table I-2
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of Food Consumption (grams)
Female Rats

Period		Corn Oil Control	2,4-dinitroanisole (DNAN) 1.56 mg/kg	3.13 mg/kg	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Days 0-7	Mean	136.2	137.4	139.8	137.6	137.6	128.3	127.9	124.0
	SD	8.32	5.61	7.77	5.78	14.14	4.71	10.59	19.63
	N	6	6	6	6	6	6	6	5
Days 7-14	Mean	112.5	115.1	115.1	119.0	120.1	115.4	114.5	118.8
	SD	6.90	8.08	2.12	6.54	9.25	4.17	6.60	9.67
	N	6	6	6	6	6	6	6	6
Net	Mean	248.7	252.4	254.9	256.5	257.7	243.7	242.4	240.4
	SD	14.15	7.17	8.50	10.58	22.23	8.61	16.35	23.91
	N	6	6	6	6	6	6	6	5

*Significantly different from corn oil control

Appendix J

Individual and Summary of 90-Day Body Mass Data

Table J-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

90-Day Individual Body Mass (grams)
Male Rats

		Day													
Group	Animal ID	0	7	14	21	28	35	42	49	56	63	70	77	84[1]	90[2]
Corn Oil	11-0097	302	348	399	448	473	509	526	550	566	593				
Control	11-0105	300	345	394	429	461	490	525	558	586	614	630	656	664	665
	11-0107	290	327	363	385	410	438	460	482	502	526	527	546	549	555
	11-0108	294	331	363	399	418	446	468	486	500	515	522	536	527	542
	11-0112	310	368	413	461	497	529	557	584	606	638	650	666	667	687
	11-0116	300	343	385	417	442	462	496	518	516	555	576	591	597	605
	11-0118	286	329	360	395	415	449	470	496	511	529	538	560	558	570
	11-0140	300	332	363	396	421	442	466	498	522	544	560	578	580	593
	11-0147	283	324	353	385	397	414	438	455	475	488	489	496	499	508
	11-0150	314	374	423	463	498	532	569	594	605	625	652	680	687	690
	Mean	**297.9**	**342.1**	**381.6**	**417.8**	**443.2**	**471.1**	**497.5**	**522.1**	**538.9**	**562.7**	**571.6**	**589.9**	**592.0**	**601.7**
	SD	**9.87**	**17.27**	**24.70**	**30.64**	**36.91**	**41.18**	**44.46**	**46.86**	**47.58**	**51.48**	**59.77**	**64.19**	**66.97**	**65.86**
1.25 mg/kg	11-0095	297	340	379	413	441	467	498	518	526	542	560	575	581	587
	11-0103	310	373	418	463	499	532	562	606	624	639	665	682	652	699
	11-0104	299	338	375	408	435	458	479	497	517	538	547	567	558	557
	11-0113	313	363	409	445	475	512	548	566	590	608	622	642	658	654
	11-0114	297	347	387	422	450	488	512	539	560	589	606	626	633	647
	11-0122	303	345	384	419	455	467	491	515	545	562	580	597	602	618
	11-0126	290	334	375	405	427	454	478	500	523	532	545	557	558	566
	11-0134	295	339	380	409	433	466	491	522	540	566	580	591	573	595
	11-0142	266	293	321	341	358	377	382	398	407	414	427	433	416	436
	11-0145	302	334	370	402	426	441	461	486	497	520	528	540	541	555
	Mean	**297.2**	**340.6**	**379.8**	**412.7**	**439.9**	**466.2**	**490.2**	**514.7**	**532.9**	**551.0**	**566.0**	**581.0**	**577.2**	**591.4**
	SD	**12.93**	**21.01**	**25.78**	**31.71**	**36.81**	**41.78**	**49.29**	**54.36**	**57.76**	**60.67**	**63.71**	**67.24**	**69.86**	**71.98**
5mg/kg	11-0100	277	311	340	366	397	423	447	469	486	504	523	538	545	550
	11-0101	306	374	423	475	518	549	586	614	640	665	683	703	714	716
	11-0115	293	340	383	409	435	464	494	518	544	568	585	589	599	608
	11-0117	320	374	419	458	497	524	548	570	588	612	626	653	660	660
	11-0124	298	324	348	377	404	411	426	440	455	466	468	484	494	502
	11-0131	293	332	367	397	420	443	467	482	497	518	530	548	546	560
	11-0135	291	337	366	400	426	448	471	495	505	518	532	539	549	546
	11-0138	294	340	378	402	430	442	472	488	503	522	534	552	552	554
	11-0141	301	345	384	422	448	465	492	512	530	548	570	589	596	595
	11-0146	295	341	379	415	452	479	500	525	539	550	566	576	574	589
	Mean	**296.8**	**341.8**	**378.7**	**412.1**	**442.7**	**464.8**	**490.3**	**511.3**	**528.7**	**547.1**	**561.7**	**577.1**	**582.9**	**588.0**
	SD	**11.09**	**19.65**	**26.61**	**33.35**	**38.49**	**43.10**	**46.97**	**50.29**	**53.47**	**57.07**	**60.01**	**62.38**	**63.64**	**61.97**
20 mg/kg	11-0106	298	334	365	390	401	417	428	437	448	463	475	484	485	494
	11-0120	308	364	405	447	475	499	527	553	569	588	607	624	626	632
	11-0121	272	290	317	347	370	397	414	434	457	485	499	507	511	521
	11-0125	300	350	393	427	460	482	506	527	544	558	576	595	587	605
	11-0127	307	354	387	420	440	470	500	530	552	580	590	610	618	630
	11-0130	291	341	386	425	464	503	527	550	566	582	600	614	615	622
	11-0133	307	350	393	422	458	480	508	530	536	562	578	590	596	597
	11-0137	286	320	348	372	393	413	432	448	455	467	473	481	477	484
	11-0139	296	347	383	418	438	467	487	512	534	550	572	583	592	594
	11-0148	317	360	415	458	500	528	562	596	619	638	650	672	672	681
	Mean	**298.2**	**341.0**	**379.2**	**412.6**	**439.9**	**465.6**	**489.1**	**511.7**	**528.0**	**547.3**	**562.0**	**576.0**	**577.9**	**586.0**
	SD	**12.91**	**21.98**	**28.86**	**33.73**	**40.50**	**43.13**	**48.97**	**54.63**	**56.87**	**57.67**	**59.64**	**64.10**	**65.02**	**64.99**
80 mg/kg	11-0099	303	335	366	393	396	416	434	452						
	11-0102	281	305	327	346	330	352	379	378	394	400	406	418	406	412
	11-0109	300	328	353	369	380	389	391	407	410	420	425	435	420	434
	11-0110	285	301	337	351	353	367	379	400	401	400	404	406	401	419
	11-0111	298	311	336	354	360	378	381	391	402	395	410	414	408	423
	11-0123	305	329	358	375	380	389	404	416	420	425	436	434	431	447
	11-0129	306	325	367	374	391	398	408	419	431	435	453	457	447	460
	11-0132	285	302	317	324	310	334	353	364	363	368	372	375	359	377
	11-0144	301	327	373	404	417	434	449	460	473	480				
	11-0149	292	314	340	358	364	378	395	408	418	432	435	433		
	Mean	**295.6**	**317.7**	**347.4**	**364.8**	**368.1**	**383.5**	**397.3**	**409.5**	**412.4**	**417.2**	**417.6**	**421.5**	**410.3**	**424.6**
	SD	**9.17**	**12.55**	**18.75**	**23.36**	**31.82**	**29.16**	**28.13**	**29.80**	**29.85**	**31.73**	**25.09**	**24.50**	**27.72**	**26.78**

Table J-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

90-Day Individual Body Weights (grams)
Female Rats

		Day													
Group	Animal ID	0	7	14	21	28	35	42	49	56	63	70	77	84[1]	90[2]
Corn Oil	11-0154	221	233	242	245	259	270	282	278	289	295	294	304	299	302
Control	11-0162	211	225	234	236	248	253	265	256	272	269	281	279	288	279
	11-0168	219	239	243	260	270	284	290	300	310	313	315	322	316	325
	11-0171	205	219	220	235	244	252	260	256	273	272	276	279	284	284
	11-0173	203	217	223	232	240	246	253	253	259	270	272	271	278	275
	11-0175	220	231	229	233	244	248	243	254	260	264	262	272	263	271
	11-0190	230	242	237	254	257	264	268	275	275	283	284	292	294	292
	11-0191	216	224	236	245	253	265	280	279	283	290	290	292	298	293
	11-0197	208	219	238	254	252	273	288	292	290	300	310	313	306	310
	11-0206	207	230	228	232	235	244	250	257	254	260	268	273	266	273
	Mean	**214.0**	**227.9**	**233.0**	**242.6**	**250.2**	**259.9**	**267.9**	**270.0**	**276.5**	**281.6**	**285.2**	**289.7**	**289.2**	**290.4**
	SD	**8.60**	**8.58**	**7.76**	**10.50**	**10.24**	**13.31**	**16.57**	**17.19**	**17.07**	**17.44**	**17.37**	**18.17**	**16.92**	**17.68**
1.25 mg/kg	11-0152	210	223	244	244	258	260	265	277	282	278	286	292	295	298
	11-0159	239	252	266	284	296	295	315	323	326	321	334	336	330	343
	11-0165	206	208	222	232	248	254	262	259	275	281	283	280	285	286
	11-0170	212	217	219	228	239	244	240	250	264	265	262	272	278	275
	11-0172	205	226	237	244	246	255	267	266	264	270	283	285	280	283
	11-0176	224	243	251	266	272	283	293	300	308	318	323	332	315	323
	11-0178	225	230	232	240	241	260	268	259	274	278	288	277	274	282
	11-0181	211	220	234	244	249	251	265	272	274	277	284	296	290	295
	11-0192	216	211	229	234	245	257	260	265	266	268	277	280	282	285
	11-0196	200	214	226	233	244	249	256	269	272	276	277	282	286	293
	Mean	**214.8**	**224.4**	**236.0**	**244.9**	**253.8**	**260.8**	**269.1**	**274.0**	**280.5**	**283.2**	**289.7**	**293.2**	**291.5**	**296.3**
	SD	**11.61**	**14.04**	**14.31**	**17.36**	**17.65**	**15.90**	**20.73**	**21.82**	**20.47**	**19.79**	**21.86**	**22.61**	**17.74**	**21.02**
5mg/kg	11-0156	218	232	244	250	263	271	279	280	290	293	300	300	303	
	11-0157	213	240	242	238	255	252	263	266	278	283	276	283	273	278
	11-0163	197	220	240	249	250	260	276	275	277	280	293	295	287	295
	11-0166	210	230	244	258	260	276	292	300	303	315	324	332	320	324
	11-0174	226	250	248	255	278	290	288	300	310	316	312	326	314	326
	11-0187	217	224	238	242	248	258	262	263	273	277	282	278	284	287
	11-0189	213	222	237	247	258	264	275	286	293	297	313	323	323	324
	11-0193	231	243	258	258	280	288	301	295	309	315	325	326	329	332
	11-0202	204	214	224	238	242	255	266	268	269	278	286	290	286	289
	11-0204	224	245	252	262	268	283	287	286	292	300	311	310	308	310
	Mean	**215.3**	**232.0**	**242.7**	**249.7**	**260.2**	**269.7**	**278.9**	**281.9**	**289.4**	**295.4**	**302.2**	**306.3**	**302.7**	**307.2**
	SD	**10.26**	**12.08**	**9.21**	**8.55**	**12.44**	**13.98**	**13.07**	**13.80**	**14.84**	**15.83**	**17.42**	**19.74**	**19.20**	**20.25**
20 mg/kg	11-0153	210	217	229	243	252	257	266	272	278	278	284	289	291	296
	11-0155	200	210	229	234	246	255	266	273	270	278	277	285	280	292
	11-0160	209	232	244	258	269	273	280	295	299	302	304	312	310	311
	11-0164	199	209	224	229	233	234	245	245	242	252	261	263	255	261
	11-0167	225	244	264	282	278	297	317	322	317	328	340	332	322	350
	11-0179	213	229	233	249	262	269	270	265	296	297	297	308	301	322
	11-0183	212	230	246	250	246	263	262	262	280	287	294	306	302	309
	11-0184	209	217	236	229	245	249	245	262	296	292	279	286	285	279
	11-0194	224	240	249	252	270	280	288	303	300	308	315	317	313	320
	11-0198	222	232	248	254	260	278	290	288	295	302	310	307	313	310
	Mean	**212.3**	**226.0**	**240.2**	**248.0**	**256.1**	**265.5**	**272.9**	**280.7**	**287.3**	**292.4**	**296.1**	**300.5**	**297.2**	**305.0**
	SD	**9.12**	**12.13**	**12.18**	**15.83**	**14.01**	**17.89**	**21.85**	**22.28**	**20.77**	**20.53**	**22.56**	**19.76**	**19.94**	**24.67**
80 mg/kg	11-0151	222	232	226											
	11-0158	207	226	233	240	255	244	258	248	269	270	260	273	259	288
	11-0177	211	229	242	259	276	286	284	288	293	310	290	308	296	314
	11-0180	201	213	224	230	247	252	255	258	271	272	266	271	268	280
	11-0182	207	210	237	248	254	262	269	271	278	278	280	282	264	264
	11-0186	216	227	240	252	257	265	272	270	277	284	284	286	268	284
	11-0188	220	221	242	242	255	264	265	272	271	276	275	279	269	280
	11-0195	222	237	242	267	262	277	282	301	328	307	310	308	303	305
	11-0200	218	231	246	258	264	246	264	268	267	275	277	278	282	280
	11-0201	215	225	236	250	258	264	266	266	275	276	282	275	280	269
	Mean	**213.9**	**225.1**	**236.8**	**249.6**	**258.7**	**262.2**	**268.3**	**271.3**	**281.0**	**283.1**	**280.4**	**284.4**	**276.6**	**284.9**
	SD	**7.16**	**8.40**	**7.24**	**11.20**	**8.12**	**13.68**	**9.79**	**15.50**	**19.22**	**14.93**	**14.35**	**14.10**	**14.95**	**15.90**

[1] rats fasted in metabolism cages one night
[2] pre-fasting weight

Table J-2
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of 90-Day Body Mass (grams)
Male Rats

		Corn Oil	DNAN in corn oil			
		Control	1.25 mg/kg	5 mg/kg	20 mg/kg	80 mg/kg
Day 0	Mean	297.9	297.2	296.8	298.2	295.6
	SD	9.87	12.93	11.09	12.91	9.17
	N	10	10	10	10	10
Day 7	Mean	342.1	340.6	341.8	341.0	317.7*
	SD	17.27	21.01	19.65	21.98	12.55
	N	10	10	10	10	10
Day 14	Mean	381.6	379.8	378.7	379.2	347.4*
	SD	24.70	25.78	26.61	28.86	18.75
	N	10	10	10	10	10
Day 21	Mean	417.8	412.7	412.1	412.6	364.8*
	SD	30.64	31.71	33.35	33.73	23.36
	N	10	10	10	10	10
Day 28	Mean	443.2	439.9	442.7	439.9	368.1*
	SD	36.91	36.81	38.49	40.50	31.82
	N	10	10	10	10	10
Day 35	Mean	471.1	466.2	464.8	465.6	383.5*
	SD	41.18	41.78	43.10	43.13	29.16
	N	10	10	10	10	10
Day 42	Mean	497.5	490.2	490.3	489.1	397.3*
	SD	44.46	49.29	46.97	48.97	28.13
	N	10	10	10	10	10
Day 49	Mean	522.1	514.7	511.3	511.7	409.5*
	SD	46.86	54.36	50.29	54.63	29.80
	N	10	10	10	10	10
Day 56	Mean	538.9	532.9	528.7	528.0	412.4*
	SD	47.58	57.76	53.47	56.87	29.85
	N	10	10	10	10	9
Day 63	Mean	562.7	551.0	547.1	547.3	417.2*
	SD	51.48	60.67	57.07	57.67	31.73
	N	10	10	10	10	9
Day 70	Mean	571.6	566.0	561.7	562.0	417.6*
	SD	59.77	63.71	60.01	59.64	25.09
	N	9	10	10	10	8
Day 77	Mean	589.9	581.0	577.1	576.0	421.5*
	SD	64.19	67.24	62.38	64.10	24.50
	N	9	10	10	10	8
Day 84	Mean	592.0	577.2	582.9	577.9	410.3*
	SD	66.97	69.86	63.64	65.02	27.72
	N	9	10	10	10	7
Day 90	Mean	601.7	591.4	588.0	586.0	424.6*
	SD	65.86	71.98	61.97	64.99	26.78
	N	9	10	10	10	7

*Significantly different from corn oil control

Table J-2
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of 90-Day Body Mass (grams)
Female Rats

		Corn Oil	DNAN in corn oil			
		Control	1.25 mg/kg	5 mg/kg	20 mg/kg	80 mg/kg
Day 0	Mean	214.0	214.8	215.3	212.3	213.9
	SD	8.60	11.61	10.26	9.12	7.16
	N	10	10	10	10	10
Day 7	Mean	227.9	224.4	232.0	226.0	225.1
	SD	8.58	14.04	12.08	12.13	8.40
	N	10	10	10	10	10
Day 14	Mean	233.0	236.0	242.7	240.2	236.8
	SD	7.76	14.31	9.21	12.18	7.24
	N	10	10	10	10	10
Day 21	Mean	242.6	244.9	249.7	248.0	249.6
	SD	10.50	17.36	8.55	15.83	11.20
	N	10	10	10	10	9
Day 28	Mean	250.2	253.8	260.2	256.1	258.7
	SD	10.24	17.65	12.44	14.01	8.12
	N	10	10	10	10	9
Day 35	Mean	259.9	260.8	269.7	265.5	262.2
	SD	13.31	15.90	13.98	17.89	13.68
	N	10	10	10	10	9
Day 42	Mean	267.9	269.1	278.9	272.9	268.3
	SD	16.57	20.73	13.07	21.85	9.79
	N	10	10	10	10	9
Day 49	Mean	270.0	274.0	281.9	280.7	271.3
	SD	17.19	21.82	13.80	22.28	15.50
	N	10	10	10	10	9
Day 56	Mean	276.5	280.5	289.4	287.3	281.0
	SD	17.07	20.47	14.84	20.77	19.22
	N	10	10	10	10	9
Day 63	Mean	281.6	283.2	295.4	292.4	283.1
	SD	17.44	19.79	15.83	20.53	14.93
	N	10	10	10	10	9
Day 70	Mean	285.2	289.7	302.2	296.1	280.4
	SD	17.37	21.86	17.42	22.56	14.35
	N	10	10	10	10	9
Day 77	Mean	289.7	293.2	306.3	300.5	284.4
	SD	18.17	22.61	19.74	19.76	14.10
	N	10	10	10	10	9
Day 84	Mean	289.2	291.5	302.7	297.2	276.6
	SD	16.92	17.74	19.20	19.94	14.95
	N	10	10	10	10	9
Day 90	Mean	290.4	296.3	307.2	305.0	284.9
	SD	17.68	21.02	20.25	24.67	15.90
	N	10	10	10	10	9

*Significantly different from corn oil control

Appendix K

Individual and Summary of 90-Day Body Mass Gain Data

Table K-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

90-Day Individual Body Weight Gains (grams)
Male Rats

		Days													
Group	Animal ID	0-7	7-14	14-21	21-28	28-35	35-42	42-49	49-56	56-63	63-70	70-77	77-84[1]	84-90[2]	total
Corn Oil Control	11-0097	46	51	49	25	36	17	24	16	27					
	11-0105	45	49	35	32	29	35	33	28	28	16	26	8	1	365
	11-0107	37	36	22	25	28	22	22	20	24	1	19	3	6	265
	11-0108	37	32	36	19	28	22	18	14	15	7	14	-9	15	248
	11-0112	58	45	48	36	32	28	27	22	32	12	16	1	20	377
	11-0116	43	42	32	25	20	34	22	-2	39	21	15	6	8	305
	11-0118	43	31	35	20	34	21	26	15	18	9	22	-2	12	284
	11-0140	32	31	33	25	21	24	32	24	22	16	18	2	13	293
	11-0147	41	29	32	12	17	24	17	20	13	1	7	3	9	225
	11-0150	60	49	40	35	34	37	25	11	20	27	28	7	3	376
	Mean	**44.2**	**39.5**	**36.2**	**25.4**	**27.9**	**26.4**	**24.6**	**16.8**	**23.8**	**12.2**	**18.3**	**2.1**	**9.7**	**304.2**
	SD	**8.88**	**8.64**	**7.94**	**7.44**	**6.56**	**6.79**	**5.25**	**8.32**	**7.97**	**8.76**	**6.42**	**5.21**	**6.00**	**56.63**
1.25 mg/kg	11-0095	43	39	34	28	26	31	20	8	16	18	15	6	6	290
	11-0103	63	45	45	36	33	30	44	18	15	26	17	-30	47	389
	11-0104	39	37	33	27	23	21	18	20	21	9	20	-9	-1	258
	11-0113	50	46	36	30	37	36	18	24	18	14	20	16	-4	341
	11-0114	50	40	35	28	38	24	27	21	29	17	20	7	14	350
	11-0122	42	39	35	36	12	24	24	30	17	18	17	5	16	315
	11-0126	44	41	30	22	27	24	22	23	9	13	12	1	8	276
	11-0134	44	41	29	24	33	25	31	18	26	14	11	-18	22	300
	11-0142	27	28	20	17	19	5	16	9	7	13	6	-17	20	170
	11-0145	32	36	32	24	15	20	25	11	23	8	12	1	14	253
	Mean	**43.4**	**39.2**	**32.9**	**27.2**	**26.3**	**24.0**	**24.5**	**18.2**	**18.1**	**15.0**	**15.0**	**-3.8**	**14.2**	**294.2**
	SD	**9.96**	**5.03**	**6.30**	**5.92**	**9.06**	**8.27**	**8.25**	**7.05**	**6.95**	**5.14**	**4.69**	**14.21**	**14.30**	**61.05**
5mg/kg	11-0100	34	29	26	31	26	24	22	17	18	19	15	7	5	273
	11-0101	68	49	52	43	31	37	28	26	25	18	20	11	2	410
	11-0115	47	43	26	26	29	30	24	26	24	17	4	10	9	315
	11-0117	54	45	39	39	27	24	22	18	24	14	27	7	0	340
	11-0124	26	24	29	27	7	15	14	15	11	2	16	10	8	204
	11-0131	39	35	30	23	23	24	15	15	21	12	18	-2	14	267
	11-0135	46	29	34	26	22	23	24	10	13	14	7	10	-3	255
	11-0138	46	38	24	28	12	30	16	15	19	12	18	0	2	260
	11-0141	44	39	38	26	17	27	20	18	18	22	19	7	-1	294
	11-0146	46	38	36	37	27	21	25	14	11	16	10	-2	15	294
	Mean	**45.0**	**36.9**	**33.4**	**30.6**	**22.1**	**25.5**	**21.0**	**17.4**	**18.4**	**14.6**	**15.4**	**5.8**	**5.1**	**291.2**
	SD	**11.23**	**7.82**	**8.40**	**6.72**	**7.80**	**5.95**	**4.67**	**5.08**	**5.30**	**5.44**	**6.77**	**5.16**	**6.23**	**55.66**
20 mg/kg	11-0106	36	31	25	11	16	11	9	11	15	12	9	1	9	196
	11-0120	56	41	42	28	24	28	26	16	19	19	17	2	6	324
	11-0121	18	27	30	23	27	17	20	23	28	14	8	4	10	249
	11-0125	50	43	34	33	22	24	21	17	14	18	19	-8	18	305
	11-0127	47	33	33	20	30	30	30	22	28	10	20	8	12	323
	11-0130	50	45	39	39	39	24	23	16	16	18	14	1	7	331
	11-0133	43	43	29	36	22	28	22	6	26	16	12	6	1	290
	11-0137	34	28	24	21	20	19	16	7	12	6	8	-4	7	198
	11-0139	51	36	35	20	29	20	25	22	16	22	11	9	2	298
	11-0148	43	55	43	42	28	34	34	23	19	12	22	0	9	364
	Mean	**42.8**	**38.2**	**33.4**	**27.3**	**25.7**	**23.5**	**22.6**	**16.3**	**19.3**	**14.7**	**14.0**	**1.9**	**8.1**	**287.8**
	SD	**11.06**	**8.79**	**6.59**	**9.96**	**6.41**	**6.87**	**7.00**	**6.46**	**5.95**	**4.81**	**5.21**	**5.24**	**4.86**	**56.40**
80 mg/kg	11-0099	32	31	27	3	20	18	18							
	11-0102	24	22	19	-16	22	27	-1	16	6	6	12	-12	6	131
	11-0109	28	25	16	11	9	2	16	3	10	5	10	-15	14	134
	11-0110	16	36	14	2	14	12	21	1	-1	4	2	-5	18	134
	11-0111	13	25	18	6	18	3	10	11	-7	15	4	-6	15	125
	11-0123	24	29	17	5	9	15	12	4	5	11	-2	-3	16	142
	11-0129	19	42	7	17	7	10	11	12	4	18	4	-10	13	154
	11-0132	17	15	7	-14	24	19	11	-1	5	4	3	-16	18	92
	11-0144	26	46	31	13	17	15	11	13	7					
	11-0149	22	26	18	6	14	17	13	10	14	3	-2		0	
	Mean	**22.1**	**29.7**	**17.4**	**3.3**	**15.4**	**13.8**	**12.2**	**7.7**	**4.8**	**8.3**	**3.9**	**-9.6**	**12.5**	**130.3**
	SD	**5.88**	**9.38**	**7.53**	**10.71**	**5.82**	**7.50**	**5.87**	**6.00**	**6.04**	**5.70**	**5.03**	**5.06**	**6.32**	**19.24**

[1] rats fasted in metabolism cages one night
[2] pre-fasting weight

Table K-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

90-Day Individual Body Weight Gains (grams)
Female Rats

		Days													
Group	Animal ID	0-7	7-14	14-21	21-28	28-35	35-42	42-49	49-56	56-63	63-70	70-77	77-84[1]	84-90[2]	total
Corn Oil	11-0154	12	9	3	14	11	12	-4	11	6	-1	10	-5	3	81
Control	11-0162	14	9	2	12	5	12	-9	16	-3	12	-2	9	-9	68
	11-0168	20	4	17	10	14	6	10	10	3	2	7	-6	9	106
	11-0171	14	1	15	9	8	8	-4	17	-1	4	3	5	0	79
	11-0173	14	6	9	8	6	7	0	6	11	2	-1	7	-3	72
	11-0175	11	-2	4	11	4	-5	11	6	4	-2	10	-9	8	51
	11-0190	12	-5	17	3	7	4	7	0	8	1	8	2	-2	62
	11-0191	8	12	9	8	12	15	-1	4	7	0	2	6	-5	77
	11-0197	11	19	16	-2	21	15	4	-2	10	10	3	-7	4	102
	11-0206	23	-2	4	3	9	6	7	-3	6	8	5	-7	7	66
	Mean	**13.9**	**5.1**	**9.6**	**7.6**	**9.7**	**8.0**	**2.1**	**6.5**	**5.1**	**3.6**	**4.5**	**-0.5**	**1.2**	**76.4**
	SD	**4.46**	**7.40**	**6.19**	**4.88**	**5.08**	**6.00**	**6.71**	**7.03**	**4.48**	**4.81**	**4.25**	**6.93**	**6.00**	**17.03**
1.25 mg/kg	11-0152	13	21	0	14	2	5	12	5	-4	8	6	3	3	88
	11-0159	13	14	18	12	-1	20	8	3	-5	13	2	-6	13	104
	11-0165	2	14	10	16	6	8	-3	16	6	2	-3	5	1	80
	11-0170	5	2	9	11	5	-4	10	14	1	-3	10	6	-3	63
	11-0172	21	11	7	2	9	12	-1	-2	6	13	2	-5	3	78
	11-0176	19	8	15	6	11	10	7	8	10	5	9	-17	8	99
	11-0178	5	2	8	1	19	8	-9	15	4	10	-11	-3	8	57
	11-0181	9	14	10	5	2	14	7	2	3	7	12	-6	5	84
	11-0192	-5	18	5	11	12	3	5	1	2	9	3	2	3	69
	11-0196	14	12	7	11	5	7	13	3	4	1	5	4	7	93
	Mean	**9.6**	**11.6**	**8.9**	**8.9**	**7.0**	**8.3**	**4.9**	**6.5**	**2.7**	**6.5**	**3.5**	**-1.7**	**4.8**	**81.5**
	SD	**7.99**	**6.19**	**5.00**	**5.09**	**5.89**	**6.48**	**7.08**	**6.42**	**4.55**	**5.25**	**6.75**	**7.12**	**4.44**	**15.30**
5mg/kg	11-0156	14	12	6	13	8	8	1	10	3	7	0	3		
	11-0157	27	2	-4	17	-3	11	3	12	5	-7	7	-10	5	65
	11-0163	23	20	9	1	10	16	-1	2	3	13	2	-8	8	98
	11-0166	20	14	14	2	16	16	8	3	12	9	8	-12	4	114
	11-0174	24	-2	7	23	12	-2	12	10	6	-4	14	-12	12	100
	11-0187	7	14	4	6	10	4	1	10	4	5	-4	6	3	70
	11-0189	9	15	10	11	6	11	11	7	4	16	10	0	1	111
	11-0193	12	15	0	22	8	13	-6	14	6	10	1	3	3	101
	11-0202	10	10	14	4	13	11	2	1	9	8	4	-4	3	85
	11-0204	21	7	10	6	15	4	-1	6	8	11	-1	-2	2	86
	Mean	**16.7**	**10.7**	**7.0**	**10.5**	**9.5**	**9.2**	**3.0**	**7.5**	**6.0**	**6.8**	**4.1**	**-3.6**	**4.6**	**92.2**
	SD	**7.12**	**5.65**	**5.77**	**8.05**	**5.42**	**5.75**	**5.70**	**4.43**	**2.91**	**7.21**	**5.57**	**6.64**	**3.43**	**17.04**
20 mg/kg	11-0153	7	12	14	9	5	9	6	6	0	6	5	2	5	86
	11-0155	10	19	5	12	9	11	7	-3	8	-1	8	-5	12	92
	11-0160	23	12	14	11	4	7	15	4	3	2	8	-2	1	102
	11-0164	10	15	5	4	1	11	0	-3	10	9	2	-8	6	62
	11-0167	19	20	18	-4	19	20	5	-5	11	12	-8	-10	28	125
	11-0179	16	4	16	13	7	1	-5	31	1	0	11	-7	21	109
	11-0183	18	16	4	-4	17	-1	20	-2	7	7	12	-4	7	97
	11-0184	8	19	-7	16	4	-4	17	34	-4	-13	7	-1	-6	70
	11-0194	16	9	3	18	10	8	15	-3	8	7	2	-4	7	96
	11-0198	10	16	6	6	18	12	-2	7	7	8	-3	6	-3	88
	Mean	**13.7**	**14.2**	**7.8**	**8.1**	**9.4**	**7.4**	**7.8**	**6.6**	**5.1**	**3.7**	**4.4**	**-3.3**	**7.8**	**92.7**
	SD	**5.40**	**5.03**	**7.63**	**7.62**	**6.48**	**7.07**	**8.63**	**14.29**	**4.86**	**7.15**	**6.28**	**4.79**	**10.36**	**18.10**
80 mg/kg	11-0151	10	-6												
	11-0158	19	7	7	15	-11	14	-10	21	1	-10	13	-14	29	81
	11-0177	18	13	17	17	10	-2	4	5	17	-20	18	-12	18	103
	11-0180	12	11	6	17	5	3	3	13	1	-6	5	-3	12	79
	11-0182	3	27	11	6	8	7	2	7	0	2	2	-18	0	57
	11-0186	11	13	12	5	8	7	-2	7	7	0	2	-18	16	68
	11-0188	1	21	0	13	9	1	7	-1	5	-1	4	-10	11	60
	11-0195	15	5	25	-5	15	5	19	27	-21	3	-2	-5	2	83
	11-0200	13	15	12	6	-18	18	4	-1	8	2	1	4	-2	62
	11-0201	10	11	14	8	6	2	0	9	1	6	-7	5	-11	54
	Mean	**11.2**	**11.7**	**11.6**	**9.1**	**3.6**	**6.1**	**3.0**	**9.7**	**2.1**	**-2.7**	**4.0**	**-7.9**	**8.3**	**71.9**
	SD	**5.77**	**8.92**	**7.09**	**7.17**	**10.76**	**6.37**	**7.73**	**9.38**	**10.19**	**8.11**	**7.52**	**8.68**	**12.20**	**15.89**

[1] rats fasted in metabolism cages one night
[2] pre-fasting weight

Appendix L

Individual and Summary of 90-Day Food Consumption Data

Table L-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

90-Day Individual Food Consumption (grams)
Male Rats

		Days													
Group	Animal ID	0-7	7-14	14-21	21-28	28-35	35-42	42-49	49-56	56-63	63-70	70-77	77-84[1]	84-90[2]	Total
Corn Oil Control	11-0097	169.6	171.5	170.9	165.6	161.6	160.7	152.8	163.4	162.4					
	11-0105	188.6	185.1	184.1	165.2	169.0	181.7	186.0	190.4	179.1	178.5	174.4	157.4	102.8	2242.3
	11-0107	169.3	165.0	150.3	151.0	155.0	151.9	154.1	152.3	153.5	138.0	146.6	124.5	97.4	1908.9
	11-0108	168.4	160.8	167.0	157.8	158.5	158.7	163.1	156.0	160.2	144.3	148.9	109.1	101.7	1954.5
	11-0112	234.2	230.3	228.4	218.4	222.6	210.4	211.4	216.0	216.8	200.9	196.7	179.3	148.6	2714.0
	11-0116	175.8	179.1	165.7	157.3	155.7	166.6	169.7	163.3	174.1	160.5	168.3	143.5	110.2	2089.8
	11-0118	170.5	161.6	165.6	153.4	157.4	156.9	165.1	154.5	164.3	148.9	153.5	124.0	108.2	1983.9
	11-0140	184.1	170.4	171.0	165.2	162.3	168.5	189.2	178.7	181.1	173.4	175.2	152.0	108.9	2180.0
	11-0147	178.2	174.6	164.4	154.5	151.0	156.1	155.3	157.3	154.7	133.0	132.5	133.1	98.8	1943.5
	11-0150	214.9	214.6	211.3	205.8	192.7	194.4	202.4	190.0	184.8	187.0	192.3	186.4	119.2	2495.8
	Mean	**185.4**	**181.3**	**177.9**	**169.4**	**168.6**	**170.6**	**174.9**	**172.2**	**173.1**	**162.7**	**165.4**	**145.5**	**110.6**	**2168.1**
	SD	**22.18**	**23.24**	**23.95**	**23.27**	**22.30**	**19.09**	**21.04**	**20.97**	**18.94**	**23.56**	**21.62**	**25.94**	**15.73**	**277.12**
1.25 mg/kg	11-0095	169.4	159.3	156.3	149.9	153.3	159.7	156.8	147.1	148.4	157.0	146.4	141.6	100.0	1945.2
	11-0103	230.9	213.3	205.2	194.5	202.5	212.2	219.1	210.5	202.1	191.4	190.8	161.1	141.2	2574.8
	11-0104	160.8	165.7	162.7	150.2	151.3	150.9	154.3	150.8	146.7	138.2	141.6	120.3	87.5	1881.0
	11-0113	197.7	190.5	184.4	181.3	184.4	191.9	182.4	186.4	179.2	174.1	179.7	169.2	120.2	2321.4
	11-0114	201.2	198.8	185.9	188.0	199.9	197.7	197.2	191.0	191.3	181.3	187.2	169.0	124.9	2413.4
	11-0122	175.4	176.2	175.2	170.9	167.6	168.5	172.0	178.1	178.6	175.8	160.5	151.9	114.1	2164.8
	11-0126	173.2	165.5	161.4	155.6	154.9	150.8	154.4	158.7	158.3	147.7	142.3	127.3	101.2	1951.3
	11-0134	176.6	168.5	169.8	159.8	158.1	160.8	167.1	159.4	171.0	151.4	155.2	120.9	104.6	2023.2
	11-0142	142.8	135.4	129.2	128.5	123.3	124.0	118.1	118.6	117.2	109.4	106.7	93.2	79.4	1525.8
	11-0145	182.8	182.9	176.4	169.0	155.1	159.1	163.0	167.8	169.5	152.6	148.9	146.6	111.8	2085.5
	Mean	**181.1**	**175.6**	**170.7**	**164.8**	**165.0**	**167.6**	**168.4**	**166.8**	**166.2**	**157.9**	**155.9**	**140.1**	**108.5**	**2088.6**
	SD	**24.25**	**22.03**	**20.39**	**20.03**	**24.31**	**26.10**	**27.28**	**26.03**	**24.58**	**23.96**	**25.24**	**24.56**	**18.09**	**299.15**
5mg/kg	11-0100	147.8	140.6	136.7	139.0	145.3	148.8	148.0	148.2	146.5	143.3	142.5	129.8	97.0	1813.5
	11-0101	213.2	207.7	210.9	207.5	196.7	212.4	193.7	186.6	185.7	177.2	174.6	161.4	118.4	2446.0
	11-0115	179.0	179.7	163.5	155.2	159.6	167.7	168.5	167.4	173.3	158.0	153.3	141.3	104.4	2070.9
	11-0117	205.4	205.3	195.7	195.6	192.2	198.9	202.7	189.0	195.4	194.9	209.1	182.6	131.4	2498.2
	11-0124	176.7	154.3	162.4	170.5	153.7	164.0	162.9	167.5	136.4	138.3	154.6	149.6	111.2	2002.1
	11-0131	173.5	177.7	171.9	162.9	160.5	164.3	170.2	163.8	166.6	154.5	149.6	136.5	105.2	2057.2
	11-0135	176.7	170.3	166.2	156.8	152.7	155.5	163.7	144.9	143.9	137.1	136.2	131.2	99.8	1935.0
	11-0138	180.1	172.2	159.5	151.4	146.8	148.9	146.0	149.7	147.1	136.0	143.6	134.3	87.3	1902.9
	11-0141	202.8	199.3	190.8	175.9	165.3	161.2	169.6	169.7	172.1	175.5	174.8	162.1	106.7	2225.8
	11-0146	196.2	191.3	189.3	191.8	197.8	195.7	199.5	195.6	192.4	180.1	170.6	152.9	123.7	2376.9
	Mean	**185.1**	**179.8**	**174.7**	**170.7**	**167.1**	**171.7**	**172.5**	**168.2**	**165.9**	**159.5**	**160.9**	**148.2**	**108.5**	**2132.9**
	SD	**19.34**	**21.81**	**21.75**	**21.90**	**20.62**	**22.41**	**19.98**	**17.73**	**21.45**	**21.19**	**21.79**	**16.93**	**13.12**	**240.46**
20 mg/kg	11-0106	172.9	168.6	169 6	157.0	149 5	154.3	156.7	144.4	145.0	145.8	142.6	133 1	106.9	1946 4
	11-0120	216.1	212.6	215.9	214.7	207.0	217.2	210.9	207.0	204 2	208.0	198.9	179.7	141.3	2633.5
	11-0121	153.2	152.5	159.4	156.0	160.4	162.2	171.6	172.2	179.0	171.7	171.0	159.0	124 4	2092.6
	11-0125	205.6	200.9	203.0	192.0	191.2	188.2	189.5	182.4	171.4	176.2	174 7	154.2	123.3	2352.6
	11-0127	195 2	184.5	182.5	180.3	177.5	187.3	189.6	190 5	194.8	178.8	180.4	173.9	129.1	2344.4
	11-0130	199 0	207.4	231.9	231.0	235.3	229.3	229.0	221 9	213.9	203.2	204 0	177.4	129.8	2713.1
	11-0133	178.5	188.7	175.7	171 7	172.2	175.1	175.5	171.0	173.8	171.8	173.8	164.1	109 5	2201.4
	11-0137	171.7	173 2	164.8	158.1	162.1	167.5	164.6	154.2	165.0	145.7	147.0	137.8	102 7	2014.4
	11-0139	192.8	192 4	188.1	181.2	185.5	183.1	194.0	193.2	192.3	177.8	174.9	159.7	122.7	2337.7
	11-0148	203.5	209.5	209.3	212.0	213.7	214.4	217.8	220.5	210.7	200.8	207.5	186.8	128.6	2635.1
	Mean	**188.9**	**189.0**	**190.0**	**185.4**	**185.4**	**187.9**	**189.9**	**185.7**	**185.0**	**178.0**	**177.5**	**162.6**	**121.8**	**2327.1**
	SD	**19.22**	**19.68**	**24.08**	**26.53**	**26.84**	**25.09**	**23.69**	**26.17**	**22.01**	**21.65**	**21.82**	**17.65**	**11.98**	**269.21**
80 mg/kg	11-0099	186.8	208.1	205.4	142.7	162.2	186.6	199.1							
	11-0102	160.5	166.8	180.5	113.4	149.5	151.6	148.2	159.7	145.7	133.1	146.7	134.6	115.2	1905.5
	11-0109	172.2	189.4	190.7	159.4	170.5	157.1	163.6	158.7	153.8	165.6	158.9	112.4	113.3	2065.6
	11-0110	151.0	177.3	193.9	150.6	176.8	161.7	167.9	155.0	141.3	143.5	151.1	131.9	113.7	2015.7
	11-0111	139.4	159.1	154.3	134.4	150.4	138.4	148.7	152.0	135.7	133.5	142.0	129.8	106.5	1824.2
	11-0123	182.9	205.7	198.0	162.1	165.3	168.4	172.7	170.9	169.8	173.9	161.6	153.4	130.5	2215.2
	11-0129	170.4	208.3	192.3	197.1	178.0	167.6	174.7	182.3	171.1	176.8	169.4	144.0	118.7	2250.7
	11-0132	164.8	182.5	177.4	131.2	164.6	172.8	173.2	161.5	149.9	146.8	148.4	137.5	111.0	2021.6
	11-0144	172.7	220.5	215.2	206.2	190.0	178.0	189.8	196.4	200.1					
	11-0149	149.7	185.6	172.2	164.1	154.4	162.2	169.3	172.1	175.2	168.9	153.8			
	Mean	**165.0**	**190.3**	**188.0**	**156.1**	**166.2**	**164.4**	**170.7**	**167.6**	**160.3**	**155.3**	**154.0**	**134.8**	**115.6**	**2042.6**
	SD	**15.06**	**19.91**	**17.50**	**28.72**	**13.01**	**13.66**	**15.80**	**14.39**	**20.44**	**18.04**	**8.91**	**12.74**	**7.58**	**153.45**

[1] rats fasted in metabolism cages one night
[2] pre-fasting

Table L-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

90-Day Individual Food Consumption (grams)
Female Rats

		Day													
Group	Animal ID	0-7	7-14	14-21	21-28	28-35	35-42	42-49	49-56	56-63	63-70	70-77	77-84[1]	84-90[2]	Total
Corn Oil Control	11-0154	119.4	107.3	107.6	107.7	121.0	118.0	119.0	108.9	109.3	102 1	113.0	89 4	79.1	1401.8
	11-0162	112.7	104.8	98.8	116.3	101.2	103.6	105.9	110.1	99.0	100.7	101.0	91.9	74.6	1320.6
	11-0168	124.5	114.0	121.5	112.8	123.0	126 9	133.7	118.1	120.1	113.1	115.6	95.9	91.7	1510.9
	11-0171	122.2	111.1	110.6	119.2	124.0	127.6	123.8	131.6	109.9	119.9	121.8	112.8	78.8	1513.3
	11-0173	105.5	105.2	110.8	103.9	116.0	117.7	117.5	118.6	117.8	111.5	116.1	95.3	86.8	1422.7
	11-0175	115.3	107.8	102.3	94.3	105.8	94.1	102.7	100.4	98.1	93.0	105.2	85.0	75.7	1279.7
	11-0190	126.5	109.7	120.7	97.9	117.1	109.2	120.2	102.7	112.8	101.6	102.0	101.4	73.0	1394.8
	11-0191	96.6	102.9	101.5	105.2	109.4	109.5	108.5	103.8	101.4	91.7	101.6	99.1	61.4	1292.6
	11-0197	98.4	104.7	105.4	102.3	127.6	120.4	115.6	109.2	107.3	102.5	108.6	94.0	55.3	1351.3
	11-0206	114.2	106.2	91.4	101.8	106.8	101.6	108.4	103.0	104.4	99.1	98.5	93.7	68.0	1297.1
	Mean	**113.5**	**107.4**	**107.1**	**106.1**	**115.2**	**112.9**	**115.5**	**110.6**	**108.0**	**103.5**	**108.3**	**95.9**	**74.4**	**1378.5**
	SD	**10.45**	**3.39**	**9.38**	**7.95**	**8.93**	**11.11**	**9.39**	**9.64**	**7.50**	**8.87**	**7.90**	**7.54**	**10.92**	**85.84**
1.25 mg/kg	11-0152	104.5	105.1	99.5	100.4	104.0	106.0	120.5	105.9	100.6	103.4	112.0	94.0	71.8	1327.7
	11-0159	136.2	129.7	128.6	121.0	131.6	141.3	127.3	123.3	122.7	122.8	122.2	106.1	95.5	1608.3
	11-0165	95.5	103.1	93.4	109.3	107.1	108.4	113.9	110.6	101.6	98.5	104.0	95.2	68.9	1309.5
	11-0170	103.0	110.3	110.3	108.4	113.7	110.7	124.6	113.1	109.7	108.5	118.9	102.1	70.2	1403.5
	11-0172	127.6	107.9	113.5	106.4	113.7	110.9	108.9	112.1	103.2	103.9	105.4	100.8	79.0	1393.3
	11-0176	127.9	119.8	122.3	117.8	137.2	119.7	129.8	119.8	119.5	115.2	121.5	104.6	83.6	1538 7
	11-0178	112.0	119.8	109.4	111.2	136.3	110.1	124.1	126.5	116.1	114.9	112.0	104.0	89.0	1485.4
	11-0181	125.9	118.4	107.7	99.2	110.9	112.1	115.3	102.2	105.3	104.6	104.8	94.3	95.4	1396.1
	11-0192	92.8	106.9	90.5	101.3	102.7	93.1	102.1	92.0	98.8	94.4	95.7	93.8	69.8	1233.9
	11-0196	104.2	101.5	95.3	97.1	112.1	106.4	114.2	106.3	96.1	94.6	97.6	86.9	69.4	1281.7
	Mean	**113.0**	**112.3**	**107.1**	**107.2**	**116.9**	**111.9**	**118.1**	**111.2**	**107.4**	**106.1**	**109.4**	**98.2**	**79.3**	**1397.8**
	SD	**15.29**	**9.18**	**12.52**	**7.97**	**13.12**	**12.30**	**8.74**	**10.33**	**9.22**	**9.35**	**9.47**	**6.22**	**10.90**	**117.83**
5mg/kg	11-0156	121.3	115.7	116.2	118.2	117.1	114.4	119.6	116.0	114.8	111.9	117.9	106.4		
	11-0157	117.0	108.9	95.8	106.3	105.8	102.7	114.2	115.4	110.7	91.5	102.9	84.4	79.5	1335.1
	11-0163	118.2	111.0	113.8	115.3	120.3	115.1	112.1	111.4	109.2	109.9	105.1	93.3	80.0	1414.7
	11-0166	134.3	123.5	123.5	123.0	140.5	138.1	142.3	133.9	136.1	126.8	125.8	103.2	83.0	1634.0
	11-0174	128.6	118.5	128.3	140.0	138.3	129.2	132.9	118.1	119.8	117.4	130.0	94.7	91.2	1587.0
	11-0187	101.2	94.9	97.9	100.1	108.7	107 0	109.2	113.0	105.6	105.5	107 7	104.3	81.2	1336.3
	11-0189	109.0	103.6	96.4	101.1	110.5	103.4	112.1	102.3	106.9	112.0	105.0	103.4	61.2	1326.9
	11-0193	127.2	122.1	114.9	123.4	121.6	126.0	116.9	121 1	115.4	113.9	120.6	111 3	79 6	1514.0
	11-0202	95.5	94.5	94.6	100.1	108.2	102.7	103.3	104.0	130.7	112.5	96.6	93.1	61.6	1297.4
	11-0204	130.0	116.1	123.2	118.5	122.2	112.5	116.1	122.0	129.3	108.4	107.1	104.0	73.3	1482.7
	Mean	**118.2**	**110.9**	**110.5**	**114.6**	**119.3**	**115.1**	**117.9**	**115.7**	**117.9**	**111.0**	**111.9**	**99.8**	**76.7**	**1436.5**
	SD	**12.86**	**10.40**	**13.07**	**12.88**	**12.13**	**12.31**	**11.53**	**9.12**	**10.77**	**8.95**	**10.97**	**8.09**	**9.86**	**123.47**
20 mg/kg	11-0153	114.5	120.7	121.1	118.3	117.8	120.9	125.1	118.1	109.5	112.2	110.9	97.3	86.4	1472.8
	11-0155	105.9	118.0	98.6	108.0	115.7	115.5	118.1	102.7	106.2	96.9	110.1	86.2	82.3	1364.2
	11-0160	119.8	122.1	127.3	118.3	118.1	121.3	132.3	121.8	112.0	118.1	124.0	105.9	85.9	1526.9
	11-0164	102.3	105.8	99.3	102 3	111.6	111.5	109.6	108.3	105.9	103.6	104.2	88.4	77.1	1329.9
	11-0167	126.5	135.6	127.5	126.2	138.5	147.8	146.3	134.3	126.3	139.2	122.2	113.9	111 8	1696.1
	11-0179	108.4	108.4	119.6	116.9	114.4	115.2	120.9	120.9	112.0	115.7	121 4	99.3	95.4	1468.4
	11-0183	124.7	128.8	124.1	116.5	130.3	123.8	139.5	129.1	126.7	128.8	136.0	120.2	100.0	1630.5
	11-0184	112.8	105.7	90.8	101.0	106.7	90.4	115.0	135 3	122.6	91.6	107.9	96.8	72 0	1348.6
	11-0194	118.3	112.1	104.2	112.2	118.3	116.3	129.4	111.5	119.4	113.1	111.8	102.4	81 7	1450.7
	11-0198	106.9	113.5	105.5	106.2	117.9	114.1	109.2	110.2	110.2	107.3	98.7	103.7	68.6	1372.0
	Mean	**114.0**	**117.1**	**111.8**	**112.6**	**118.9**	**117.7**	**124.5**	**119.2**	**115.1**	**112.7**	**114.9**	**101.4**	**86.1**	**1466.0**
	SD	**8.22**	**9.94**	**13.56**	**8.07**	**9.13**	**14.05**	**12.41**	**11.19**	**7.98**	**14.17**	**11.48**	**10.43**	**13.17**	**122.70**
80 mg/kg	11-0151	110.5	108.2												
	11-0158	116.5	128.8	101.5	121.1	114.7	112.7	111.7	121.9	111.9	102.1	119.8	96.5	89.2	1448.4
	11-0177	108.3	139.5	154.1	152.2	154.8	137.3	148.3	136.4	147.3	101.5	126.9	103.7	94.2	1704.5
	11-0180	102.0	113.7	114.6	120.6	133.8	129.5	127.5	124.1	125.0	114.0	118.9	107.1	83.2	1514.0
	11-0182	95.5	127.6	144.3	122.3	118.0	112.1	128.8	125.4	123.3	120.5	130.8	100.7	67.7	1517.0
	11-0186	104.1	117.7	123.8	123.3	119.8	114.5	127.5	114.9	120.8	122.4	118.8	98.0	86.3	1491.9
	11-0188	105.5	123.6	112.5	116.2	117.7	124.1	130.0	118.2	119.3	119.4	124.9	103.9	83.7	1499.0
	11-0195	123.0	121.1	129.3	122.3	127.9	124.6	143.6	143.0	105.7	116.8	122.8	123.3	74.1	1577.5
	11-0200	118.1	129.1	131.1	132.2	81.7	131.2	142.6	109.7	125.8	127.3	125.0	113.5	71.9	1539.2
	11-0201	103.0	114.3	118.2	117.5	119.3	106.6	120.6	113.5	115.1	99.3	80.9	110.5	58.3	1377.1
	Mean	**108.7**	**122.4**	**125.5**	**125.3**	**120.9**	**121.4**	**131.2**	**123.0**	**121.6**	**113.7**	**118.8**	**106.4**	**78.7**	**1518.7**
	SD	**8.43**	**9.27**	**16.34**	**11.05**	**19.24**	**10.37**	**11.74**	**10.86**	**11.66**	**10.25**	**14.74**	**8.43**	**11.50**	**89.81**

Table L-2
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of Food Consumption (grams)
Male Rats

		Corn Oil	DNAN in corn oil			
		Control	1.25 mg/kg	5 mg/kg	20 mg/kg	80 mg/kg
Days 0-7	Mean	185.4	181.1	185.1	188.9	165.0
	SD	22.18	24.25	19.34	19.22	15.06
	N	10	10	10	10	10
Days 7-14	Mean	181.3	175.6	179.8	189.0	190.3
	SD	23.24	22.03	21.81	19.68	19.91
	N	10	10	10	10	10
Days 14-21	Mean	177.9	170.7	174.7	190.0	188.0
	SD	23.95	20.39	21.75	24.08	17.50
	N	10	10	10	10	10
Days 21-28	Mean	169.4	164.8	170.7	185.4	156.1
	SD	23.27	20.03	21.90	26.53	28.72
	N	10	10	10	10	10
Days 28-35	Mean	168.6	165.0	167.1	185.4	166.2
	SD	22.30	24.31	20.62	26.84	13.01
	N	10	10	10	10	10
Days 35-42	Mean	170.6	167.6	171.7	187.9	164.4
	SD	19.09	26.10	22.41	25.09	13.66
	N	10	10	10	10	10
Days 42-49	Mean	174.9	168.4	172.5	189.9	170.7
	SD	21.04	27.28	19.98	23.69	15.80
	N	10	10	10	10	10
Days 49-56	Mean	172.2	166.8	168.2	185.7	167.6
	SD	20.97	26.03	17.73	26.17	14.39
	N	10	10	10	10	9
Days 56-63	Mean	173.1	166.2	165.9	185.0	160.3
	SD	18.94	24.58	21.45	22.01	20.44
	N	10	10	10	10	9
Days 63-70	Mean	162.7	157.9	159.5	178.0	155.3
	SD	23.56	23.96	21.19	21.65	18.04
	N	9	10	10	10	8
Days 70-77	Mean	165.4	155.9	160.9	177.5	154.0
	SD	21.62	25.24	21.79	21.82	8.91
	N	9	10	10	10	8
Days 77-84	Mean	145.5	140.1	148.2	162.6	134.8
	SD	25.94	24.56	16.93	17.65	12.74
	N	9	10	10	10	7
Days 84-90	Mean	110.6	108.5	108.5	121.8	115.6
	SD	15.73	18.09	13.12	11.98	7.58
	N	9	10	10	10	7
Net	Mean	2168.1	2088.6	2132.9	2327.1	2042.6
	SD	277.12	299.15	240.46	269.21	153.45
	N	9	10	10	10	7

*Significantly different from corn oil control

Table L-2
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of Food Consumption (grams)
Female Rats

		Corn Oil	DNAN in corn oil			
		Control	1.25 mg/kg	5 mg/kg	20 mg/kg	80 mg/kg
Days 0-7	Mean	113.5	113.0	118.2	114.0	108.7
	SD	10.45	15.29	12.86	8.22	8.43
	N	10	10	10	10	10
Days 7-14	Mean	107.4	112.3	110.9	117.1	122.4*
	SD	3.39	9.18	10.40	9.94	9.27
	N	10	10	10	10	10
Days 14-21	Mean	107.1	107.1	110.5	111.8	125.5*
	SD	9.38	12.52	13.07	13.56	16.34
	N	10	10	10	10	9
Days 21-28	Mean	106.1	107.2	114.6	112.6	125.3*
	SD	7.95	7.97	12.88	8.07	11.05
	N	10	10	10	10	9
Days 28-35	Mean	115.2	116.9	119.3	118.9	120.9
	SD	8.93	13.12	12.13	9.13	19.24
	N	10	10	10	10	9
Days 35-42	Mean	112.9	111.9	115.1	117.7	121.4
	SD	11.11	12.30	12.31	14.05	10.37
	N	10	10	10	10	9
Days 42-49	Mean	115.5	118.1	117.9	124.5	131.2*
	SD	9.39	8.74	11.53	12.41	11.74
	N	10	10	10	10	9
Days 49-56	Mean	110.6	111.2	115.7	119.2	123.0
	SD	9.64	10.33	9.12	11.19	10.86
	N	10	10	10	10	9
Days 56-63	Mean	108.0	107.4	117.9	115.1	121.6*
	SD	7.50	9.22	10.77	7.98	11.66
	N	10	10	10	10	9
Days 63-70	Mean	103.5	106.1	111.0	112.7	113.7
	SD	8.87	9.35	8.95	14.17	10.25
	N	10	10	10	10	9
Days 70-77	Mean	108.3	109.4	111.9	114.9	118.8
	SD	7.90	9.47	10.97	11.48	14.74
	N	10	10	10	10	9
Days 77-84	Mean	95.9	98.2	99.8	101.4	106.4
	SD	7.54	6.22	8.09	10.43	8.43
	N	10	10	10	10	9
Days 84-90	Mean	74.4	79.3	76.7	86.1	78.7
	SD	10.92	10.90	9.86	13.17	11.50
	N	10	10	10	10	9
Net	Mean	1378.5	1397.8	1436.5	1466.0	1518.7
	SD	85.84	117.83	123.47	122.70	89.81
	N	10	10	10	10	9

*Significantly different from corn oil control

Appendix M

Individual and Summary of Feed Conversion Efficiency Data

Table M-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

90-Day Individual Feed Efficiency
Male Rats

		Week													
Group	Animal ID	1	2	3	4	5	6	7	8	9	10	11	12	13	total
	11-0097	0.27	0.30	0.29	0.15	0.22	0.11	0.16	0.10	0.17					
Corn Oil	11-0105	0.24	0.26	0.19	0.19	0.17	0.19	0.18	0.15	0.16	0.09	0.15	0.05	0.01	0.16
Control	11-0107	0.22	0.22	0.15	0.17	0.18	0.14	0.14	0.13	0.16	0.01	0.13	0.02	0.06	0.14
	11-0108	0.22	0.20	0.22	0.12	0.18	0.14	0.11	0.09	0.09	0.05	0.09	-0.08	0.15	0.13
	11-0112	0.25	0.20	0.21	0.16	0.14	0.13	0.13	0.10	0.15	0.06	0.08	0.01	0.13	0.14
	11-0116	0.24	0.23	0.19	0.16	0.13	0.20	0.13	-0.01	0.22	0.13	0.09	0.04	0.07	0.15
	11-0118	0.25	0.19	0.21	0.13	0.22	0.13	0.16	0.10	0.11	0.06	0.14	-0.02	0.11	0.14
	11-0140	0.17	0.18	0.19	0.15	0.13	0.14	0.17	0.13	0.12	0.09	0.10	0.01	0.12	0.13
	11-0147	0.23	0.17	0.19	0.08	0.11	0.15	0.11	0.13	0.08	0.01	0.05	0.02	0.09	0.12
	11-0150	0.28	0.23	0.19	0.17	0.18	0.19	0.12	0.06	0.11	0.14	0.15	0.04	0.03	0.15
	Mean	**0.238**	**0.218**	**0.203**	**0.146**	**0.166**	**0.154**	**0.140**	**0.097**	**0.137**	**0.071**	**0.110**	**0.011**	**0.086**	**0.140**
	SD	**0.0299**	**0.0400**	**0.0351**	**0.0322**	**0.0370**	**0.0315**	**0.0240**	**0.0466**	**0.0419**	**0.0482**	**0.0338**	**0.0404**	**0.0477**	**0.0136**
	11-0095	0.25	0.24	0.22	0.19	0.17	0.19	0.13	0.05	0.11	0.11	0.10	0.04	0.06	0.15
1.25 mg/kg	11-0103	0.27	0.21	0.22	0.19	0.16	0.14	0.20	0.09	0.07	0.14	0.09	-0.19	0.33	0.15
	11-0104	0.24	0.22	0.20	0.18	0.15	0.14	0.12	0.13	0.14	0.07	0.14	-0.07	-0.01	0.14
	11-0113	0.25	0.24	0.20	0.17	0.20	0.19	0.10	0.13	0.10	0.08	0.11	0.09	-0.03	0.15
	11-0114	0.25	0.20	0.19	0.15	0.19	0.12	0.14	0.11	0.15	0.09	0.11	0.04	0.11	0.15
	11-0122	0.24	0.22	0.20	0.21	0.07	0.14	0.14	0.17	0.10	0.10	0.11	0.03	0.14	0.15
	11-0126	0.25	0.25	0.19	0.14	0.17	0.16	0.14	0.14	0.06	0.09	0.08	0.01	0.08	0.14
	11-0134	0.25	0.24	0.17	0.15	0.21	0.16	0.19	0.11	0.15	0.09	0.07	-0.15	0.21	0.15
	11-0142	0.19	0.21	0.15	0.13	0.15	0.04	0.14	0.08	0.06	0.12	0.06	-0.18	0.25	0.11
	11-0145	0.18	0.20	0.18	0.14	0.10	0.13	0.15	0.07	0.14	0.05	0.08	0.01	0.13	0.12
	Mean	**0.238**	**0.224**	**0.192**	**0.164**	**0.158**	**0.141**	**0.144**	**0.108**	**0.108**	**0.094**	**0.095**	**-0.037**	**0.127**	**0.140**
	SD	**0.0308**	**0.0195**	**0.0199**	**0.0254**	**0.0436**	**0.0425**	**0.0303**	**0.0370**	**0.0367**	**0.0251**	**0.0239**	**0.1032**	**0.1140**	**0.0131**
	11-0100	0.23	0.21	0.19	0.22	0.18	0.16	0.15	0.11	0.12	0.13	0.11	0.05	0.05	0.15
5mg/kg	11-0101	0.32	0.24	0.25	0.21	0.16	0.17	0.14	0.14	0.13	0.10	0.11	0.07	0.02	0.17
	11-0115	0.26	0.24	0.16	0.17	0.18	0.18	0.14	0.16	0.14	0.11	0.03	0.07	0.09	0.15
	11-0117	0.26	0.22	0.20	0.20	0.14	0.12	0.11	0.10	0.12	0.07	0.13	0.04		0.14
	11-0124	0.15	0.16	0.18	0.16	0.05	0.09	0.09	0.09	0.08	0.01	0.10	0.07	0.07	0.10
	11-0131	0.22	0.20	0.17	0.14	0.14	0.15	0.09	0.09	0.13	0.08	0.12	-0.01	0.13	0.13
	11-0135	0.26	0.17	0.20	0.17	0.14	0.15	0.15	0.07	0.09	0.10	0.05	0.08	-0.03	0.13
	11-0138	0.26	0.22	0.15	0.18	0.08	0.20	0.11	0.10	0.13	0.09	0.13		0.02	0.14
	11-0141	0.22	0.20	0.20	0.15	0.10	0.17	0.12	0.11	0.10	0.13	0.11	0.04	-0.01	0.13
	11-0146	0.23	0.20	0.19	0.19	0.14	0.11	0.13	0.07	0.06	0.09	0.06	-0.01	0.12	0.12
	Mean	**0.241**	**0.204**	**0.189**	**0.179**	**0.131**	**0.150**	**0.122**	**0.103**	**0.111**	**0.091**	**0.094**	**0.043**	**0.052**	**0.136**
	SD	**0.0440**	**0.0267**	**0.0268**	**0.0269**	**0.0429**	**0.0344**	**0.0237**	**0.0273**	**0.0267**	**0.0331**	**0.0357**	**0.0348**	**0.0565**	**0.0178**
	11-0106	0.21	0.18	0.15	0.07	0.11	0.07	0.06	0.08	0.10	0.08	0.06	0.01	0.08	0.10
20 mg/kg	11-0120	0.26	0.19	0.19	0.13	0.12	0.13	0.12	0.08	0.09	0.09	0.09	0.01	0.04	0.12
	11-0121	0.12	0.18	0.19	0.15	0.17	0.10	0.12	0.13	0.16	0.08	0.05	0.03	0.08	0.12
	11-0125	0.24	0.21	0.17	0.17	0.12	0.13	0.11	0.09	0.08	0.10	0.11	-0.05	0.15	0.13
	11-0127	0.24	0.18	0.18	0.11	0.17	0.16	0.16	0.12	0.14	0.06	0.11	0.05	0.09	0.14
	11-0130	0.25	0.22	0.17	0.17	0.17	0.10	0.10	0.07	0.07	0.09	0.07	0.01	0.05	0.12
	11-0133	0.24	0.23	0.17	0.21	0.13	0.16	0.13	0.04	0.15	0.09	0.07	0.04	0.01	0.13
	11-0137	0.20	0.16	0.15	0.13	0.12	0.11	0.10	0.05	0.07	0.04	0.05	-0.03	0.07	0.10
	11-0139	0.26	0.19	0.19	0.11	0.16	0.11	0.13	0.11	0.08	0.12	0.06	0.06	0.02	0.13
	11-0148	0.21	0.26	0.21	0.20	0.13	0.16	0.16	0.10	0.09	0.06	0.11		0.07	0.14
	Mean	**0.223**	**0.200**	**0.175**	**0.145**	**0.136**	**0.124**	**0.117**	**0.087**	**0.105**	**0.082**	**0.078**	**0.012**	**0.066**	**0.123**
	SD	**0.0434**	**0.0300**	**0.0196**	**0.0430**	**0.0243**	**0.0292**	**0.0293**	**0.0314**	**0.0324**	**0.0241**	**0.0236**	**0.0349**	**0.0396**	**0.0138**
	11-0099	0.17	0.15	0.13	0.02	0.12	0.10	0.09							
80 mg/kg	11-0102	0.15	0.13	0.11	-0.14	0.15	0.18	-0.01	0.10	0.04	0.05	0.08	-0.09	0.05	0.07
	11-0109	0.16	0.13	0.08	0.07	0.05	0.01	0.10	0.02	0.07	0.03	0.06	-0.13	0.12	0.06
	11-0110	0.11	0.20	0.07	0.01	0.08	0.07	0.13	0.01	-0.01	0.03	0.01	-0.04	0.16	0.07
	11-0111	0.09	0.16	0.12	0.04	0.12	0.02	0.07	0.07	-0.05	0.11	0.03	-0.05	0.14	0.07
	11-0123	0.13	0.14	0.09	0.03	0.05	0.09	0.07	0.02	0.03	0.06	-0.01	-0.02	0.12	0.06
	11-0129	0.11	0.20	0.04	0.09	0.04	0.06	0.06	0.07	0.02	0.10	0.02	-0.07	0.11	0.07
	11-0132	0.10	0.08	0.04	-0.11	0.15	0.11	0.06	-0.01	0.03	0.03	0.02	-0.12	0.16	0.05
	11-0144	0.15	0.21	0.14	0.06	0.09	0.08	0.06	0.07	0.03					
	11-0149	0.15	0.14	0.10	0.04	0.09	0.10	0.08	0.06	0.08	0.02	-0.01			
	Mean	**0.133**	**0.155**	**0.092**	**0.012**	**0.094**	**0.083**	**0.070**	**0.045**	**0.028**	**0.053**	**0.026**	**-0.073**	**0.124**	**0.064**
	SD	**0.0275**	**0.0397**	**0.0358**	**0.0753**	**0.0388**	**0.0469**	**0.0340**	**0.0355**	**0.0386**	**0.0361**	**0.0331**	**0.0421**	**0.0372**	**0.0083**

Table M-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

90-Day Individual Feed Efficiency
Female Rats

		Week													
Group	Animal ID	1	2	3	4	5	6	7	8	9	10	11	12	13	total
	11-0154	0.10	0.08	0.03	0.13	0.09	0.10	-0.03	0.10	0.05	-0.01	0.09	-0.06	0.04	0.06
Corn Oil	11-0162	0.12	0.09	0.02	0.10	0.05	0.12	-0.08	0.15	-0.03	0.12	-0.02	0.10	-0.12	0.05
Control	11-0168	0.16	0.04	0.14	0.09	0.11	0.05	0.07	0.08	0.02	0.02	0.06	-0.06	0.10	0.07
	11-0171	0.11	0.01	0.14	0.08	0.06	0.06	-0.03	0.13	-0.01	0.03	0.02	0.04		0.05
	11-0173	0.13	0.06	0.08	0.08	0.05	0.06		0.05	0.09	0.02	-0.01	0.07	-0.03	0.05
	11-0175	0.10	-0.02	0.04	0.12	0.04	-0.05	0.11	0.06	0.04	-0.02	0.10	-0.11	0.11	0.04
	11-0190	0.09	-0.05	0.14	0.03	0.06	0.04	0.06		0.07	0.01	0.08	0.02	-0.03	0.04
	11-0191	0.08	0.12	0.09	0.08	0.11	0.14	-0.01	0.04	0.07		0.02	0.06	-0.08	0.06
	11-0197	0.11	0.18	0.15	-0.02	0.16	0.12	0.03	-0.02	0.09	0.10	0.03	-0.07	0.07	0.08
	11-0206	0.20	-0.02	0.04	0.03	0.08	0.06	0.06	-0.03	0.06	0.08	0.05	-0.07	0.10	0.05
	Mean	**0.122**	**0.049**	**0.087**	**0.071**	**0.083**	**0.069**	**0.020**	**0.062**	**0.047**	**0.038**	**0.042**	**-0.008**	**0.017**	**0.055**
	SD	**0.0358**	**0.0704**	**0.0521**	**0.0454**	**0.0386**	**0.0554**	**0.0630**	**0.0601**	**0.0410**	**0.0493**	**0.0396**	**0.0744**	**0.0856**	**0.0109**
	11-0152	0.12	0.20		0.14	0.02	0.05	0.10	0.05	-0.04	0.08	0.05	0.03	0.04	0.07
1.25 mg/kg	11-0159	0.10	0.11	0.14	0.10	-0.01	0.14	0.06	0.02	-0.04	0.11	0.02	-0.06	0.14	0.06
	11-0165	0.02	0.14	0.11	0.15	0.06	0.07	-0.03	0.14	0.06	0.02	-0.03	0.05	0.01	0.06
	11-0170	0.05	0.02	0.08	0.10	0.04	-0.04	0.08	0.12	0.01	-0.03	0.08	0.06	-0.04	0.04
	11-0172	0.16	0.10	0.06	0.02	0.08	0.11	-0.01	-0.02	0.06	0.13	0.02	-0.05	0.04	0.06
	11-0176	0.15	0.07	0.12	0.05	0.08	0.08	0.05	0.07	0.08	0.04	0.07	-0.16	0.10	0.06
	11-0178	0.04	0.02	0.07	0.01	0.14	0.07	-0.07	0.12	0.03	0.09	-0.10	-0.03	0.09	0.04
	11-0181	0.07	0.12	0.09	0.05	0.02	0.12	0.06	0.02	0.03	0.07	0.11	-0.06	0.05	0.06
	11-0192	-0.05	0.17	0.06	0.11	0.12	0.03	0.05	0.01	0.02	0.10	0.03	0.02	0.04	0.06
	11-0196	0.13	0.12	0.07	0.11	0.04	0.07	0.11	0.03	0.04	0.01	0.05	0.05	0.10	0.07
	Mean	**0.080**	**0.105**	**0.090**	**0.084**	**0.059**	**0.071**	**0.041**	**0.057**	**0.025**	**0.060**	**0.032**	**-0.015**	**0.057**	**0.058**
	SD	**0.0673**	**0.0588**	**0.0285**	**0.0484**	**0.0457**	**0.0506**	**0.0590**	**0.0549**	**0.0406**	**0.0479**	**0.0607**	**0.0703**	**0.0509**	**0.0102**
	11-0156	0.12	0.10	0.05	0.11	0.07	0.07	0.01	0.09	0.03	0.06		0.03		
5mg/kg	11-0157	0.23	0.02	-0.04	0.16	-0.03	0.11	0.03	0.10	0.05	-0.08	0.07	-0.12	0.06	0.05
	11-0163	0.19	0.18	0.08	0.01	0.08	0.14	-0.01	0.02	0.03	0.12	0.02	-0.09	0.10	0.07
	11-0166	0.15	0.11	0.11	0.02	0.11	0.12	0.06	0.02	0.09	0.07	0.06	-0.12	0.05	0.07
	11-0174	0.19	-0.02	0.05	0.16	0.09	-0.02	0.09	0.08	0.05	-0.03	0.11	-0.13	0.13	0.06
	11-0187	0.07	0.15	0.04	0.06	0.09	0.04	0.01	0.09	0.04	0.05	-0.04	0.06	0.04	0.05
	11-0189	0.08	0.14	0.10	0.11	0.05	0.11	0.10	0.07	0.04	0.14	0.10		0.02	0.08
	11-0193	0.09	0.12		0.18	0.07	0.10	-0.05	0.12	0.05	0.09	0.01	0.03	0.04	0.07
	11-0202	0.10	0.11	0.15	0.04	0.12	0.11	0.02	0.01	0.07	0.07	0.04	-0.04	0.05	0.07
	11-0204	0.16	0.06	0.08	0.05	0.12	0.04	-0.01	0.05	0.06	0.10	-0.01	-0.02	0.03	0.06
	Mean	**0.139**	**0.098**	**0.070**	**0.090**	**0.078**	**0.081**	**0.024**	**0.065**	**0.050**	**0.059**	**0.040**	**-0.044**	**0.057**	**0.064**
	SD	**0.0539**	**0.0608**	**0.0539**	**0.0632**	**0.0441**	**0.0477**	**0.0462**	**0.0378**	**0.0193**	**0.0672**	**0.0485**	**0.0712**	**0.0369**	**0.0104**
	11-0153	0.06	0.10	0.12	0.08	0.04	0.07	0.05	0.05		0.05	0.05	0.02	0.06	0.06
20 mg/kg	11-0155	0.09	0.16	0.05	0.11	0.08	0.10	0.06	-0.03	0.08	-0.01	0.07	-0.06	0.15	0.07
	11-0160	0.19	0.10	0.11	0.09	0.03	0.06	0.11	0.03	0.03	0.02	0.06	-0.02	0.01	0.07
	11-0164	0.10	0.14	0.05	0.04	0.01	0.10		-0.03	0.09	0.09	0.02	-0.09	0.08	0.05
	11-0167	0.15	0.15	0.14	-0.03	0.14	0.14	0.03	-0.04	0.09	0.09	-0.07	-0.09	0.25	0.07
	11-0179	0.15	0.04	0.13	0.11	0.06	0.01	-0.04	0.26	0.01		0.09	-0.07	0.22	0.07
	11-0183	0.14	0.12	0.03	-0.03	0.13	-0.01	0.14	-0.02	0.06	0.05	0.09	-0.03	0.07	0.06
	11-0184	0.07	0.18	-0.08	0.16	0.04	-0.04	0.15	0.25	-0.03	-0.14	0.06	-0.01	-0.08	0.05
	11-0194	0.14	0.08	0.03	0.16	0.08	0.07	0.12	-0.03	0.07	0.06	0.02	-0.04	0.09	0.07
	11-0198	0.09	0.14	0.06	0.06	0.15	0.11	-0.02	0.06	0.06	0.07	-0.03	0.06	-0.04	0.06
	Mean	**0.119**	**0.121**	**0.064**	**0.074**	**0.077**	**0.059**	**0.067**	**0.052**	**0.050**	**0.031**	**0.037**	**-0.033**	**0.079**	**0.063**
	SD	**0.0413**	**0.0426**	**0.0651**	**0.0684**	**0.0492**	**0.0566**	**0.0684**	**0.1124**	**0.0411**	**0.0724**	**0.0516**	**0.0474**	**0.1056**	**0.0089**
	11-0151	0.09	-0.06												
80 mg/kg	11-0158	0.16	0.05	0.07	0.12	-0.10	0.12	-0.09	0.17	0.01	-0.10	0.11	-0.15	0.33	0.06
	11-0177	0.17	0.09	0.11	0.11	0.06	-0.01	0.03	0.04	0.12	-0.20	0.14	-0.12	0.19	0.06
	11-0180	0.12	0.10	0.05	0.14	0.04	0.02	0.02	0.10	0.01	-0.05	0.04	-0.03	0.14	0.05
	11-0182	0.03	0.21	0.08	0.05	0.07	0.06	0.02	0.06		0.02	0.02	-0.18		0.04
	11-0186	0.11	0.11	0.10	0.04	0.07	0.06	-0.02	0.06	0.06		0.02	-0.18	0.19	0.05
	11-0188	0.01	0.17		0.11	0.08	0.01	0.05	-0.01	0.04	-0.01	0.03	-0.10	0.13	0.04
	11-0195	0.12	0.04	0.19	-0.04	0.12	0.04	0.13	0.19	-0.20	0.03	-0.02	-0.04	0.03	0.05
	11-0200	0.11	0.12	0.09	0.05	-0.22	0.14	0.03	-0.01	0.06	0.02	0.01	0.04	-0.03	0.04
	11-0201	0.10	0.10	0.12	0.07	0.05	0.02		0.08	0.01	0.06	-0.09	0.05	-0.19	0.04
	Mean	**0.101**	**0.093**	**0.101**	**0.072**	**0.018**	**0.051**	**0.022**	**0.076**	**0.013**	**-0.030**	**0.029**	**-0.079**	**0.098**	**0.047**
	SD	**0.0497**	**0.0723**	**0.0431**	**0.0566**	**0.1069**	**0.0514**	**0.0623**	**0.0702**	**0.0932**	**0.0837**	**0.0665**	**0.0862**	**0.1577**	**0.0084**

Appendix N

Individual and Summary of 14-Day Organ Mass Data

Table N-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

14-Day Individual Organ Mass
Male Rats

ABSOLUTE ORGAN MASS (GRAMS)

Group	Animal ID	Body Mass	Adrenals	Brain	Heart	Kidneys	Epididymides	Liver	Spleen	Testes	Thymus
	10-1635	341	0 068	1.913	1.288	2.714	0 980	11.374	0.685	3.204	0 457
Corn Oil	10-1643	352	0.069	1.921	1 421	2 476	0.939	12 342	0 680	3 227	0.501
Control	10-1645	354	0.081	2 051	1.335	3 106	0.977	13.379	0.894	3 459	0.682
	10-1662	350	0.073	2 109	1.599	2 932	0.825	13.580	0.831	2.901	0.548
	10-1664	336	0.627	2.085	1.469	2.471	0.908	11.635	0 599	2.939	0.564
	10-1675	345	0.064	1.845	1.245	3.219	0.825	12.255	0.685	2.771	0 525
	Mean	**346.3**	**0.1637**	**1.9873**	**1.3928**	**2.8197**	**0.9090**	**12.4275**	**0.7290**	**3.0835**	**0.5462**
	SD	**8.95**	**0.22708**	**0.10824**	**0.13061**	**0.31778**	**0.07025**	**0.89539**	**0.11027**	**0.25611**	**0.07843**
	10-1642	347	0.093	2.021	1.478	2.674	0.801	12.843	0.712	3.372	0.615
1.58 mg/kg	10-1651	329	0.076	1 980	1 319	2 680	1.086	11 357	0.727	3.280	0.375
	10-1656	323	0.073	1.945	1.118	2.608	1.038	11.165	0.571	3 543	0.445
	10-1660	355	0.080	2.027	1.658	3 334	0.909	13.673	0.862	3.035	0.620
	10-1672	334	0.062	2.028	1.343	2.742	1.119	10 680	0.683	3.300	0.516
	10-1678	358	0 079	1.997	1.52	3.037	1.072	13 688	0.843	3 183	0.749
	Mean	**341.0**	**0.0772**	**1.9997**	**1.4080**	**2.6458**	**1.0042**	**12.2343**	**0.7330**	**3.2655**	**0.5533**
	SD	**14.41**	**0.01011**	**0.03262**	**0.18771**	**0.28257**	**0.12328**	**1.33266**	**0.10767**	**0.17163**	**0.13525**
	10-1648	336	0 076	1.914	1.367	3.065	0.937	12.643	0.598	2.858	0.246
3.13 mg/kg	10-1652	337	0.078	1.866	1.321	2.611	0.869	13.080	0.652	2.758	0.597
	10-1657	332	0.074	2.021	1.203	3.215	0.784	12.888	0.531	2.954	0.593
	10-1661	341	0.059	2.021	1.568	3.143	1.082	12.618	0.760	3.246	0.530
	10-1666	334	0.075	2.091	1.313	2.945	1.002	11.550	0.761	3.367	0.796
	10-1683	333	0.088	1.764	1.339	2.791	0.901	11.301	0.770	3.417	0.465
	Mean	**335.5**	**0.0750**	**1.9462**	**1.3518**	**2.9617**	**0.9292**	**12.3467**	**0.6787**	**3.1000**	**0.5378**
	SD	**3.27**	**0.00934**	**0.12088**	**0.11978**	**0.22808**	**0.10413**	**0.73761**	**0.10076**	**0.27926**	**0.18096**
	10-1638	359	0.064	1.978	1.209	3.151	0.855	13.002	0.806	2.69	0.453
6.25 mg/kg	10-1639	336	0.067	2.008	1.206	2.954	0.891	12.439	0.797	3.065	0.547
	10-1641	337	0.075	2.037	1.194	2.565	0.915	13.236	0.756	3.539	0.449
	10-1644	329	0.057	1.978	1.336	2.731	0.825	12.279	0.684	2.959	0.487
	10-1670	325	0.084	1.934	1.339	2 704	1.020	11.159	0.763	3.171	0.418
	10-1680	307	0.056	1.986	1.145	2 432	1.043	10.131	0.781	3.505	0.513
	Mean	**332.2**	**0.0672**	**1.9868**	**1.2382**	**2.7562**	**0.9248**	**12.0410**	**0.7645**	**3.1548**	**0.4778**
	SD	**17.05**	**0.01080**	**0.03439**	**0.08032**	**0.28077**	**0.08844**	**1.18259**	**0.04383**	**0.32648**	**0.04719**
	10-1654	339	0.066	1 906	1.389	3.031	1.085	11.260	0.883	3.305	0.476
12.5 mg/kg	10-1659	333	0.078	1.969	1.375	3.524	0.888	13.193	0.575	3.138	0.423
	10-1663	358	0.063	2.118	1.373	3.345	0.887	12.736	0.878	3.412	0.503
	10-1665	345	0.074	1.949	1.374	3.523	0.942	13.378	0.855	3.127	0.721
	10-1676	338	0.071	1.989	1.501	2.952	0.894	13.347	0.690	3.024	0.592
	10-1681	354	0.067	2.008	1.357	3.141	1.053	12.489	0.845	3.205	0.589
	Mean	**344.5**	**0.0698**	**1.9898**	**1.3948**	**3.2527**	**0.9582**	**12.7338**	**0.7543**	**3.2018**	**0.5507**
	SD	**9.77**	**0.00558**	**0.07195**	**0.05299**	**0.24788**	**0.08882**	**0.80423**	**0.12250**	**0.13865**	**0.10615**
	10-1636	324	0 055	1 916	1.339	2.599	0.758	10.769	0 848	2 664	0 439
25 mg/kg	10-1640	329	0.058	2.005	1.406	3 055	0.969	13.933	0 786	3 126	0 642
	10-1658	321	0.079	2 070	1 237	2 993	0.928	11 749	0 785	3 305	0 460
	10-1671	334	0.069	1 829	1.322	2.811	0.903	11.987	0 691	3 192	0 424
	10-1673	354	0.081	2.062	1.693	3 404	0.989	13.295	0 778	2.992	0 722
	10-1677	321	0.060	1.852	1 351	2.639	1.004	11.082	0.906	3.091	0 379
	Mean	**330.5**	**0.0670**	**1.9557**	**1.3913**	**2.9168**	**0.9252**	**12.1358**	**0.7990**	**3.0617**	**0.5110**
	SD	**12.57**	**0.01112**	**0.10505**	**0.15761**	**0.30054**	**0.09017**	**1.24291**	**0.07255**	**0.22099**	**0.13745**
	10-1650	319	0.066	1.918	1 291	3 416	0.858	11.683	0 874	3.041	0 348
50 mg/kg	10-1653	306	0.075	1.851	1.293	2 906	0.983	11.337	0.659	3.085	0 455
	10-1668	304	0.060	1.974	1.213	2.739	0.941	11.804	0 713	2.836	0.557
	10-1669	317	0.065	2.039	1.125	3.023	1.037	11.372	0.608	3.536	0.521
	10-1679	324	0.065	1.979	1.373	2.816	0.902	11.998	0.764	3.287	0.457
	10-1684	333	0.084	1.975	1.382	3.385	0.858	12.400	0.730	3 163	0.423
	Mean	**317.2**	**0.0692**	**1.9580**	**1.2795**	**3.0475**	**0.9298**	**11.7657**	**0.7247**	**3.1580**	**0.4602**
	SD	**10.94**	**0.00875**	**0.06414**	**0.09783**	**0.28951**	**0.07146**	**0.40085**	**0.09158**	**0.23759**	**0.07359**
	10-1637	305	0.078	1.953	1 205	2 786	0.920	12.389	0.940	2.959	0.481
100 mg/kg	10-1646	299	0.080	2.024	1.238	3.003	0.688	13.248	0.800	1.866	0.435
	10-1647	315	0.059	2.058	1.358	3.302	0 768	12.618	0.841	2.38	0.506
	10-1649	295	0.060	1.971	1.203	3.065	0.914	12.582	0.763	2 773	0.481
	10-1655	306	0.067	2 085	1.342	3.041	1.053	12.381	1.023	3 418	0.542
	10-1674	322	0.064	1.977	1.435	3.226	0.800	13.503	0.800	2.830	0.454
	Mean	**307.0**	**0.0680**	**2.0113**	**1.2968**	**3.0705**	**0.8572**	**12.7888**	**0.8812**	**2.7043**	**0.4832**
	SD	**10.02**	**0.00901**	**0.05286**	**0.09547**	**0.18120**	**0.13088**	**0.47308**	**0.09993**	**0.52986**	**0.03785**

Table N-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

14-Day Individual Organ Mass
Female Rats

ABSOLUTE ORGAN MASS (GRAMS)

Group	Animal ID	Body Weight	Adrenals	Brain	Heart	Kidneys	Liver	Ovaries	Spleen	Thymus	Uterus
	10-1693	196	0.068	1.717	0.793	1.401	6.150	0.088	0.368	0.391	0.346
Corn Oil	10-1697	226	0.126	1.929	0.957	1.824	7.860	0.190	0.494	0.359	0.561
Control	10-1703	220	0.080	1.841	1.209	1.991	7.863	0.125	0.502	0.512	0.386
	10-1713	213	0.082	1.832	0.885	1.645	7.261	0.131	0.436	0.387	0.398
	10-1718	220	0.080	1.923	0.967	1.736	7.245	0.188	0.456	0.450	0.648
	10-1722	236	0.074	1.877	0.963	2.060	7.931	0.164	0.519	0.400	0.656
	Mean	**218.5**	**0.0850**	**1.8532**	**0.9823**	**1.7762**	**7.3850**	**0.1477**	**0.4825**	**0.4165**	**0.4992**
	SD	**13.44**	**0.02074**	**0.07789**	**0.13824**	**0.24024**	**0.68006**	**0.04009**	**0.05553**	**0.05539**	**0.13934**
	10-1685	213	0.063	1.923	0.979	1.662	7.527	0.138	0.484	0.379	0.430
1.56 mg/kg	10-1690	221	0.101	1.913	0.816	1.818	7.777	0.178	0.514	0.399	0.582
	10-1692	231	0.077	1.830	0.856	1.891	7.960	0.109	0.500	0.630	0.380
	10-1700	229	0.090	2.114	0.895	1.830	7.850	0.169	0.469	0.520	0.498
	10-1701	238	0.086	1.841	0.948	2.050	8.062	0.141	0.529	0.517	0.514
	10-1709	218	0.104	1.878	0.864	1.768	7.889	0.180	0.450	0.382	0.345
	Mean	**225.0**	**0.0868**	**1.9165**	**0.8930**	**1.8365**	**7.8442**	**0.1525**	**0.4910**	**0.4712**	**0.4582**
	SD	**9.27**	**0.01530**	**0.10368**	**0.06093**	**0.12968**	**0.18331**	**0.02798**	**0.02822**	**0.10136**	**0.08916**
	10-1695	228	0.067	1.964	0.891	1.752	7.887	0.157	0.547	0.380	0.475
3.13 mg/kg	10-1699	225	0.089	1.952	0.860	1.899	8.730	0.143	0.554	0.521	0.380
	10-1705	230	0.109	1.762	ND	1.867	8.761	0.142	0.521	ND	0.536
	10-1714	226	0.096	2.019	0.943	1.918	8.328	0.174	0.496	0.541	0.434
	10-1719	221	0.081	1.825	0.894	1.556	7.163	0.184	0.438	0.288	0.595
	10-1723	214	0.098	1.930	0.907	1.887	7.610	0.089	0.549	0.487	0.663
	Mean	**224.0**	**0.0900**	**1.9087**	**0.8990**	**1.8132**	**8.0798**	**0.1482**	**0.5175**	**0.4434**	**0.5138**
	SD	**5.76**	**0.01464**	**0.09600**	**0.03004**	**0.13898**	**0.63981**	**0.03345**	**0.04470**	**0.10679**	**0.10500**
	10-1702	223	0.086	1.835	0.939	1.852	7.242	0.105	0.541	0.493	0.496
6.25 mg/kg	10-1707	233	0.095	1.850	0.889	1.837	7.422	0.213	0.613	0.359	0.524
	10-1711	220	0.100	1.960	0.977	1.918	7.524	0.135	0.489	0.382	0.483
	10-1715	225	0.102	1.931	1.022	1.965	7.928	0.193	0.715	0.328	0.661
	10-1729	219	0.081	1.863	0.953	1.681	8.075	0.165	0.624	0.362	0.477
	10-1730	232	0.110	1.851	0.941	2.277	8.882	0.109	0.521	0.667	0.541
	Mean	**225.3**	**0.0957**	**1.8817**	**0.9535**	**1.9217**	**7.8455**	**0.1533**	**0.5838**	**0.4352**	**0.5303**
	SD	**5.96**	**0.01071**	**0.05107**	**0.04421**	**0.19905**	**0.59678**	**0.04451**	**0.08300**	**0.12652**	**0.06654**
	10-1687	211	0.088	1.901	0.899	1.955	7.844	0.128	0.642	0.419	0.551
12.5 mg/kg	10-1694	218	0.082	1.938	0.906	1.902	9.484	0.166	0.507	0.446	0.362
	10-1725	213	0.085	1.760	1.021	1.748	7.372	0.139	0.611	0.595	0.409
	10-1726	238	0.071	1.863	0.978	1.925	8.890	0.152	0.505	0.450	0.820
	10-1733	216	0.082	1.893	1.116	1.983	7.703	0.159	0.543	0.264	0.482
	10-1734	226	0.066	1.896	0.947	1.861	7.823	0.175	0.636	0.441	0.502
	Mean	**220.7**	**0.0790**	**1.8755**	**0.9778**	**1.8957**	**8.1660**	**0.1535**	**0.5740**	**0.4358**	**0.5243**
	SD	**10.35**	**0.00759**	**0.06143**	**0.08158**	**0.08373**	**0.81548**	**0.01769**	**0.06332**	**0.10533**	**0.15747**
	10-1691	216	0.079	1.934	0.960	1.836	7.970	0.180	0.767	0.293	0.713
25 mg/kg	10-1704	215	0.075	1.924	0.996	1.913	8.144	0.154	0.532	0.231	0.436
	10-1708	201	0.098	1.858	0.828	1.869	7.365	0.132	0.558	0.431	0.598
	10-1720	213	0.095	1.834	0.764	1.917	7.583	0.125	0.567	0.523	0.756
	10-1727	222	0.093	1.847	0.885	1.918	8.938	0.157	0.592	0.514	0.518
	10-1732	216	0.052	1.848	0.843	1.768	7.414	0.170	0.544	0.340	0.825
	Mean	**213.8**	**0.0820**	**1.8742**	**0.8793**	**1.8702**	**7.9023**	**0.1530**	**0.5833**	**0.3887**	**0.6410**
	SD	**6.97**	**0.01734**	**0.04327**	**0.08650**	**0.05987**	**0.59410**	**0.02126**	**0.08752**	**0.11994**	**0.14918**
	10-1688	222	0.072	1.939	0.820	2.103	9.301	0.139	0.603	0.432	0.902
50 mg/kg	10-1706	225	0.071	1.968	0.910	2.061	8.929	0.192	0.622	0.266	0.698
	10-1710	227	0.077	1.903	0.856	1.984	8.569	0.161	0.710	0.467	0.334
	10-1716	203	0.058	1.844	0.742	1.769	7.150	0.126	0.544	0.483	0.374
	10-1717	203	0.070	1.866	0.813	1.981	7.953	0.157	0.647	0.461	0.645
	10-1731	231	0.087	1.785	0.934	2.165	9.240	0.144	0.753	0.298	0.525
	Mean	**218.5**	**0.0725**	**1.8842**	**0.8482**	**2.0105**	**8.5237**	**0.1532**	**0.6465**	**0.4045**	**0.6130**
	SD	**12.36**	**0.00948**	**0.06858**	**0.07009**	**0.13775**	**0.83822**	**0.02283**	**0.07533**	**0.09716**	**0.23970**
	10-1686	209	0.056	1.811	0.865	1.719	7.432	0.090	0.739	0.396	0.453
100 mg/kg	10-1689	210	0.078	1.945	0.723	1.928	9.202	0.090	0.804	0.374	0.520
	10-1696	200	0.068	1.841	0.865	1.944	7.637	0.166	0.733	0.340	0.405
	10-1712	208	0.073	1.937	1.013	2.107	8.134	0.122	1.045	0.379	0.405
	10-1724	209	0.087	1.830	0.848	2.008	8.607	0.126	0.788	0.351	0.475
	10-1728	230	0.076	1.901	0.872	2.196	9.804	0.163	0.792	0.348	0.511
	Mean	**211.0**	**0.0730**	**1.8775**	**0.8643**	**1.9837**	**8.4893**	**0.1265**	**0.8168**	**0.3647**	**0.4615**
	SD	**10.00**	**0.01043**	**0.05772**	**0.09209**	**0.16469**	**0.91852**	**0.03341**	**0.11555**	**0.02167**	**0.05002**

Table N-2
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

14-Day Individual % Body Mass Organ Mass
Male Rats

% BODY WEIGHT ORGAN MASS

Group	Animal ID	Adrenals	Brain	Heart	Kidneys	Epididymides	Liver	Spleen	Testes	Thymus
	10-1635	0.0199	0.5610	0 3777	0 7959	0.2874	3.3355	0.2009	0.9396	0 1340
Corn Oil	10-1643	0.0196	0 5457	0.4037	0.7034	0 2668	3.5063	0 1932	0.9168	0.1423
Control	10-1645	0.0229	0 5794	0.3771	0.8774	0.2760	3.7794	0.2525	0.9771	0 1927
	10-1662	0.0209	0.6026	0.4569	0.8377	0.2357	3.8800	0.2374	0.8289	0.1566
	10-1664	0.1866	0.6205	0.4372	0 7354	0 2702	3.4628	0.1783	0.8747	0.1679
	10-1675	0.0186	0.5348	0.3609	0.9330	0.2391	3.5522	0.1986	0.8032	0.1522
	Mean	0.04807	0.57400	0.40224	0.81381	0.26254	3.58601	0.21014	0.89004	0.15780
	SD	0.067883	0.033216	0.037620	0.088587	0.020703	0.204584	0.028516	0.066744	0.020755
	10-1642	0.0268	0.5824	0.4259	0 7706	0.2308	3.7012	0.2052	0.9718	0 1772
1.56 mg/kg	10-1651	0.0231	0 6018	0.4009	0 8146	0.3301	3 4520	0 2210	0.9970	0 1140
	10-1656	0.0226	0.6022	0.3461	0 8074	0.3214	3.4567	0.1768	1.0969	0 1378
	10-1660	0 0225	0.5710	0.4670	0.9392	0.2561	3.8515	0 2428	0 8549	0 1746
	10-1672	0.0186	0.6072	0.4021	0 8210	0.3350	3.1976	0 2045	0.9880	0 1545
	10-1678	0 0221	0.5578	0.4246	0.8483	0.2994	3.8235	0.2355	0.8891	0.2092
	Mean	0.02281	0.58707	0.41112	0.83351	0.29547	3.58040	0.21429	0.96626	0.16122
	SD	0.002826	0.019930	0.039843	0.057503	0.042650	0.255155	0.024055	0.085862	0.033368
	10-1648	0.0226	0.5696	0.4068	0 9122	0.2789	3.7628	0 1780	0.8506	0.0732
3.13 mg/kg	10-1652	0.0231	0.5537	0.3920	0.7748	0.2579	3.8813	0.1935	0.8184	0.1772
	10-1657	0.0223	0.6087	0.3623	0.9684	0.2361	3.8819	0.1599	0.8898	0.1786
	10-1661	0.0173	0.5927	0.4598	0.9217	0.3173	3.7003	0.2229	0.9519	0.1554
	10-1666	0.0225	0.6260	0.3931	0.8817	0.3000	3.4581	0.2278	1.0081	0.2383
	10-1683	0.0264	0.5297	0.4021	0.8381	0.2706	3.3937	0.2312	1.0261	0.1396
	Mean	0.02237	0.58009	0.40270	0.88282	0.27679	3.67988	0.20222	0.92414	0.16039
	SD	0.002926	0.035867	0.031986	0.088360	0.029097	0.209859	0.029588	0.084842	0.054298
	10-1638	0.0178	0.5510	0.3368	0.8777	0.2382	3.6217	0.2245	0.7493	0.1262
6.25 mg/kg	10-1639	0.0199	0.5976	0.3589	0.8792	0.2652	3.7021	0.2372	0.9122	0.1628
	10-1641	0.0223	0.6045	0.3543	0.7611	0.2715	3.9276	0.2243	1.0501	0.1332
	10-1644	0.0173	0.6012	0.4061	0.8301	0.2508	3.7322	0.2079	0.8994	0.1480
	10-1670	0.0258	0.5951	0.4120	0.8320	0.3138	3.4335	0.2348	0.9757	0.1286
	10-1680	0.0182	0.6469	0.3730	0.7922	0.3397	3.3000	0.2544	1.1417	0.1671
	Mean	0.02024	0.59937	0.37351	0.82871	0.27987	3.61953	0.23052	0.95474	0.14433
	SD	0.003283	0.030504	0.029913	0.046809	0.039016	0.224105	0.015613	0.135330	0.017734
	10-1654	0.0195	0.5622	0.4097	0.8941	0.3201	3.3215	0.2015	0.9749	0.1404
12.5 mg/kg	10-1659	0.0234	0.5913	0.4129	1.0583	0.2667	3.9619	0.1727	0.9423	0 1270
	10-1663	0.0176	0.5916	0.3835	0.9344	0.2478	3.5575	0.2453	0.9531	0.1405
	10-1665	0.0214	0.5649	0.3983	1 0212	0.2730	3.8777	0.2478	0.9064	0.2090
	10-1676	0.0210	0.5885	0.4441	0.8734	0.2645	3.9488	0.2041	0.8947	0.1751
	10-1681	0.0189	0.5672	0.3833	0.8873	0.2975	3.5280	0.2387	0.9054	0.1664
	Mean	0.02031	0.57763	0.40531	0.94476	0.27825	3.69923	0.21834	0.92946	0.15974
	SD	0.002071	0.014184	0.022752	0.077191	0.026064	0.266524	0.030265	0.031981	0.030068
	10-1636	0.0170	0.5914	0.4133	0 8022	0.2340	3.3238	0 2617	0.8222	0 1355
25 mg/kg	10-1640	0 0176	0.6094	0.4274	0 9286	0.2945	4.2350	0 2389	0 9502	0 1951
	10-1658	0 0246	0 6449	0 3854	0 9324	0.2891	3.6601	0 2445	1.0296	0 1433
	10-1671	0.0207	0.5476	0 3958	0.8416	0.2704	3.5889	0 2069	0.9557	0 1269
	10-1673	0 0229	0.5825	0.4782	0.9616	0.2794	3.7556	0.2198	0.8452	0 2040
	10-1677	0.0187	0.5769	0.4209	0 8221	0.3128	3.4523	0 2822	0.9629	0.1181
	Mean	0.02024	0.59211	0.42015	0.88141	0.28001	3.66929	0.24235	0.92763	0.15382
	SD	0.003037	0.032821	0.032491	0.087277	0.026765	0.316365	0.027390	0.078541	0.036517
	10-1650	0.0207	0.6013	0 4047	1.0708	0.2690	3 6624	0.2740	0.9533	0 1091
50 mg/kg	10-1653	0.0245	0.6049	0 4225	0.9497	0.3212	3 7049	0.2154	1.0082	0 1487
	10-1668	0.0197	0.6493	0 3990	0.9010	0.3095	3.8829	0.2345	0.9329	0.1832
	10-1669	0.0205	0 6432	0.3549	0.9536	0.3271	3.5874	0 1918	1.1155	0 1644
	10-1679	0.0201	0.6108	0.4238	0.8691	0.2784	3 7031	0.2358	1.0145	0 1410
	10-1684	0.0252	0.5931	0 4150	1.0165	0.2577	3.7237	0 2192	0.9498	0 1270
	Mean	0.02179	0.61710	0.40332	0.96013	0.29382	3.71073	0.22845	0.99569	0.14557
	SD	0.002419	0.023399	0.025653	0.073982	0.029230	0.097421	0.027438	0.087374	0.028364
	10-1637	0.0256	0.6403	0.3951	0.9134	0 3016	4.0620	0.3082	0 9702	0 1577
100 mg/kg	10-1646	0.0268	0 6769	0.4140	1.0043	0.2301	4.4308	0.2676	0 6241	0 1455
	10-1647	0.0187	0.6533	0.4311	1.0483	0.2438	4.0057	0.2670	0.7556	0 1606
	10-1649	0.0203	0.6681	0 4078	1.0390	0.3098	4.2651	0.2586	0.9400	0 1631
	10-1655	0.0219	0.6814	0.4386	0.9938	0.3441	4.0461	0 3343	1.1170	0.1771
	10-1674	0.0199	0.6140	0.4457	1.0019	0.2484	4.1935	0 2484	0.8789	0.1410
	Mean	0.02219	0.65588	0.42204	1.00011	0.27966	4.16718	0.28069	0.88095	0.15750
	SD	0.003260	0.025483	0.019528	0.047752	0.045307	0.162211	0.033221	0.172553	0.012982

Table N-2
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

14-Day Individual % Body Weight Organ Mass
Female Rats

% BODY WEIGHT ORGAN MASS

Group	Animal ID	Adrenals	Brain	Heart	Kidneys	Liver	Ovaries	Spleen	Thymus	Uterus
	10-1693	0.0347	0 8760	0.4046	0.7148	3 1378	0 0449	0.1878	0.1995	0.1765
Corn Oil	10-1697	0.0558	0 8535	0.4235	0.8071	3.4779	0.0841	0 2186	0.1588	0.2482
Control	10-1703	0.0364	0.8368	0.5495	0.9050	3 5741	0.0568	0.2282	0.2327	0 1755
	10-1713	0.0385	0.8601	0 4155	0.7723	3 4089	0.0615	0 2047	0.1817	0.1869
	10-1718	0.0364	0.8741	0.4395	0.7891	3.2932	0.0855	0.2073	0.2045	0.2945
	10-1722	0.0314	0.7953	0.4081	0.8729	3.3606	0.0695	0.2199	0.1695	0.2780
	Mean	0.03884	0.84932	0.44011	0.81019	3.37540	0.06704	0.21107	0.19113	0.22660
	SD	0.008821	0.030102	0.055044	0.069151	0.151365	0.015878	0.014326	0.028757	0.053713
	10-1685	0.0296	0.9028	0 4596	0.7803	3 5338	0.0648	0.2272	0.1779	0.2019
1.56 mg/kg	10-1690	0.0457	0.8656	0 3692	0 8226	3 5190	0.0805	0.2326	0.1805	0.2633
	10-1692	0.0333	0.7922	0 3706	0 8186	3.4459	0.0472	0 2165	0.2727	0 1645
	10-1700	0.0393	0 9231	0 3908	0.7991	3 4279	0.0738	0.2048	0.2271	0 2175
	10-1701	0.0361	0 7735	0.3983	0.8613	3 3874	0.0592	0.2223	0.2172	0 2160
	10-1709	0.0477	0.8615	0 3963	0.8110	3 6188	0.0826	0.2064	0.1752	0.1583
	Mean	0.03863	0.85313	0.39748	0.81550	3.48881	0.06802	0.21829	0.20846	0.20357
	SD	0.007058	0.059403	0.032957	0.027189	0.084459	0.013590	0.011190	0.038390	0.038737
	10-1695	0.0294	0.8614	0.3908	0.7684	3.4592	0.0689	0.2399	0 1667	0.2083
3.13 mg/kg	10-1699	0.0396	0.8676	0.3822	0.8440	3.8800	0.0636	0.2462	0.2316	0.1689
	10-1705	0.0474	0.7681	ND	0.8117	3.8091	0.0617	0.2265	ND	0.2330
	10-1714	0.0425	0.8934	0.4173	0.8487	3.6850	0.0770	0.2195	0.2394	0.1920
	10-1719	0.0367	0.8258	0.4045	0.7041	3.2412	0.0833	0.1982	0.1303	0.2692
	10-1723	0.0458	0.9019	0.4238	0.8818	3.5561	0.0416	0.2565	0.2276	0.3096
	Mean	0.04021	0.85268	0.40372	0.80978	3.60509	0.06600	0.23114	0.19910	0.23022
	SD	0.006605	0.050186	0.017448	0.064429	0.236518	0.014470	0.020942	0.048139	0.052064
	10-1702	0.0386	0.8229	0.4211	0.8305	3.2475	0.0471	0.2426	0.2211	0.2224
6.25 mg/kg	10-1707	0.0408	0.7940	0.3815	0 7884	3.1854	0.0914	0.2631	0.1541	0.2249
	10-1711	0.0455	0.8908	0.4441	0.8718	3.4200	0.0614	0.2223	0.1736	0.2195
	10-1715	0.0453	0.8582	0.4542	0.8733	3.5236	0.0858	0.3178	0.1458	0.2938
	10-1729	0.0370	0.8507	0.4352	0.7676	3.6872	0.0753	0.2849	0.1744	0.2178
	10-1730	0.0474	0.7978	0.4056	0.9815	3.8284	0.0470	0.2246	0.2875	0.2332
	Mean	0.04242	0.83575	0.42362	0.85218	3.46203	0.06799	0.25921	0.19275	0.23527
	SD	0.004236	0.037728	0.026796	0.078474	0.249260	0.019191	0.037275	0.053260	0.029183
	10-1687	0.0322	0.9009	0.4261	0.9265	3.7175	0.0607	0.3043	0.1986	0 2611
12.5 mg/kg	10-1694	0.0376	0.8890	0.4156	0.8725	4.3505	0.0771	0.2326	0.2046	0.1752
	10-1725	0.0399	0.8263	0.4793	0.8207	3 4610	0.0653	0.2869	0.2793	0.1920
	10-1726	0.0298	0.7828	0.4109	0.8088	3.7353	0.0639	0 2122	0.1891	0.3445
	10-1733	0.0380	0.8764	0.5167	0.9181	3.5662	0.0736	0.2514	0.1222	0.2231
	10-1734	0.0377	0.8325	0.4154	0.8162	3.4311	0.0768	0.2789	0.1934	0.2202
	Mean	0.03568	0.85131	0.44399	0.86046	3.71026	0.06954	0.28103	0.19787	0.23604
	SD	0.003921	0.045147	0.043755	0.052982	0.338026	0.007135	0.035060	0.050008	0.060754
	10-1691	0 0366	0.8954	0 4444	0.8500	3 6898	0.0833	0.3551	0 1356	0.3301
25 mg/kg	10-1704	0 0349	0 8949	0.4633	0.8898	3 7879	0 0716	0.2474	0 1074	0 2028
	10-1708	0.0488	0.9244	0 4119	0 9299	3 6642	0 0657	0.2776	0 2144	0.2975
	10-1720	0.0446	0.8610	0 3587	0 9000	3 5601	0 0587	0.2662	0 2455	0 3549
	10-1727	0.0419	0.8320	0.3986	0.8640	4.0261	0 0707	0.2667	0 2315	0.2333
	10-1732	0.0241	0.8556	0.3903	0.8185	3.4324	0.0787	0.2519	0.1574	0 3819
	Mean	0.03646	0.87720	0.41121	0.87535	3.69342	0.07146	0.27748	0.18200	0.30010
	SD	0.006704	0.033595	0.037865	0.039466	0.203171	0.008840	0.039565	0.056311	0.070054
	10-1688	0.0324	0 8734	0 3694	0.9473	4.1896	0 0626	0.2716	0.1946	0.4063
50 mg/kg	10-1706	0.0316	0.8747	0 4044	0.9160	3.9684	0 0853	0.2764	0.1182	0.3102
	10-1710	0.0339	0.8383	0 3780	0 8740	3.7749	0 0709	0.3128	0.2057	0.1471
	10-1716	0.0286	0.9084	0.3655	0.8714	3.5222	0.0621	0.2680	0.2379	0.1842
	10-1717	0.0345	0.9192	0.4005	0.9759	3.9177	0.0773	0.3187	0.2369	0.4163
	10-1731	0.0377	0.7727	0.4043	0.9372	4.0000	0.0623	0.3260	0.1290	0.2273
	Mean	0.03310	0.86445	0.36702	0.92030	3.89548	0.07010	0.29559	0.18707	0.28191
	SD	0.003057	0.053308	0.018113	0.041599	0.226794	0.009654	0.026294	0.052136	0.114030
	10-1686	0.0268	0.8665	0.4139	0.8225	3.5560	0.0431	0 3536	0.1895	0.2167
100 mg/kg	10-1689	0.0371	0.9262	0.3443	0 9181	4.3819	0.0429	0.3829	0.1781	0.2476
	10-1696	0.0340	0.9205	0.4325	0.9720	3.8185	0.0830	0.3665	0.1700	0.2025
	10-1712	0.0351	0.9313	0.4870	1.0130	3.9106	0.0587	0.5024	0.1822	0.1947
	10-1724	0.0416	0.8756	0.4057	0.9608	4.1182	0.0612	0.3770	0 1679	0.2273
	10-1728	0.0330	0.8265	0.3791	0.9548	4.2626	0.0709	0.3443	0.1513	0.2222
	Mean	0.03462	0.89109	0.41043	0.94019	4.00796	0.05995	0.38779	0.17317	0.21850
	SD	0.004895	0.041769	0.048459	0.085251	0.305448	0.015692	0.057942	0.013321	0.018779

Table N-3
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

14-Day Individual % Brain Weight Organ Mass
Male Rats

% BRAIN WEIGHT ORGAN MASS

Group	Animal ID	Adrenals	Heart	Kidneys	Epididymides	Liver	Spleen	Testes	Thymus
	10-1635	3.5546	67.3288	141.8714	51.2284	594.5635	35.8076	167.4856	23.8892
Corn Oil	10-1643	3.5919	73.9719	128.8912	48.8808	642.4779	35.3982	167.9854	26.0802
Control	10-1645	3.9493	65.0902	151.4383	47.6353	652.3159	43.5885	168.6494	33.2521
	10-1662	3.4614	75.8179	139.0232	39.1181	643.9071	39.4026	137.5533	25.9839
	10-1664	30.0719	70.4556	118.5132	43.5492	558.0336	28.7290	140.9592	27.0504
	10-1675	3.4688	67.4797	174.4715	44.7154	664.2276	37.1274	150.1897	28.4553
	Mean	**8.01632**	**70.02402**	**142.36815**	**45.85453**	**625.92094**	**36.67555**	**155.47046**	**27.45182**
	SD	**10.806488**	**4.181429**	**19.365012**	**4.317417**	**40.874724**	**4.918572**	**14.381650**	**3.210599**
	10-1642	4.6017	73.1321	132.3107	39.6338	635.4775	35.2301	166.8481	30.4305
1.56 mg/kg	10-1651	3.8384	66.6162	135.3535	54.8485	573.5859	36.7172	165.6566	18.9394
	10-1656	3.7532	57.4807	134.0874	53.3676	574.0360	29.3573	182.1594	22.8792
	10-1660	3.9467	81.7958	164.4795	44.8446	674.5437	42.5259	149.7287	30.5871
	10-1672	3.0572	66.2229	135.2071	55.1775	526.6272	33.6785	162.7219	25.4438
	10-1678	3.9559	76.1142	152.0781	53.6805	685.4281	42.2133	159.3891	37.5063
	Mean	**3.85886**	**70.22697**	**142.25274**	**50.25876**	**611.61639**	**36.62038**	**164.41728**	**27.63103**
	SD	**0.494805**	**8.584561**	**13.083876**	**6.462741**	**63.338985**	**5.089303**	**10.640860**	**6.587928**
	10-1648	3.9707	71.4211	160.1358	48.9551	660.5538	31.2435	149.3208	12.8527
3.13 mg/kg	10-1652	4.1601	70.7931	139.9250	46.5702	700.9646	34.9411	147.8028	31.9936
	10-1657	3.6616	59.5250	159.0797	38.7927	637.7041	26.2741	146.1653	29.3419
	10-1661	2.9193	77.5854	155.5171	53.5379	624.3444	37.6051	160.6136	26.2246
	10-1666	3.5868	62.7929	140.8417	47.9197	552.3673	36.3941	161.0234	38.0679
	10-1683	4.9887	75.9070	158.2200	51.0771	640.6463	43.6508	193.7075	26.3605
	Mean	**3.88453**	**69.67076**	**152.28653**	**47.80876**	**636.09875**	**35.01811**	**159.77222**	**27.47354**
	SD	**0.690481**	**7.156768**	**9.350855**	**5.052814**	**48.944892**	**5.898108**	**17.841100**	**8.399174**
	10-1638	3.2356	61.1223	159.3023	43.2255	657.3306	40.7482	135.9960	22.9019
6.25 mg/kg	10-1639	3.3367	60.0598	147.1116	44.3725	619.4721	39.6912	152.6394	27.2410
	10-1641	3.6819	58.6156	125.9205	44.9190	649.7791	37.1134	173.7359	22.0422
	10-1644	2.8817	67.5430	138.0688	41.7088	620.7786	34.5804	149.5956	24.6208
	10-1670	4.3433	69.2347	139.8139	52.7404	576.9907	39.4519	163.9607	21.6132
	10-1680	2.8197	57.6536	122.4572	52.5176	510.1208	39.3253	176.4854	25.8308
	Mean	**3.38315**	**62.37150**	**138.77903**	**46.58084**	**605.74532**	**38.48507**	**158.73549**	**24.04188**
	SD	**0.586394**	**4.839800**	**13.593797**	**4.812693**	**54.745428**	**2.251487**	**15.525210**	**2.234571**
	10-1654	3.4627	72.8751	159.0241	56.9255	590.7660	35.8342	173.3998	24.9738
12.5 mg/kg	10-1659	3.9614	69.8324	178.9741	45.0990	670.0356	29.2026	159.3702	21.4830
	10-1663	2.9745	64.8253	157.9320	41.8791	601.3220	41.4542	161.0954	23.7488
	10-1665	3.7968	70.4977	180.7594	48.3325	686.4033	43.8687	160.4413	36.9933
	10-1676	3.5696	75.4651	148.4163	44.9472	671.0407	34.6908	152.0362	29.7637
	10-1681	3.3367	67.5797	156.4243	52.4402	621.9622	42.0817	159.6116	29.3327
	Mean	**3.51698**	**70.17921**	**163.58837**	**48.27060**	**640.25495**	**37.85536**	**160.99240**	**27.71588**
	SD	**0.348807**	**3.764352**	**13.159634**	**5.554307**	**40.854332**	**5.584859**	**6.913890**	**5.587484**
	10-1636	2.8706	69.8852	135.6472	39.5616	562.0564	44.2589	139.0397	22.9123
25 mg/kg	10-1640	2.8928	70.1247	152.3691	48.3292	694.9127	39.2020	155.9102	32.0200
	10-1658	3.8164	59.7585	144.5894	44.8309	567.5845	37.9227	159.6618	22.2222
	10-1671	3.7726	72.2799	153.6905	49.3712	655.3855	37.7802	174.5216	23.1821
	10-1673	3.9282	82.1048	165.0824	47.9631	644.7624	37.7304	145.1018	35.0145
	10-1677	3.2397	72.9482	142.4946	54.2117	598.3801	48.9201	166.9006	20.4644
	Mean	**3.42005**	**71.18353**	**148.97887**	**47.37795**	**620.51380**	**40.96904**	**158.85597**	**25.98924**
	SD	**0.480018**	**7.168380**	**10.315876**	**4.888825**	**53.005475**	**4.628227**	**13.246263**	**5.998094**
	10-1650	3.4411	67.3097	178.1022	44.7341	609.1241	45.5683	158.5506	18.1439
50 mg/kg	10-1653	4.0519	69.8541	156.9962	53.1064	612.4797	35.6024	166.6667	24.5813
	10-1668	3.0395	61.4488	136.7538	47.6697	597.9737	36.1196	143.6677	28.2168
	10-1669	3.1878	55.1741	148.2590	50.8583	557.7244	29.8185	173.4183	25.5517
	10-1679	3.2845	69.3785	142.2941	45.5786	606.2658	38.6054	166.0940	23.0925
	10-1684	4.2532	69.9747	171.3924	43.4430	627.8481	36.9620	160.1519	21.4177
	Mean	**3.54299**	**65.52332**	**155.98628**	**47.56502**	**801.90263**	**37.11269**	**161.42486**	**23.50066**
	SD	**0.493990**	**6.005524**	**15.947576**	**3.755381**	**23.782931**	**5.103334**	**10.181555**	**3.487607**
	10-1637	3.9939	61.6999	142.6523	47.1070	634.3574	48.1311	151.5105	24.6288
100 mg/kg	10-1646	3.9526	61.1660	148.3696	33.9921	654.5455	39.5257	92.1937	21.4921
	10-1647	2.8669	65.9864	160.4470	37.3178	613.1195	40.8649	115.6463	24.5870
	10-1649	3.0441	61.0350	155.5048	46.3724	638.3562	38.7113	140.6900	24.4039
	10-1655	3.2134	64.3645	145.8513	50.5036	593.8129	49.0647	163.9329	25.9952
	10-1674	3.2372	72.5847	163.1765	40.4654	683.0046	40.4654	143.1462	22.9641
	Mean	**3.38468**	**64.47277**	**152.86693**	**42.62837**	**636.19934**	**42.79365**	**134.51991**	**24.01183**
	SD	**0.475135**	**4.439550**	**8.297560**	**6.380818**	**31.191781**	**4.587293**	**28.130475**	**1.564973**

Table N-3
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

14-Day Individual % Brain Weight Organ Mass
Female Rats

% BRAIN WEIGHT ORGAN MASS

Group	Animal ID	Adrenals	Heart	Kidneys	Liver	Ovaries	Spleen	Thymus	Uterus
	10-1693	3.9604	46.1852	81.5958	358.1829	5.1252	21.4327	22.7723	20.1514
Corn Oil	10-1697	6.5319	49.6112	94.5568	407.4650	9.8497	25.6091	18.6107	29.0824
Control	10-1703	4.3455	65.6708	108.1477	427.1048	6.7898	27.2678	27.8110	20.9669
	10-1713	4.4760	48.3079	89.7926	396.3428	7.1507	23.7991	21.1245	21.7249
	10-1718	4.1602	50.2860	90.2756	376.7551	9.7764	23.7129	23.4009	33.6973
	10-1722	3.9425	51.3053	109.7496	422.5360	8.7373	27.6505	21.3106	34.9494
	Mean	**4.56939**	**51.89440**	**95.88635**	**398.06442**	**7.90484**	**24.91204**	**22.50499**	**26.76206**
	SD	**0.984034**	**6.977037**	**11.107596**	**28.751310**	**1.871713**	**2.379776**	**3.083041**	**6.880905**
	10-1685	3.2761	50.9100	86.4275	391.4197	7.1763	25.1690	19.7088	22.3609
1.56 mg/kg	10-1690	5.2787	42.6555	95.0340	406.5342	9.3048	26.8688	20.8573	30.4234
	10-1692	4.2077	46.7760	103.3333	434.9727	5.9563	27.3224	34.4262	20.7650
	10-1700	4.2573	42.3368	86.5658	371.3340	7.9943	22.1854	24.5979	23.5572
	10-1701	4.6714	51.4938	111.3525	437.9142	7.6589	28.7344	28.0826	27.9196
	10-1709	5.5378	46.0064	94.1427	420.0745	9.5847	23.9617	20.3408	18.3706
	Mean	**4.53833**	**46.69841**	**96.14263**	**410.37488**	**7.94587**	**25.70695**	**24.66893**	**23.89947**
	SD	**0.818253**	**3.913735**	**9.740402**	**25.908726**	**1.354078**	**2.402559**	**5.739812**	**4.507789**
	10-1695	3.4114	45.3666	89.2057	401.5784	7.9939	27.8513	19.3483	24.1853
3.13 mg/kg	10-1699	4.5594	44.0574	97.2848	447.2336	7.3258	28.3811	26.6906	19.4672
	10-1705	6.1862	ND	105.9591	497.2191	8.0590	29.5687	ND	30.4200
	10-1714	4.7548	46.7063	94.9975	412.4814	8.6181	24.5666	26.7954	21.4958
	10-1719	4.4384	48.9863	85.2603	392.4932	10.0822	24.0000	15.7808	32.6027
	10-1723	5.0777	46.9948	97.7720	394.3005	4.6114	28.4456	25.2332	34.3523
	Mean	**4.73798**	**46.42228**	**95.07992**	**424.21770**	**7.78174**	**27.13556**	**22.76985**	**27.08723**
	SD	**0.904918**	**1.849993**	**7.230581**	**41.013886**	**1.809555**	**2.288099**	**4.954773**	**6.197735**
	10-1702	4.6866	51.1717	100.9264	394.6594	5.7221	29.4823	26.8665	27.0300
6.25 mg/kg	10-1707	5.1351	48.0541	99.2973	401.1892	11.5135	33.1351	19.4054	28.3243
	10-1711	5.1020	49.8469	97.8571	383.8776	6.8878	24.9490	19.4898	24.6429
	10-1715	5.2822	52.9259	101.7607	410.5645	9.9948	37.0274	16.9860	34.2310
	10-1729	4.3478	51.1541	90.2308	433.4407	8.8567	33.4944	20.5046	25.8039
	10-1730	5.8427	50.8374	123.0146	479.8487	5.8887	28.1469	36.0346	29.2274
	Mean	**5.08277**	**50.68501**	**102.18117**	**417.26334**	**8.14393**	**31.03919**	**23.21447**	**28.17657**
	SD	**0.543966**	**1.620107**	**11.005520**	**34.949792**	**2.358899**	**4.339580**	**7.102277**	**3.411819**
	10-1687	3.5771	47.2909	102.8406	412.6249	6.7333	33.7717	22.0410	28.9847
12.5 mg/kg	10-1694	4.2312	46.7492	98.1424	489.3705	8.6687	26.1610	23.0134	19.7110
	10-1725	4.8295	58.0114	99.3182	418.8636	7.8977	34.7159	33.8068	23.2386
	10-1726	3.8111	52.4960	103.3280	477.1873	8.1589	27.1068	24.1546	44.0150
	10-1733	4.3317	58.9540	104.7544	406.9202	8.3994	28.6846	13.9461	25.4622
	10-1734	4.5311	49.8946	98.0506	412.1707	9.2202	33.5090	23.2350	26.4489
	Mean	**4.21861**	**52.23269**	**101.07235**	**436.18955**	**8.17971**	**30.65817**	**23.38616**	**27.97676**
	SD	**0.460765**	**5.284887**	**2.917686**	**36.873042**	**0.841681**	**3.768764**	**6.328725**	**8.457792**
	10-1691	4.0848	49.6381	94.9328	412.0993	9.3071	39.6587	15.1499	36.8666
25 mg/kg	10-1704	3.8981	51.7672	99.4283	423.2848	8.0042	27.6507	12.0062	22.6611
	10-1708	5.2745	44.5640	100.5920	396.3940	7.1044	30.0323	23.1970	32.1851
	10-1720	5.1799	41.6576	104.5256	413.4678	6.8157	30.9160	28.5189	41.2214
	10-1727	5.0352	47.9155	103.8441	483.9199	8.5003	32.0520	27.8289	28.0455
	10-1732	2.8139	45.8169	95.6710	401.1905	9.1991	29.4372	18.3983	44.6429
	Mean	**4.38107**	**46.85988**	**99.83230**	**421.72804**	**8.15514**	**31.62450**	**20.84954**	**34.27043**
	SD	**0.983477**	**3.654964**	**4.004592**	**31.921147**	**1.044705**	**4.203422**	**6.778033**	**8.252483**
	10-1688	3.7133	42.2898	108.4580	479.6802	7.1686	31.0985	22.2795	46.5188
50 mg/kg	10-1706	3.6077	46.2398	104.7256	453.7093	9.7561	31.6057	13.5163	35.4675
	10-1710	4.0462	45.0867	104.2564	450.2890	8.4603	37.3095	24.5402	17.5512
	10-1716	3.1453	40.2386	95.9328	387.7440	6.8330	29.5011	26.1931	20.2820
	10-1717	3.7513	43.5691	106.1629	426.2058	8.4137	34.6731	25.7771	45.2840
	10-1731	4.8739	52.3249	121.2885	517.6471	8.0672	42.1849	16.6947	29.4118
	Mean	**3.85831**	**44.95818**	**106.80403**	**452.54592**	**8.11650**	**34.39546**	**21.50013**	**32.41922**
	SD	**0.578001**	**4.177387**	**8.269722**	**44.438641**	**1.043373**	**4.729723**	**5.234496**	**12.255677**
	10-1686	3.0922	47.7637	94.9199	410.3810	4.9696	40.8062	21.8664	25.0138
100 mg/kg	10-1689	4.0103	37.1722	99.1260	473.1105	4.6272	41.3368	19.2288	26.7352
	10-1696	3.6936	46.9853	105.5948	414.8289	9.0168	39.6153	18.4682	21.9989
	10-1712	3.7687	52.2974	108.7765	419.9277	6.2984	53.9494	19.5663	20.9086
	10-1724	4.7541	46.3388	109.7268	470.3279	6.9945	43.0601	19.1803	25.9563
	10-1728	3.9979	45.8706	115.5181	515.7286	8.5744	41.6623	18.3062	26.8806
	Mean	**3.88614**	**46.07133**	**105.61034**	**450.71743**	**6.74685**	**43.43834**	**19.43603**	**24.58224**
	SD	**0.540817**	**4.934950**	**7.502882**	**42.368593**	**1.811441**	**5.258348**	**1.284145**	**2.538179**

Appendix O

Individual and Summary of 90-Day Organ Mass Data

Toxicological Study No. 87-XE-0DBP-10, Subchronic toxicity of DNAN, Sept 2010-March 2011

Table O-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

90-Day Individual Organ Mass
Male Rats

ABSOLUTE ORGAN MASS (GRAMS)

Group	Animal ID	Body Mass	Adrenals	Brain	Heart	Kidneys	Epididymides	Liver	Spleen	Testes	Thymus
	11-0097										
Corn Oil	11-0105	650	0.062	2.045	1.670	4.246	1.737	21.531	0.821	3.614	0.404
Control	11-0107	546	0.061	2.138	1.516	4.001	1.460	17.553	0.725	2.977	0.423
	11-0108	530	0.057	2.146	1.616	3.896	1.690	16.060	0.677	3.165	0.484
	11-0112	672	0.061	2.115	1.642	4.426	1.688	21.950	0.962	3.814	0.663
	11-0116	588	0.074	2.208	1.621	4.052	1.763	18.193	0.866	3.265	0.443
	11-0118	560	0.082	2.162	1.526	3.626	1.523	17.415	0.892	3.515	0.428
	11-0140	580	0.064	2.056	1.906	4.165	1.731	18.367	1.088	3.108	0.506
	11-0147	490	0.078	2.003	1.517	3.592	1.701	13.620	0.850	3.629	0.335
	11-0150	666	0.069	2.288	1.872	4.118	1.647	21.840	0.811	3.885	0.787
	Mean	**586.9**	**0.0676**	**2.1290**	**1.6540**	**4.0136**	**1.6600**	**18.5032**	**0.8547**	**3.4413**	**0.4970**
	SD	**63.79**	**0.00868**	**0.08763**	**0.14498**	**0.27417**	**0.10240**	**2.83282**	**0.12193**	**0.32410**	**0.14140**
	11-0095	567	0.050	2.089	1.673	4.093	1.729	17.652	0.937	3.756	0.405
1.25 mg/kg	11-0103	669	0.059	2.144	1.945	4.401	1.917	22.606	0.925	3.855	0.632
	11-0104	543	0.072	1.999	1.624	3.039	1.607	16.184	0.894	3.641	0.470
	11-0113	640	0.097	2.157	1.950	4.036	1.677	19.866	0.935	3.811	0.473
	11-0114	638	0.057	2.078	1.714	3.778	1.652	21.128	0.876	3.606	0.548
	11-0122	598	0.072	2.304	1.792	4.343	1.691	19.011	0.969	3.544	0.540
	11-0126	548	0.043	2.128	1.749	4.400	1.876	15.898	0.680	3.253	0.293
	11-0134	576	0.086	2.066	1.688	4.811	2.071	18.950	0.862	3.654	0.486
	11-0142	418	0.046	2.083	1.231	2.868	1.493	12.207	0.634	3.171	0.309
	11-0145	540	0.06	2.057	1.706	4.124	1.706	15.547	0.822	3.301	0.334
	Mean	**573.7**	**0.0642**	**2.1105**	**1.7072**	**3.9893**	**1.7419**	**17.9049**	**0.8534**	**3.5592**	**0.4490**
	SD	**70.89**	**0.01749**	**0.08205**	**0.19992**	**0.61233**	**0.16781**	**3.05012**	**0.11233**	**0.24011**	**0.11208**
	11-0100	531	0.053	2.014	1.498	3.415	1.483	15.698	0.664	3.098	0.403
5 mg/kg	11-0101	697	0.084	2.207	3.207	4.665	1.640	20.345	1.077	3.381	0.629
	11-0115	592	0.050	2.085	1.525	4.155	1.694	17.850	0.794	3.650	0.504
	11-0117	642	0.064	2.311	2.085	4.777	1.920	20.070	0.929	3.544	0.418
	11-0124	490	0.086	2.115	1.617	3.764	1.647	16.059	0.790	3.711	0.452
	11-0131	540	0.053	2.191	1.666	3.869	1.787	16.646	0.845	3.647	0.443
	11-0135	536	0.051	2.249	1.666	3.479	1.565	16.485	1.022	3.156	0.441
	11-0138	536	0.079	2.253	1.709	3.970	1.721	14.233	0.984	3.332	0.376
	11-0141	578	0.055	2.072	1.655	3.876	1.530	18.390	0.852	3.058	0.527
	11-0146	577	0.052	2.141	1.748	4.007	1.551	18.619	0.830	3.281	0.483
	Mean	**571.9**	**0.0627**	**2.1638**	**1.8376**	**3.9977**	**1.6538**	**17.4395**	**0.8787**	**3.3858**	**0.4676**
	SD	**60.56**	**0.01462**	**0.09397**	**0.50717**	**0.44382**	**0.13247**	**1.96032**	**0.12420**	**0.24171**	**0.07282**
	11-0106	475	0.079	2.142	1.639	3.986	1.534	15.162	0.727	3.604	0.39
20 mg/kg	11-0120	611	0.055	2.148	1.916	4.976	2.255	22.186	1.123	4.093	0.363
	11-0121	508	0.048	1.963	1.456	4.144	1.479	19.251	0.767	2.909	0.419
	11-0125	583	0.057	2.037	1.984	4.276	1.554	21.864	1.142	3.045	0.444
	11-0127	613	0.052	2.207	1.944	4.867	2.112	20.558	0.902	3.948	0.627
	11-0130	607	0.052	2.089	1.711	4.830	1.759	20.780	1.061	3.494	0.398
	11-0133	578	0.056	2.156	1.709	4.923	1.882	18.701	1.148	3.566	0.327
	11-0137	470	0.061	2.195	1.740	3.890	1.279	15.056	0.897	3.222	0.422
	11-0139	582	0.057	1.980	1.485	5.102	1.652	20.673	0.885	3.360	0.482
	11-0148	653	0.073	2.303	1.838	4.882	1.846	20.842	0.980	3.654	0.377
	Mean	**568.0**	**0.0590**	**2.1220**	**1.7422**	**4.5876**	**1.7352**	**19.5073**	**0.9632**	**3.4895**	**0.4249**
	SD	**62.36**	**0.00973**	**0.10610**	**0.18238**	**0.45873**	**0.29825**	**2.53870**	**0.15276**	**0.37169**	**0.08311**
	11-0099										
80 mg/kg	11-0102	391	0.054	1.994	1.312	3.593	0.927	12.022	0.925	1.181	0.248
	11-0109	401	0.070	2.265	1.374	3.938	1.060	16.802	1.037	1.514	0.316
	11-0110	388	0.058	2.063	1.334	3.345	1.177	16.356	0.859	1.379	0.260
	11-0111	400	0.042	2.150	1.382	3.822	0.998	15.084	1.259	1.336	0.294
	11-0123	423	0.059	2.145	1.598	4.279	0.929	16.833	0.999	1.079	0.317
	11-0129	436	0.047	2.102	1.465	3.470	1.113	17.037	1.089	1.477	0.300
	11-0132	361	0.064	2.015	1.319	3.548	0.765	13.107	0.939	1.064	0.223
	11-0144										
	11-0149										
	Mean	**400.0**	**0.0563**	**2.1049**	**1.3977**	**3.7136**	**0.9956**	**15.3201**	**1.0153**	**1.2900**	**0.2797**
	SD	**24.40**	**0.00960**	**0.09257**	**0.10251**	**0.32118**	**0.13733**	**2.01398**	**0.13163**	**0.18378**	**0.03636**

Toxicological Study No. 87-XE-0DBP-10, Subchronic toxicity of DNAN, Sept 2010-March 2011

Table O-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

90-Day Individual Organ Mass
Female Rats

ABSOLUTE ORGAN MASS (GRAMS)

Group	Animal ID	Body Weight	Adrenals	Brain	Heart	Kidneys	Liver	Ovaries	Spleen	Thymus	Uterus
	11-0154	291	0.076	1.932	1.149	1.997	9.395	0.173	0.611	0.268	0.531
Corn Oil	11-0162	270	0.055	1.877	0.949	2.106	8.566	0.136	0.538	0.290	0.533
Control	11-0168	307	0.088	1.956	1.147	2.156	9.904	0.211	0.491	0.337	0.873
	11-0171	270	0.073	2.000	0.950	1.717	10.124	0.131	0.507	0.282	0.883
	11-0173	265	0.101	1.867	0.937	2.186	9.049	0.108	0.473	0.265	0.893
	11-0175	260	0.061	2.428	1.072	1.752	8.384	0.154	0.446	0.216	1.129
	11-0190	280	0.074	1.901	1.009	1.846	8.045	0.183	0.538	0.416	0.717
	11-0191	287	0.060	1.979	1.026	1.938	8.402	0.151	0.561	0.334	0.603
	11-0197	300	0.069	1.903	1.079	1.881	7.855	0.160	0.518	0.347	0.639
	11-0206	263	0.054	1.760	0.892	1.804	7.649	0.135	0.672	0.333	0.696
	Mean	**279.3**	**0.0711**	**1.9603**	**1.0210**	**1.9383**	**8.7373**	**0.1542**	**0.5355**	**0.3088**	**0.7497**
	SD	**16.36**	**0.01490**	**0.17766**	**0.08964**	**0.16794**	**0.85131**	**0.02949**	**0.06668**	**0.05601**	**0.19145**
	11-0152	283	0.071	2.070	1.081	2.036	8.426	0.089	0.523	0.313	0.894
1.25 mg/kg	11-0159	322	0.079	1.969	1.083	2.250	10.037	0.174	0.549	0.384	0.896
	11-0165	273	0.081	1.871	0.992	1.775	9.542	0.149	0.585	0.277	0.724
	11-0170	260	0.063	1.977	0.979	1.884	8.341	0.132	0.413	0.183	0.883
	11-0172	281	0.072	1.791	0.910	1.735	7.697	0.147	0.545	0.276	0.582
	11-0176	313	0.085	1.888	1.218	2.073	10.313	0.159	0.606	0.379	0.589
	11-0178	277	0.074	1.955	1.087	2.217	9.235	0.168	0.478	0.265	0.526
	11-0181	280	0.045	1.859	1.169	1.914	8.303	0.148	0.572	0.324	1.148
	11-0192	269	0.063	1.899	1.118	1.861	9.034	0.079	0.474	0.267	0.785
	11-0196	280	0.081	2.019	0.971	1.757	8.286	0.177	0.685	0.276	1.049
	Mean	**283.8**	**0.0714**	**1.9298**	**1.0608**	**1.9502**	**8.9214**	**0.1422**	**0.5430**	**0.2944**	**0.8076**
	SD	**19.14**	**0.01189**	**0.08330**	**0.09633**	**0.18604**	**0.85277**	**0.03367**	**0.07679**	**0.05908**	**0.20564**
	11-0156	293	0.099	1.987	1.106	1.949	11.106	0.169	0.631	0.199	0.520
5 mg/kg	11-0157	270	0.062	1.959	1.057	2.050	10.255	0.181	1.538*	0.318	0.591
	11-0163	281	0.070	1.816	1.042	2.055	8.865	0.136	0.617	0.325	0.792
	11-0166	316	0.085	1.882	1.151	2.113	9.132	1.98*	0.702	0.229	0.641
	11-0174	309	0.071	2.355	1.063	2.212	9.341	0.159	0.625	0.296	0.989
	11-0187	280	0.066	1.895	1.047	1.981	8.544	0.141	0.532	0.289	0.505
	11-0189	305	0.073	1.969	1.043	2.023	8.200	0.144	0.696	0.381	1.073
	11-0193	321	0.083	2.039	1.205	2.313	9.491	0.156	0.643	0.401	0.657
	11-0202	280	0.054	2.000	1.026	1.963	8.760	0.148	0.699	0.212	0.519
	11-0204	300	0.076	1.952	1.123	1.902	9.057	0.146	0.720	0.382	0.702
	Mean	**295.5**	**0.0739**	**1.9854**	**1.0863**	**2.0561**	**9.2751**	**0.1533**	**0.6517**	**0.3032**	**0.6989**
	SD	**17.35**	**0.01276**	**0.14504**	**0.05815**	**0.12658**	**0.85344**	**0.01446**	**0.05931**	**0.07261**	**0.19763**
	11-0153	281	0.072	1.993	1.008	2.055	8.898	0.149	0.575	0.303	1.083
20 mg/kg	11-0155	277	0.062	1.947	0.986	1.996	8.113	0.177	0.711	0.289	1.486
	11-0160	299	0.075	1.930	1.111	2.300	9.894	0.159	0.727	0.270	1.428
	11-0164	250	0.061	1.888	0.946	1.998	7.498	0.127	0.497	0.232	0.524
	11-0167	328	0.084	2.127	1.270	2.596	10.973	0.165	0.775	0.368	0.569
	11-0179	300	0.090	1.877	1.271	2.282	11.344	0.172	0.817	0.406	1.339
	11-0183	296	0.082	2.013	1.055	2.326	11.767	0.113	0.786	0.332	0.888
	11-0184	268	0.067	1.964	0.920	1.948	7.755	0.105	0.501	0.325	0.765
	11-0194	307	0.064	1.969	1.173	2.184	12.515	0.158	0.707	0.307	0.787
	11-0198	300	0.054	1.870	1.020	2.283	9.362	0.120	0.649	0.409	0.548
	Mean	**290.6**	**0.0711**	**1.9578**	**1.0760**	**2.1968**	**9.8119**	**0.1445**	**0.6745**	**0.3241**	**0.9417**
	SD	**22.06**	**0.01156**	**0.07680**	**0.12642**	**0.20094**	**1.77452**	**0.02604**	**0.11554**	**0.05704**	**0.37073**
	11-0151										
80 mg/kg	11-0158	261	0.059	1.769	0.948	2.231	10.113	0.119	0.892	0.238	0.519
	11-0177	291	0.050	2.004	1.058	2.652	11.689	0.142	0.907	0.259	0.577
	11-0180	257	0.059	1.839	1.060	2.277	9.644	0.132	0.922	0.292	1.373
	11-0182	246	0.065	1.839	0.893	1.853	9.741	0.113	0.692	0.213	0.440
	11-0186	266	0.056	1.847	1.278	2.115	10.827	0.112	1.165	0.279	0.561
	11-0188	260	0.057	1.917	0.993	2.243	9.403	0.119	0.804	0.206	0.782
	11-0195	287	0.050	2.104	1.090	2.522	10.122	0.149	1.191	0.223	0.510
	11-0200	265	0.050	2.036	0.958	2.072	9.393	0.118	0.961	0.256	0.945
	11-0201	257	0.051	1.903	0.966	1.867	9.191	0.151	1.133	0.235	0.816
	Mean	**265.6**	**0.0552**	**1.9176**	**1.0271**	**2.2036**	**10.0137**	**0.1283**	**0.9630**	**0.2446**	**0.7248**
	SD	**14.53**	**0.00533**	**0.10947**	**0.11339**	**0.26728**	**0.80123**	**0.01551**	**0.16966**	**0.02928**	**0.29549**

* dropped from analysis

Table O-2
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

90-Day Individual % Body Mass Organ Mass
Male Rats

% BODY MASS ORGAN MASS

Group	Animal ID	Adrenals	Brain	Heart	Kidneys	Epididymides	Liver	Spleen	Testes	Thymus
	11-0097									
Corn Oil	11-0105	0.0095	0.3146	0.2569	0.6532	0.2672	3.3125	0.1263	0.5560	0.0622
Control	11-0107	0.0112	0.3916	0.2777	0.7328	0.2674	3.2148	0.1328	0.5452	0.0775
	11-0108	0.0108	0.4049	0.3049	0.7351	0.3189	3.0302	0.1277	0.5972	0.0913
	11-0112	0.0091	0.3147	0.2443	0.6586	0.2512	3.2664	0.1432	0.5676	0.0987
	11-0116	0.0126	0.3755	0.2757	0.6891	0.2998	3.0940	0.1473	0.5553	0.0753
	11-0118	0.0146	0.3861	0.2725	0.6475	0.2720	3.1098	0.1593	0.6277	0.0764
	11-0140	0.0110	0.3545	0.3286	0.7181	0.2984	3.1667	0.1876	0.5359	0.0872
	11-0147	0.0159	0.4088	0.3096	0.7331	0.3471	2.7796	0.1735	0.7406	0.0684
	11-0150	0.0104	0.3435	0.2811	0.6183	0.2473	3.2793	0.1218	0.5833	0.1182
	Mean	**0.01168**	**0.36602**	**0.28348**	**0.68732**	**0.28549**	**3.13926**	**0.14660**	**0.58986**	**0.08391**
	SD	**0.002297**	**0.036049**	**0.026543**	**0.044335**	**0.033105**	**0.164467**	**0.022776**	**0.063245**	**0.017114**
	11-0095	0.0088	0.3684	0.2951	0.7219	0.3049	3.1132	0.1653	0.6624	0.0714
1.25 mg/kg	11-0103	0.0088	0.3205	0.2907	0.6578	0.2865	3.3791	0.1383	0.5762	0.0945
	11-0104	0.0133	0.3681	0.2991	0.5597	0.2959	2.9805	0.1646	0.6705	0.0866
	11-0113	0.0152	0.3370	0.3047	0.6306	0.2620	3.1041	0.1461	0.5955	0.0739
	11-0114	0.0089	0.3257	0.2687	0.5922	0.2589	3.3116	0.1373	0.5652	0.0859
	11-0122	0.0120	0.3853	0.2997	0.7263	0.2828	3.1791	0.1620	0.5926	0.0903
	11-0126	0.0078	0.3883	0.3192	0.8029	0.3423	2.9011	0.1241	0.5936	0.0535
	11-0134	0.0149	0.3587	0.2931	0.8352	0.3595	3.2899	0.1497	0.6344	0.0844
	11-0142	0.0110	0.4983	0.2945	0.6861	0.3572	2.9203	0.1517	0.7586	0.0739
	11-0145	0.0111	0.3809	0.3159	0.7637	0.3159	2.8791	0.1522	0.6113	0.0619
	Mean	**0.01119**	**0.37313**	**0.29805**	**0.69764**	**0.30662**	**3.10580**	**0.14912**	**0.62604**	**0.07762**
	SD	**0.002631**	**0.050214**	**0.014052**	**0.089540**	**0.036621**	**0.182507**	**0.013241**	**0.058166**	**0.013066**
	11-0100	0.0100	0.3793	0.2821	0.6431	0.2793	2.9563	0.1250	0.5834	0.0759
5 mg/kg	11-0101	0.0121	0.3166	0.4601	0.6693	0.2353	2.9189	0.1545	0.4851	0.0902
	11-0115	0.0084	0.3522	0.2576	0.7019	0.2861	3.0152	0.1341	0.6166	0.0851
	11-0117	0.0100	0.3600	0.3248	0.7441	0.2991	3.1262	0.1447	0.5520	0.0651
	11-0124	0.0176	0.4316	0.3300	0.7682	0.3361	3.2773	0.1612	0.7573	0.0922
	11-0131	0.0098	0.4057	0.3085	0.7165	0.3309	3.0826	0.1565	0.6754	0.0820
	11-0135	0.0095	0.4196	0.3108	0.6491	0.2920	3.0756	0.1907	0.5888	0.0823
	11-0138	0.0147	0.4203	0.3188	0.7407	0.3211	2.6554	0.1836	0.6216	0.0701
	11-0141	0.0095	0.3585	0.2863	0.6706	0.2647	3.1817	0.1474	0.5291	0.0912
	11-0146	0.0090	0.3711	0.3029	0.6945	0.2688	3.2269	0.1438	0.5686	0.0837
	Mean	**0.01106**	**0.38149**	**0.31821**	**0.69978**	**0.29134**	**3.05161**	**0.15416**	**0.59779**	**0.08180**
	SD	**0.002913**	**0.036867**	**0.054461**	**0.042407**	**0.031707**	**0.179710**	**0.020398**	**0.076780**	**0.009022**
	11-0106	0.0166	0.4509	0.3451	0.8392	0.3229	3.1920	0.1531	0.7587	0.0821
20 mg/kg	11-0120	0.0090	0.3516	0.3136	0.8144	0.3691	3.6311	0.1838	0.6699	0.0594
	11-0121	0.0094	0.3864	0.2866	0.8157	0.2911	3.7896	0.1510	0.5726	0.0825
	11-0125	0.0098	0.3494	0.3403	0.7334	0.2666	3.7503	0.1959	0.5223	0.0762
	11-0127	0.0085	0.3600	0.3171	0.7940	0.3445	3.3537	0.1471	0.6440	0.1023
	11-0130	0.0086	0.3442	0.2819	0.7957	0.2898	3.4234	0.1748	0.5756	0.0656
	11-0133	0.0097	0.3730	0.2957	0.8517	0.3256	3.2355	0.1986	0.6170	0.0566
	11-0137	0.0130	0.4670	0.3702	0.8277	0.2721	3.2034	0.1909	0.6855	0.0898
	11-0139	0.0098	0.3402	0.2552	0.8766	0.2838	3.5521	0.1521	0.5773	0.0828
	11-0148	0.0112	0.3527	0.2815	0.7476	0.2827	3.1917	0.1501	0.5596	0.0577
	Mean	**0.01055**	**0.37754**	**0.30871**	**0.80961**	**0.30483**	**3.43226**	**0.16973**	**0.61826**	**0.07549**
	SD	**0.002517**	**0.045210**	**0.035253**	**0.044229**	**0.033897**	**0.234667**	**0.021150**	**0.071352**	**0.015272**
	11-0099									
80 mg/kg	11-0102	0.0138	0.5100	0.3355	0.9189	0.2371	3.0747	0.2366	0.3020	0.0634
	11-0109	0.0175	0.5648	0.3426	0.9820	0.2643	4.1900	0.2586	0.3776	0.0788
	11-0110	0.0149	0.5317	0.3438	0.8621	0.3034	4.2155	0.2214	0.3554	0.0670
	11-0111	0.0105	0.5375	0.3455	0.9555	0.2495	3.7710	0.3148	0.3340	0.0735
	11-0123	0.0139	0.5071	0.3778	1.0116	0.2196	3.9794	0.2362	0.2551	0.0749
	11-0129	0.0108	0.4821	0.3360	0.7959	0.2553	3.9076	0.2498	0.3388	0.0688
	11-0132	0.0177	0.5582	0.3654	0.9828	0.2119	3.6307	0.2601	0.2947	0.0618
	11-0144									
	11-0149									
	Mean	**0.01417**	**0.52734**	**0.34952**	**0.92984**	**0.24873**	**3.82413**	**0.25391**	**0.32251**	**0.06975**
	SD	**0.002864**	**0.029542**	**0.015935**	**0.076949**	**0.030575**	**0.391577**	**0.030144**	**0.041323**	**0.006258**

Toxicological Study No. 87-XE-0DBP-10, Subchronic toxicity of DNAN, Sept 2010-March 2011

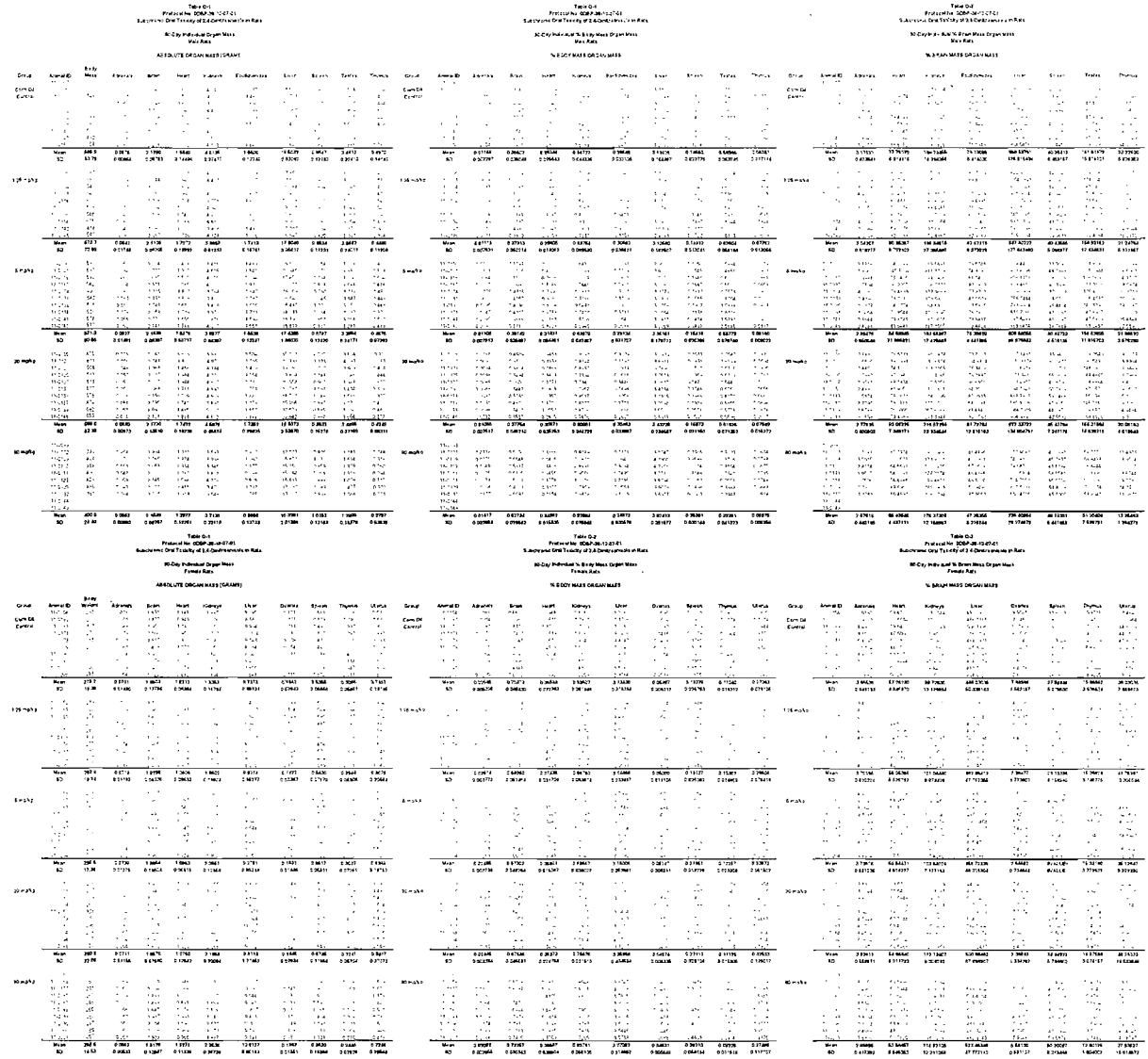

Table O-3
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

90-Day Individual % Brain Mass Organ Mass
Male Rats

% BRAIN MASS ORGAN MASS

Group	Animal ID	Adrenals	Heart	Kidneys	Epididymides	Liver	Spleen	Testes	Thymus
	11-0097								
Corn Oil	11-0105	3.0318	81.6626	207.6284	84.9389	1052.8606	40.1467	176.7237	19.7555
Control	11-0107	2.8531	70.9074	187.1375	68.2881	821.0009	33.9102	139.2423	19.7848
	11-0108	2.6561	75.3029	181.5471	78.7512	748.3691	31.5471	147.4837	22.5536
	11-0112	2.8842	77.6359	209.2671	79.8109	1037.8251	45.4846	180.3310	31.3475
	11-0116	3.3514	73.4149	183.5145	79.8460	823.9583	39.2210	147.8714	20.0634
	11-0118	3.7928	70.5828	167.7151	70.4440	805.5042	41.2581	162.5809	19.7965
	11-0140	3.1128	92.7043	202.5778	84.1926	893.3366	52.9183	151.1673	24.6109
	11-0147	3.8942	75.7364	179.3310	84.9226	679.9800	42.4363	181.1782	16.7249
	11-0150	3.0157	81.8182	179.9825	71.9843	954.5455	35.4458	169.7990	34.3969
	Mean	**3.17691**	**77.75170**	**188.74455**	**78.13095**	**868.59781**	**40.26313**	**161.81972**	**23.22600**
	SD	**0.423841**	**6.914916**	**14.398355**	**6.414030**	**126.915438**	**6.459157**	**15.918101**	**5.926959**
	11-0095	2.3935	80.0862	195.9311	82.7669	844.9976	44.8540	179.7989	19.3873
1.25 mg/kg	11-0103	2.7519	90.7183	205.2705	89.4123	1054.3843	43.1437	179.8041	29.4776
	11-0104	3.6018	81.2406	152.0260	80.3902	809.6048	44.7224	182.1411	23.5118
	11-0113	4.4970	90.4033	187.1117	77.7469	921.0014	43.3472	176.6806	21.9286
	11-0114	2.7430	82.4832	181.8094	79.4995	1016.7469	42.1559	173.5322	26.3715
	11-0122	3.1250	77.7778	188.4983	73.3941	825.1302	42.0573	153.8194	23.4375
	11-0126	2.0207	82.1898	206.7669	88.1579	747.0865	31.9549	152.8665	13.7688
	11-0134	4.1626	81.7038	232.8654	100.2420	917.2314	41.7231	176.8635	23.5237
	11-0142	2.2084	59.0975	137.6860	71.6755	586.0298	30.4369	152.2324	14.8344
	11-0145	2.9169	82.9363	200.4861	82.9363	755.8094	39.9611	160.4764	16.2372
	Mean	**3.04207**	**80.86367**	**188.84516**	**82.62216**	**847.80222**	**40.43565**	**168.82152**	**21.24784**
	SD	**0.819277**	**8.702102**	**27.365880**	**8.372028**	**137.642480**	**5.088977**	**12.434831**	**5.111867**
	11-0100	2.6316	74.3793	169.5631	73.6346	779.4439	32.9692	153.8232	20.0099
5 mg/kg	11-0101	3.8061	145.3104	211.3729	74.3090	921.8396	48.7993	153.1944	28.5002
	11-0115	2.3981	73.1415	199.2806	81.2470	856.1151	38.0815	175.0600	24.1727
	11-0117	2.7694	90.2207	206.7071	83.0809	868.4552	40.1990	153.3535	18.0874
	11-0124	4.0662	76.4539	177.9669	77.8723	759.2908	37.3522	175.4610	21.3712
	11-0131	2.4190	76.0383	176.5860	81.5609	759.7444	38.5669	166.4537	20.2191
	11-0135	2.2677	74.0774	154.6910	69.5865	732.9924	45.4424	140.3290	19.6087
	11-0138	3.5064	75.8544	176.2095	76.3870	631.7355	43.6751	147.8917	16.6889
	11-0141	2.6544	79.8745	187.0656	73.8417	887.5483	41.1197	147.5869	25.4344
	11-0146	2.4288	81.6441	187.1555	72.4428	869.6404	38.7669	153.2461	22.5596
	Mean	**2.89476**	**84.69945**	**184.65982**	**76.39628**	**806.68055**	**40.49723**	**156.63995**	**21.66520**
	SD	**0.650048**	**21.885921**	**17.425669**	**4.441965**	**88.976503**	**4.510135**	**11.815703**	**3.570290**
	11-0106	3.6881	76.5173	186.0878	71.6153	707.8431	33.9402	168.2540	18.2073
20 mg/kg	11-0120	2.5605	89.1993	231.6574	104.9814	1032.8678	52.2812	190.5493	16.8994
	11-0121	2.4452	74.1722	211.1055	75.3439	980.6928	39.0728	148.1915	21.3449
	11-0125	2.7982	97.3981	209.9165	76.2887	1073.3432	56.0628	149.4845	21.7968
	11-0127	2.3561	88.0834	220.5256	95.6955	931.4907	40.8700	178.8854	28.4096
	11-0130	2.4892	81.9052	231.2111	84.2030	994.7343	50.7899	167.2571	19.0522
	11-0133	2.5974	79.2672	228.3395	87.2913	867.3933	53.2468	165.3989	15.1670
	11-0137	2.7790	79.2711	177.2210	58.2688	685.9226	40.8656	146.7882	19.2255
	11-0139	2.8788	75.0000	257.6768	83.4343	1044.0909	44.6970	169.6970	24.3434
	11-0148	3.1698	79.8089	211.9844	80.1563	904.9935	42.5532	158.6626	16.3700
	Mean	**2.77625**	**82.06226**	**216.57254**	**81.72784**	**922.33722**	**45.43794**	**164.31684**	**20.08160**
	SD	**0.400609**	**7.348171**	**23.234544**	**12.915163**	**134.904701**	**7.247176**	**14.039216**	**4.019649**
	11-0099								
80 mg/kg	11-0102	2.7081	65.7974	180.1906	46.4895	602.9087	46.3892	59.2277	12.4373
	11-0109	3.0905	60.6623	173.8631	46.7991	741.8102	45.7837	66.8433	13.9514
	11-0110	2.8114	64.6631	162.1425	57.0528	792.8260	41.6384	66.8444	12.6030
	11-0111	1.9535	64.2791	177.7674	46.4186	701.5814	58.5581	62.1395	13.6744
	11-0123	2.7506	74.4988	199.4872	43.3100	784.7552	46.5734	50.3030	14.7786
	11-0129	2.2360	69.6955	165.0809	52.9496	810.5138	51.8078	70.2664	14.2721
	11-0132	3.1762	65.4591	176.0794	37.9653	650.4715	46.6005	52.8040	11.0670
	11-0144								
	11-0149								
	Mean	**2.67518**	**66.43646**	**176.37302**	**47.28355**	**726.40954**	**48.19301**	**61.20404**	**13.25483**
	SD	**0.440186**	**4.437111**	**12.159963**	**6.215244**	**78.274676**	**5.441869**	**7.526791**	**1.284378**

Table O-3
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

90-Day Individual % Brain Mass Organ Mass
Female Rats

% BRAIN MASS ORGAN MASS

Group	Animal ID	Adrenals	Heart	Kidneys	Liver	Ovaries	Spleen	Thymus	Uterus
	11-0154	3.9337	59.4720	103.3644	486.2836	8.9545	31.6253	13.8716	27.4845
Corn Oil	11-0162	2.9302	50.5594	112.2003	456.3665	7.2456	28.6628	15.4502	28.3964
Control	11-0168	4.4990	58.6401	110.2249	506.3395	10.7873	25.1022	17.2290	44.6319
	11-0171	3.6500	47.5000	85.8500	506.2000	6.5500	25.3500	14.1000	44.1500
	11-0173	5.4097	50.1875	117.0862	484.6813	5.7847	25.3348	14.1939	47.8307
	11-0175	2.5124	44.1516	72.1582	345.3048	6.3427	18.3690	8.8962	46.4992
	11-0190	3.8927	53.0773	97.1068	423.1983	9.6265	28.3009	21.8832	37.7170
	11-0191	3.0318	51.8444	97.9282	424.5579	7.6301	28.3477	16.8772	30.4699
	11-0197	3.6259	56.6999	98.8439	412.7693	8.4078	27.2202	18.2344	33.5786
	11-0206	3.0682	50.6818	102.5000	434.6023	7.6705	38.1818	18.9205	39.5455
	Mean	**3.65536**	**52.28140**	**99.72630**	**448.03035**	**7.89996**	**27.64946**	**15.96562**	**38.03036**
	SD	**0.849193**	**4.845970**	**13.129894**	**50.336143**	**1.562167**	**5.078500**	**3.536634**	**7.689823**
	11-0152	3.4300	52.2222	98.3575	407.0531	4.2995	25.2657	15.1208	43.1884
1.25 mg/kg	11-0159	4.0122	55.0025	114.2712	509.7511	8.8370	27.8822	19.5023	45.5053
	11-0165	4.3292	53.0198	94.8691	509.9947	7.9637	31.2667	14.8049	38.6959
	11-0170	3.1866	49.5195	95.2959	421.9019	6.6768	20.8902	9.2564	44.6636
	11-0172	4.0201	50.8096	96.8733	429.7599	8.2077	30.4299	15.4104	32.4958
	11-0176	4.5021	64.5127	109.7987	546.2394	8.4216	32.0975	20.0742	31.1970
	11-0178	3.7852	55.6010	113.4015	472.3785	8.5934	24.4501	13.5550	26.9054
	11-0181	2.4207	62.8833	102.9586	446.6380	7.9613	30.7692	17.4287	61.7536
	11-0192	3.3175	58.8731	97.9989	475.7241	4.1601	24.9605	14.0600	41.3375
	11-0196	4.0119	48.0931	87.0233	410.4012	8.7667	33.9277	13.6701	51.9564
	Mean	**3.70155**	**55.05368**	**101.08480**	**462.98419**	**7.38877**	**28.19398**	**15.28828**	**41.76991**
	SD	**0.620324**	**5.526792**	**8.879426**	**47.762355**	**1.773807**	**4.158980**	**3.145275**	**10.305548**
	11-0156	4.9824	55.6618	98.0876	558.9331	8.5053	31.7564	10.0151	26.1701
5 mg/kg	11-0157	3.1649	53.9561	104.6452	523.4814	9.2394	#VALUE!	16.2328	30.1685
	11-0163	3.8546	57.3789	113.1608	488.1608	7.4890	33.9758	17.8965	43.6123
	11-0166	4.5165	61.1583	112.2742	485.2285	ND	37.3007	12.1679	34.0595
	11-0174	3.0149	45.1380	93.9278	396.6454	6.7516	26.5393	12.5690	41.9958
	11-0187	3.4828	55.2507	104.5383	450.8707	7.4406	28.0739	15.2507	26.6491
	11-0189	3.7075	52.9711	102.7425	416.4551	7.3134	35.3479	19.3499	54.4947
	11-0193	4.0706	59.0976	113.4380	465.4733	7.6508	31.5351	19.6665	32.2217
	11-0202	2.7000	51.3000	98.1500	438.0000	7.4000	34.9500	10.6000	25.9500
	11-0204	3.8934	57.5307	97.4385	463.9857	7.4795	36.8852	19.5697	35.9631
	Mean	**3.73876**	**54.94431**	**103.84028**	**468.72338**	**7.69662**	**#VALUE!**	**15.33180**	**35.12847**
	SD	**0.691036**	**4.514227**	**7.127183**	**48.315304**	**0.734844**	**#VALUE!**	**3.779932**	**9.222390**
	11-0153	3.6126	50.5770	103.1109	446.4626	7.4762	28.8510	15.2032	54.3402
20 mg/kg	11-0155	3.1844	50.6420	102.5167	416.6923	9.0909	36.5177	14.8433	76.3225
	11-0160	3.8860	57.5648	119.1710	512.6425	8.2383	37.6684	13.9896	73.9896
	11-0164	3.2309	50.1059	105.8263	397.1398	6.7267	26.3242	12.2881	27.7542
	11-0167	3.9492	59.7085	122.0498	515.8909	7.7574	36.4363	17.3014	26.7513
	11-0179	4.7949	67.7144	121.5770	604.3687	9.1636	43.5269	21.6303	71.3372
	11-0183	4.0735	52.4093	115.5489	584.5504	5.6135	39.0462	16.4928	44.1133
	11-0184	3.4114	46.8432	99.1853	394.8574	5.3462	25.5092	16.5479	38.9511
	11-0194	3.2504	59.5734	110.9192	635.6018	8.0244	35.9066	15.5917	39.9695
	11-0198	2.8877	54.5455	122.0856	500.6417	6.4171	34.7059	21.8717	29.3048
	Mean	**3.62811**	**54.96840**	**112.19907**	**500.88483**	**7.38543**	**34.44922**	**16.57599**	**48.28339**
	SD	**0.559971**	**6.211723**	**9.009292**	**87.098907**	**1.334262**	**5.788860**	**3.074157**	**19.533548**
	11-0151								
80 mg/kg	11-0158	3.3352	53.5896	126.1164	571.6789	6.7270	50.4240	13.4539	29.3386
	11-0177	2.4950	52.7944	132.3353	583.2834	7.0858	45.2595	12.9242	28.7924
	11-0180	3.2083	57.6400	123.8173	524.4154	7.1778	50.1359	15.8782	74.6601
	11-0182	3.5345	48.5590	100.7613	529.6900	6.1446	37.6291	11.5824	23.9260
	11-0186	3.0319	69.1933	114.5100	586.1938	6.0639	63.0753	15.1056	30.3736
	11-0188	2.9734	51.7997	117.0057	490.5060	6.2076	41.9405	10.7460	40.7929
	11-0195	2.3764	51.8061	119.8669	481.0837	7.0817	56.6065	10.5989	24.2395
	11-0200	2.4558	47.0530	101.7682	461.3458	5.7957	47.2004	12.5737	46.4145
	11-0201	2.6800	50.7620	98.1083	482.9743	7.9348	59.5376	12.3489	42.8797
	Mean	**2.89895**	**53.68857**	**114.92105**	**523.46348**	**6.69100**	**50.20097**	**12.80129**	**37.93527**
	SD	**0.417392**	**6.546363**	**12.211066**	**47.773110**	**0.691107**	**8.319488**	**1.804002**	**16.014572**

Table O-4
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of 90-Day Organ Mass
Male Rats

Absolute Organ Mass (grams)

		Corn Oil	DNAN in corn oil			
		Control	1.25 mg/kg	5 mg/kg	20 mg/kg	80 mg/kg
Body Weight	Mean	586.9	573.7	571.9	568.0	400.0
	SD	63.79	70.89	60.56	62.36	24.40
	N	9	10	10	10	7
Adrenals	Mean	0.0676	0.0642	0.0627	0.0590	0.0563
	SD	0.00868	0.01749	0.01462	0.00973	0.00960
	N	9	10	10	10	7
Brain	Mean	2.1290	2.1105	2.1638	2.1220	2.1049
	SD	0.08763	0.08205	0.09397	0.10610	0.09257
	N	9	10	10	10	7
Heart	Mean	1.6540	1.7072	1.8376	1.7422	1.3977
	SD	0.14498	0.19992	0.50717	0.18238	0.10251
	N	9	10	10	10	7
Kidneys	Mean	4.0136	3.9893	3.9977	4.5876	3.7136
	SD	0.27417	0.61233	0.44382	0.45873	0.32118
	N	9	10	10	10	7
Epididymides	Mean	1.6600	1.7419	1.6538	1.7352	0.9956*
	SD	0.10240	0.16781	0.13247	0.29825	0.13733
	N	9	10	10	10	7
Liver	Mean	18.5032	17.9049	17.4395	19.5073	15.3201
	SD	2.83282	3.05012	1.96032	2.53870	2.01398
	N	9	10	10	10	7
Spleen	Mean	0.8547	0.8534	0.8787	0.9632	1.0153
	SD	0.12193	0.11233	0.12420	0.15276	0.13163
	N	9	10	10	10	7
Testes	Mean	3.4413	3.5592	3.3858	3.4895	1.2900*
	SD	0.32410	0.24011	0.24171	0.37169	0.18378
	N	9	10	10	10	7
Thymus	Mean	0.4970	0.4490	0.4676	0.4249	0.2797*
	SD	0.14140	0.11208	0.07282	0.08311	0.03636
	N	9	10	10	10	7

*Significantly different from corn oil control

Table O-4
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of 90-Day Organ Mass
Female Rats

Absolute Organ Mass (grams)

		Corn Oil	DNAN in corn oil			
		Control	1.25 mg/kg	5 mg/kg	20 mg/kg	80 mg/kg
Body Weight	Mean	279.3	283.8	295.5	290.6	265.6
	SD	16.36	19.14	17.35	22.06	14.53
	N	10	10	10	10	9
Adrenals	Mean	0.0711	0.0714	0.0739	0.0711	0.0552*
	SD	0.01490	0.01189	0.01276	0.01156	0.00533
	N	10	10	10	10	9
Brain	Mean	1.9603	1.9298	1.9854	1.9578	1.9176
	SD	0.17766	0.08330	0.14504	0.07680	0.10947
	N	10	10	10	10	9
Heart	Mean	1.0210	1.0608	1.0863	1.0760	1.0271
	SD	0.08964	0.09633	0.05815	0.12642	0.11339
	N	10	10	10	10	9
Kidneys	Mean	1.9383	1.9502	2.0561	2.1968*	2.2036*
	SD	0.16794	0.18604	0.12658	0.20094	0.26728
	N	10	10	10	10	9
Liver	Mean	8.7373	8.9214	9.2751	9.8119	10.0137
	SD	0.85131	0.85277	0.85344	1.77452	0.80123
	N	10	10	10	10	9
Ovaries	Mean	0.1542	0.1422	0.1533	0.1445	0.1283
	SD	0.02949	0.03367	0.01446	0.02604	0.01551
	N	10	10	9	10	9
Spleen	Mean	0.5355	0.5430	0.7403	0.6745	0.9630*
	SD	0.06668	0.07679	0.28581	0.11554	0.16966
	N	10	10	10	10	9
Thymus	Mean	0.3088	0.2944	0.3032	0.3241	0.2446
	SD	0.05601	0.05908	0.07261	0.05704	0.02928
	N	10	10	10	10	9
Uterus	Mean	0.7497	0.8076	0.6989	0.9417	0.7248
	SD	0.19145	0.20564	0.19763	0.37073	0.29549
	N	10	10	10	10	9

*Significantly different from corn oil control

Table O-5
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of 90-Day % Body Mass Organ Masss
Male Rats

% Body Mass Organ Mass

		Corn Oil	DNAN in corn oil			
		Control	1.25 mg/kg	5 mg/kg	20 mg/kg	80 mg/kg
Adrenals	Mean	0.01168	0.01119	0.01106	0.01055	0.01417
	SD	0.002297	0.002631	0.002913	0.002517	0.002864
	N	9	10	10	10	7
Brain	Mean	0.36602	0.37313	0.38149	0.37754	0.52734*
	SD	0.036049	0.050214	0.036867	0.045210	0.029542
	N	9	10	10	10	7
Heart	Mean	0.28348	0.29805	0.31821	0.30871	0.34952*
	SD	0.026543	0.014052	0.054461	0.035253	0.015935
	N	9	10	10	10	7
Kidneys	Mean	0.68732	0.69764	0.69978	0.80961*	0.92984*
	SD	0.044335	0.089540	0.042407	0.044229	0.076949
	N	9	10	10	10	7
Epididymides	Mean	0.28549	0.30662	0.29134	0.30483	0.24873
	SD	0.033105	0.036621	0.031707	0.033897	0.030575
	N	9	10	10	10	7
Liver	Mean	3.13926	3.10580	3.05161	3.43226	3.82413*
	SD	0.164467	0.182507	0.179710	0.234667	0.391577
	N	9	10	10	10	7
Spleen	Mean	0.14660	0.14912	0.15416	0.16973	0.25391*
	SD	0.022776	0.013241	0.020398	0.021150	0.030144
	N	9	10	10	10	7
Testes	Mean	0.58986	0.62604	0.59779	0.61826	0.32251*
	SD	0.063245	0.058166	0.076780	0.071352	0.041323
	N					
Thymus	Mean	0.08391	0.07762	0.08180	0.07549	0.06975
	SD	0.017114	0.013066	0.009022	0.015272	0.006258
	N	9	10	10	10	7

*Significantly different from corn oil control

Table O-5
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of 90-Day % Body Mass Organ Masss
Female Rats

% Body Mass Organ Mass

		Corn Oil	DNAN in corn oil			
		Control	1.25 mg/kg	5 mg/kg	20 mg/kg	80 mg/kg
Adrenals	Mean	0.02546	0.02514	0.02495	0.02446	0.02092
	SD	0.005325	0.003772	0.003738	0.003394	0.002955
	N	10	10	10	10	9
Brain	Mean	0.70474	0.68260	0.67302	0.67646	0.72257
	SD	0.086430	0.052454	0.049264	0.046597	0.030343
	N	10	10	10	10	9
Heart	Mean	0.36544	0.37435	0.36801	0.36973	0.38687
	SD	0.022263	0.031709	0.015087	0.024758	0.038604
	N	10	10	10	10	9
Kidneys	Mean	0.69507	0.68760	0.69667	0.75575	0.82781*
	SD	0.061948	0.053574	0.038022	0.031510	0.065106
	N	10	10	10	10	9
Liver	Mean	3.13436	3.14488	3.15005	3.35966	3.77092*
	SD	0.318394	0.233497	0.363891	0.434694	0.214893
	N	10	10	10	10	9
Ovaries	Mean	0.05497	0.05000	0.05247	0.04974	0.04831
	SD	0.008312	0.011105	0.006311	0.008335	0.005049
	N	10	10	9	10	9
Spleen	Mean	0.19228	0.19137	0.25371	0.23110	0.36213*
	SD	0.026753	0.025082	0.112472	0.029108	0.058144
	N	10	10	10	10	9
Thymus	Mean	0.11040	0.10302	0.10257	0.11126	0.09225
	SD	0.018312	0.014859	0.023308	0.015905	0.011516
	N					
Uterus	Mean	0.27063	0.28606	0.23573	0.32533	0.27486
	SD	0.078105	0.076418	0.061502	0.129017	0.117707
	N	10	10	10	10	9

*Significantly different from corn oil control

Table O-6
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of 90-Day % Brain Mass Organ Masss
Male Rats

% Brain Mass Organ Mass

		Corn Oil	DNAN in corn oil			
		Control	1.25 mg/kg	5 mg/kg	20 mg/kg	80 mg/kg
Adrenals	Mean	3.17691	3.04207	2.89476	2.77625	2.67518
	SD	0.423841	0.819277	0.650048	0.400609	0.440186
	N	9	10	10	10	7
Heart	Mean	77.75170	80.86367	84.69945	82.06226	66.43646
	SD	6.914916	8.702102	21.885921	7.348171	4.437111
	N	9	10	10	10	7
Kidneys	Mean	188.74455	188.84516	184.65982	216.57254*	176.37302
	SD	14.398355	27.365880	17.425669	23.234544	12.159963
	N	9	10	10	10	7
Epididymides	Mean	78.13095	82.62216	76.39628	81.72784	47.28355*
	SD	6.414030	8.372028	4.441965	12.915163	6.215244
	N					
Liver	Mean	868.59781	847.80222	806.68055	922.33722	726.40954
	SD	126.915438	137.642480	88.976503	134.904701	78.274676
	N	9	10	10	10	7
Spleen	Mean	40.26313	40.43565	40.49723	45.43794	48.19301
	SD	6.459157	5.088977	4.510135	7.247176	5.441869
	N	9	10	10	10	7
Testes	Mean	161.81972	168.82152	156.63995	164.31684	61.20404*
	SD	15.918101	12.434831	11.815703	14.039216	7.526791
	N	9	10	10	10	7
Thymus	Mean	23.22600	21.24784	21.66520	20.08160	13.25483*
	SD	5.926959	5.111867	3.570290	4.019649	1.284378
	N	9	10	10	10	7

*Significantly different from corn oil control

Table O-6
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of 90-Day % Brain Mass Organ Masss
Female Rats

% Brain Mass Organ Mass

		Corn Oil	DNAN in corn oil			
		Control	1.25 mg/kg	5 mg/kg	20 mg/kg	80 mg/kg
Adrenals	Mean	3.65536	3.70155	3.73876	3.62811	2.89895
	SD	0.849193	0.620324	0.691036	0.559971	0.417392
	N	10	10	10	10	9
Heart	Mean	52.28140	55.05368	54.94431	54.96840	53.68857
	SD	4.845970	5.526792	4.514227	6.211723	6.546363
	N	10	10	10	10	9
Kidneys	Mean	99.72630	101.08480	103.84028	112.19907	114.92105*
	SD	13.129894	8.879426	7.127183	9.009292	12.211066
	N	10	10	10	10	9
Liver	Mean	448.03035	462.98419	468.72338	500.88483	523.46348
	SD	50.336143	47.762355	48.315304	87.098907	47.773110
	N	10	10	10	10	9
Ovaries	Mean	7.89996	7.38877	7.69662	7.38543	6.69100
	SD	1.562167	1.773807	0.734844	1.334262	0.691107
	N	10	10	9	10	9
Spleen	Mean	27.64946	28.19398	37.48737	34.44922	50.20097*
	SD	5.078500	4.158980	14.844764	5.788860	8.319488
	N	10	10	10	10	9
Thymus	Mean	15.96562	15.28828	15.33180	16.57599	12.80129
	SD	3.536634	3.145275	3.779932	3.074157	1.804002
	N	10	10	10	10	9
Uterus	Mean	38.03036	41.76991	35.12847	48.28339	37.93527
	SD	7.689823	10.305548	9.222390	19.533548	16.014572
	N	10	10	10	10	9

*Significantly different from corn oil control

Appendix P

Individual and Summary of 14-Day Clinical Chemistry Data

Toxicological Study No. 87-XE-0DBP-10, Subchronic toxicity of DNAN, Sept 2010-March 2011

Table P-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

14-Day Individual Clinical Chemistry
Male Rats

Group	Animal ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	CA (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (mg/dL)	GLU (mg/dL)	LDH (U/L)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	K (mmol/L)	Cl (mmol/L)
	10-1635	2.9	407	55	99	23	11.4	60	0.7	3.1	146	268	10.7	< 0.1	6.0	9.9	101
Corn Oil	10-1643	2.9	343	50	77	17	11.5	54	0.6	3.2	126	221	9.2	< 0.1	6.1	6.6	103
Control	10-1645	3.1	356	50	86	16	11.1	41	0.5	2.9	142	294	9.2	< 0.1	6.0	7.1	101
	10-1662	3.3	273	52	73	17	11.1	61	0.6	3.2	141	259	10.8	< 0.1	6.4	6.8	101
	10-1664	3.2	340	51	66	15	10.8	46	0.6	3.1	127	283	10.5	< 0.1	6.2	6.6	102
	10-1675	3.1	309	40	60	12	11.3	69	0.6	3.3	146	267	9.0	< 0.1	6.4	6.9	101
	Mean	**3.08**	**338.0**	**49.7**	**76.8**	**16.7**	**11.2**	**55.2**	**0.60**	**3.13**	**138.0**	**265.3**	**9.90**	**0.10**	**6.18**	**7.32**	**101.5**
	SD	**0.160**	**45.12**	**5.09**	**14.08**	**3.61**	**0.25**	**10.34**	**0.063**	**0.137**	**9.14**	**25.10**	**0.849**	**0.000**	**0.183**	**1.280**	**0.84**
	10-1642	3.2	261	60	65	15	11.2	37	0.8	3.1	156	195	11.1	< 0.1	6.3	9.1	104
1.56 mg/kg	10-1651	3.4	259	54	79	25	11.5	56	0.6	3.0	175	271	9.6	< 0.1	6.5	6.1	102
	10-1656	3.2	299	57	78	13	10.8	41	0.8	3.2	105	257	11.2	< 0.1	6.5	10.3	102
	10-1660	3.2	386	68	93	20	11.7	62	0.5	3.0	154	397	9.9	< 0.1	6.2	6.7	102
	10-1672	3.2	329	56	93	18	11.8	64	0.6	3.2	163	263	9.9	< 0.1	6.5	7.2	103
	10-1678	3.5	303	49	84	13	11.4	64	0.6	3.2	118	402	8.1	< 0.1	6.7	6.1	98
	Mean	**3.28**	**306.2**	**57.3**	**82.0**	**17.3**	**11.4**	**54.0**	**0.65**	**3.12**	**145.2**	**297.5**	**9.97**	**0.10**	**6.45**	**7.58**	**101.8**
	SD	**0.133**	**47.37**	**6.38**	**10.58**	**4.68**	**0.36**	**12.05**	**0.122**	**0.098**	**27.40**	**83.50**	**1.134**	**0.000**	**0.176**	**1.733**	**2.04**
	10-1648	3.4	301	49	90	12	11.2	60	0.5	2.9	148	324	7.8	0.2	6.3	5.9	102
3.13 mg/kg	10-1652	2.9	232	51	76	13	10.3	47	0.9	3.2	98	184	10.9	< 0.1	6.0	10.5	102
	10-1657	3.2	268	54	73	12	10.9	50	0.7	3.2	132	206	9.8	< 0.1	6.3	6.8	99
	10-1661	3.0	289	55	100	12	10.9	55	0.5	2.8	117	354	8.5	< 0.1	5.9	7.0	100
	10-1666	3.3	338	56	84	14	11.4	60	0.5	3.1	145	303	9.0	< 0.1	6.3	6.8	103
	10-1683	3.1	319	61	72	10	11.3	37	0.6	3.1	120	277	8.8	< 0.1	6.3	6.3	102
	Mean	**3.15**	**291.2**	**54.3**	**82.5**	**12.2**	**11.0**	**51.5**	**0.62**	**3.05**	**126.7**	**274.7**	**9.13**	**0.12**	**6.18**	**7.22**	**101.3**
	SD	**0.187**	**37.70**	**4.18**	**11.02**	**1.33**	**0.40**	**8.83**	**0.160**	**0.164**	**18.86**	**67.04**	**1.084**	**0.041**	**0.183**	**1.658**	**1.51**
	10-1638	3.0	418	59	94	14	11.1	51	0.6	3.0	138	216	10.8	< 0.1	6.0	8.2	102
6.25 mg/kg	10-1639	3.6	229	47	82	16	11.7	66	0.6	3.1	166	301	9.9	< 0.1	6.8	6.8	102
	10-1641	3.2	304	61	87	17	10.8	64	0.9	3.1	140	211	9.0	< 0.1	6.2	9.0	102
	10-1644	3.2	322	61	89	17	10.3	27	0.6	3.1	113	226	8.4	< 0.1	6.2	6.4	103
	10-1670	3.5	335	53	95	21	11.6	64	0.6	3.1	137	315	7.9	< 0.1	6.5	5.6	101
	10-1680	3.5	328	58	82	16	11.1	57	0.6	2.8	146	456	9.2	< 0.1	6.3	6.2	102
	Mean	**3.33**	**322.7**	**56.5**	**88.2**	**16.8**	**11.1**	**54.8**	**0.65**	**3.03**	**140.0**	**287.5**	**9.20**	**0.10**	**6.33**	**7.03**	**102.0**
	SD	**0.234**	**60.65**	**5.50**	**5.64**	**2.32**	**0.52**	**14.74**	**0.122**	**0.121**	**17.05**	**93.89**	**1.041**	**0.000**	**0.280**	**1.299**	**0.63**
	10-1654	3.3	319	65	97	20	11.4	64	0.6	3.0	136	312	10.9	< 0.1	6.3	8.5	102
12.50 mg/kg	10-1659	3.4	334	80	133	26	11.5	46	0.6	3.3	119	488	8.9	< 0.1	6.7	6.3	99
	10-1663	3.0	266	51	81	14	10.5	30	0.6	3.0	98	204	9.2	< 0.1	6.0	6.7	102
	10-1665	3.1	273	48	58	14	11.2	31	0.5	3.0	111	243	9.4	< 0.1	6.2	6.2	100
	10-1676	3.4	297	49	55	15	11.4	79	0.7	3.4	172	238	8.7	< 0.1	6.9	6.4	101
	10-1681	3.2	223	55	81	15	12.0	68	0.6	3.3	170	271	8.8	< 0.1	6.4	7.1	102
	Mean	**3.23**	**285.3**	**58.0**	**84.2**	**17.3**	**11.3**	**53.0**	**0.60**	**3.17**	**134.3**	**292.7**	**9.32**	**0.10**	**6.42**	**6.87**	**101.0**
	SD	**0.163**	**40.12**	**12.43**	**28.65**	**4.80**	**0.49**	**20.42**	**0.063**	**0.186**	**30.96**	**102.29**	**0.818**	**0.000**	**0.331**	**0.864**	**1.26**
	10-1636	3.3	355	53	102	20	11.1	47	0.6	3.1	141	239	8.4	< 0.1	6.4	6.7	102
25 mg/kg	10-1640	3.4	319	53	87	16	11.0	53	0.8	2.8	133	285	11.3	< 0.1	6.2	ND	ND
	10-1658	3.2	241	67	93	16	11.3	47	0.5	2.7	147	269	9.9	< 0.1	5.9	6.6	102
	10-1671	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	6.7	100
	10-1673	3.7	381	63	128	16	11.5	41	0.5	3.2	120	649	8.1	< 0.1	6.9	6.1	99
	10-1677	3.5	328	55	78	16	11.1	62	0.6	2.9	139	235	9.4	< 0.1	6.4	6.4	101
	Mean	**3.42**	**324.8**	**58.2**	**97.6**	**16.8**	**11.2**	**50.0**	**0.60**	**2.94**	**136.0**	**335.4**	**9.42**	**0.10**	**6.36**	**6.50**	**100.8**
	SD	**0.192**	**52.77**	**6.42**	**19.11**	**1.79**	**0.20**	**7.94**	**0.122**	**0.207**	**10.25**	**176.54**	**1.279**	**0.000**	**0.365**	**0.255**	**1.30**
	10-1650	3.3	308	59	93	19	10.9	66	0.6	3.2	118	247	7.7	< 0.1	6.6	5.8	98
50 mg/kg	10-1653	3.6	270	63	105	20	11.6	52	0.5	2.8	118	372	8.2	0.1	6.4	5.9	99
	10-1668	3.8	242	56	98	18	11.6	50	0.6	3.3	138	294	8.2	0.1	7.1	6.9	99
	10-1669	3.4	327	116	114	20	11.0	54	0.8	3.2	103	181	8.7	< 0.1	6.6	6.2	101
	10-1679	3.4	241	61	145	22	11.5	52	0.5	3.2	142	725	8.5	< 0.1	6.6	6.4	101
	10-1684	3.3	218	54	87	15	11.4	48	0.8	3.5	134	324	8.4	< 0.1	6.8	6.8	101
	Mean	**3.47**	**267.7**	**68.2**	**107.0**	**19.0**	**11.3**	**53.7**	**0.63**	**3.20**	**125.5**	**357.2**	**8.28**	**0.10**	**6.68**	**6.33**	**99.8**
	SD	**0.197**	**42.40**	**23.66**	**20.85**	**2.37**	**0.31**	**6.38**	**0.137**	**0.228**	**14.96**	**191.72**	**0.343**	**0.000**	**0.240**	**0.455**	**1.33**
	10-1637	3.5	339	52	94	18	11.0	52	0.8	3.8	122	224	7.9	< 0.1	7.3	5.4	97
100 mg/kg	10-1646	3.8	320	64	83	28	10.9	48	0.9	3.0	149	257	11.0	< 0.1	6.8	7.4	99
	10-1647	3.5	338	65	102	18	11.4	35	0.6	2.8	151	211	10.1	< 0.1	6.3	7.3	100
	10-1649	3.6	237	60	73	17	11.1	48	0.7	3.0	139	179	10.4	< 0.1	6.6	6.8	102
	10-1655	3.3	331	82	112	21	11.3	40	0.6	3.4	122	336	9.1	< 0.1	6.7	7.0	100
	10-1674	3.0	247	64	104	13	11.5	66	0.5	3.2	107	530	8.2	< 0.1	6.3	5.9	100
	Mean	**3.45**	**302.0**	**64.5**	**94.7**	**19.2**	**11.2**	**48.2**	**0.68**	**3.20**	**131.7**	**289.5**	**9.45**	**0.10**	**6.67**	**6.63**	**99.7**
	SD	**0.274**	**47.07**	**9.83**	**14.47**	**5.04**	**0.24**	**10.70**	**0.147**	**0.358**	**17.45**	**129.40**	**1.250**	**0.000**	**0.372**	**0.807**	**1.63**

Toxicological Study No. 87-XE-0DBP-10, Subchronic toxicity of DNAN, Sept 2010-March 2011

Table P-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

14-Day Individual Clinical Chemistry
Female Rats

Group	Animal ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	CA (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (mg/dL)	GLU (mg/dL)	LDH (U/L)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	K (mmol/L)	Cl (mmol/L)
	10-1693	3.5	190	61	114	21	11.0	46	0.7	2.9	113	232	9.6	< 0.1	6.4	8.2	100
Corn Oil	10-1697	3.7	190	43	91	15	10.9	42	0.6	3.0	148	242	10.6	< 0.1	6.7	7.9	102
Control	10-1703	3.8	190	53	96	16	11.6	26	0.7	3.1	123	313	7.9	< 0.1	6.9	6.1	99
	10-1713	3.8	142	69	98	15	11.2	62	0.5	2.6	115	270	6.5	< 0.1	6.3	5.5	100
	10-1718	3.7	217	43	88	17	11.1	47	0.6	3.0	127	357	9.8	< 0.1	6.7	7.5	101
	10-1722	3.3	214	75	96	21	11.2	35	0.5	2.8	142	268	9.3	0.2	6.1	9.0	104
	Mean	**3.63**	**190.5**	**57.3**	**97.2**	**17.5**	**11.2**	**43.0**	**0.60**	**2.90**	**128.0**	**280.3**	**8.95**	**0.12**	**6.52**	**7.37**	**101.0**
	SD	**0.197**	**26.86**	**13.35**	**9.04**	**2.81**	**0.24**	**12.17**	**0.089**	**0.179**	**14.25**	**46.92**	**1.490**	**0.041**	**0.299**	**1.323**	**1.79**
	10-1685	3.4	214	62	79	20	11.4	46	0.5	2.7	142	265	10.0	0.1	6.1	8.5	105
1.56 mg/kg	10-1690	3.1	158	39	104	21	10.8	58	0.7	3.3	149	295	7.7	< 0.1	6.4	6.3	102
	10-1692	3.9	189	46	99	23	11.4	80	0.6	2.9	129	248	9.9	< 0.1	6.7	8.8	102
	10-1700	3.8	136	71	78	20	11.6	42	0.6	2.7	162	214	9.2	< 0.1	6.4	7.6	104
	10-1701	3.5	245	52	89	19	11.8	58	0.6	3.0	161	256	9.5	< 0.1	6.5	7.6	104
	10-1709	4.2	145	49	79	22	11.6	57	0.5	2.9	131	302	8.9	0.2	7.2	7.7	102
	Mean	**3.65**	**181.2**	**53.2**	**88.0**	**20.8**	**11.4**	**56.8**	**0.58**	**2.92**	**145.7**	**263.3**	**9.20**	**0.12**	**6.55**	**7.75**	**103.2**
	SD	**0.394**	**42.66**	**11.55**	**11.31**	**1.47**	**0.34**	**13.24**	**0.075**	**0.223**	**14.28**	**32.32**	**0.844**	**0.041**	**0.373**	**0.873**	**1.33**
	10-1695	3.6	161	43	54	18	10.9	52	0.5	3.1	109	254	7.6	0.3	6.7	6.1	107
3.13 mg/kg	10-1699	3.4	248	60	89	20	11.2	55	0.6	3.3	120	268	8.0	< 0.1	6.7	5.9	103
	10-1705	3.9	117	55	103	21	11.4	37	0.6	3.0	122	469	9.0	< 0.1	7.0	8.1	100
	10-1714	4.1	156	50	71	22	11.4	70	0.6	3.5	100	250	7.2	0.1	7.6	6.0	101
	10-1719	3.9	162	48	70	19	11.7	56	0.5	3.3	129	269	10.4	0.2	7.2	8.0	103
	10-1723	3.7	172	53	85	16	11.5	47	0.6	3.0	167	195	8.5	< 0.1	6.7	7.0	105
	Mean	**3.77**	**169.3**	**51.5**	**78.7**	**19.3**	**11.4**	**52.8**	**0.57**	**3.20**	**124.5**	**284.2**	**8.45**	**0.15**	**6.98**	**6.85**	**103.2**
	SD	**0.250**	**42.98**	**5.89**	**17.21**	**2.16**	**0.27**	**10.91**	**0.052**	**0.200**	**23.21**	**94.53**	**1.148**	**0.084**	**0.366**	**1.009**	**2.56**
	10-1702	3.6	230	66	96	20	11.5	38	0.6	2.8	110	338	8.8	< 0.1	6.4	8.2	102
6.25 mg/kg	10-1707	3.6	178	50	75	17	10.8	24	0.6	2.8	99	196	8.1	< 0.1	6.5	6.3	99
	10-1711	4.2	244	56	83	19	12.0	79	0.5	3.0	162	223	9.7	< 0.1	7.2	7.5	101
	10-1715	3.9	114	59	100	18	11.0	46	0.7	3.5	111	258	7.2	0.1	7.4	6.1	104
	10-1729	3.9	221	54	95	19	11.8	49	0.5	3.4	139	421	10.9	0.4	7.3	8.8	101
	10-1730	3.9	164	46	96	12	12.0	58	0.5	3.0	246	347	9.8	< 0.1	6.9	7.2	102
	Mean	**3.85**	**191.8**	**55.2**	**90.8**	**17.5**	**11.5**	**49.0**	**0.57**	**3.08**	**144.5**	**297.2**	**9.08**	**0.15**	**6.95**	**7.35**	**101.5**
	SD	**0.226**	**49.10**	**7.00**	**9.66**	**2.88**	**0.52**	**18.63**	**0.082**	**0.299**	**54.81**	**85.74**	**1.326**	**0.122**	**0.423**	**1.052**	**1.64**
	10-1687	3.9	191	54	83	15	11.2	74	0.5	3.2	130	345	7.4	0.3	7.0	6.5	106
12.50 mg/kg	10-1694	3.8	149	61	111	18	11.3	78	0.5	3.3	121	449	7.4	< 0.1	7.1	5.4	100
	10-1725	3.9	128	62	104	17	11.6	44	0.6	2.8	100	303	7.0	0.3	6.7	6.3	101
	10-1726	4.0	201	55	79	20	11.7	72	0.6	3.3	194	215	8.9	0.2	7.3	6.9	102
	10-1733	3.8	121	61	127	24	11.2	45	0.6	2.9	117	420	8.0	< 0.1	6.7	7.1	102
	10-1734	3.4	94	54	87	18	11.3	39	0.5	2.5	123	232	9.0	< 0.1	5.9	7.9	102
	Mean	**3.80**	**147.3**	**57.8**	**98.5**	**18.7**	**11.4**	**58.7**	**0.55**	**3.00**	**130.8**	**327.3**	**7.95**	**0.18**	**6.78**	**6.68**	**102.2**
	SD	**0.210**	**41.72**	**3.87**	**18.72**	**3.08**	**0.21**	**17.75**	**0.055**	**0.322**	**32.53**	**95.92**	**0.838**	**0.098**	**0.492**	**0.840**	**2.04**
	10-1691	3.6	135	46	82	18	11.4	55	0.6	2.7	194	367	9.7	0.2	6.3	5.8	103
25 mg/kg	10-1704	3.5	187	51	76	18	11.5	66	0.5	3.2	151	243	9.1	< 0.1	6.7	8.2	104
	10-1708	3.6	141	69	97	23	11.3	54	0.6	2.9	115	240	8.5	< 0.1	6.5	6.9	100
	10-1720	3.8	195	56	97	12	11.7	42	0.5	3.1	152	280	8.3	< 0.1	6.9	8.3	103
	10-1727	3.8	209	47	109	19	11.6	82	0.6	2.8	150	371	9.0	0.2	6.5	6.5	102
	10-1732	3.3	137	66	88	13	11.4	55	0.5	3.3	157	252	8.7	< 0.1	6.6	7.4	103
	Mean	**3.60**	**167.3**	**55.8**	**91.5**	**17.2**	**11.5**	**59.0**	**0.55**	**3.00**	**153.2**	**292.2**	**8.88**	**0.13**	**6.58**	**7.18**	**102.5**
	SD	**0.190**	**33.31**	**9.75**	**11.91**	**4.07**	**0.15**	**13.59**	**0.055**	**0.237**	**25.12**	**61.18**	**0.500**	**0.052**	**0.204**	**0.979**	**1.38**
	10-1688	3.6	284	76	105	20	11.1	72	0.6	3.3	107	265	7.6	< 0.1	6.8	6.2	101
50 mg/kg	10-1706	3.5	272	78	84	16	11.2	88	0.5	3.3	130	368	8.7	0.2	6.8	7.2	106
	10-1710	3.6	152	78	119	26	11.0	68	0.6	3.2	119	332	8.7	0.4	6.8	7.3	101
	10-1716	3.9	216	70	94	18	12.0	85	0.6	3.0	143	241	8.2	< 0.1	6.9	6.7	101
	10-1717	3.8	169	76	106	23	11.5	76	0.6	3.2	125	308	8.0	< 0.1	7.0	5.9	101
	10-1731	3.5	131	88	102	27	11.7	68	0.5	3.6	116	522	7.8	0.5	7.1	6.6	98
	Mean	**3.65**	**204.0**	**77.7**	**101.7**	**21.7**	**11.4**	**76.2**	**0.57**	**3.27**	**123.3**	**339.3**	**8.17**	**0.23**	**6.90**	**6.65**	**101.3**
	SD	**0.164**	**63.92**	**5.85**	**11.84**	**4.41**	**0.39**	**8.59**	**0.052**	**0.197**	**12.44**	**100.42**	**0.459**	**0.175**	**0.126**	**0.547**	**2.58**
	10-1686	3.7	143	101	155	26	11.0	64	0.6	2.7	135	473	7.7	< 0.1	6.5	6.0	97
100 mg/kg	10-1689	3.9	119	70	87	19	11.6	72	0.6	2.8	137	206	9.2	< 0.1	6.6	7.3	104
	10-1696	3.8	98	79	87	27	11.4	57	0.6	3.3	104	196	8.1	0.4	7.2	6.4	98
	10-1712	4.0	119	63	103	25	11.1	74	0.5	2.9	119	397	7.9	< 0.1	6.9	6.1	101
	10-1724	3.9	135	83	113	24	11.3	57	0.6	3.1	116	223	7.9	< 0.1	7.0	5.8	99
	10-1728	4.0	138	84	100	32	11.7	69	0.7	3.0	155	223	8.7	0.2	7.1	8.5	104
	Mean	**3.88**	**125.3**	**80.0**	**107.5**	**25.5**	**11.4**	**65.5**	**0.60**	**2.97**	**127.7**	**286.3**	**8.25**	**0.17**	**6.88**	**6.68**	**100.5**
	SD	**0.117**	**16.69**	**13.08**	**25.31**	**4.23**	**0.27**	**7.40**	**0.063**	**0.216**	**18.22**	**118.09**	**0.579**	**0.121**	**0.279**	**1.034**	**3.02**

Table P-2
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of 14-Day Clinical Chemistry
Male Rats

		Corn Oil	2,4-dinitroanisole (DNAN)						
		Control	1.56 mg/kg	3.13 mg/kg	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
ALB	Mean	3.08	3.28	3.15	3.33	3.23	3.42	3.47*	3.45*
(g/dL)	SD	0.160	0.133	0.187	0.234	0.163	0.192	0.197	0.274
	N	6	6	6	6	6	6	6	6
ALKP	Mean	338.0	306.2	291.2	322.7	285.3	324.8	267.7	302.0
(U/L)	SD	45.12	47.37	37.70	60.65	40.12	52.77	42.40	47.07
	N	6	6	6	6	6	6	6	6
ALT	Mean	49.7	57.3	54.3	56.5	58.0	58.2	68.2	64.5
(U/L)	SD	5.09	6.38	4.18	5.50	12.43	6.42	23.66	9.83
	N	6	6	6	6	6	6	6	6
AST	Mean	76.8	82.0	82.5	88.2	84.2	97.6	107.0	94.7
(U/L)	SD	14.08	10.58	11.02	5.64	28.65	19.11	20.85	14.47
	N	6	6	6	6	6	6	6	6
BUN	Mean	16.7	17.3	12.2	16.8	17.3	16.8	19.0	19.2
(mg/dL)	SD	3.61	4.68	1.33	2.32	4.80	1.79	2.37	5.04
	N	6	6	6	6	6	6	6	6
CA	Mean	11.2	11.4	11.0	11.1	11.3	11.2	11.3	11.2
(mg/dL)	SD	0.25	0.36	0.40	0.52	0.49	0.20	0.31	0.24
	N	6	6	6	6	6	6	6	6
CHOL	Mean	55.2	54.0	51.5	54.8	53.0	50.0	53.7	48.2
(mg/dL)	SD	10.34	12.05	8.83	14.74	20.42	7.94	6.38	10.70
	N	6	6	6	6	6	6	6	6
CREA	Mean	0.60	0.65	0.62	0.65	0.60	0.60	0.63	0.68
(mg/dL)	SD	0.063	0.122	0.160	0.122	0.063	0.122	0.137	0.147
	N	6	6	6	6	6	6	6	6
GLOB	Mean	3.13	3.12	3.05	3.03	3.17	2.94	3.20	3.20
(mg/dL)	SD	0.137	0.098	0.164	0.121	0.186	0.207	0.228	0.358
	N	6	6	6	6	6	6	6	6
GLU	Mean	138.0	145.2	126.7	140.0	134.3	136.0	125.5	131.7
(mg/dL)	SD	9.14	27.40	18.86	17.05	30.96	10.25	14.96	17.45
	N	6	6	6	6	6	6	6	6
LDH	Mean	265.3	297.5	274.7	287.5	292.7	335.4	357.2	289.5
(U/L)	SD	25.10	83.50	67.04	93.89	102.29	176.54	191.72	129.40
	N	6	6	6	6	6	6	6	6
PHOS	Mean	9.90	9.97	9.13	9.20	9.32	9.42	8.28	9.45
(mg/dL)	SD	0.849	1.134	1.084	1.041	0.818	1.279	0.343	1.250
	N	6	6	6	6	6	6	6	6
TBIL	Mean	0.10	0.10	0.12	0.10	0.10	0.10	0.10	0.10
(mg/dL)	SD	0.000	0.000	0.041	0.000	0.000	0.000	0.000	0.000
	N	6	6	6	6	6	6	6	6
TP	Mean	6.18	6.45	6.18	6.33	6.42	6.36	6.68	6.67
(g/dL)	SD	0.183	0.176	0.183	0.280	0.331	0.365	0.240	0.372
	N	6	6	6	6	6	6	6	6
K	Mean	7.32	7.58	7.22	7.03	6.87	6.50	6.33	6.63
(mmol/L)	SD	1.280	1.733	1.658	1.299	0.864	0.255	0.455	0.807
	N	6	6	6	6	6	6	6	6
Cl	Mean	101.5	101.8	101.3	102.0	101.0	100.8	99.8	99.7
(mmol/L)	SD	0.84	2.04	1.51	0.63	1.26	1.30	1.33	1.63
	N	6	6	6	6	6	6	6	6

*Significantly different from corn oil control

Table P-2
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of 14-Day Clinical Chemistry
Female Rats

		Corn Oil	2,4-dinitroanisole (DNAN)						
		Control	1.56 mg/kg	3.13 mg/kg	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
ALB	Mean	3.63	3.65	3.77	3.85	3.80	3.60	3.65	3.88
(g/dL)	SD	0.197	0.394	0.250	0.226	0.210	0.190	0.164	0.117
	N	6	6	6	6	6	6	6	6
ALKP	Mean	190.5	181.2	169.3	191.8	147.3	167.3	204.0	125.3
(U/L)	SD	26.86	42.66	42.98	49.10	41.72	33.31	63.92	16.69
	N	6	6	6	6	6	6	6	6
ALT	Mean	57.3	53.2	51.5	55.2	57.8	55.8	77.7*	80.0*
(U/L)	SD	13.35	11.55	5.89	7.00	3.87	9.75	5.85	13.08
	N	6	6	6	6	6	6	6	6
AST	Mean	97.2	88.0	78.7	90.8	98.5	91.5	101.7	107.5
(U/L)	SD	9.04	11.31	17.21	9.66	18.72	11.91	11.84	25.31
	N	6	6	6	6	6	6	6	6
BUN	Mean	17.5	20.8	19.3	17.5	18.7	17.2	21.7	25.5*
(mg/dL)	SD	2.81	1.47	2.16	2.88	3.08	4.07	4.41	4.23
	N	6	6	6	6	6	6	6	6
CA	Mean	11.2	11.4	11.4	11.5	11.4	11.5	11.4	11.4
(mg/dL)	SD	0.24	0.34	0.27	0.52	0.21	0.15	0.39	0.27
	N	6	6	6	6	6	6	6	6
CHOL	Mean	43.0	56.8	52.8	49.0	58.7	59.0	76.2*	65.5
(mg/dL)	SD	12.17	13.24	10.91	18.63	17.75	13.59	8.59	7.40
	N	6	6	6	6	6	6	6	6
CREA	Mean	0.60	0.58	0.57	0.57	0.55	0.55	0.57	0.60
(mg/dL)	SD	0.089	0.075	0.052	0.082	0.055	0.055	0.052	0.063
	N	6	6	6	6	6	6	6	6
GLOB	Mean	2.90	2.92	3.20	3.08	3.00	3.00	3.27	2.97
(mg/dL)	SD	0.179	0.223	0.200	0.299	0.322	0.237	0.197	0.216
	N	6	6	6	6	6	6	6	6
GLU	Mean	128.0	145.7	124.5	144.5	130.8	153.2	123.3	127.7
(mg/dL)	SD	14.25	14.28	23.21	54.81	32.53	25.12	12.44	18.22
	N	6	6	6	6	6	6	6	6
LDH	Mean	280.3	263.3	284.2	297.2	327.3	292.2	339.3	286.3
(U/L)	SD	46.92	32.32	94.53	85.74	95.92	61.18	100.42	118.09
	N	6	6	6	6	6	6	6	6
PHOS	Mean	8.95	9.20	8.45	9.08	7.95	8.88	8.17	8.25
(mg/dL)	SD	1.490	0.844	1.148	1.326	0.838	0.500	0.459	0.579
	N	6	6	6	6	6	6	6	6
TBIL	Mean	0.12	0.12	0.15	0.15	0.18	0.13	0.23	0.17
(mg/dL)	SD	0.041	0.041	0.084	0.122	0.098	0.052	0.175	0.121
	N	6	6	6	6	6	6	6	6
TP	Mean	6.52	6.55	6.98	6.95	6.78	6.58	6.90	6.88
(g/dL)	SD	0.299	0.373	0.366	0.423	0.492	0.204	0.126	0.279
	N	6	6	6	6	6	6	6	6
K	Mean	7.37	7.75	6.85	7.35	6.68	7.18	6.65	6.68
(mmol/L)	SD	1.323	0.873	1.009	1.052	0.840	0.979	0.547	1.034
	N	6	6	6	6	6	6	6	6
Cl	Mean	101.0	103.2	103.2	101.5	102.2	102.5	101.3	100.5
(mmol/L)	SD	1.79	1.33	2.56	1.64	2.04	1.38	2.58	3.02
	N	6	6	6	6	6	6	6	6

*Significantly different from corn oil control

Appendix Q

Individual and Summary of 90-Day Clinical Chemistry Data

Table Q-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

90-Day Individual Clinical Chemistry
Male Rats

Group	Animal ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	CA (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (mg/dL)	GLU (mg/dL)	LDH (U/L)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
	11-0097	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Corn Oil	11-0105	3.4	139	54	52	17	11.6	85	0.7	3.6	165	269	9.9	< 0.1	7.0	148	10.2	101
Control	11-0107	3.6	178	78	78	18	10.9	62	0.6	3.3	157	315	7.0	< 0.1	6.9	152	6.6	102
	11-0108	3.6	305	79	64	16	11.3	64	0.6	3.5	151	188	8.8	< 0.1	7.1	154	7.2	104
	11-0112	3.4	133	45	50	19	11.3	120	0.5	3.4	152	297	7.5	< 0.1	6.8	152	6.5	103
	11-0116	3.0	139	47	49	16	11.3	76	0.6	3.3	150	255	10.1	< 0.1	6.3	151	9.0	103
	11-0118	3.4	134	70	69	15	11.5	66	0.6	3.2	185	228	8.5	< 0.1	6.6	150	8.2	101
	11-0140	3.1	100	95	102	18	11.5	73	0.6	3.4	143	382	8.1	< 0.1	6.6	151	7.1	104
	11-0147	3.3	214	79	60	14	11.5	48	0.5	3.2	157	300	7.8	< 0.1	6.4	152	6.9	104
	11-0150	3.4	234	62	80	15	11.6	71	0.7	3.6	157	299	9.6	< 0.1	7.0	153	7.8	102
	Mean	**3.36**	**175.1**	**67.7**	**67.1**	**16.4**	**11.4**	**73.9**	**0.60**	**3.39**	**157.4**	**281.4**	**8.59**	**0.10**	**6.74**	**151.44**	**7.72**	**102.7**
	SD	**0.201**	**64.78**	**16.87**	**17.37**	**1.67**	**0.22**	**20.11**	**0.071**	**0.154**	**12.00**	**55.42**	**1.098**	**0.000**	**0.283**	**1.740**	**1.230**	**1.22**
	11-0095	3.4	118	56	62	16	11.2	47	0.7	3.2	135	216	8.8	< 0.1	6.6	150	9.5	102
1.25 mg/kg	11-0103	3.8	137	63	65	18	11.7	86	0.6	3.5	176	300	7.7	< 0.1	7.3	153	6.2	100
	11-0104	3.1	140	107	268	13	10.7	53	0.8	3.4	191	1170	8.9	< 0.1	6.5	152	7.1	105
	11-0113	3.3	131	59	89	16	11.8	53	0.6	3.5	153	293	8.2	< 0.1	6.9	151	7.6	101
	11-0114	3.8	251	52	50	14	11.9	86	0.6	3.6	179	254	8.1	< 0.1	7.4	155	6.0	102
	11-0122	3.3	135	62	66	16	11.8	66	0.7	3.8	179	288	9.7	< 0.1	7.1	151	9.1	102
	11-0126	3.3	108	59	70	14	11.3	56	0.6	3.3	148	370	8.6	< 0.1	6.5	153	8.4	105
	11-0134	3.6	232	79	72	15	12.2	65	0.5	3.9	184	297	8.6	< 0.1	7.5	155	6.8	104
	11-0142	3.4	130	58	56	13	11.4	44	0.5	3.2	173	321	7.9	< 0.1	6.6	151	7.9	105
	11-0145	3.3	200	67	61	15	11.5	55	0.5	3.5	157	285	8.2	< 0.1	6.8	153	5.7	103
	Mean	**3.43**	**158.2**	**66.2**	**85.9**	**15.0**	**11.6**	**61.1**	**0.61**	**3.49**	**167.5**	**379.4**	**8.47**	**0.10**	**6.92**	**152.40**	**7.43**	**102.9**
	SD	**0.231**	**50.33**	**16.10**	**64.83**	**1.56**	**0.42**	**14.78**	**0.099**	**0.233**	**18.10**	**280.63**	**0.581**	**0.000**	**0.382**	**1.713**	**1.305**	**1.79**
	11-0100	3.3	86	57	58	14	10.8	68	0.6	3.8	119	250	7.2	< 0.1	7.1	154	6.3	101
5 mg/kg	11-0101	3.3	144	69	74	15	11.2	62	0.5	3.5	157	318	7.3	< 0.1	6.8	153	6.0	105
	11-0115	3.4	103	48	62	13	11.6	69	0.6	3.3	156	196	7.6	< 0.1	6.7	152	6.9	102
	11-0117	3.6	144	63	70	21	11.4	51	0.6	3.5	135	377	7.7	< 0.1	7.1	153	6.6	104
	11-0124	3.3	169	59	62	13	11.2	47	0.5	3.1	157	302	8.0	< 0.1	6.4	154	6.9	105
	11-0131	3.5	111	41	42	15	11.6	74	0.5	3.4	165	261	7.7	< 0.1	6.9	153	6.2	103
	11-0135	3.1	128	231	168	16	11.4	50	0.7	3.5	149	394	9.6	< 0.1	6.6	148	9.5	103
	11-0138	3.5	213	120	125	16	11.2	58	0.5	3.5	131	509	7.5	< 0.1	6.9	154	6.4	102
	11-0141	3.6	158	50	70	16	11.5	57	0.7	3.6	174	273	7.6	< 0.1	7.2	153	6.1	102
	11-0146	3.6	138	86	52	19	11.7	64	0.6	3.5	157	294	8.4	< 0.1	7.1	151	7.1	102
	Mean	**3.42**	**139.4**	**82.4**	**78.3**	**15.8**	**11.4**	**60.0**	**0.58**	**3.47**	**150.0**	**317.4**	**7.86**	**0.10**	**6.88**	**152.50**	**6.80**	**102.9**
	SD	**0.169**	**36.20**	**56.96**	**38.49**	**2.53**	**0.27**	**8.97**	**0.079**	**0.183**	**16.77**	**89.04**	**0.700**	**0.000**	**0.257**	**1.841**	**1.019**	**1.37**
	11-0106	3.5	281	69	94	17	11.1	52	0.7	3.5	147	304	6.9	< 0.1	7.0	154	6.8	102
20 mg/kg	11-0120	3.6	146	49	56	14	11.3	58	0.6	3.2	143	299	7.8	< 0.1	6.8	154	6.1	101
	11-0121	3.5	164	55	104	18	11.2	52	0.6	3.3	152	293	7.9	< 0.1	6.8	152	6.4	102
	11-0125	3.5	85	60	64	14	11.3	48	0.6	3.2	144	229	7.3	< 0.1	6.7	152	6.1	100
	11-0127	3.7	127	73	60	14	11.7	43	0.6	3.5	140	362	7.0	< 0.1	7.2	155	6.3	104
	11-0130	3.4	185	79	66	17	11.3	57	0.6	3.6	135	265	7.1	< 0.1	7.0	155	6.3	102
	11-0133	3.4	186	66	58	16	12.0	55	0.6	3.5	201	319	8.4	< 0.1	6.9	155	6.8	104
	11-0137	3.4	96	56	54	17	11.3	40	0.6	3.2	136	296	7.7	< 0.1	6.6	153	6.3	102
	11-0139	3.6	204	68	55	14	11.3	60	0.5	3.2	147	269	7.0	< 0.1	6.8	151	6.2	104
	11-0148	3.5	99	80	59	17	11.8	60	0.7	3.7	166	284	7.5	< 0.1	7.2	156	6.4	101
	Mean	**3.51**	**157.3**	**65.5**	**67.0**	**15.8**	**11.4**	**52.5**	**0.61**	**3.39**	**151.1**	**292.0**	**7.46**	**0.10**	**6.90**	**153.70**	**6.37**	**102.2**
	SD	**0.099**	**60.14**	**10.38**	**17.44**	**1.62**	**0.29**	**6.96**	**0.057**	**0.191**	**19.64**	**35.17**	**0.488**	**0.000**	**0.200**	**1.636**	**0.250**	**1.40**
	11-0099	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
80 mg/kg	11-0102	2.6	160	94	64	19	11.0	44	0.6	3.6	139	197	7.1	< 0.1	6.2	152	7.4	106
	11-0109	3.9	177	92	68	17	11.0	33	0.6	3.5	99	227	7.3	< 0.1	7.4	155	6.2	106
	11-0110	4.0	136	78	56	24	11.7	58	0.9	3.9	175	233	11.1	< 0.1	7.8	152	11.3	103
	11-0111	3.9	289	100	94	18	11.1	55	0.6	3.0	128	383	8.1	< 0.1	6.9	152	7.2	104
	11-0123	3.5	95	79	66	14	11.4	47	0.5	3.6	123	349	7.6	< 0.1	7.1	153	7.1	104
	11-0129	3.5	305	82	77	19	11.3	50	0.6	3.5	196	275	9.6	< 0.1	7.0	157	7.0	108
	11-0132	4.0	105	107	121	19	11.1	60	0.6	3.4	122	283	8.6	< 0.1	7.5	155	7.5	107
	11-0144	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	11-0149	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	**3.63**	**181.0**	**90.3**	**78.0**	**18.6**	**11.2**	**49.6**	**0.63**	**3.50**	**140.3**	**278.1**	**8.49**	**0.10**	**7.13**	**153.71**	**7.67**	**105.4**
	SD	**0.502**	**84.35**	**11.09**	**22.47**	**2.99**	**0.26**	**9.32**	**0.125**	**0.271**	**33.66**	**67.44**	**1.435**	**0.000**	**0.515**	**1.976**	**1.655**	**1.81**

(ND = NO DATA)

Toxicological Study No. 87-XE-0DBP-10, Subchronic toxicity of DNAN, Sept 2010-March 2011

Table Q-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

90-Day Individual Clinical Chemistry
Female Rats

Group	Animal ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	CA (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (mg/dL)	GLU (mg/dL)	LDH (U/L)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
	11-0154	3.8	77	77	88	23	11.6	70	0.7	3.5	107	231	8.8	< 0.1	7.2	150	9.7	105
Corn Oil	11-0162	3.6	171	83	79	19	10.7	45	0.6	3.2	119	331	5.9	< 0.1	6.8	153	6.5	104
Control	11-0168	4.2	86	69	71	15	11.5	81	0.6	3.8	147	345	6.9	0.3	8.0	154	7.2	102
	11-0171	4.2	240	84	79	22	11.5	80	0.6	3.3	139	398	6.8	< 0.1	7.5	149	8.5	103
	11-0173	3.8	89	96	108	13	11.1	51	0.6	3.3	170	313	8.7	< 0.1	7.1	150	9.4	107
	11-0175	4.0	77	60	71	23	10.9	64	0.7	3.2	134	466	8.1	< 0.1	7.2	149	9.2	104
	11-0190	3.5	93	55	60	15	11.3	72	0.6	3.4	112	211	6.8	< 0.1	7.0	154	5.5	102
	11-0191	3.9	63	58	58	19	11.7	65	0.5	3.3	132	283	7.6	< 0.1	7.2	151	6.9	102
	11-0197	4.3	56	57	60	14	11.6	55	0.5	3.1	123	278	7.6	< 0.1	7.4	153	6.6	105
	11-0206	3.4	74	58	77	15	10.8	55	0.6	3.5	118	334	7.7	< 0.1	6.9	150	7.3	105
	Mean	**3.87**	**102.6**	**69.7**	**75.1**	**17.8**	**11.3**	**63.8**	**0.60**	**3.36**	**130.1**	**319.0**	**7.49**	**0.12**	**7.23**	**151.30**	**7.68**	**103.9**
	SD	**0.309**	**57.71**	**14.42**	**15.15**	**3.88**	**0.37**	**12.19**	**0.067**	**0.201**	**18.73**	**75.51**	**0.910**	**0.063**	**0.343**	**2.003**	**1.427**	**1.66**
	11-0152	4.7	59	65	63	18	11.7	71	0.6	3.2	168	309	7.9	< 0.1	7.9	149	8.9	102
1.25 mg/kg	11-0159	4.2	95	70	71	17	11.2	72	0.7	3.3	143	221	6.6	< 0.1	7.5	152	8.3	103
	11-0165	3.8	86	55	61	16	11.0	83	0.7	3.3	147	307	6.8	< 0.1	7.1	151	7.4	105
	11-0170	4.3	58	60	62	14	11.4	67	0.6	3.1	135	273	8.4	< 0.1	7.4	150	8.7	104
	11-0172	4.3	70	84	93	30	11.8	62	0.7	3.4	148	391	7.8	< 0.1	7.7	153	7.3	105
	11-0176	4.3	58	52	52	19	11.5	88	0.6	3.2	133	265	7.7	< 0.1	7.5	151	6.9	103
	11-0178	4.0	91	79	89	21	11.3	68	0.6	3.2	147	233	8.7	< 0.1	7.2	150	9.5	107
	11-0181	4.4	61	56	60	28	11.6	75	0.7	3.4	110	300	7.7	0.1	7.9	154	6.7	101
	11-0192	4.9	62	66	91	21	12.3	84	0.6	3.2	165	1552	7.7	< 0.1	8.1	152	8.3	105
	11-0196	3.7	76	64	64	15	11.3	65	0.6	3.6	171	281	7.1	< 0.1	7.4	152	6.9	106
	Mean	**4.26**	**71.6**	**65.1**	**70.6**	**19.9**	**11.5**	**73.5**	**0.64**	**3.29**	**146.7**	**413.2**	**7.64**	**0.10**	**7.57**	**151.40**	**7.89**	**104.1**
	SD	**0.369**	**14.48**	**10.32**	**14.84**	**5.34**	**0.37**	**8.81**	**0.052**	**0.145**	**18.46**	**402.88**	**0.657**	**0.000**	**0.323**	**1.506**	**0.976**	**1.85**
	11-0156	4.1	152	81	92	17	11.1	84	0.6	3.4	129	288	6.3	1.3	7.5	152	8.0	104
5 mg/kg	11-0157	3.6	73	65	88	27	10.9	69	0.6	3.0	107	226	6.0	< 0.1	6.7	150	5.9	106
	11-0163	4.2	84	67	64	26	11.3	54	0.7	3.2	127	378	7.3	< 0.1	7.4	150	8.0	104
	11-0166	4.0	112	79	94	21	11.5	78	0.6	3.5	137	328	7.0	< 0.1	7.5	153	6.6	104
	11-0174	4.3	63	57	50	13	11.4	75	0.6	3.1	143	345	5.7	< 0.1	7.3	153	6.6	105
	11-0187	3.9	63	72	89	23	10.9	70	0.6	3.1	97	231	6.3	< 0.1	6.9	154	6.2	102
	11-0189	4.0	82	52	61	12	11.8	72	0.6	3.1	150	240	7.5	< 0.1	7.1	153	7.1	103
	11-0193	3.9	75	76	96	27	11.4	59	0.7	3.2	133	346	9.0	< 0.1	7.1	149	8.6	104
	11-0202	3.8	68	78	61	17	11.4	74	0.6	3.2	128	288	7.6	< 0.1	7.0	152	6.6	103
	11-0204	3.9	55	82	101	13	12.5	82	0.6	3.2	178	596	9.9	0.6	7.0	153	8.1	106
	Mean	**3.97**	**82.7**	**70.9**	**79.6**	**19.6**	**11.4**	**71.7**	**0.62**	**3.20**	**132.9**	**326.6**	**7.26**	**0.27**	**7.15**	**151.90**	**7.17**	**104.1**
	SD	**0.200**	**29.01**	**10.38**	**18.43**	**5.99**	**0.47**	**9.42**	**0.042**	**0.149**	**22.31**	**108.36**	**1.338**	**0.395**	**0.268**	**1.663**	**0.932**	**1.29**
	11-0153	4.1	99	100	79	18	11.5	79	0.6	3.5	129	439	7.3	0.2	7.6	159	8.4	109
20 mg/kg	11-0155	3.9	62	48	57	17	11.5	78	0.6	3.2	162	338	8.0	< 0.1	7.1	150	7.8	102
	11-0160	3.8	66	74	62	17	10.7	56	0.7	2.8	122	255	7.0	< 0.1	6.6	150	7.8	104
	11-0164	3.8	92	96	94	18	11.0	68	0.6	3.4	110	641	7.7	< 0.1	7.2	151	7.4	101
	11-0167	4.5	99	48	66	18	11.4	86	0.6	3.2	174	320	8.4	< 0.1	7.7	151	6.8	104
	11-0179	4.3	43	74	72	20	11.5	57	0.7	3.3	129	317	6.9	< 0.1	7.6	152	6.4	101
	11-0183	4.8	65	84	98	22	12.1	99	0.7	3.5	151	415	7.3	0.2	8.3	152	6.7	102
	11-0184	4.0	62	48	75	18	11.6	75	0.5	3.5	117	241	6.6	< 0.1	7.5	154	6.8	103
	11-0194	4.9	53	81	76	24	12.1	113	0.7	3.5	154	262	7.0	0.3	8.4	151	8.0	102
	11-0198	4.0	75	78	75	15	11.7	72	0.7	3.4	123	301	6.8	0.3	7.5	154	6.8	102
	Mean	**4.21**	**71.6**	**73.1**	**75.4**	**18.7**	**11.5**	**78.3**	**0.64**	**3.33**	**137.1**	**354.9**	**7.30**	**0.16**	**7.55**	**152.40**	**7.29**	**103.0**
	SD	**0.401**	**19.30**	**19.28**	**12.86**	**2.63**	**0.43**	**17.64**	**0.070**	**0.221**	**21.49**	**118.63**	**0.572**	**0.084**	**0.532**	**2.716**	**0.677**	**2.36**
	11-0151	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
80 mg/kg	11-0158	3.9	145	77	74	21	11.2	70	0.7	3.5	112	350	7.6	< 0.1	7.4	150	6.9	100
	11-0177	4.6	105	76	86	29	11.5	101	0.7	3.2	121	333	6.8	0.4	7.8	150	6.9	101
	11-0180	3.9	74	70	68	18	11.3	75	0.6	3.1	125	199	8.0	< 0.1	7.0	151	7.2	102
	11-0182	4.1	148	81	78	25	11.3	58	0.5	3.4	149	409	8.5	< 0.1	7.5	154	6.4	106
	11-0186	4.4	100	99	79	25	11.6	87	0.7	3.3	134	251	7.6	< 0.1	7.7	154	7.6	105
	11-0188	4.0	126	75	77	24	11.2	63	0.6	3.7	90	183	7.4	0.3	7.7	154	6.3	104
	11-0195	4.0	60	144	105	22	11.3	70	0.6	2.9	90	417	6.8	< 0.1	6.9	148	6.9	101
	11-0200	4.4	82	94	79	33	11.7	78	0.7	3.0	130	245	6.7	0.1	7.4	152	6.8	104
	11-0201	3.8	56	91	54	20	11.4	73	0.6	3.3	123	240	6.2	< 0.1	7.1	151	7.2	103
	Mean	**4.12**	**99.6**	**89.7**	**77.8**	**24.1**	**11.4**	**75.0**	**0.63**	**3.27**	**119.3**	**291.9**	**7.29**	**0.16**	**7.39**	**151.56**	**6.91**	**102.9**
	SD	**0.277**	**34.58**	**22.59**	**13.64**	**4.65**	**0.18**	**12.83**	**0.071**	**0.250**	**19.47**	**87.67**	**0.724**	**0.113**	**0.326**	**2.128**	**0.401**	**2.03**

(ND = NO DATA)

Table Q-2
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of 90-Day Clinical Chemistry
Male Rats

		Corn Oil	2,4-dinitroanisole (DNAN)			
		Control	1.25 mg/kg	5 mg/kg	20 mg/kg	80 mg/kg
ALB	Mean	3.36	3.43	3.42	3.51	3.63
(g/dL)	SD	0.201	0.231	0.169	0.099	0.502
	N	9	10	10	10	7
ALKP	Mean	175.1	158.2	139.4	157.3	181.0
(U/L)	SD	64.78	50.33	36.20	60.14	84.35
	N	9	10	10	10	7
ALT	Mean	67.7	66.2	82.4	65.5	90.3
(U/L)	SD	16.87	16.10	56.96	10.38	11.09
	N	9	10	10	10	7
AST	Mean	67.1	85.9	78.3	67.0	78.0
(U/L)	SD	17.37	64.83	38.49	17.44	22.47
	N	9	10	10	10	7
BUN	Mean	16.4	15.0	15.8	15.8	18.6
(mg/dL)	SD	1.67	1.56	2.53	1.62	2.99
	N	9	10	10	10	7
CA	Mean	11.4	11.6	11.4	11.4	11.2
(mg/dL)	SD	0.22	0.42	0.27	0.29	0.26
	N	9	10	10	10	7
CHOL	Mean	73.9	61.1	60.0	52.5*	49.6*
(mg/dL)	SD	20.11	14.78	8.97	6.96	9.32
	N	9	10	10	10	7
CREA	Mean	0.60	0.61	0.58	0.61	0.63
(mg/dL)	SD	0.071	0.099	0.079	0.057	0.125
	N	9	10	10	10	7
GLOB	Mean	3.39	3.49	3.47	3.39	3.50
(mg/dL)	SD	0.154	0.233	0.183	0.191	0.271
	N	9	10	10	10	7
GLU	Mean	157.4	167.5	150.0	151.1	140.3
(mg/dL)	SD	12.00	18.10	16.77	19.64	33.66
	N	9	10	10	10	7
LDH	Mean	281.4	379.4	317.4	292.0	278.1
(U/L)	SD	55.42	280.63	89.04	35.17	67.44
	N	9	10	10	10	7
PHOS	Mean	8.59	8.47	7.86	7.46	8.49
(mg/dL)	SD	1.098	0.581	0.700	0.488	1.435
	N	9	10	10	10	7
TBIL	Mean	0.10	0.10	0.10	0.10	0.10
(mg/dL)	SD	1.47E-17	1.46E-17	1.46E-17	1.46E-17	1.50E-17
	N	9	10	10	10	7
TP	Mean	6.74	6.92	6.88	6.90	7.13
(g/dL)	SD	0.283	0.382	0.257	0.200	0.515
	N	9	10	10	10	7
Na	Mean	151.44	152.40	152.50	153.70	153.71
(mmol/L)	SD	1.740	1.713	1.841	1.636	1.976
	N	9	10	10	10	7
K	Mean	7.72	7.43	6.80	6.37	7.67
(mmol/L)	SD	1.230	1.305	1.019	0.250	1.655
	N	9	10	10	10	7
Cl	Mean	102.67	102.90	102.90	102.20	105.43*
(mmol/L)	SD	1.225	1.792	1.370	1.398	1.813
	N	9	10	10	10	7

*Significantly different from corn oil control

Table Q-2
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of 90-Day Clinical Chemistry
Female Rats

		Corn Oil	2,4-dinitroanisole (DNAN)			
		Control	1.25 mg/kg	5 mg/kg	20 mg/kg	80 mg/kg
ALB	Mean	3.87	4.26	3.97	4.21	4.12
(g/dL)	SD	0.309	0.369	0.200	0.401	0.277
	N	10	10	10	10	9
ALKP	Mean	102.6	71.6	82.7	71.6	99.6
(U/L)	SD	57.71	14.48	29.01	19.30	34.58
	N	10	10	10	10	9
ALT	Mean	69.7	65.1	70.9	73.1	89.7
(U/L)	SD	14.42	10.32	10.38	19.28	22.59
	N	10	10	10	10	9
AST	Mean	75.1	70.6	79.6	75.4	77.8
(U/L)	SD	15.15	14.84	18.43	12.86	13.64
	N	10	10	10	10	9
BUN	Mean	17.8	19.9	19.6	18.7	24.1
(mg/dL)	SD	3.88	5.34	5.99	2.63	4.65
	N	10	10	10	10	9
CA	Mean	11.3	11.5	11.4	11.5	11.4
(mg/dL)	SD	0.37	0.37	0.47	0.43	0.18
	N	10	10	10	10	9
CHOL	Mean	63.8	73.5	71.7	78.3	75.0
(mg/dL)	SD	12.19	8.81	9.42	17.64	12.83
	N	10	10	10	10	9
CREA	Mean	0.60	0.64	0.62	0.64	0.63
(mg/dL)	SD	0.067	0.052	0.042	0.070	0.071
	N	10	10	10	10	9
GLOB	Mean	3.36	3.29	3.20	3.33	3.27
(mg/dL)	SD	0.201	0.145	0.149	0.221	0.250
	N	10	10	10	10	9
GLU	Mean	130.1	146.7	132.9	137.1	119.3
(mg/dL)	SD	18.73	18.46	22.31	21.49	19.47
	N	10	10	10	10	9
LDH	Mean	319.0	413.2	326.6	354.9	291.9
(U/L)	SD	75.51	402.88	108.36	118.63	87.67
	N	10	10	10	10	9
PHOS	Mean	7.49	7.64	7.26	7.30	7.29
(mg/dL)	SD	0.910	0.657	1.338	0.572	0.724
	N	10	10	10	10	9
TBIL	Mean	0.12	0.10	0.27	0.16	0.16
(mg/dL)	SD	6.32E-02	1.46E-17	3.95E-01	8.43E-02	1.13E-01
	N	10	10	10	10	9
TP	Mean	7.23	7.57	7.15	7.55	7.39
(g/dL)	SD	0.343	0.323	0.268	0.532	0.326
	N	10	10	10	10	9
Na	Mean	151.30	151.40	151.90	152.40	151.56
(mmol/L)	SD	2.003	1.506	1.663	2.716	2.128
	N	10	10	10	10	9
K	Mean	7.68	7.89	7.17	7.29	6.91
(mmol/L)	SD	1.427	0.976	0.932	0.677	0.401
	N	10	10	10	10	9
Cl	Mean	103.90	104.10	104.10	103.00	102.89
(mmol/L)	SD	1.663	1.853	1.287	2.357	2.028
	N	10	10	10	10	9

*Significantly different from corn oil control

Appendix R

Individual and Summary of 14-Day Hematology Data

Table R-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

14-Day Individual Hematology
Male Rats

Group	Animal ID	WBC (K/uL)	NEU (%N)	LYM (%L)	MONO (%M)	EOS (%E)	BASO (%B)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)
	10-1662	23.10	9.380	83.700	4.300	0.520	2.130	7.39	14.60	42.5	57.6	19.8	34.4	14.9	1281.0	4.44
Corn Oil	10-1664	14.30	15.900	74.900	5.780	1.230	2.170	7.57	14.90	43.9	57.9	19.7	34.0	16.3	1131.0	4.72
Control	10-1675	21.20	5.440	86.600	4.740	0.957	2.230	7.29	14.30	42.0	57.6	19.7	34.1	15.5	1241.0	4.77
	10-1635	19.00	12.900	78.500	6.380	0.479	1.770	7.79	15.50	46.6	59.7	19.9	33.3	17.2	1248.0	4.36
	10-1643	20.60	15.500	76.100	6.480	0.332	1.620	7.28	14.70	43.3	59.5	20.2	34.0	16.7	1182.0	4.52
	10-1645	22.60	9.590	85.300	3.010	0.726	1.340	6.99	14.30	42.4	60.7	20.4	33.7	16.3	1450.0	4.69
	Mean	**20.133**	**11.4517**	**80.8500**	**5.1150**	**0.7073**	**1.8767**	**7.385**	**14.717**	**43.45**	**58.83**	**19.95**	**33.92**	**16.15**	**1255.50**	**4.583**
	SD	**3.2110**	**4.05394**	**4.98949**	**1.35215**	**0.33585**	**0.35786**	**0.2736**	**0.4491**	**1.688**	**1.311**	**0.288**	**0.376**	**0.829**	**109.224**	**0.1669**
	10-1642	13.30	11.700	80.500	4.670	0.723	2.360	7.56	15.40	45.7	60.4	20.4	33.7	14.8	1263.0	4.75
1.56 mg/kg	10-1656	16.10	19.000	73.900	4.220	0.750	2.140	7.69	15.10	44.3	57.7	19.6	34.0	16.8	1258.0	4.55
	10-1678	17.40	5.700	87.600	4.260	0.387	2.070	7.69	14.90	44.6	58.0	19.4	33.5	16.7	1188.0	4.61
	10-1651	14.30	11.500	81.800	4.390	0.837	1.400	8.14	15.70	46.6	57.3	19.2	33.6	16.0	1178.0	4.49
	10-1660	26.50	11.000	80.900	4.570	0.372	3.160	7.40	15.30	44.6	60.3	20.7	34.2	14.8	1338.0	4.47
	10-1672	23.30	8.270	83.300	5.150	0.295	2.980	8.22	16.70	48.6	59.2	20.3	34.3	16.5	1384.0	4.71
	Mean	**18.483**	**11.1950**	**81.3333**	**4.5433**	**0.5607**	**2.3517**	**7.783**	**15.517**	**45.73**	**58.82**	**19.93**	**33.88**	**15.93**	**1268.17**	**4.597**
	SD	**5.2667**	**4.47336**	**4.46079**	**0.34442**	**0.23448**	**0.64468**	**0.3262**	**0.6401**	**1.649**	**1.347**	**0.612**	**0.331**	**0.920**	**81.217**	**0.1150**
	10-1652	14.20	10.700	78.000	6.490	0.767	4.050	7.47	15.60	45.5	60.9	21.0	34.4	15.1	1034.0	5.15
3.13 mg/kg	10-1657	12.50	7.420	87.300	3.390	0.284	1.610	7.49	14.80	44.1	58.9	19.8	33.6	16.2	1318.0	4.33
	10-1683	18.40	8.380	80.800	7.440	0.597	2.740	7.08	14.90	43.9	56.4	19.1	33.9	15.5	1202.0	4.58
	10-1648	14.90	11.000	79.200	7.160	0.869	1.770	7.59	15.30	45.1	59.4	20.2	34.0	16.3	1352.0	4.68
	10-1661	16.90	11.300	74.000	8.390	1.060	5.220	7.59	15.00	43.7	57.6	19.7	34.2	16.8	1174.0	5.13
	10-1666	25.70	9.590	84.300	3.500	0.545	2.020	7.81	15.60	45.2	57.9	20.0	34.5	15.8	1295.0	4.18
	Mean	**17.100**	**9.7317**	**80.6000**	**6.0617**	**0.6870**	**2.9017**	**7.505**	**15.200**	**44.58**	**58.52**	**19.97**	**34.10**	**15.95**	**1229.17**	**4.675**
	SD	**4.6925**	**1.58185**	**4.70956**	**2.11711**	**0.27178**	**1.44540**	**0.2414**	**0.3521**	**0.770**	**1.569**	**0.628**	**0.335**	**0.609**	**117.631**	**0.4014**
	10-1641	12.50	6.490	85.500	5.710	0.452	1.820	7.14	14.40	41.8	58.6	20.1	34.4	15.6	1170.0	4.58
6.25 mg/kg	10-1644	14.10	13.900	79.000	4.020	1.290	1.830	7.03	14.20	42.2	60.0	20.2	33.7	14.7	1053.0	4.83
	10-1680	25.70	7.780	84.600	4.130	0.837	2.610	8.15	16.10	46.7	57.2	19.7	34.4	15.6	489.0	4.77
	10-1638	19.60	14.500	75.000	7.540	0.801	2.220	7.30	14.90	43.7	59.9	20.4	34.1	15.2	1123.0	4.69
	10-1639	18.20	8.390	82.000	6.100	0.614	2.880	7.95	15.50	46.4	58.3	19.5	33.4	16.6	1149.0	4.51
	10-1670	10.90	16.900	74.400	5.880	0.408	2.360	7.72	15.30	45.6	59.0	19.8	33.5	16.2	975.0	4.92
	Mean	**16.833**	**11.3267**	**80.0833**	**5.5633**	**0.7337**	**2.2867**	**7.548**	**15.067**	**44.40**	**58.83**	**19.95**	**33.92**	**15.65**	**993.17**	**4.717**
	SD	**5.4698**	**4.29768**	**4.74823**	**1.32313**	**0.32386**	**0.42255**	**0.4582**	**0.7118**	**2.136**	**1.052**	**0.339**	**0.445**	**0.680**	**257.101**	**0.1544**
	10-1663	14.60	7.780	79.900	7.330	0.592	4.400	6.88	13.80	41.0	59.6	20.1	33.7	15.3	1094.0	4.35
12.50 mg/kg	10-1665	19.60	6.730	85.200	4.790	0.798	2.500	7.62	14.80	43.7	57.3	19.4	33.9	17.9	1264.0	5.15
	10-1676	20.40	6.630	83.600	5.980	0.756	3.000	8.16	15.80	46.2	56.6	19.4	34.2	16.0	1045.0	4.72
	10-1654	22.20	7.240	81.700	6.860	1.200	2.980	7.91	15.70	46.2	58.4	19.9	34.0	15.8	1054.0	4.40
	10-1659	10.50	12.000	80.400	4.570	0.486	2.590	7.25	14.70	42.5	58.7	20.3	34.6	15.7	1272.0	4.65
	10-1681	21.40	10.000	77.700	7.870	1.210	3.210	7.54	14.80	43.8	58.1	19.6	33.8	16.5	1321.0	4.34
	Mean	**18.117**	**8.3967**	**81.4167**	**6.2333**	**0.8403**	**3.1133**	**7.560**	**14.933**	**43.90**	**58.12**	**19.78**	**34.03**	**16.20**	**1175.00**	**4.602**
	SD	**4.5880**	**2.15275**	**2.69475**	**1.35559**	**0.30402**	**0.68491**	**0.4572**	**0.7367**	**2.049**	**1.057**	**0.376**	**0.327**	**0.921**	**123.894**	**0.3129**
	10-1640	20.60	12.500	78.700	6.840	0.616	1.390	8.35	15.80	46.3	55.5	19.0	34.2	17.6	1215.0	4.63
25 mg/kg	10-1671	17.40	14.600	76.600	5.360	0.225	3.230	8.06	15.40	45.4	56.3	19.1	33.9	15.5	1098.0	4.39
	10-1677	31.80	7.150	76.800	7.920	0.600	7.570	7.65	15.30	45.9	60.0	20.0	33.4	16.7	1018.0	4.56
	10-1636	15.50	11.700	77.000	7.850	0.393	3.100	7.57	14.70	43.6	57.6	19.5	33.8	15.7	1309 0	5.13
	10-1658	21.50	11.900	79.800	4.880	0.562	2.820	7.61	15.60	45.2	59.3	20.5	34.5	17.7	1174.0	4.87
	10-1673	16.70	6.530	85.100	4.460	0.341	3.530	7.87	15.20	45.9	58.2	19.4	33.2	15.2	1204.0	5.47
	Mean	**20.583**	**10.7300**	**79.0000**	**6.2183**	**0.4562**	**3.6067**	**7.852**	**15.333**	**45.38**	**57.82**	**19.58**	**33.83**	**16.40**	**1169.67**	**4.842**
	SD	**5.9617**	**3.18986**	**3.24160**	**1.52079**	**0.16008**	**2.08062**	**0.3068**	**0.3777**	**0.958**	**1.722**	**0.571**	**0.484**	**1.092**	**100.802**	**0.4017**
	10-1650	16.00	14.500	78.000	4.760	0.707	2.110	7.60	15.00	44.1	57.9	19.7	34.0	17.3	1161.0	4.62
50 mg/kg	10-1669	14.70	10.300	84.000	3.190	0.574	1.940	7.71	14.40	43.3	56.1	18.7	33.4	15.9	1190.0	5.36
	10-1684	18.70	9.870	79.800	6.840	0.441	3.040	7.90	14.90	43.9	55.6	18.9	33.9	17.1	1076.0	4.60
	10-1653	18.00	15.500	75.400	5.450	0.754	2.930	7.92	15.40	44.7	56.5	19.5	34.5	17.9	1188.0	4.87
	10-1668	22.90	4.680	87.800	4.150	0.655	2.750	8.26	16.20	47.1	57.1	19.6	34.3	17.3	1105.0	4.56
	10-1679	25.00	5.550	86.500	4.950	0.579	2.440	7.88	15.80	46.8	59.4	20.1	33.8	17.6	989.0	4.46
	Mean	**19.217**	**10.0667**	**81.9167**	**4.8900**	**0.6183**	**2.5350**	**7.878**	**15.283**	**44.98**	**57.10**	**19.42**	**33.98**	**17.18**	**1118.17**	**4.745**
	SD	**3.9877**	**4.44264**	**4.94466**	**1.23079**	**0.11187**	**0.44743**	**0.2252**	**0.6524**	**1.590**	**1.381**	**0.523**	**0.387**	**0.688**	**78.111**	**0.3304**
	10-1637	16.80	10.100	82.100	4.900	0.395	2.470	7.48	15.00	44.7	59.7	20.1	33.7	16.8	965.0	4.97
100 mg/kg	10-1646	15.80	11.600	79.800	5.880	0.434	2.290	8.29	15.90	46.5	56.1	19.2	34.2	18.3	1111.0	5.00
	10-1649	14.20	7.470	80.000	6.380	0.958	5.180	7.80	14.50	43.6	55.9	18.6	33.3	16.1	1111.0	4.67
	10-1647	20.10	10.400	81.000	5.080	0.320	3.220	7.91	15.30	45.1	56.9	19.3	33.9	16.9	1054.0	4.84
	10-1655	22.00	5.970	81.100	7.320	0.582	5.030	7.50	14.40	43.0	57.4	19.1	33.4	18.6	1267.0	4.60
	10-1674	13.20	6.830	82.100	5.780	0.511	4.740	7.52	14.30	42.3	56.3	19.0	33.8	16.8	834.0	5.59
	Mean	**17.017**	**8.7283**	**81.0167**	**5.8900**	**0.5333**	**3.8217**	**7.750**	**14.900**	**44.20**	**57.05**	**19.22**	**33.72**	**17.25**	**1057.00**	**4.945**
	SD	**3.4167**	**2.26795**	**0.98675**	**0.88695**	**0.22703**	**1.31784**	**0.3187**	**0.6229**	**1.534**	**1.411**	**0.496**	**0.331**	**0.977**	**147.061**	**0.3537**

Toxicological Study No. 87-XE-0DBP-10, Subchronic toxicity of DNAN, Sept 2010-March 2011

Table R-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

14-Day Individual Hematology
Female Rats

Group	Animal ID	WBC (K/uL)	NEU (%N)	LYM (%L)	MONO (%M)	EOS (%E)	BASO (%B)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)
	10-1697	7.39	14.100	80.600	3.880	0.879	0.480	6.97	14.00	41.8	59.9	20.1	33.5	15.5	1182.0	4.44
Corn Oil	10-1718	6.80	12.000	80.300	5.650	1.050	0.968	7.51	13.80	42.1	56.1	18.3	32.7	14.7	1405.0	4.99
Control	10-1722	17.60	6.350	85.700	5.450	0.788	1.720	7.56	14.70	42.3	55.9	19.4	34.7	16.3	1429.0	4.74
	10-1693	14.70	13.300	77.000	4.940	0.677	4.060	7.82	14.90	42.3	54.0	19.0	35.2	16.3	1214.0	4.11
	10-1703	10.20	6.750	86.300	4.120	0.874	1.930	7.78	15.10	42.5	54.6	19.3	35.4	16.6	1320.0	4.28
	10-1713	17.50	4.190	88.600	5.060	0.708	1.440	7.62	14.90	42.1	55.2	19.6	35.4	15.3	823.0	4.51
	Mean	**12.365**	**9.4483**	**83.0833**	**4.8500**	**0.8293**	**1.7663**	**7.543**	**14.567**	**42.18**	**55.95**	**19.28**	**34.48**	**15.78**	**1228.83**	**4.512**
	SD	**4.8915**	**4.18366**	**4.43956**	**0.71077**	**0.13623**	**1.24003**	**0.3061**	**0.5354**	**0.240**	**2.089**	**0.605**	**1.130**	**0.733**	**222.048**	**0.3166**
	10-1685	11.40	5.650	88.600	4.090	0.965	0.724	7.95	15.30	46.3	58.2	19.3	33.1	14.9	1345.0	4.70
1.56 mg/kg	10-1690	10.30	9.600	84.400	4.630	0.617	0.743	7.23	14.30	42.6	58.9	19.8	33.5	15.4	1085.0	4.25
	10-1709	13.90	7.570	86.500	4.350	0.716	0.854	7.58	14.90	43.0	56.7	19.7	34.7	15.7	1232.0	4.57
	10-1692	18.00	9.150	83.500	3.770	0.713	2.870	7.72	15.30	44.3	57.4	19.8	34.5	14.8	1317.0	4.81
	10-1700	17.80	8.920	82.800	4.530	0.987	2.790	7.83	15.30	44.5	56.8	19.6	34.5	14.5	1247.0	4.79
	10-1701	16.80	4.590	89.700	3.110	0.784	1.810	7.50	14.70	42.4	56.5	19.7	34.8	14.9	1324.0	4.77
	Mean	**14.700**	**7.5800**	**85.9167**	**4.0800**	**0.7970**	**1.6318**	**7.835**	**14.967**	**43.85**	**57.42**	**19.65**	**34.18**	**15.03**	**1258.33**	**4.648**
	SD	**3.3407**	**2.04968**	**2.81810**	**0.56833**	**0.14867**	**1.01246**	**0.2568**	**0.4131**	**1.484**	**0.954**	**0.187**	**0.705**	**0.437**	**96.053**	**0.2138**
	10-1695	9.22	12.400	78.200	6.670	1.440	1.260	7.19	14.50	41.8	58.1	20.1	34.6	16.2	1522.0	4.67
3.13 mg/kg	10-1714	7.99	10.700	83.600	3.640	0.673	1.370	7.43	14.10	42.4	57.0	19.0	33.4	14.8	1268.0	4.93
	10-1719	8.48	12.500	77.900	7.150	1.070	1.370	7.92	15.40	44.1	55.7	19.4	34.9	15.9	1157.0	4.52
	10-1699	7.94	6.260	88.100	2.460	0.440	2.770	7.14	13.70	39.0	54.7	19.1	35.0	14.6	1419.0	4.60
	10-1705	13.40	5.220	90.100	1.960	0.775	1.970	7.49	14.30	42.4	56.6	19.1	33.8	15.4	1115.0	4.65
	10-1723	16.50	7.260	86.200	4.050	1.080	1.450	6.47	13.80	35.8	55.3	21.3	38.5	14.3	1078.0	4.92
	Mean	**10.588**	**9.0567**	**84.0167**	**4.3217**	**0.9130**	**1.6983**	**7.273**	**14.300**	**40.92**	**56.23**	**19.67**	**35.03**	**15.20**	**1259.83**	**4.715**
	SD	**3.5478**	**3.20950**	**5.09644**	**2.14923**	**0.35515**	**0.58167**	**0.4816**	**0.6164**	**3.006**	**1.242**	**0.896**	**1.812**	**0.756**	**178.184**	**0.1707**
	10-1707	8.35	6.170	88.300	3.660	0.606	1.250	7.54	14.30	42.7	56.6	19.0	33.5	15.7	1212.0	4.30
6.25 mg/kg	10-1715	8.93	17.400	78.200	2.560	0.121	1.660	7.20	14.30	41.5	57.6	19.8	34.4	15.1	1211.0	4.34
	10-1729	23.40	6.550	84.900	6.210	0.552	1.820	7.59	15.30	44.5	58.6	20.1	34.4	15.0	1099.0	4.57
	10-1702	20.40	12.200	81.900	3.310	0.780	1.740	8.27	15.00	42.6	51.5	18.2	35.3	15.4	1387.0	4.37
	10-1711	19.30	4.740	89.200	3.600	0.733	1.700	8.10	15.60	44.3	54.7	19.2	35.2	15.7	1189.0	4.90
	10-1730	18.10	4.820	90.500	3.210	0.286	1.190	7.14	13.90	40.7	57.0	19.4	34.1	13.9	760.0	4.87
	Mean	**16.413**	**8.6467**	**85.5000**	**3.7583**	**0.5130**	**1.5600**	**7.640**	**14.733**	**42.72**	**56.00**	**19.28**	**34.48**	**15.13**	**1143.00**	**4.558**
	SD	**6.2754**	**5.09279**	**4.75689**	**1.26357**	**0.25886**	**0.26930**	**0.4614**	**0.6653**	**1.500**	**2.554**	**0.665**	**0.679**	**0.671**	**209.601**	**0.2698**
	10-1687	14.40	10.700	79.500	7.440	0.485	1.900	7.62	14.90	43.5	57.1	19.5	34.2	14.6	1098.0	4.77
12.50 mg/kg	10-1733	19.30	9.380	82.000	5.900	0.781	1.920	7.86	15.10	43.7	55.5	19.2	34.5	16.1	745.0	4.67
	10-1734	23.70	6.330	86.500	4.850	0.408	1.960	7.51	14.60	43.0	57.3	19.4	33.9	15.6	887.0	3.98
	10-1694	9.90	5.630	90.300	1.860	0.236	1.990	6.44	13.00	37.4	58.0	20.2	34.7	14.0	1229.0	4.53
	10-1725	11.10	3.680	84.400	7.200	0.556	4.210	7.99	15.30	44.4	55.5	19.2	34.5	15.4	1029.0	4.62
	10-1726	17.70	7.050	82.700	5.850	0.653	3.730	7.73	14.80	42.5	55.0	19.1	34.8	16.1	1115.0	4.41
	Mean	**16.017**	**7.1283**	**84.2333**	**5.5167**	**0.5198**	**2.6183**	**7.525**	**14.617**	**42.42**	**56.40**	**19.43**	**34.43**	**15.30**	**1017.17**	**4.497**
	SD	**5.2316**	**2.55368**	**3.78717**	**2.03010**	**0.19054**	**1.05840**	**0.5580**	**0.8280**	**2.540**	**1.220**	**0.403**	**0.333**	**0.844**	**174.434**	**0.2813**
	10-1691	16.00	14.200	73.700	8.110	1.390	2.570	7.24	14.40	42.3	58.4	20.0	34.2	14.7	1188.0	4.87
25 mg/kg	10-1704	14.50	9.870	80.300	7.490	1.020	1.280	8.12	14.90	44.2	54.4	18.4	33.7	16.6	1217.0	4.57
	10-1732	16.70	8.920	84.000	3.970	0.688	2.410	7.60	14.40	41.6	54.7	19.0	34.7	16.8	1197.0	4.79
	10-1708	11.00	5.200	85.800	4.870	0.342	3.760	7.90	14.20	42.0	53.2	18.0	33.8	16.2	1260.0	4.74
	10-1720	18.50	9.720	81.400	5.560	0.483	2.880	7.96	14.80	43.0	54.0	18.6	34.3	16.1	1381.0	4.03
	10-1727	25.70	10.200	83.100	4.280	1.010	1.410	7.15	14.20	41.2	57.6	19.9	34.5	14.5	1123.0	4.97
	Mean	**17.067**	**9.6850**	**81.3833**	**5.7133**	**0.8222**	**2.3850**	**7.662**	**14.483**	**42.38**	**55.38**	**18.98**	**34.20**	**15.82**	**1227.67**	**4.862**
	SD	**4.9245**	**2.87702**	**4.23104**	**1.71633**	**0.38999**	**0.93190**	**0.3996**	**0.2994**	**1.082**	**2.104**	**0.816**	**0.390**	**0.979**	**87.336**	**0.3372**
	10-1706	13.20	7.880	83.800	5.310	0.996	2.060	7.25	13.70	40.9	56.5	18.9	33.5	16.8	1196.0	4.75
50 mg/kg	10-1710	16.80	9.030	79.100	7.860	1.490	2.500	7.50	14.00	41.8	55.7	18.7	33.6	16.7	1200.0	4.97
	10-1731	17.20	6.250	80.300	8.210	0.963	4.320	7.41	14.10	41.8	56.4	19.0	33.7	17.2	1320.0	5.28
	10-1688	8.32	16.700	74.500	5.090	0.484	3.260	7.55	14.20	41.9	55.6	18.8	33.8	16.2	1091.0	4.36
	10-1716	15.10	3.920	83.100	8.480	0.995	3.510	6.97	13.60	40.3	57.8	19.5	33.7	16.9	1218.0	4.34
	10-1717	12.40	12.200	76.800	6.650	0.275	4.100	7.09	13.50	39.6	55.8	19.1	34.2	17.4	1158.0	4.55
	Mean	**13.837**	**9.3300**	**79.6000**	**6.9333**	**0.8672**	**3.2917**	**7.295**	**13.850**	**41.05**	**56.30**	**19.00**	**33.75**	**16.87**	**1197.17**	**4.708**
	SD	**3.3043**	**4.54974**	**3.59110**	**1.48284**	**0.43070**	**0.88355**	**0.2324**	**0.2881**	**0.952**	**0.825**	**0.283**	**0.243**	**0.418**	**75.282**	**0.3665**
	10-1696	10.20	9.610	83.900	4.310	0.864	1.360	6.26	12.50	38.7	61.9	19.9	32.2	26.2	1154.0	4.83
100 mg/kg	10-1712	8.39	14.500	73.900	7.370	0.926	3.310	6.62	12.90	38.4	58.0	19.4	33.5	28.1	1298.0	4.54
	10-1728	15.30	7.360	80.100	7.140	0.814	4.540	6.67	12.30	37.7	56.5	18.4	32.5	24.2	1434.0	5.27
	10-1686	14.20	12.400	82.700	2.740	0.657	1.500	6.49	12.30	37.4	57.6	19.0	32.9	24.3	539.0	5.20
	10-1689	16.90	10.900	74.700	7.290	0.505	6.610	6.20	11.60	35.0	56.5	18.8	33.2	19.9	1117.0	4.80
	10-1724	10.60	10.800	76.200	8.720	0.512	3.740	6.52	13.20	40.4	62.0	20.2	32.6	24.4	1135.0	4.71
	Mean	**12.598**	**10.9283**	**78.5833**	**6.2617**	**0.7130**	**3.5100**	**6.460**	**12.467**	**37.93**	**58.75**	**19.28**	**32.82**	**24.52**	**1112.83**	**4.892**
	SD	**3.3414**	**2.42532**	**4.24755**	**2.24997**	**0.18175**	**1.97111**	**0.1907**	**0.5538**	**1.780**	**2.549**	**0.682**	**0.479**	**2.726**	**306.243**	**0.2853**

Table R-2
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of 14-Day Hematology
Male Rats

		Corn Oil	2,4-dinitroanisole (DNAN)						
		Control	1.56 mg/kg	3.13 mg/kg	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
WBC	Mean	20.133	18.483	17.100	16.833	18.117	20.583	19.217	17.017
(K/uL)	SD	3.2110	5.2667	4.6925	5.4698	4.5880	5.9617	3.9877	3.4167
	N	6	6	6	6	6	6	6	6
NEU	Mean	11.4517	11.1950	9.7317	11.3267	8.3967	10.7300	10.0667	8.7283
(%N)	SD	4.05394	4.47336	1.56185	4.29768	2.15275	3.18986	4.44264	2.26795
	N	6	6	6	6	6	6	6	6
LYM	Mean	80.8500	81.3333	80.6000	80.0833	81.4167	79.0000	81.9167	81.0167
(%L)	SD	4.98949	4.46079	4.70956	4.74823	2.69475	3.24160	4.94466	0.98675
	N	6	6	6	6	6	6	6	6
MONO	Mean	5.1150	4.5433	6.0617	5.5633	6.2333	6.2183	4.8900	5.8900
(%M)	SD	1.35215	0.34442	2.11711	1.32313	1.35559	1.52079	1.23079	0.88695
	N	6	6	6	6	6	6	6	6
EOS	Mean	0.7073	0.5607	0.6870	0.7337	0.8403	0.4562	0.6183	0.5333
(%E)	SD	0.33585	0.23448	0.27178	0.32386	0.30402	0.16008	0.11187	0.22703
	N	6	6	6	6	6	6	6	6
BASO	Mean	1.8767	2.3517	2.9017	2.2867	3.1133	3.6067	2.5350	3.8217
(%B)	SD	0.35786	0.64468	1.44540	0.42255	0.88491	2.08062	0.44743	1.31784
	N	6	6	6	6	6	6	6	6
RBC	Mean	7.385	7.783	7.505	7.548	7.560	7.852	7.878	7.750
(M/uL)	SD	0.2736	0.3262	0.2414	0.4582	0.4572	0.3068	0.2252	0.3187
	N	6	6	6	6	6	6	6	6
HGB	Mean	14.717	15.517	15.200	15.067	14.933	15.333	15.283	14.900
(g/dL)	SD	0.4491	0.6401	0.3521	0.7118	0.7367	0.3777	0.6524	0.6229
	N	6	6	6	6	6	6	6	6
HCT	Mean	43.45	45.73	44.58	44.40	43.90	45.38	44.98	44.20
(%)	SD	1.688	1.649	0.770	2.136	2.049	0.958	1.590	1.534
	N	6	6	6	6	6	6	6	6
MCV	Mean	58.83	58.82	58.52	58.83	58.12	57.82	57.10	57.05
(fL)	SD	1.311	1.347	1.569	1.052	1.057	1.722	1.381	1.411
	N	6	6	6	6	6	6	6	6
MCH	Mean	19.95	19.93	19.97	19.95	19.78	19.58	19.42	19.22
(pg)	SD	0.288	0.612	0.628	0.339	0.376	0.571	0.523	0.496
	N	6	6	6	6	6	6	6	6
MCHC	Mean	33.92	33.88	34.10	33.92	34.03	33.83	33.98	33.72
(g/dL)	SD	0.376	0.331	0.335	0.445	0.327	0.484	0.387	0.331
	N	6	6	6	6	6	6	6	6
RDW	Mean	16.15	15.93	15.95	15.65	16.20	16.40	17.18	17.25
(%)	SD	0.829	0.920	0.609	0.680	0.921	1.092	0.688	0.977
	N	6	6	6	6	6	6	6	6
PLT	Mean	1255.50	1268.17	1229.17	993.17*	1175.00	1169.67	1118.17	1057.00
(K/uL)	SD	109.224	81.217	117.631	257.101	123.894	100.802	78.111	147.061
	N	6	6	6	6	6	6	6	6
MPV	Mean	4.583	4.597	4.675	4.717	4.602	4.842	4.745	4.945
(fL)	SD	0.1669	0.1150	0.4014	0.1544	0.3129	0.4017	0.3304	0.3537
	N	6	6	6	6	6	6	6	6

*Significantly different from corn oil control

Table R-2
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of 14-Day Hematology
Female Rats

		Corn Oil	2,4-dinitroanisole (DNAN)						
		Control	1.56 mg/kg	3.13 mg/kg	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
WBC	Mean	12.365	14.700	10.588	16.413	16.017	17.067	13.837	12.598
(K/uL)	SD	4.8915	3.3407	3.5478	6.2754	5.2316	4.9245	3.3043	3.3414
	N	6	6	6	6	6	6	6	6
NEU	Mean	9.4483	7.5800	9.0567	8.6467	7.1283	9.6850	9.3300	10.9283
(%N)	SD	4.18366	2.04968	3.20950	5.09279	2.55368	2.87702	4.54974	2.42532
	N	6	6	6	6	6	6	6	6
LYM	Mean	83.0833	85.9167	84.0167	85.5000	84.2333	81.3833	79.6000	78.5833
(%L)	SD	4.43956	2.81810	5.09644	4.75689	3.78717	4.23104	3.59110	4.24755
	N	6	6	6	6	6	6	6	6
MONO	Mean	4.8500	4.0800	4.3217	3.7583	5.5167	5.7133	6.9333	6.2617
(%M)	SD	0.71077	0.56833	2.14923	1.26357	2.03010	1.71633	1.48284	2.24997
	N	6	6	6	6	6	6	6	6
EOS	Mean	0.8293	0.7970	0.9130	0.5130	0.5198	0.8222	0.8672	0.7130
(%E)	SD	0.13623	0.14867	0.35515	0.25886	0.19054	0.38999	0.43070	0.18175
	N	6	6	6	6	6	6	6	6
BASO	Mean	1.7663	1.6318	1.6983	1.5600	2.6183	2.3850	3.2917	3.5100
(%B)	SD	1.24003	1.01246	0.58167	0.26930	1.05840	0.93190	0.88355	1.97111
	N	6	6	6	6	6	6	6	6
RBC	Mean	7.543	7.635	7.273	7.640	7.525	7.662	7.295	6.460*
(M/uL)	SD	0.3061	0.2568	0.4816	0.4614	0.5580	0.3998	0.2324	0.1907
	N	6	6	6	6	6	6	6	6
HGB	Mean	14.567	14.967	14.300	14.733	14.617	14.483	13.850	12.467*
(g/dL)	SD	0.5354	0.4131	0.6164	0.6653	0.8280	0.2994	0.2881	0.5538
	N	6	6	6	6	6	6	6	6
HCT	Mean	42.18	43.85	40.92	42.72	42.42	42.38	41.05	37.93*
(%)	SD	0.240	1.484	3.006	1.500	2.540	1.082	0.952	1.780
	N	6	6	6	6	6	6	6	6
MCV	Mean	55.95	57.42	56.23	56.00	56.40	55.38	56.30	58.75
(fL)	SD	2.089	0.954	1.242	2.554	1.220	2.104	0.825	2.549
	N	6	6	6	6	6	6	6	6
MCH	Mean	19.28	19.65	19.67	19.28	19.43	18.98	19.00	19.28
(pg)	SD	0.605	0.187	0.896	0.665	0.403	0.816	0.283	0.682
	N	6	6	6	6	6	6	6	6
MCHC	Mean	34.48	34.18	35.03	34.48	34.43	34.20	33.75	32.82*
(g/dL)	SD	1.130	0.705	1.812	0.679	0.333	0.390	0.243	0.479
	N	6	6	6	6	6	6	6	6
RDW	Mean	15.78	15.03	15.20	15.13	15.30	15.82	16.87	24.52*
(%)	SD	0.733	0.437	0.756	0.671	0.844	0.979	0.418	2.726
	N	6	6	6	6	6	6	6	6
PLT	Mean	1228.83	1258.33	1259.83	1143.00	1017.17	1227.67	1197.17	1112.83
(K/uL)	SD	222.048	96.053	178.184	209.601	174.434	87.338	75.282	306.243
	N	6	6	6	6	6	6	6	6
MPV	Mean	4.512	4.648	4.715	4.558	4.497	4.662	4.708	4.892
(fL)	SD	0.3166	0.2138	0.1707	0.2698	0.2813	0.3372	0.3685	0.2853
	N	6	6	6	6	6	6	6	6

*Significantly different from corn oil control

Appendix S

Individual and Summary of 90-Day Hematology Data

Table S-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

90-Day Individual Hematology
Male Rats

Group	Animal ID	WBC (K/uL)	NEU (%)	LYM (%)	MONO (%)	EOS (%)	BASO (%)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)	NOTES
	11-0097	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
Corn Oil	11-0105	11.20	10.600	78.100	8.040	1.060	2.200	8.81	14.70	42.8	48.6	16.7	34.4	17.7	984.0	4.25	
Control	11-0107	10.70	10.700	79.400	6.970	1.600	1.290	8.41	13.80	40.2	47.9	16.5	34.4	17.9	1379.0	4.57	
	11-0108	12.80	8.670	86.100	3.700	0.982	0.500	8.45	15.30	45.0	53.3	18.1	34.0	15.9	854.0	4.19	
	11-0112	14.60	8.790	83.500	4.780	1.550	1.360	7.84	13.80	39.8	50.7	17.5	34.6	18.1	1261.0	4.95	
	11-0116	10.60	12.400	78.900	4.320	1.720	2.710	8.17	14.00	41.1	50.2	17.1	34.1	17.3	922.0	4.40	
	11-0118	20.40	6.670	72.800	12.000	1.380	7.160	8.41	14.70	43.4	51.6	17.5	33.9	16.9	1000.0	4.31	
	11-0140	18.90	10.900	80.800	5.680	0.879	1.720	7.91	14.10	41.7	52.7	17.8	33.8	17.1	956.0	4.86	
	11-0147	13.40	9.510	81.700	6.910	0.896	0.969	8.63	15.50	44.3	51.3	17.9	34.9	16.5	1005.0	5.02	
	11-0150	11.40	10.000	79.700	7.840	1.450	1.020	8.68	14.40	42.1	48.4	16.6	34.2	19.5	1228.0	5.04	
	Mean	**13.778**	**9.8044**	**80.1111**	**6.6933**	**1.2797**	**2.1032**	**8.368**	**14.478**	**42.27**	**50.52**	**17.30**	**34.26**	**17.43**	**1065.44**	**4.621**	
	SD	**3.6010**	**1.64461**	**3.70791**	**2.51727**	**0.32658**	**2.01057**	**0.3353**	**0.6241**	**1.776**	**1.918**	**0.598**	**0.354**	**1.039**	**178.471**	**0.3485**	
	11-0095	14.00	9.410	86.600	1.650	0.705	1.670	9.27	15.50	45.3	48.9	16.7	34.2	18.2	897.0	4.27	
1.25 mg/kg	11-0103	14.40	10.300	80.600	6.350	1.220	1.530	9.38	15.70	45.4	48.4	16.7	34.6	17.8	1012.0	4.42	
	11-0104	14.00	9.260	80.400	6.170	1.680	2.500	8.92	15.00	44.0	49.3	16.8	34.0	17.1	1034.0	4.98	
	11-0113	15.70	8.940	79.000	8.570	1.570	1.940	9.53	15.70	45.2	47.5	16.5	34.7	18.2	1025.0	4.17	
	11-0114	16.10	7.000	82.600	7.120	2.010	1.240	8.60	14.90	43.8	51.0	17.3	34.0	17.0	1109.0	4.75	
	11-0122	19.40	7.910	83.800	6.200	0.695	1.370	8.45	14.60	42.7	50.6	17.3	34.1	15.7	988.0	4.85	
	11-0126	13.60	11.100	79.900	6.460	1.240	1.330	8.74	14.90	43.5	49.7	17.1	34.4	16.9	991.0	4.48	
	11-0134	17.30	9.880	78.400	6.350	2.590	2.800	9.28	15.60	44.2	47.6	16.8	35.2	18.2	1342.0	4.46	
	11-0142	15.20	7.250	81.500	8.190	1.390	1.630	8.75	14.90	43.1	49.2	17.0	34.5	17.2	1163.0	4.52	
	11-0145	15.70	6.000	81.000	6.930	1.760	4.260	8.22	14.90	42.9	52.2	18.1	34.6	17.3	953.0	4.56	
	Mean	**15.540**	**8.7050**	**81.3800**	**6.3990**	**1.4860**	**2.0270**	**8.914**	**15.170**	**44.01**	**49.44**	**17.03**	**34.43**	**17.36**	**1051.40**	**4.546**	
	SD	**1.7728**	**1.61569**	**2.42798**	**1.86715**	**0.57632**	**0.93595**	**0.4359**	**0.4084**	**1.007**	**1.493**	**0.460**	**0.374**	**0.782**	**126.475**	**0.2513**	
	11-0100	10.90	8.030	82.600	6.890	0.495	1.980	8.39	14.30	41.9	50.0	17.1	34.1	17.6	868.0	5.36	
5 mg/kg	11-0101	13.90	8.910	82.100	4.350	1.120	3.480	8.68	14.80	43.4	50.0	17.1	34.1	17.5	884.0	4.14	
	11-0115	12.10	11.100	75.600	10.400	1.080	1.840	8.09	13.90	41.5	51.3	17.2	33.5	18.9	1296.0	4.43	
	11-0117	14.50	9.280	77.000	10.200	2.020	1.490	9.17	15.30	44.7	48.8	16.7	34.2	19.4	977.0	4.22	
	11-0124	14.20	7.760	84.500	3.910	1.160	2.700	9.07	16.20	46.4	51.2	17.8	34.8	18.6	818.0	4.61	
	11-0131	15.00	8.920	80.700	7.790	1.050	1.520	8.92	15.70	45.5	51.0	17.6	34.5	18.6	969.0	4.67	
	11-0135	15.40	14.300	72.800	10.400	1.360	1.050	8.79	14.60	41.7	47.4	16.6	35.1	19.4	986.0	4.95	
	11-0138	15.20	8.580	80.200	8.110	1.600	1.460	9.57	15.40	44.2	46.2	16.1	34.9	19.7	986.0	4.80	
	11-0141	16.50	10.800	81.100	5.990	0.981	1.080	9.71	14.90	43.0	44.3	15.4	34.7	19.7	937.0	5.20	
	11-0146	18.80	8.120	68.000	15.500	1.700	6.670	9.04	14.70	42.6	47.1	16.2	34.4	16.4	1006.0	4.61	
	Mean	**14.650**	**9.5800**	**78.4600**	**8.3540**	**1.2566**	**2.3270**	**8.943**	**14.980**	**43.49**	**48.73**	**16.78**	**34.43**	**18.58**	**972.70**	**4.699**	
	SD	**2.1854**	**1.99717**	**5.09514**	**3.44377**	**0.43023**	**1.69892**	**0.4927**	**0.6812**	**1.674**	**2.393**	**0.733**	**0.474**	**1.097**	**129.123**	**0.3933**	
	11-0106	14.30	10.800	76.400	9.100	1.760	1.950	10.20	15.00	43.0	42.0	14.7	34.9	20.7	1104.0	4.65	
20 mg/kg	11-0120	16.70	9.230	77.800	9.070	1.140	2.770	9.53	14.90	42.8	44.9	15.7	34.9	20.7	1022.0	4.35	
	11-0121	17.80	6.820	86.000	5.480	1.200	0.499	9.03	14.90	43.3	48.0	16.5	34.4	19.4	1126.0	4.95	
	11-0125	13.70	13.700	77.200	4.900	1.500	2.750	8.89	14.90	43.6	49.1	16.7	34.1	23.1	917.0	4.60	
	11-0127	17.50	11.100	69.900	14.800	1.560	2.630	9.57	15.30	44.2	46.2	16.0	34.6	21.7	910.0	4.72	
	11-0130	16.10	8.390	79.000	9.680	2.210	0.720	9.04	14.80	43.9	48.6	16.4	33.8	20.3	914.0	4.56	
	11-0133	19.10	6.770	81.800	8.500	1.150	1.750	9.25	15.10	43.6	47.1	16.3	34.6	21.1	1066.0	5.49	
	11-0137	20.50	4.360	87.000	6.720	1.100	0.781	9.03	15.50	45.4	50.2	17.2	34.2	19.4	823.0	4.88	
	11-0139	16.50	6.830	79.200	9.940	1.660	2.370	9.41	15.00	43.2	45.9	15.9	34.8	21.1	846.0	5.98	
	11-0148	13.70	8.640	82.400	4.220	1.550	3.230	10.10	16.00	46.8	46.3	15.8	34.1	22.0	995.0	5.45	
	Mean	**16.590**	**8.6640**	**79.6700**	**8.2410**	**1.4830**	**1.9450**	**9.405**	**15.140**	**43.98**	**46.83**	**16.12**	**34.44**	**20.95**	**972.30**	**4.963**	
	SD	**2.2630**	**2.69009**	**4.96880**	**3.10443**	**0.35002**	**0.97833**	**0.4548**	**0.3688**	**1.235**	**2.349**	**0.676**	**0.381**	**1.139**	**106.100**	**0.5146**	
	11-0099	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
80 mg/kg	11-0102	15.10	6.350	82.800	9.010	0.604	1.220	7.94	14.10	42.5	53.6	17.8	33.2	22.9	802.0	4.46	
	11-0109	11.90	11.400	82.600	3.790	0.920	1.330	8.16	13.70	41.2	50.5	16.8	33.2	24.2	750.0	5.19	
	11-0110	19.70	7.800	83.500	7.060	0.473	1.190	8.49	14.90	44.7	52.6	17.5	33.4	23.3	1020.0	5.14	
	11-0111	17.50	11.200	79.900	4.200	1.230	3.480	8.71	13.90	42.0	48.2	16.0	33.1	26.0	1064.0	5.85	
	11-0123	14.70	7.080	75.800	14.100	0.860	2.210	8.09	14.40	42.2	52.1	17.8	34.2	25.4	838.0	4.79	
	11-0129	13.30	7.290	79.100	8.520	1.190	3.880	8.74	15.00	45.3	51.9	17.2	33.2	23.2	1034.0	4.89	
	11-0132	16.00	12.900	73.000	11.100	1.070	1.910	8.34	14.50	43.9	52.7	17.3	32.9	22.3	827.0	5.70	
	11-0144	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
	11-0149	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
	Mean	**15.457**	**9.1457**	**79.5286**	**8.2543**	**0.9067**	**2.1743**	**8.353**	**14.357**	**43.11**	**51.66**	**17.20**	**33.31**	**23.90**	**905.00**	**5.146**	
	SD	**2.5973**	**2.60558**	**3.93180**	**3.66755**	**0.28680**	**1.10135**	**0.3091**	**0.4894**	**1.529**	**1.793**	**0.635**	**0.418**	**1.364**	**129.328**	**0.4945**	

(ND = NO DATA)

Toxicological Study No. 87-XE-0DBP-10, Subchronic toxicity of DNAN, Sept 2010-March 2011

Table S-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

90-Day Individual Hematology
Female Rats

Group	Animal ID	WBC (K/uL)	NEU (%)	LYM (%)	MONO (%)	EOS (%)	BASO (%)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)	NOTES
	11-0154	10.00	15.200	77.800	2.420	1.430	3.100	8.50	15.80	46.1	54.2	18.5	34.2	17.8	992.0	4.62	
Corn Oil	11-0162	5.85	4.320	88.000	4.300	2.580	0.774	8.28	14.90	44.1	53.2	18.0	33.8	17.1	1172.0	4.57	
Control	11-0168	ND	ND	ND	ND	ND	ND	7.98	14.00	41.2	51.6	17.5	34.0	16.6	1012.0	4.66	WBC error
	11-0171	8.81	13.100	77.900	4.380	0.966	3.620	7.67	13.50	39.4	51.4	17.7	34.4	16.9	1090.0	4.74	
	11-0173	7.05	7.750	86.800	2.410	1.220	1.830	7.89	13.80	40.4	51.2	17.5	34.2	17.1	985.0	4.54	
	11-0175	8.05	15.600	76.900	5.390	1.060	1.090	7.76	14.00	41.6	53.6	18.1	33.8	16.8	1100.0	4.72	
	11-0190	7.26	5.900	86.400	4.750	0.699	2.210	8.39	14.60	42.9	51.1	17.4	34.0	15.9	960.0	4.42	
	11-0191	12.10	3.870	86.500	6.300	2.140	1.140	7.93	13.80	40.2	50.7	17.4	34.4	16.8	1045.0	4.25	
	11-0197	12.30	5.620	87.000	5.240	1.080	1.050	8.01	14.70	42.5	53.0	18.3	34.5	15.5	949.0	4.61	
	11-0206	7.64	6.150	89.300	2.470	0.757	1.300	7.52	13.40	39.1	52.0	17.8	34.3	17.0	945.0	4.76	
	Mean	**8.784**	**8.6122**	**84.0667**	**4.1844**	**1.3258**	**1.7904**	**7.993**	**14.250**	**41.75**	**52.20**	**17.82**	**34.16**	**16.75**	**1025.00**	**4.589**	
	SD	**2.2534**	**4.69568**	**4.98698**	**1.44117**	**0.63590**	**0.99846**	**0.3152**	**0.7427**	**2.197**	**1.206**	**0.391**	**0.250**	**0.645**	**75.364**	**0.1570**	
	11-0152	10.70	6.170	85.000	5.620	1.690	1.540	7.62	13.70	40.5	53.2	17.9	33.7	16.0	1176.0	4.94	
1.25 mg/kg	11-0159	5.99	7.580	82.800	6.930	1.610	1.140	8.16	14.80	43.6	53.4	18.2	34.0	16.1	932.0	4.41	
	11-0165	9.53	10.800	84.000	1.980	1.270	1.960	8.55	14.70	43.1	50.4	17.1	34.0	16.3	955.0	4.13	
	11-0170	6.41	7.210	81.700	8.720	1.190	1.210	7.81	14.20	41.1	52.6	18.1	34.5	15.8	989.0	4.57	
	11-0172	11.00	7.770	81.200	8.340	1.590	1.130	8.17	14.80	43.5	53.3	18.1	34.0	16.1	1139.0	4.96	
	11-0176	9.61	7.670	83.300	6.000	1.780	1.210	7.63	13.80	41.3	54.1	18.1	33.3	17.1	1102.0	4.50	
	11-0178	6.56	9.030	82.500	3.180	2.880	2.370	7.72	13.70	40.7	52.7	17.7	33.6	16.1	1055.0	4.19	
	11-0181	5.98	14.900	76.900	4.270	1.400	2.490	8.10	14.60	42.4	52.3	18.0	34.4	16.9	1027.0	4.76	
	11-0192	14.30	9.640	84.500	2.850	0.916	2.080	7.58	13.40	39.3	51.9	17.7	34.2	16.6	1152.0	4.92	
	11-0196	16.50	5.640	88.000	4.630	0.926	0.809	7.69	14.30	42.0	54.7	18.6	34.1	14.7	856.0	4.69	
	Mean	**9.658**	**8.6410**	**82.9900**	**5.2520**	**1.5252**	**1.5939**	**7.903**	**14.200**	**41.75**	**52.86**	**17.95**	**33.98**	**16.17**	**1038.30**	**4.607**	
	SD	**3.6270**	**2.68685**	**2.88230**	**2.28991**	**0.56394**	**0.58790**	**0.3234**	**0.5207**	**1.418**	**1.197**	**0.395**	**0.365**	**0.662**	**105.618**	**0.3013**	
	11-0156	5.63	12.800	75.200	7.260	1.940	2.810	8.11	13.70	40.6	50.1	16.9	33.7	16.9	939.0	4.11	
5 mg/kg	11-0157	8.69	6.050	83.100	7.540	0.642	2.650	6.72	12.10	35.4	52.6	18.0	34.2	18.4	886.0	4.37	
	11-0163	12.90	9.940	82.800	3.100	1.560	2.620	8.40	15.40	44.6	53.1	18.3	34.5	17.8	1105.0	4.54	
	11-0166	16.40	9.150	82.400	5.840	0.796	1.800	8.06	15.30	43.8	54.4	18.9	34.8	16.9	1002.0	4.44	
	11-0174	7.70	7.280	83.600	6.630	1.680	0.817	8.12	14.30	41.8	51.5	17.6	34.1	16.4	872.0	5.56	
	11-0187	9.41	9.940	74.100	9.460	1.360	5.160	8.13	14.20	41.3	50.9	17.4	34.3	16.3	1023.0	4.62	
	11-0189	17.60	4.100	87.100	6.250	1.040	1.510	8.38	15.50	45.3	54.1	18.5	34.3	15.5	1055.0	4.79	
	11-0193	14.50	4.970	88.000	3.400	1.090	2.490	8.17	15.40	45.4	55.5	18.9	34.0	15.5	839.0	4.73	
	11-0202	13.90	3.660	90.700	2.570	0.946	2.100	8.26	14.50	42.0	50.9	17.6	34.5	15.4	989.0	4.20	
	11-0204	13.70	9.000	79.000	7.370	1.170	3.500	7.97	14.50	41.6	52.2	18.2	34.8	15.9	660.0	5.74	
	Mean	**12.043**	**7.6890**	**82.6000**	**5.9420**	**1.2224**	**2.5457**	**8.032**	**14.490**	**42.18**	**52.53**	**18.03**	**34.32**	**16.50**	**937.00**	**4.710**	
	SD	**3.9580**	**2.97153**	**5.33667**	**2.24088**	**0.40905**	**1.18558**	**0.4804**	**1.0429**	**2.950**	**1.748**	**0.657**	**0.346**	**1.013**	**128.943**	**0.5412**	
	11-0153	9.14	11.400	83.400	2.350	1.010	1.840	9.14	15.10	44.9	49.2	16.5	33.5	18.1	898.0	4.53	
20 mg/kg	11-0155	17.20	4.380	89.600	3.950	0.826	1.190	8.41	15.10	43.9	52.2	17.9	34.3	15.5	873.0	4.10	
	11-0160	15.30	3.680	87.900	5.500	0.749	2.140	8.25	14.00	41.8	50.6	17.0	33.5	18.7	895.0	4.65	
	11-0164	11.60	12.400	79.400	5.770	0.919	1.550	8.24	15.20	44.2	53.6	18.5	34.5	17.1	832.0	4.37	
	11-0167	10.30	6.010	86.800	3.520	1.400	2.250	8.61	14.50	43.6	50.7	16.8	33.2	19.1	901.0	5.12	
	11-0179	12.80	9.440	78.900	9.080	1.300	1.300	7.70	13.30	38.8	50.4	17.3	34.3	20.0	1049.0	4.85	
	11-0183	15.60	7.760	81.100	8.360	0.753	2.040	7.10	12.60	37.3	52.6	17.7	33.7	16.6	1045.0	4.25	
	11-0184	11.50	5.310	85.800	6.530	1.320	1.040	8.84	14.50	42.0	47.5	16.4	34.6	17.9	767.0	4.65	
	11-0194	14.40	12.000	72.700	10.600	1.210	3.550	8.43	13.40	39.4	46.7	15.9	34.0	18.7	1193.0	4.77	
	11-0198	13.60	17.500	72.400	7.740	1.220	1.150	8.03	14.20	42.1	52.5	17.7	33.6	19.5	1188.0	5.10	
	Mean	**13.144**	**8.9880**	**81.8000**	**6.3400**	**1.0707**	**1.8050**	**8.275**	**14.190**	**41.80**	**50.60**	**17.17**	**33.92**	**18.12**	**964.10**	**4.639**	
	SD	**2.5439**	**4.37801**	**6.03177**	**2.63200**	**0.24852**	**0.75456**	**0.5769**	**0.8724**	**2.542**	**2.261**	**0.796**	**0.485**	**1.388**	**146.819**	**0.3386**	
	11-0151	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
80 mg/kg	11-0158	15.60	9.010	76.000	11.900	1.280	1.800	7.22	13.60	40.5	56.1	18.8	33.4	20.5	902.0	5.88	
	11-0177	15.30	7.940	79.700	8.450	1.110	2.760	6.99	13.20	39.0	55.8	18.8	33.7	21.7	1131.0	4.64	
	11-0180	14.00	3.170	86.600	7.560	0.811	1.810	7.04	12.70	37.7	53.5	18.0	33.7	19.4	967.0	4.80	
	11-0182	15.90	4.390	86.500	6.470	0.524	2.110	6.96	12.40	38.9	55.9	17.9	31.9	19.9	1033.0	4.84	
	11-0186	17.20	7.100	84.900	6.280	0.619	1.070	6.30	12.40	37.8	60.0	19.7	32.7	21.1	1191.0	5.89	
	11-0188	11.90	9.920	83.200	5.200	0.512	1.150	6.52	12.40	37.7	57.8	19.0	32.8	21.6	1212.0	4.68	
	11-0195	23.70*	7.080*	85.300*	5.950*	0.420*	1.280*	6.69*	11.40*	36.0*	53.8*	17.1*	31.7*	23.0*	56.3*	ND	platelet outlier
	11-0200	12.90	8.040	77.800	11.800	1.160	1.240	6.37	11.80	35.9	56.4	18.5	32.8	21.1	992.0	5.11	
	11-0201	15.00	3.600	85.600	7.710	0.844	2.210	6.55	11.60	35.9	54.8	17.8	32.5	23.3	1069.0	5.09	
	Mean	**14.725**	**6.6463**	**82.5375**	**8.1713**	**0.8575**	**1.7688**	**6.744**	**12.513**	**37.93**	**56.29**	**18.56**	**32.94**	**21.08**	**1062.13**	**5.116**	
	SD	**1.7119**	**2.57963**	**4.15484**	**2.47948**	**0.29847**	**0.59126**	**0.3477**	**0.6621**	**1.561**	**1.945**	**0.648**	**0.625**	**1.203**	**109.751**	**0.5037**	

(ND = NO DATA)
*Data dropped from analysis

Table S-2
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of 90-Day Hematology
Male Rats

		Corn Oil	2,4-dinitroanisole (DNAN)			
		Control	1.25 mg/kg	5 mg/kg	20 mg/kg	80 mg/kg
WBC	Mean	13.778	15.540	14.650	16.590	15.457
(K/uL)	SD	3.6010	1.7728	2.1854	2.2630	2.5973
	N	9	10	10	10	7
NEU	Mean	9.8044	8.7050	9.5800	8.6640	9.1457
(%N)	SD	1.64461	1.61569	1.99717	2.69009	2.60558
	N	9	10	10	10	7
LYM	Mean	80.1111	81.3800	78.4600	79.6700	79.5286
(%L)	SD	3.70791	2.42798	5.09514	4.96880	3.93180
	N	9	10	10	10	7
MONO	Mean	6.6933	6.3990	8.3540	8.2410	8.2543
(%M)	SD	2.51727	1.86715	3.44377	3.10443	3.66755
	N	9	10	10	10	7
EOS	Mean	1.2797	1.4860	1.2566	1.4830	0.9067
(%E)	SD	0.32658	0.57632	0.43023	0.35002	0.28680
	N	9	10	10	10	7
BASO	Mean	2.1032	2.0270	2.3270	1.9450	2.1743
(%B)	SD	2.01057	0.93595	1.69892	0.97833	1.10135
	N	9	10	10	10	7
RBC	Mean	8.368	8.914	8.943*	9.405*	8.353
(M/uL)	SD	0.3353	0.4359	0.4927	0.4548	0.3091
	N	9	10	10	10	7
HGB	Mean	14.478	15.170	14.980	15.140	14.357
(g/dL)	SD	0.6241	0.4084	0.6812	0.3688	0.4894
	N	9	10	10	10	7
HCT	Mean	42.27	44.01	43.49	43.98	43.11
(%)	SD	1.776	1.007	1.674	1.235	1.529
	N	9	10	10	10	7
MCV	Mean	50.52	49.44	48.73	46.83*	51.66
(fL)	SD	1.918	1.493	2.393	2.349	1.793
	N	9	10	10	10	7
MCH	Mean	17.30	17.03	16.78	16.12*	17.20
(pg)	SD	0.598	0.460	0.733	0.676	0.635
	N	9	10	10	10	7
MCHC	Mean	34.26	34.43	34.43	34.44	33.31*
(g/dL)	SD	0.354	0.374	0.474	0.381	0.418
	N	9	10	10	10	7
RDW	Mean	17.43	17.36	18.58	20.95*	23.90*
(%)	SD	1.039	0.782	1.097	1.139	1.364
	N	9	10	10	10	7
PLT	Mean	1065.44	1051.40	972.70	972.30	905.00
(K/uL)	SD	178.471	126.475	129.123	106.100	129.328
	N	9	10	10	10	7
MPV	Mean	4.621	4.546	4.699	4.963	5.146
(fL)	SD	0.3485	0.2513	0.3933	0.5146	0.4945
	N	9	10	10	10	7

*Significantly different from corn oil control

Table S-2
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of 90-Day Hematology
Female Rats

		Corn Oil	2,4-dinitroanisole (DNAN)			
		Control	1.25 mg/kg	5 mg/kg	20 mg/kg	80 mg/kg
WBC	Mean	8.784	9.658	12.043	13.144*	14.725*
(K/uL)	SD	2.2534	3.6270	3.9580	2.5439	1.7119
	N	9	10	10	10	8
NEU	Mean	8.6122	8.6410	7.6890	8.9880	6.6463
(%N)	SD	4.69568	2.68685	2.97153	4.37801	2.57963
	N	9	10	10	10	8
LYM	Mean	84.0667	82.9900	82.6000	81.8000	82.5375
(%L)	SD	4.98698	2.88230	5.33667	6.03177	4.15484
	N	9	10	10	10	8
MONO	Mean	4.1844	5.2520	5.9420	6.3400	8.1713*
(%M)	SD	1.44117	2.28991	2.24088	2.63200	2.47948
	N	9	10	10	10	8
EOS	Mean	1.3258	1.5252	1.2224	1.0707	0.8575
(%E)	SD	0.63590	0.56394	0.40905	0.24852	0.29847
	N	9	10	10	10	8
BASO	Mean	1.7904	1.5939	2.5457	1.8050	1.7688
(%B)	SD	0.99846	0.58790	1.18558	0.75456	0.59126
	N	9	10	10	10	8
RBC	Mean	7.993	7.903	8.032	8.275	6.744*
(M/uL)	SD	0.3152	0.3234	0.4804	0.5769	0.3477
	N	10	10	10	10	8
HGB	Mean	14.250	14.200	14.490	14.190	12.513*
(g/dL)	SD	0.7427	0.5207	1.0429	0.8724	0.6621
	N	10	10	10	10	8
HCT	Mean	41.75	41.75	42.18	41.80	37.93*
(%)	SD	2.197	1.418	2.950	2.542	1.561
	N	10	10	10	10	8
MCV	Mean	52.20	52.86	52.53	50.60	56.29*
(fL)	SD	1.206	1.197	1.748	2.261	1.945
	N	10	10	10	10	8
MCH	Mean	17.82	17.95	18.03	17.17	18.56
(pg)	SD	0.391	0.395	0.657	0.796	0.648
	N	10	10	10	10	8
MCHC	Mean	34.16	33.98	34.32	33.92	32.94*
(g/dL)	SD	0.250	0.365	0.346	0.485	0.625
	N	10	10	10	10	8
RDW	Mean	16.75	16.17	16.50	18.12*	21.08*
(%)	SD	0.645	0.662	1.013	1.388	1.203
	N	10	10	10	10	8
PLT	Mean	1025.00	1038.30	937.00	964.10	1062.13
(K/uL)	SD	75.364	105.618	128.943	146.819	109.751
	N	10	10	10	10	8
MPV	Mean	4.589	4.607	4.710	4.639	5.116*
(fL)	SD	0.1570	0.3013	0.5412	0.3386	0.5037
	N	10	10	10	10	8

*Significantly different from corn oil control

Appendix T

Neurobehavioral Evaluation Report

Neurobehavioral Analysis in Rats Orally Dosed with 2,4-Dinitroanisole

Protocol No.: 0DBP-38-10-07-01

Prepared by:
Theresa Hanna and Emily May Lent

5 October 2011

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

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

1. The statistical analyses were conducted by the US Army Public Health Command statisticians and it is not known if they were conducted in compliance with 40 CFR Part 792.

Submitted By:

Theresa Hanna

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Date: 5 October 2011

Emily May Lent

EMILY MAY LENT
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Date: 5 Oct 2011

The following were responsible for the conduct of this study and preparation of this report:

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ABSTRACT

Male and female Sprague-Dawley rats were orally dosed with 2,4-dinitroanisole (DNAN) at 0, 1.25, 5, 20, and 80 mg/kg-day for 90-days. Testing with a functional observational battery including home cage, handheld, and open field observations, was conducted weekly. Motor activity and sensory functions were assessed during week 12 of the study. DNAN produced neuromuscular toxicity, characterized by hunched body position, hind limb impairment, and walking on toes or ataxia, in both males and females given 80 mg/kg-day. Activity levels were also depressed for both sexes in the 80 mg/kg-day group. The 80 mg/kg-day dose groups also exhibited behavioral effects; however, the effects in males were evident in an increase in excitability/affective responses, while in females there was a depression in sensorimotor responses. This study suggests that neurotoxicity should be considered in the evaluation of DNAN.

INTRODUCTION

The functional observational battery (FOB) employs a suite of over 30 non-invasive tests of neurological function and behavior to screen compounds for neurotoxicity. FOB measures are typically grouped into three functional domains: neuromuscular, autonomic, and behavioral (Moser 1991; Tilson and Moser 1992; Boucard et al. 2010). Neuromuscular endpoints include incoordination, gait and seizure activity. Autonomic endpoints include pupil response, salivation and breathing. Behavioral endpoints include grooming, reactivity to handling and arousal level. Additional behavioral tests can be performed to evaluate sensory function including reaction to touch and tail pinch. In addition to FOB, neurotoxicity screening often employs automated assessments of motor activity (Kulig et al. 1996). FOB and motor activity yield a large amount of data that must be integrated to provide an accurate assessment of neurotoxicity. Composite scores which combine subsets of endpoints based on functional domains have been used to integrate data (Boucard et al. 2010; Kulig et al. 1996).

METHODS

Animals and Treatments

Fifty young adult male and fifty female Sprague-Dawley rats were obtained from Charles River Laboratories, Wilmington, Massachusetts. The animals were acclimatized for five days after their arrival in this facility. Animals were seven weeks old and females and males weighed 214.1 ± 9.14 and 297.1 ± 10.88 grams, respectively, at the start of dosing. were maintained at 71.1 ± 1.23°F, 46.2 ± 3.35% relative humidity, with a 12-hour light/dark cycle. A certified pesticide-free rodent chow (Harlan Teklad®, 8728C Certified Rodent Diet) and drinking quality water were available *ad libitum*. Rats were housed individually in suspended polycarbonate boxes with ALPHA-dri® bedding. (Teklad® is a registered trademark of Harlan, Teklad; ALPHA-dri® is a registered trademark with Shepard Specialty Papers.)

Ten males and ten females were distributed into four treatment groups and a vehicle control group using a stratified random method, with stratification based on body weight taken three days prior to study start. The dose groups were: corn oil control, 1.25, 5, 20, 80 mg/kg-day. The DNAN solution/suspensions and corn oil control were administered daily via oral gavage for 90 days.

Experimental Testing

Potential neurotoxic effects of 2,4-dinitroanisole (DNAN) were evaluated using the FOB and motor activity assessment. The FOB protocol used in this study followed the methods described in McDaniel et al. (1993). Animals were divided into two subsets for each sex, using a stratified random procedure based on dose group. The FOB was conducted on each animal prior to initiation of dosing and weekly thereafter, with one subset of animals being assessed per day. The order of animals evaluated each day was randomly determined prior to study initiation. The FOB was performed at the same time each morning prior to dosing. Each rat was removed from its cage and held by the observer to conduct the handheld observation of reactivity and appearance. The rat was then placed on a cart to conduct the

open arena observations of gait, arousal, rears, and excretions. Home cage observations were performed weekly on all animals on the same day. During week eleven of dosing, sensorimotor responses were tested after the open arena observations. Observations and FOB was performed by the same evaluator throughout the study; the evaluator was blind to the treatment groups. Motor activity was measured after week eleven of dosing using an open field chamber with automated detection devices.

Home Cage Observation: The home cage observations included signs of agitation, convulsions, tremors, posture, mutilation, and the area mutilated. Each rat was assigned a number corresponding with the observed response. Agitation and mutilation were scored as present (1) or absent (2), area mutilated was only described if present. Convulsions and tremors were scored as absent (1), slight (2), or severe (3). Posture was scored for the following positions: lying down (1), sit/stand (2), rearing (3), flattened (4), lying down with limbs up (5), crouched with head down (6), and/ or head bobbing (7), animals demonstrated one or more body postures in one observation.

Handheld Observation: Each animal was removed from the home cage and the following observations were recorded: ease of removal (ER), reactivity to handling (RH), lacrimation (LAC), salivation (SAL), barbering (BAR), piloerection (PIL), paprebral closure (PC) of left and right eye, exothalmus (EXO), and pupillary status (PS) of the left and right eye. ER describes the removal of the rat from the home cage and was scored 1-6: very easy, easy, moderately difficult, rat flinches, difficult, and very difficult. RH was scored 1 – 5: very low, low, moderately low, moderately high and high. Tearing from the eye (LAC), salivation (SAL), eye bulging (EXO), and absence of hair from the forelimbs due to excessive grooming (BAR) were scored as present (1) or absent (2). PC described the eye lid and was scored for left and right eye as normal (1), squinted (2), or closed (3). PS was scored for left and right eye as normal (1), constricted (2), or dilated (3).

Open Arena Observation: Open Arena was conducted following the handheld observations. Each rat was placed on a 36" x 24" cart lined with paper. The rat was allowed to move freely around the arena for three minutes. During this time observations were scored by an observer blind to the treatment groups. The following observations were recorded: number of rears and grooms, arousal, gait, fecal boli, fecal description, and urine. Rears were defined as the front limbs being lifted from the floor, supported or unsupported. Grooms were defined as any licking, biting, or scratching. Arousal was scored: very low (1), low (some head/body movement and exploration) (2), normal (3), high (slight excitement, sudden darting/freezing) (4), and very high (hyper alert, excited, sudden bouts of running/movement) (5). Gait, the movement/coordination of the rat, was scored: normal (1), too little movement to determine gait (2), ataxia (3), hind limb impairment (4), forelimb impairment (5), walking on toes (6), hunched (7), body drags (8), no movement (9) and unable to move (10). Fecal boli was the absence (1) or presence (2) of fecal matter. If fecal boli was present, fecal description was scored: normal (1), diarrhea (2), soft (3), mucoid (4), and bloody (5). After the three minute assessment the rat was returned to the home cage and the arena cleaned prior to assessment of subsequent rats.

Sensory Motor: Reactivity to different types of stimuli was evaluated with the elicited responses during week 11 of the study. They were performed following the open area assessment. Each rat was scored for reaction to the approach of a closed pen, auditory startle response to a loud click, tail pinch response, pinna response and pupillary response to a pen light. Approach was scored: no reaction (1), slow approach (2), approaches energetically (3), jumps/avoids (4), freezes (5), bizarre/attack (6). Auditory/startle was scored: no reaction (1), slight (ear flick) (2), energetic/vocalize (3), jumps (4), freezes (5) and bizarre/attacks (6). Tail pinch was scored as response (1) or no visible response (2). Pinna response was scored as response (1) or no visible response (2). Pupillary response was scored as eye constricts (1) or does not constrict (2). Righting reflex was measured by placing the rat on its back on a padded surface. The rat was scored on how quickly it turned over onto its feet. Righting reflex was scored: normal (1), impaired (greater than 2 seconds to right) (2), and totally impaired (remains on back or side) (3). To score aerial righting, the rat was held in the air at 20 centimeters with its back horizontal to a padded surface. The rat was released and scored on its ability to turn over to land on its feet. Aerial righting was scored: normal (1), slightly uncoordinated (2), lands on side (3), and lands on back (4). To

measure hind limb landing foot splay, the back feet of each rat were moistened with water. The rat was held by the scruff of the neck and the base of the tail and dropped from 20 centimeters onto a cage pad to show foot impressions. Foot splay was measured as the distance between the centers of the foot prints, to the nearest 0.5 centimeter. This was repeated twice and the measures were averaged. Forelimb and hind limb grip strength was assessed following these measurements. Grip strength was measured using Chatillon Digital Force Meters (Model DFM-10®) that were verified using standard weights. The force meters were set to measure the peak force in kilograms, trials were reposted twice and the average was calculated. Forelimb test: the animal was held by the base of the tail and allowed to place forepaws on the grate, the animal was pulled away from the grate at a continuous rate until grip was released and the reading was recorded. For the hind limb test, the animal was held by the base of the tail and allowed to grasp the grate with hind paws, the animal was pulled away form the grate at a continuous rate until grip was released, and the reading was recorded. (Chatillon® is a registered trademark of Ametek Inc.)

Motor Activity: Motor activity was assessed using a SmartFrame® Open Field Activity System. The system consisted of four Plexiglas motor activity chambers (41 x 41 x 38 cm) each surrounded by a frame containing 32 evenly spaced (16x and 16y, 2.5 cm apart) infrared photocells. The floor of each chamber was equipped with a hole board containing nine holes equipped with infrared photocells to detect nose poke activity. Activity was measured as basic movement, immobility, x and y ambulation, and nose pokes based on the number of photobeam breaks recorded using the MotorMonitor® sortware (Version 4.14). After acclimation to the test room for at least 30 minutes, animals were removed from the home cage and placed individually into an open field arena for 15 minutes. Data was collected automatically by the system at fifteen equally spaced times while each rat was within the enclosure. After completion of the test, the rat was returned to its home cage and the chamber cleaned prior to testing of subsequent animals. Functioning of the software and chambers was verified prior to each test session by manually disrupting the beams and running a software diagnostic test. (SmartFrame® and MotorMonitor® are registered trademarks of Hamilton Kinder).

Statistical Analyses

Two types of data were collected in this study, continuous/count variables or categorical variables. The continuous/count variables were either measurements or counts of a specified action. The categorical variables were either presence absence of a response or a severity of occurrence. Due to the low frequency of grooms, this count variable was converted to a categorical (presence/absence) variable for analysis. For the motor activity data, the fifteen interval recordings were averaged to get one single number per rat. The nose pokes response was calculated by totaling the nine nose poke recordings per interval and then taking an average over the fifteen minute interval. For continuous data, an analysis of variance (ANOVA) was used to test for differences between treatment groups, separately for each sex. If the ANOVA revealed significant differences, a Dunnett C test was used if variances were homogenous and a Dunnett's t3 test if variances differed between treatment groups. Levene's test was used to test the homogeneity of variance among treatment groups. For categorical data, Fisher's exact test was used to test for differences between treatment groups at each week, for each sex. If significant differences were observed, then a Mann-Whitney test was conducted to compare pairs of treatment groups. SPSS® 16.0 (Chicago, Illinois) and SAS® 9.2 were used for all statistical analyses. Statistical significance was defined as $P<0.05$. Details of the statistical analyses can be found in Appendix A. (SPSS® is a registered trademark of IBM Corp.; SAS® is a registered trademark of SAS Institute Inc.)

RESULTS

Home Cage Observation: There were no differences among treatment groups in any of the home cage parameters: agitation, convulsions, tremors, posture, mutilation, and the area mutilated.

Handheld Observation:

Males
There were no differences among treatment groups for lacrimation, salivation, piloerection, paprebral closures, exothalmus and pupillary status. The 80 mg/kg-day dose group had fewer rats that were classified as very easy to remove from the cage at weeks one ($P = 0.0154$), five ($P = 0.0046$), seven ($P = 0.0497$) and eight ($P < 0.001$). To help show the drastic difference in observed responses between the 80 mg/kg-day group and the other four dose groups, an average ease of removal score was calculated for each rat. The 80 mg/kg-day group had the seven highest average scores, pointing towards more difficult ease of removal for this dose group. Reactivity to handling also differed among treatment groups at weeks two ($P = 0.0485$), three ($P = 0.0495$), six ($P = 0.0218$), seven ($P < 0.001$), nine ($P = 0.0422$) and ten ($P = 0.0061$). The 80 mg/kg-day group had fewer low and more moderately high reactivity to handling observations than the other dose groups. The 80 mg/kg-day group had six of the seven highest 11 week average reactivity to handling scores, indicating that reactivity was higher, in general, for rats in this dose group. For both ease of removal and reactivity to handling, responses for rat 102 differed from the remaining rats in the 80 mg/kg-day, with rat 102 appearing less affected by the treatment than the other rats.

Females
There were no differences among treatment groups for ease of removal, reactivity to handling, lacrimation, salivation, piloerection, paprebral closures, exothalmus and pupillary status. At week 6, the 80 mg/kg-day dose group had more barbering ($P = 0.0289$) observations than the control group. Barbering was, however, present in all dose groups (80 = 5, 20 = 2, 5 = 5, 1.25 = 4, control = 1 rat) during the 11 week study and did not differ between dose groups at any other time point.

Open Arena Observation:

Males
There were no differences found in grooms, rears, arousal, fecal boli, fecal description, and urine. The 80 mg/kg-day group had fewer normal gait observations than other dose groups at weeks five ($P = 0.002$), nine ($P = 0.011$) and 11 ($P = 0.015$). Generally rats in the 80 mg/kg-day dose group had too little movement to determine gait; however, at week 11 more hunched body position was observed in this group. Additional gait observations included ataxia, hind limb impairment, and walking on toes. If a rat was recorded as either having hind limb impairment, walking on toes or hunched body position, the rat usually displayed all three characteristics.

Females
There were no differences found in grooms, arousal, fecal boli, fecal description, and urine. Females in the 80 mg/kg-day dose group had fewer normal gait observations at weeks nine ($P < 0.001$), ten ($P = 0.050$) and 11 ($P = 0.013$). Similar to the males, females in the 80 mg/kg-day had fewer normal observations and more hunched body position was observed at weeks ten and 11. As with males, hind limb impairment, walking on toes or hunched body position, when observed, typically occurred together. Rats in the 80 mg/kg-day group reared less often than those in the other dose groups at weeks six ($P = 0.004$), seven ($P = 0.020$) and ten ($P = 0.040$).

Sensory Motor:

Males
There were no differences among treatment groups in any of the sensory motor responses: approach, auditory startle response, tail pinch, pinna response, pupillary response, righting reflex, aerial righting, landing foot splay, forelimb grip strength, and hindlimb grip strength.

Females

There were no differences for auditory startle response, pinna response, pupillary response, righting reflex, aerial righting, landing foot splay, forelimb grip strength, and hindlimb grip strength. Tail pinch and approach differed among the five dose groups for females (P = 0.020 and P = 0.024, respectively). The 80 mg/kg-day dose group had fewer response observations for tail pinch and fewer slow approach observations for the approach variable. Nine of the ten animals in the 80 mg/kg-day dose group had no reaction responses for the approach variable. There were three, two, three, and four no reaction responses in the control, 1.25, 5, 20 mg/kg-day groups, respectively. Three animals in the 80 mg/kg-day group had no response to the tail pinch whereas all animals in all other dose groups showed a response with the exception of one animal in the 20 mg/kg-day group.

Motor Activity:

Males

There were no differences among treatment groups in basic movement, immobility, X and Y ambulation. Mean number of nose pokes was lower (P = 0.009) in the 80 mg/kg-day group than the other dose groups.

Females

There were no differences among treatment groups in basic movement, immobility, X and Y ambulation. Mean number of nose pokes was lower (P = 0.014) in the 80 mg/kg-day group than the other dose groups.

DISCUSSION

Treatment with DNAN at 80 mg/kg-day resulted in neurobehavioral alterations in both male and female rats. However, the pattern of observed effects differed between the sexes. In males, neuromuscular function, activity, and excitability/affective domains were altered; whereas in females, neuromuscular function, activity, and sensorimotor domains were altered (Baird et al. 1997, Kulig et al. 1996, Boucard et al. 2010). Neuromuscular function was altered for both males and females, with rats given 80 mg/kg-day DNAN generally, exhibiting hunched body position, hind limb impairment, and walking on toes or ataxia. Activity was altered for both males and females in the 80 mg/kg-day group, with both sexes exhibiting a decrease in nose poke activity. Females additionally exhibited a decrease in rearing activity. The 80 mg/kg-day dose groups also exhibited behavioral effects; however, the effects in males were evident in excitability/affective responses while in females the sensorimotor responses were affected. Males in the 80 mg/kg-day dose group were difficult to remove from the cage and had high reactivity to handling scores, indicating either a neurobehavioral effect of DNAN resulting in excitability or a systemic toxicological effect of DNAN resulting in pain and aversion to handling. Females in the 80 mg/kg-day dose group had reduced scores, relative to the other dose groups, for approach response and tail pinch response.

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APPENDIX A

STATISTICAL ANALYSIS

Statistical Analysis of Subchronic Oral Toxicity of 2,4-Dinitroanisole (DNAN) in Rats (Rattus norvegicus) Data

Shane Hall

August 2, 2011

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Introduction

A 90-day subchronic oral dosage study involving 50 rats per gender was run to test the effects that DNAN has on rats. The 50 rats (per gender) were divided into 5 dose groups (0, 1.25, 5, 20, 80 mg/kg-day). Observational measurements from week 1 to week 11 were recorded and used to construct five datasets available for statistical analysis. The five datasets were elicit response, homecage observations, motor activity, open arena, and hand-held observations. The purpose of the statistical analysis was to see if any of the dose groups responded differently than the other dose groups to being repeatedly dosed with DNAN, when comparing their observational behaviors.

Conclusions

Table 5 (shown in the analysis section) shows all of the variables that had any statistically significant findings. There were a total of four significant differences found in the measured variables that were only collected at the end of the study (3-female, 1-male). The significant differences from the one-time observed datasets were from the variables: approach, tail pinch, and pokes. Out of the variables that were measured weekly, twenty-one significant differences were found (13-male, 8-female), in a total of only 5 variables. These significant differences were from the variables: barbering, ease of removal, reactivity to handling, rears and gait.

In terms of the general differences of doses in the study, the rats in the control dose along with doses 1.25, 5 and even 20 mg/kg-day, all generally had the same reactions to being exposed to DNAN. This was apparent from their almost equal response distribution to a high percentage of the observed variables. The dose 80 mg/kg-day rats appeared to react and behave drastically different than the rats receiving the other four doses. Even though the main interest was comparing dose 0 to the exposed animals, dose 0 turned out to be very similar in the observed and measured results to the other doses, excluding dose 80. Therefore, it appears that rats were not affected by being exposed to DNAN until they received a large dosage of the substance. In terms of comparing genders, dose 80 males seemed to be more reactive to human interaction compared to females. This indication comes from the significance results of ease of removal and reactivity to handling.

Background

Elicit Response

At the end of week 11, observations and measurements of 10 variables (7 categorical, 3 continuous) were made by the lab technician. The categorical variables were approach, auditory, papillary, pinna, tail pinch, surface right, and aerial right. Each categorical variable was recorded using a nominal scale, meaning that the response categories were in no particular order. Along with the seven categorical variables, the continuous variables were foot splay, forelimb grip and hind limb grip. They were calculated by measuring either the splay or grip twice, then taking the average of those two numbers. Table 1 shows the list of options for each categorical variable.

TABLE 1. Elicit Response

VARIABLE	OBSERVED CATEGORY	
Approach	1-no reaction	4-jumps/avoids
	2-slow approach	5-freezes
	3-approaches/energetic	6-bizarre/attack
Auditory	1-no reaction	4-jumps
	2-slight	5-freezes
	3-energetic	6-bizarre/attack
Pupillary	1-eye constricts	
	2-eye does not constrict	
Pinna	1-visible response	
	2-no visible response	
Tail Pinch	1-response	
	2-no response	
Surface Right	1-normal	
	2-impaired, greater than 2 sec	
	3-totally impaired, remains on side or back	
Aerial Right	1-normal	
	2-slightly uncoordinated	
	3-lands on side	
	4-lands on back	

Homecage Observations

Homecage observations were recorded weekly from week 1 to week 11. The rats were observed for signs of abnormalities with agitation, convulsions, tremors, posture and mutilation. Each rat was assigned a number associated with a response, according to what the lab technician observed. All five variables were categorical. Table 2 shows the levels for each categorical response variable.

TABLE 2. Homecage Observations

VARIABLE	OBSERVED CATEGORY	
Agitation	1-yes	
	2-no	
Convulsions	1-not present	
	2-slight convulsions	
	3-severe convulsions	
Tremors	1-not present	
	2-slight tremors	
	3-severe tremors	
Posture	1-lying down	4-flattened
	2-sit/stand	5-lying on side
	3-rearing	6-crouched
	7-head bobbing	
Mutilation	1-yes	
	2-no	

Motor Activity

Measurements related to a rat's movement and activities were taken for each rat. Each rat was free to roam, in one of four enclosures, on their own. Sensors monitored their movements and actions. The sensors recorded basic movements, immobility, fine movements, x ambulation and y ambulation as well as the number of pokes each rat had at each of the 9 holes. The sensor's readings were taken and recorded at fifteen equally spaced times while the rat was within the enclosure for a one-time period at the end of the study.

Handheld Observations

Handheld observations were recorded weekly for the 11 weeks. The observed variables in this dataset were ease of removal, reactivity to handling, lacrimination, salivation, barbering, piloerection, palpebral closure, exophthalmus, and pupilary state. All nine observation variables were categorical. The four variables with the non-yes/no options are considered ordinal, meaning they were ranked on degree of severity. Table 3 shows the levels for each observed categorical variable.

TABLE 3. Handheld Observations

VARIABLE	OBSERVED CATEGORY	
Ease of removal	1-very easy	4-rat flinches
	2-easy	5-difficult
	3-moderately difficult	6-very difficult
Reactivity to handling	1-very low	4-moderately high
	2-low	5-high
	3-moderately low	
Lacrimation	1-yes	
	2-no	
Salavation	1-yes	
	2-no	
Barbering	1-yes	
	2-no	
Piloerection	1-yes	
	2-no	
Palpebral closure each eye	1-eye wide open	
	2-squinted	
	3-completely closed	
Exophthalmus	1-yes	
	2-no	
Pupilary status	1-normal	
	2-dilated	
	3-constricted	

Open Arena

While placed in an open, free to move arena, rats were observed by the lab technician for rears, grooms, arousals, gait, fecal boli, and urination. If fecal boli was present, a description of the substance was documented. Rears and grooms were recorded as the number of times a rat reared or groomed itself in three minutes. The other four variables were categorical. Table 4 displays the levels for each categorical variable.

TABLE 4. Open Arena Observations

VARIABLE	OBSERVED CATEGORY	
Arousal	1-very low	4-normal
	2-low	5-high
	3-somewhat low	6-very high
Gait	1-normal	6-walking on toes
	2-too little movement to determine gait	7-hunched body position
	3-ataxia	8-body drags
	4-hind limb impairment	9-no movement
	5-fore limb impairment	10-unable to move
Fecal boli	1-yes	
	2-no	
Fecal description	1-normal	4-muciod
	2-diarrhea	5-bloody
	3-soft	
Urine	1-yes	
	2-no	

Statistical Analysis Results

Table 5 displays all of the statistically significant results that were found from all five datasets. All p-values are less than .05, signifying that the significance level used to determine if there was a significant difference between doses was 95%. Homecage observations was the only dataset without a statistically significant variable. The statistically significant variables were: approach and tail pinch – elicit response, pokes – motor activity, barbering, ease of removal, and reactivity to handling – handheld observation, rears and gait – open arena. Further descriptions of the statistical methods can be found in the Statistical Analysis Procedures.

TABLE 5. Summary of Significance testing results

VARIABLE MEASURED	GENDER	WEEK	P-VALUE	STATISTICALLY SIGNIFICANT RESULTS	RESULT DIFFERENCE
Approach	Female	--	0.024	Doses 0, 1.25 and 5 are significantly different from dose 80	Dose 80 has less slow approach observations than other noted doses
Tail Pinch	Female	--	0.020	Doses 0, 1.25 and 5 are significantly different from dose 80	Dose 80 has less response observations than other noted doses
Pokes	Male	--	0.009	Doses 0, 5 and 20 are significantly different from dose 80	Dose 80 has significant lower mean/median than other noted doses
	Female	--	0.014	Doses 0, 1.25, 5, and 20 are significantly different from dose 80	
Barbering	Female	Week 6	0.029	Dose 0 is significantly different from dose 80	Dose 80 has less no observations than other noted doses
Ease of Removal	Male	Week 1	0.015	Doses 0, 1.25 and 5 are significantly different from dose 80	Dose 80 has less very easy observations than other noted doses
	Male	Week 5	0.005	Doses 0, 1.25, 5, and 20 are significantly different from dose 80	
	Male	Week 7	0.050	Doses 0, 1.25 and 5 are significantly different from dose 80	
	Male	Week 8	0.000	Doses 0, 1.25, 5, and 20 are significantly different from dose 80	
Reactivity to Handling	Male	Week 2	0.049	Dose 1.25 is significantly different from dose 80.	Dose 80 has less low observations and more moderately high compared to other noted doses
	Male	Week 3	0.050	Doses 0 and 20 are significantly different from dose 80	
	Male	Week 6	0.022	Dose 5 is significantly different from dose 80	
	Male	Week 7	0.000	Doses 0, 1.25 and 5 are significantly different from dose 80	
	Male	Week 9	0.042	Doses 0 and 20 are significantly different from dose 80	
	Male	Week 10	0.006	Doses 0, 5 and 20 are significantly different from dose 80	
Rears	Female	Week 6	0.004	Doses 0 and 5 are significantly different from dose 80	Dose 80 has significant lower mean/median than other noted doses
	Female	Week 7	0.020	Doses 0 and 20 are significantly different from dose 80	
	Female	Week 10	0.040	Dose 5 is significantly different from dose 80	
Gait	Female	Week 9	0.000	Dose 1.25 is significantly different from dose 80.	Dose 80 has less normal observations than other noted doses
	Female	Week 10	0.050	Doses 1.25, 5 and 20 are significantly different from dose 80	Dose 80 has less normal observations and more hunched body positions than other noted doses
	Female	Week 11	0.013	Doses 0, 1.25, 5, and 20 are significantly different from dose 80	Dose 80 has less normal observations and more hunched body positions than other noted doses
	Male	Week 5	0.002	Doses 0, 5 and 20 are significantly different from dose 80	Dose 80 has less normal observations and more with too little movement than other noted doses
	Male	Week 9	0.011	Doses 1.25 and 5 are significantly different from dose 80	Dose 80 has less normal observations than other noted doses
	Male	Week 11	0.015	Dose 0 and 1.25 are significantly different from dose 80	Dose 80 has less normal observations and more hunched body positions than other noted doses

Elicit Response

Males

There were no statistically significant results at the .05 alpha level for the elicit response dataset in the males. Approach (.4262), auditory (.5493) and tail pinch (.1702) all had p-values well above .05. The other categorical variables of the elicit response dataset - pupillar, pinna, surface right, and aerial right - all had no variation in the results. Single response variables were pupillar - "eye constricts", pinna - "visible response", surface right and aerial right - "normal". Approach mainly had either no reaction or slow approach and the responses and auditory either had no or slight reaction. Tail pinch had both response and no response observations. Foot splay and grip p-values were between .211 and .677, all greater than the .05 cutoff, meaning they are not determined statistically significant.

Females

Tail pinch (.0204) and approach (.0243) were statistically significant in terms of comparing distribution of responses across the five doses for the females. For both observed variables, the lowest four doses had significantly different responses than dose 80. Dose 80 had less slow approach observations for approach, and dose 80 had less response observations for tail pinch.

Auditory had some variation within the responses, however the p-value was well above statistical significance at .05. The other categorical variables of the elicit response dataset- pupillar, pinna, surface right and aerial right- all had no variation in the results. Single response variables were pupillar - "eye constricts", pinna - "visible response", surface right and aerial right - "normal". There were also no statistically significant difference between dose group measurements for the three foot splay and grip measurements. P-values for these variables were between .178 and .452.

Homecage Observations

Males

Males had p-values for posture ranging from .114 to .843, resulting in no statistically significant differences (distributions) among the dose groups for any of the weeks. Posture was the only observed variable that consisted of any variation within the responses, making it the only variable that has a chance to have a statistically different distribution of responses between dose groups. Posture responses consisted mainly of lying down or sit/stand along with a few crouched observations. This was true for all eleven weeks. The homogeneous response variables were agitation-no, convulsions-not present, tremors-not present, and mutilations-no.

Females

Females had p-values for posture ranging from .149 to .806, resulting in no statistically significant differences (results) among the dose groups for any of the weeks. Posture was the only observed variable that had any variation within the responses, making it the only variable that had a chance to have a statistically different distribution of responses between dose groups. Posture responses consisted mainly of lying down or sit/stand along with a few crouched observations. This was true for all eleven weeks. The homogeneous response variables were agitation-no, convulsions-not present, tremors-not present, and mutilations-no.

Motor Activity

Males

The average number of times a male rat poked his nose at one of the nine holes was found to be significantly different between at least two of the dose groups with a p-value of .009. Doses 0, 5, and 20 all had significantly higher median pokes than dose 80, at the 95% confidence level.

Basic movement, fine movement, x ambulation, y ambulation and immobility all had dose means that were not significantly different from one another at the alpha=.05 level (95% confidence), i.e. their p-values were greater than .05.

Females

Pokes was also found to be significantly different between at least two of the female dose groups with a p-value of .014. Follow up testing revealed that all four lower doses all had significantly higher median pokes than dose 80, at the 95% confidence level.

Basic movement, fine movement, x ambulation, y ambulation and immobility all had dose means that were not significantly different from one another at the alpha=.05 level (95% confidence), i.e. their p-values were greater than .05.

Handheld Observations

Males

Males exhibited much more variation of responses in ease of removal than females did. Week 1 (.0154), week 5 (.0046), week 7 (.0497) and week 8 (.000) all had significant differences in distributions between dosage groups, with their corresponding p-values in parenthesis. At Week 1, 5, 7 and 8, dose groups 0, 1.25 and 5 are all significantly different in terms of the distribution of responses, when compared to dose 80. At week 5 and week 8, dose 20 is also significantly different from dose 80. The difference in distribution for all four weeks was that dose 80 had fewer rats that were classified as very easy to remove.

In terms of ease of removal, the major difference between dose 80 and the four lower doses can be further noted. Considering all 11 weeks, only one dose 80 rat was classified as very easy for all 11 weeks. In comparison, dose 20 and dose 5 had 5 rats, dose 1.25 had 6 and dose 0 had 3 rats. To help show the drastic difference in observed responses between dose 80 and the other four doses, an average ease of removal was calculated for each rat. Dose 80 had the 7 highest averages, pointing towards more difficult ease of removal for dose 80 rats. If rat 99, who died in week 8 is also included, dose 80 had the 8 highest averages. Rat 102 was the only rat in dose 80 that did not appear to be affected by the high dosage amount.

Six out of the eleven weeks have significantly different dose distributions for reactivity to handling. Week 2 (.0485), week 3 (.0495), week 6 (.0218), week 7 (.000), week 9 (.0422) and week 10 (.0061) are all statistically different at a 95% confidence level. During these weeks, dose 80 had fewer low and more moderately high observations than the other doses.

Averaging the 11 weeks of reactivity to handling together for one rat, dose 80 has 6 out of 7 highest averages, meaning that the reactivity is higher, in general for dose 80 rats. As was the case in ease of removal, Rat 102 appeared to be the only rat in dose 80 not dramatically affected by the high dosage amount for reactivity to handling.

Barbering results showed that there was variation in the yes/no responses but not enough to have statistically different results between dose groups. Only 11 (22%) of the rats displayed barbering at any point during the eleven weeks. All 5 doses were accounted for in the 11 rats (2 rats at dose 80; 1 at dose 20; 3 at dose 5; 2 at dose 1.25; and 3 at dose 0).

Lacrimination, salivation, piloerection, palpebral closure, exophthalmus, and pupilary status all have recorded observations that were constant across all rats in all 11 weeks, for both genders. All single response variables were: lacrimination – no, salivation – no, piloerection – no, palpebral closure – eyes wide open, exophthalmus – no, pupilary status – normal.

Females

Barbering was the only observational variable in the handheld observation dataset that had any statistically significant results. Week 6 was significant in terms of one dose group having a different distribution of yes/no responses than another group (p-value .0289). At week 6, dose 80 had less no observations that dose 0.

Barbering was present in 17 females (34%) sometime during the 11 week study. All five doses were accounted for within the 17 females (80-5, 20-2, 5-5, 1.25-4, 0-1). Dose 80 had 4 to 5 rats consistently barbering from week 5 to week 11.

There were no statistically significant results for ease of removal or reactivity to handling for the female gender, unlike for males. Very little variation between yes/no responses for ease of removal was found for any of the eleven weeks. Week 1 actually had the most variation, but a p-value well above .05 showed that none of the eleven weeks had statistically different dose distributions. The majority of the responses for reactivity to handling were low or moderately low, however the five dose groups had very similar outcomes, leading to no statistically significant results.

Lacrimination, salivation, piloerection, palpebral closure, exophthalmus, and pupilary status all have recorded observations that were constant across all rats in all 11 weeks, for both genders. All responses were: lacrimination – no, salivation – no, piloerection – no, palpebral closure – 1, exophthalmus – no, pupilary status – normal.

Open Arena

Males

Gait response results were statistically significant in week 5, 9 and 11. The p-values for the corresponding weeks were .002, .011 and .015. In these weeks, dose 80 had less normal observations than other dose groups. Generally, the response was either normal or too little movement to determine. There were some observations that were ataxia, hind limb impairment, walking on toes or hunched body position. If a rat was recorded as either having hind limb impairment, walking on toes or hunched body position, the rat usually displayed all three characteristics.

Grooms and rears had no statistically significant results comparing the dose groups' distributions within any of the weeks. These two variables were calculated by the amount of times the rat performed the particular action. For rears, week 10 was the closest to having a significant difference, but the calculated p-value (.211) was well above .05. Grooms was a count variable that was converted into either yes-the rat reared at least once or no-the rat never reared. This was done because a majority of the rats didn't perform any grooms during the observational period. As an observational note on grooms, dose 80 had 8 out of the 14 occurrences of at least 3 rats grooming themselves for a particular dose during a given week. For males, the rats that groomed themselves the most weeks were: rat 107 (dose 0, 8 weeks), rat 142 (dose 1.25, 10 weeks), rat 144 (dose 80, 8 weeks) and rat 146 (dose 5, 6 weeks).

Statistical testing on the distribution responses of fecal boli, urine, and arousal all proved to be non-significant. The majority of the responses for arousal were low to normal, and fecal boli and urine both had yes/no responses.

Females

Even though males had no significant results for rears, females had significantly different means/medians for weeks 6, 7 and 10, with p-values of .004, .020 and .040. In each of these weeks, dose 80 had a significantly lower amount of rears than the other doses, specifically dose 0 and dose 5.

Gait responses were significantly different for females at weeks 9, 10 and 11, at the 95% confidence level. P-values were .000 (week 9), .050 (week 10), and .013 (week 11). In all three weeks, dose 80 had less normal observations. The female responses were generally the same observational categories as males. The responses were either normal or too little movement to determine. There were some observations that were ataxia, hind limb impairment, walking on

toes or hunched body position. If a rat was recorded as either having hind limb impairment, walking on toes or hunched body position, the rat usually displayed all three characteristics.

For females, grooms were also categorized into a yes/no variable. No statistically significant results were found comparing the five doses; however some observational notes were found. Dose 80 had 6 out of the 9 occurrences of at least 3 rats grooming themselves for a particular dose during a given week. Even though there appeared to be a random pattern of which dose group groomed themselves the most, a few of the rats groomed themselves a majority of the weeks. For females, the rats that groomed themselves the most weeks were: rat 173 (dose 0, 9 weeks), rat 177 (dose 80, 8 weeks), rat 186 (dose 80, 7 weeks) and rat 196 (dose 1.25, 7 weeks).

Statistical testing on the distribution responses of fecal boli, urine, and arousal all proved to be non-significant. The majority of the responses for arousal were low to normal, and fecal boli and urine both had yes/no responses.

Statistical Analysis Procedures

The purpose of analyzing the collected data was to see if the means/distributions/responses of the five dose groups (0, 1.25, 5, 20, 80 mg/kg) were statistically different, in terms of the observed or measured variables. In particular, the interest was to see if any of the actual exposed/dosed groups (1.25, 5, 20, 80) were statistically different from the non-dosed rats (dose 0). Male and female datasets were analyzed separately to properly account for the known variation between the two genders.

In the statistical results of this document, the terms p-value, alpha and 95% confidence level are mentioned. To claim that there was a statistical difference between dose groups, an alpha of .05 was used. An alpha of .05 means 95% of the time the difference in means or distributions that we observed is true for the entire population, and doesn't happen just by chance. The .05 is the 5% chance or 1 in 20 chances that the difference we observed was not actually true when extrapolating to the entire population. The greater the alpha, the easier it becomes to claim that two groups are statistically different. The term p-value is also referenced in this document and that is the chance of observing a difference as large as is observed even if the two means are identical (i.e., the chance that the difference could have occurred randomly). Therefore, a smaller p-value means more evidence that the two population means or distributions truly are not equal. The p-value is the statistical result of the test and is compared to the already determined alpha level. If the calculated p-value of the test is less than already specified alpha, there is a statistically significant result for that test. The p-value of .05 directly correlates with a 95% confidence level.

Two types of data were collected in this observational study. The collected variables were either continuous/count variables or categorical variables. The categorical variables had distinct responses like yes/no or a range of severity like very low to very high. The continuous/count variables had either numbers that were measurements (any number on the number line) or counts of how many times a rat did a specified action (number of grooms).

For the categorical variables, statistical significance means that we can claim, with 95%

DEPARTMENT OF THE ARMY
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MCHB-IP-TEP

19 June 2012

MEMORANDUM FOR Environmental Acquisition and Logistics Sustainment Program (AMSRD-FE/Kimberly A. Watts), U.S. Army Research, Development and Engineering Command, 3072 Aberdeen Blvd., Aberdeen Proving Ground, MD 21005

SUBJECT: Toxicology Study No. 87-XE-0DBP-10, Protocol No. 0DBP-38-10-07-01, The subchronic oral toxicity of 2,4-dinitroanisole (DNAN) in rats, September 2010 – March 2011

1. Electronic copy of the subject report is enclosed.

2. Please contact us if this report or any of our services did not meet your expectations.

3. The point of contact is Dr. Emily May Lent, Toxicology Portfolio, Toxicity Evaluation Program, at 410-436-3980, DSN 584-3980, or FAX at 410-436-6710. She may also be reached b electronic mail at usaphctoxinfo@amedd.army.mil.

FOR THE DIRECTOR:

Encl

CHRIS E. HANSON
COL, VC
Portfolio Director, Toxicology

certainty, that at least two of the dose groups have a significantly different distribution of their responses within the categorical response variable.

For the continuous variables, statistical significance means that we are claiming, with 95% certainty, that one of the dose groups has a significantly different average/median "Y" than another dose group, whichever "Y" that may be.

Different analyses were executed for the two types of measured variables within this study. For all categorical variables, no matter what observational dataset, or how many response options the variable has, the analysis was the same. The same held for all of the continuous/count data.

Analysis of the Continuous Data

SPSS software was used to test if the mean of the measured variable is statistically different between any of the five doses. In other words, the average of all the rats in one dose was compared to the other doses' averages, and if one of the comparisons is statistically different, then SPSS noted a difference in the results. For the mobility dataset, the fifteen interval recordings were averaged to get one single number per rat. The pokes response was calculated by totaling the nine poke recordings per interval. Then, the interval totals were averaged to get one single poke number per rat. Once each rat had one continuous number assigned to it, analysis could proceed.

To test differences between multiple groups' means, SPSS used an analysis of variance (ANOVA) test. ANOVA test compares the averages of the five dose groups to see if they are significantly different, but also accounts for the variation within each dose group. In running an ANOVA test, the data must first be checked to see if it is normally distributed, and that the variances between dose groups are statistically equal.

For simplicity, a normal distribution means that if a histogram of the data were to be plotted, the data would form a bell shaped curve (most of the observations appear in the middle and less observations appear as the data reaches its minimum and maximum). The Shapiro-Wilk test deteremines if the data is normally distributed. If the p-value of the test statistic is greater than .05, then it can be concluded that the data is normally distributed. If the data is not normally distributed, two approaches can be taken to solve this problem. The first is run a different type of test (non-parametric), which will be mentioned later. The other option is to identify the outlier in the dataset and remove it. Typically if the data is not normally distributed, there is an extreme observation (either the minimum or maximum) that is causing the violation to this rule. After removing the outlier, the ANOVA process can continue.

The assumption of homogeneous variances means that the five dose groups are assumed to have variation (variances) that are statistically equal. The Levene's test measures this, and if the rule is followed, the Levene's statistic will have a p-value greater than .05. If the rule is violated, a different post hoc test can be run, which will be discussed later.

If the two assumptions have been met, the next step is to actually run the ANOVA test. Again, ANOVA tests to see if any two of the dose group means are statistically different from each other. The ANOVA test will output a p-value, and if that p-value is less than our cutoff of .05, we conclude that at least two of the groups are statistically different from one another, in terms of their dose group means. If the p-value is less than .05, a post hoc test called Dunnett's C test will be run. This particular test is to see if any of the dose groups are statistically different

from the control, which in this case is dose 0. If the p-value of this test is less than .05, it can be concluded that the particular dose testing against dose 0 is statistically different from dose 0. As mentioned before, if the homogeneity of variance assumption is violated, a different post hoc test can be run to account for this violation. This time Dunnett's t3 test would be run.

As mentioned before, if the normality assumption is violated and no outlier is removed, a non-parametric Kruskal-Wallis test can be run. The purpose of this test is identical to the ANOVA test, however this procedure ranks the data from minimum to maximum and tests to see if the medians are significantly different between at least two of the dose groups. A .05 cutoff for the p-value will again be used to check for significant differences between doses.

Categorical Data Analysis

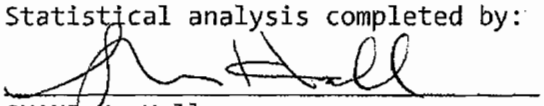
The first step in analyzing categorical data is set the data up into a contingency table. A contingency table displays the x variable (dose groups) as the rows, and the y variable subgroups (observational variable) as the columns. SPSS was used to create the contingency table, displaying the five dose groups as the rows, and the categories of the observed categorical variable as the columns. Each cell in the contingency table contains the number of observations present for that particular dose group and that particular observational outcome group. Then row percents are calculated by dividing the number of observations in that particular cell divided by the total number in that dose group. To test and see if one dose group is statistically different, the distribution of the row percentages is compared across doses.

A typical categorical data test is the Chi-square test, however one of its assumptions is that the expected counts of each cell is greater than 5. To find the expected count, take the row total times the column total and divide that number by the overall total. If this is violated, the Chi-square p-value is not accurate. To adjust for this violation, a Fisher's exact test can be run. Since SPSS does not have the capability of running a Fisher's exact test, SAS was used for this test. This test also tests the distribution of the outcome variable and sees if it is equal throughout the five doses. If the p-value of this test is less than .05, then two of the doses have a significantly different distribution of the categorical outcome variable. To follow up, a non-parametric Mann-Whitney test can be run to test two of the distributions at a time. This test again uses the ranks of the data and sees if they are significantly different from each other. If the p-value of this test is less than .05, then the two groups can be considered statistically different, in terms of their distribution of responses.

Suggestions

For future studies involving rat observational data, a suggestion is to limit the amount of data collected, at least the amount for statistical analyses. This project had over 30 variables observed or measured. Many of these variables had eleven weeks of results. From an analysis standpoint, the goal was to see if there was a difference in doses comparing a measured/observed variable. With so many variables, most multiplied by eleven weeks of observations, compounded by the relatively small sample size of only ten rats per group, there is a high percentage chance that a significant difference between dose groups will be found, just by random chance.

Statistical analysis completed by:



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Statistician
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Appendix U

Individual and Summary of 90-Day Urinalysis Data

Table U-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of 90-Day Urinalysis Results
Male Rats

		Corn Oil	DNAN in corn oil			
		Control	1.25 mg/kg	5 mg/kg	20 mg/kg	80 mg/kg
Volume	Mean	7.44	8.35	8.75	15.00*	12.64
	SD	2.910	2.174	1.990	7.386	8.688
	N	9	10	10	10	7
Color[a]	Mean	4.67	4.80	5.20	6.10*	8.00*
	SD	1.000	0.632	0.422	0.876	1.000
	N	9	10	10	10	7
Appearance[a]	Mean	1.78	1.90	1.80	2.00	1.00
	SD	1.042	1.197	1.033	1.054	0.000
	N	9	10	10	10	7
Glucose[a]	Mean	0.00	0.00	0.00	10.00	14.29
	SD	0.000	0.000	0.000	31.623	37.796
	N	9	10	10	10	7
Bilirubin[a]	Mean	1.11	0.80	0.80	0.70	3.00*
	SD	1.054	1.033	1.033	1.160	1.000
	N	9	10	10	10	7
Ketone[a]	Mean	0.22	0.40	0.30	0.30	0.71
	SD	0.441	0.843	0.675	0.675	1.113
	N	9	10	10	10	7
Specific Gravity	Mean	1.033	1.032	1.030	1.024*	1.028
	SD	0.0046	0.0031	0.0041	0.0075	0.0103
	N	9	10	10	10	7
Blood[a]	Mean	0.00	0.00	0.00	0.00	0.00
	SD	0.000	0.000	0.000	0.000	0.000
	N	9	10	10	10	7
pH	Mean	6.61	6.95	6.75	6.90	6.71
	SD	0.220	0.369	0.264	0.316	0.267
	N	9	10	10	10	7
Protein (mg/dl)	Mean	316.7	58.0	44.0	38.8	41.7
	SD	641.48	36.15	29.51	24.75	28.58
	N	9	10	10	8	6
Urobilinogen (mg/dl)	Mean	0.20	0.20	0.20	0.20	0.20
	SD	0.000	0.000	0.000	0.000	0.000
	N	9	10	10	10	7
Nitrites	Mean	0.00	0.00	0.00	0.00	0.00
	SD	0.000	0.000	0.000	0.000	0.000
	N	9	10	10	10	7
Leucocytes[a]	Mean	0.22	0.30	0.10	0.10	0.14
	SD	0.667	0.675	0.316	0.316	0.378
	N	9	10	10	10	7

*Significantly different from corn oil control
[a]data coded for analysis

Table U-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of 90-Day Urinalysis Results
Female Rats

		Corn Oil	DNAN in corn oil			
		Control	1.25 mg/kg	5 mg/kg	20 mg/kg	80 mg/kg
Volume	Mean	6.45	6.85	8.50	11.05	20.67*
	SD	2.499	3.000	2.261	5.500	10.296
	N	10	10	10	10	9
Color[a]	Mean	2.80	2.70	3.00	4.60*	6.56*
	SD	0.919	0.483	0.000	1.174	1.236
	N	10	10	10	10	9
Appearance[a]	Mean	1.30	1.60	1.20	1.10	1.00
	SD	0.675	1.265	0.422	0.316	0.000
	N	10	10	10	10	9
Glucose[a]	Mean	0.00	0.00	0.00	0.00	11.11
	SD	0.000	0.000	0.000	0.000	33.333
	N	10	10	10	10	9
Bilirubin[a]	Mean	0.20	0.20	0.20	0.30	0.44
	SD	0.632	0.632	0.632	0.675	0.882
	N	10	10	10	10	9
Ketone[a]	Mean	0.00	0.10	0.00	0.00	0.00
	SD	0.000	0.316	0.000	0.000	0.000
	N	10	10	10	10	9
Specific Gravity	Mean	1.026	1.026	1.022	1.020	1.014*
	SD	0.0070	0.0065	0.0061	0.0077	0.0070
	N	10	10	10	10	9
Blood[a]	Mean	0.00	0.00	0.00	0.00	0.00
	SD	0.000	0.000	0.000	0.000	0.000
	N	10	10	10	10	9
pH	Mean	6.70	6.80	6.90	6.70	6.78
	SD	0.350	0.422	0.394	0.258	0.507
	N	10	10	10	10	9
Protein (mg/dl)	Mean	30.0	30.0	30.0	30.0	30.0
	SD	0.00	0.00	0.00	0.00	0.00
	N	5	5	1	2	1
Urobilinogen (mg/dl)	Mean	0.20	0.20	0.20	0.20	0.20
	SD	0.000	0.000	0.000	0.000	0.000
	N	10	10	10	10	9
Nitrites	Mean	0.00	0.00	0.00	0.00	0.00
	SD	0.000	0.000	0.000	0.000	0.000
	N	10	10	10	10	9
Leucocytes[a]	Mean	0.00	0.00	0.00	0.00	0.00
	SD	0.000	0.000	0.000	0.000	0.000
	N	10	10	10	10	9

*Significantly different from corn oil control

Appendix V

Individual and Summary of 90-Day Sperm Analysis Data

Table V-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

90-Day Individual Sperm Analysis
Male Rats

Group	Animal ID	Ave. Count	Total Sperm in sample (millions)	Concentration (million/ml)	Sample Volume (ml)	Tissue Weight (g)	Sperm/gram (millions/gram)	Percent Motile	Percent Progressive	Motile Sperm (Millions/gram)	Progressive Sperm (Millions/gram)
Corn Oil	11-0097	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Control	11-0105	48.0	0.10	0.45	0.200	0.024	3.70	18.00	4.50	0.67	0.17
	11-0107	89.0	0.15	0.85	0.200	0.016	10.25	14.50	9.50	1.49	0.97
	11-0108	102.0	0.20	0.95	0.200	0.021	9.00	21.00	7.50	1.89	0.68
	11-0112	154.0	0.25	1.40	0.200	0.020	14.25	45.00	21.50	6.41	3.06
	11-0116	101.5	0.20	0.95	0.200	0.024	7.85	12.00	3.50	0.94	0.27
	11-0118	88.5	0.20	0.80	0.200	0.018	9.05	26.50	6.50	2.40	0.59
	11-0140	18.5	0.00	0.15	0.200	0.020	1.70	0.00	0.00	0.00	0.00
	11-0147	130.0	0.25	1.20	0.200	0.020	12.00	36.00	16.50	4.32	1.98
	11-0150	66.0	0.10	0.60	0.200	0.017	7.15	30.50	13.50	2.18	0.97
	Mean	**88.61**	**0.161**	**0.817**	**0.2000**	**0.0200**	**8.328**	**22.611**	**9.222**	**2.255**	**0.965**
	SD	**40.943**	**0.0821**	**0.3791**	**0.00000**	**0.00278**	**3.8760**	**13.5618**	**6.8242**	**1.9885**	**0.9815**
1.25 mg/kg-d	11-0095	187.0	0.35	1.75	0.200	0.020	17.30	49.50	20.50	8.56	3.55
	11-0103	115.5	0.20	1.10	0.200	0.022	9.70	10.00	3.50	0.97	0.34
	11-0104	119.5	0.20	1.10	0.200	0.020	11.00	25.00	12.50	2.75	1.38
	11-0113	95.0	0.20	0.90	0.200	0.018	9.75	29.00	7.50	2.83	0.73
	11-0114	88.0	0.15	0.85	0.200	0.021	7.75	13.00	2.50	1.01	0.19
	11-0122	216.0	0.40	2.00	0.200	0.018	20.10	37.50	14.50	7.54	2.91
	11-0126	141.0	0.30	1.30	0.200	0.025	10.45	45.00	18.50	4.70	1.93
	11-0134	34.0	0.10	0.30	0.200	0.022	2.90	22.00	6.00	0.64	0.17
	11-0142	110.5	0.20	1.00	0.200	0.020	10.25	34.00	13.50	3.49	1.38
	11-0145	133.5	0.25	1.25	0.200	0.099	13.75	41.50	17.50	5.71	2.41
	Mean	**124.00**	**0.235**	**1.155**	**0.2000**	**0.0285**	**11.295**	**30.650**	**11.650**	**3.819**	**1.500**
	SD	**50.907**	**0.0914**	**0.4734**	**0.00000**	**0.02486**	**4.8245**	**13.2687**	**6.4207**	**2.7700**	**1.1846**
5 mg/kg-d	11-0100	41.0	0.10	0.40	0.200	0.017	4.45	23.00	5.00	1.02	0.22
	11-0101	301.5	0.55	2.80	0.200	0.023	24.25	63.50	27.50	15.40	6.67
	11-0115	50.0	0.10	0.45	0.200	0.019	4.90	33.00	19.00	1.62	0.93
	11-0117	204.0	0.35	1.90	0.200	0.023	16.40	43.50	14.00	7.13	2.30
	11-0124	117.0	0.20	1.10	0.200	0.018	12.00	43.50	19.00	5.22	2.28
	11-0131	45.0	0.10	0.40	0.200	0.025	3.30	16.50	5.50	0.54	0.18
	11-0135	258.5	0.50	2.40	0.200	0.022	21.70	52.00	21.00	11.28	4.56
	11-0138	102.5	0.20	0.95	0.200	0.026	7.30	40.00	16.00	2.92	1.17
	11-0141	17.0	0.00	0.20	0.200	0.017	1.80	9.00	0.00	0.16	0.00
	11-0146	90.5	0.15	0.85	0.200	0.021	7.95	18.00	7.00	1.43	0.56
	Mean	**122.70**	**0.225**	**1.145**	**0.2000**	**0.0211**	**10.405**	**34.200**	**13.400**	**4.673**	**1.886**
	SD	**98.696**	**0.1830**	**0.9127**	**0.00000**	**0.00325**	**7.9142**	**17.3833**	**8.6916**	**5.1504**	**2.1796**
20 mg/kg-d	11-0106	158.0	0.30	1.45	0.200	0.020	14.60	32.00	13.50	4.67	1.97
	11-0120	58.5	0.10	0.55	0.200	0.018	6.00	19.00	12.50	1.14	0.75
	11-0121	173.0	0.30	1.60	0.200	0.021	15.25	49.50	20.50	7.55	3.13
	11-0125	80.0	0.15	0.75	0.200	0.018	8.20	37.00	17.50	3.03	1.44
	11-0127	71.5	0.10	0.65	0.200	0.031	4.25	27.00	8.50	1.15	0.36
	11-0130	50.5	0.10	0.45	0.200	0.023	4.05	16.50	4.50	0.67	0.18
	11-0133	90.5	0.15	0.85	0.200	0.016	10.45	31.50	12.00	3.29	1.25
	11-0137	83.0	0.15	0.75	0.200	0.023	6.65	27.00	9.50	1.80	0.63
	11-0139	48.0	0.10	0.45	0.200	0.023	3.85	18.50	5.50	0.71	0.21
	11-0148	171.0	0.30	1.55	0.200	0.022	14.35	50.00	19.00	7.18	2.73
	Mean	**98.40**	**0.175**	**0.905**	**0.2000**	**0.0215**	**8.765**	**30.800**	**12.300**	**3.119**	**1.265**
	SD	**49.639**	**0.0890**	**0.4537**	**0.00000**	**0.00414**	**4.5868**	**11.9378**	**5.4782**	**2.5793**	**1.0484**
80 mg/kg-d	11-0099	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	11-0102	7.0	0.00	0.10	0.200	0.018	0.70	0.00	0.00	0.00	0.00
	11-0109	6.0	0.00	0.10	0.200	0.019	0.60	0.00	0.00	0.00	0.00
	11-0110	0.0	0.00	0.00	0.200	0.018	0.00	0.00	0.00	0.00	0.00
	11-0111	0.0	0.00	0.00	0.200	0.027	0.00	0.00	0.00	0.00	0.00
	11-0123	7.0	0.00	0.10	0.200	0.029	0.40	0.00	0.00	0.00	0.00
	11-0129	3.0	0.00	0.00	0.200	0.016	0.30	0.00	0.00	0.00	0.00
	11-0132	7.5	0.00	0.10	0.200	0.024	0.60	0.00	0.00	0.00	0.00
	11-0144	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	11-0149	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mean	**4.36**	**0.000**	**0.057**	**0.2000**	**0.0216**	**0.371**	**0.000**	**0.000**	**0.000**	**0.000**
	SD	**3.326**	**0.0000**	**0.0535**	**0.00000**	**0.00506**	**0.2870**	**0.0000**	**0.0000**	**0.0000**	**0.0000**

ND=No Data

Table V-2
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of 90-Day Sperm Analysis
Male Rats

		Corn Oil	DNAN in corn oil			
		Control	1.25 mg/kg	5 mg/kg	20 mg/kg	80 mg/kg
Sperm/tissue	**Mean**	8.328	11.295	10.405	8.765	0.371*
(Millions/gram)	**SD**	3.8760	4.8245	7.9142	4.5868	0.2870
	N	9	10	10	10	7
Motile Sperm	**Mean**	2.255	3.819	4.673	3.119	0.000*
(Millions/gram)	**SD**	1.9885	2.7700	5.1504	2.5793	0.0000
	N	9	10	10	10	7
Progressive Sperm	**Mean**	0.965	1.500	1.886	1.265	0.000*
(Millions/gram)	**SD**	0.9815	1.1846	2.1796	1.0484	0.0000
	N	9	10	10	10	7

*Significantly different from corn oil control

Appendix W

Histopathology Report

Pathology report for

0DBP-38-10-07-01

The Subchronic Oral Toxicity of 2,4-Dinitroanisole (DNAN) in Rats (*Rattus norvegicus*)

01 September 2011

Prepared by:
Shannon M. Wallace, DVM, Diplomate, ACVP
LTC, VC

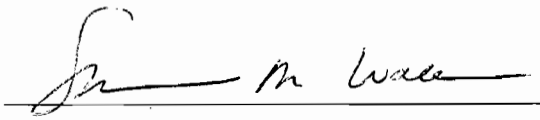
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The portion of the study described in this contributing scientist report, including the tissue processing piece conducted at the United States Army Medical Research Institute of Chemical Defense's Comparative Pathology Branch, was conducted in compliance with Title 40, Code of Federal Regulations (CFR) Part 792, Good Laboratory Practice Standards.

Comments:

1) The Quality Assurance Unit (QSO) inspected the Histopathology Evaluation Procedures on 9/14/2011 and reported the results of the inspection to Management and the Study Director on 9/23/2011.

2) Also, A Facility/Process based inspection was conducted on 10/06/2011 to fulfill the requirements of section 8.1 of PTOX/MRICD SOP 1.0 which states "USAPHC's QSO will inspect (MRICD tissue processing) at intervals adequate to ensure the (quality and) integrity of the study data for the portion of the study conducted at MRICD-Comparative Pathology Branch (CPB)" The results of this inspection were reported to Management on 10/19/2011.



14 JUN 2012

Shannon M. Wallace, DVM, Diplomate, ACVP

Date

Veterinary Pathologist

The following were responsible for the conduct of this study and preparation of this report:

S. M. Wallace — 14 JUNE 2012

Shannon M. Wallace, DVM, Diplomate, ACVP — Date

Veterinary Pathologist

TABLE OF CONTENTS

INTRODUCTION

The purpose of this study is to determine the oral toxicity of 2,4-Dinitroanisole (DNAN), an insensitive, energetic material used in explosive formulations. Three oral gavage dose studies on adult Sprague-Dawley rats were performed to accomplish this, approximate lethal dose, 14-day repeat-dose, and 90-day repeat-dose. Histopathologic examination was performed on five concentrations of DNAN suspended in corn oil dosed via oral gavage needle for 7days per week for 90 days. Complete histopathologic examination was performed on 0-dose and 80-dose males and females. Selected tissues were examined in all remaining groups.

METHODS

Necropsies were performed at US Army Public Health Command (USAPHC), Portfolio of Toxicology (PTOX). Tissues were collected and appropriately preserved in 10% buffered formalin, selectively trimmed and placed in cassettes labeled with protocol number, animal identification number and laboratory assigned accession number. Cassettes were placed in labeled formalin filled bottles and transported to the US Army Institute of Chemical Defense (USAMRICD) for processing. Tissues were routinely processed and paraffin embedded. All processed and embedded tissues were microtomed at 5*u*m thick and automatically stained with hematoxylin and eosin and coverslipped. The pathologist examined slides for compound-induced histopathologic changes via light microscopy. The prevelance and severity of findings were graded as compared to controls. Findings were assigned as none, minimal, mild, moderate or severe. The description and criteria of severity grades per particular organs can be reviewed in Appendix B.

RESULTS

Based on incidence and severity, a finding associated with DNAN exposure was severe and moderate degeneration and atrophy of testicular seminiferous tubules. Seminiferous tubular degeneration is characterized by partial depletion and degeneration of germ cells. Seminiferous tubular atrophy is characterized by shrunken tubules with loss of all germ cells with only the innermost lining of supporting Sertoli cells remaining. Microscopically, seminiferous tubules of the 80-dose group were moderately to severely degenerate, retaining only Sertoli cells, spermatogonia and early spermatocytes. Absent germ cell layers included all spermatid and late spermatocyte stages resulting in the absence of mature sperm in seminiferous tubules. Testes additionally demonstrated moderate to numerous numbers of atrophic tubules. Findings within the epididymides can be a direct reflection of the changes within the testes. The epididymides of all 80-dose males examined demonstrated moderate to severe aspermia with eosinophilic cellular tubular debris. Few sperm were noted in the tail of individual animals. Epididymal findings are a direct reflection of the disruption of spermatogenesis in the testes.

Table 1. Prevalence and Severity of Selected Testicular Findings										
Dose group	0		1.25		5		20		80	
Sex	M	F	M	F	M	F	M	F	M	F
Number examined	10[b]	N/A	Ne	N/A	NE	N/A	10	N/A	9[a]	N/A
Testes: Degeneration and atrophy										
None	10						10			
Minimal										
Mild										
Moderate									5	
Severe									4	

[a]= 11-0149 pre-term death included (Severe atrophy and degeneration); 11-0144 pre-term death included (Severe degeneration with few atrophic tubules); 11-0099 not examined
[b] = 11-0097 pre-term death
NE: Not examined
N/A: Not applicable

A finding present in the spleens of all male and female rats on study was hemosiderin and hemosiderin-laden macrophages. Excess iron from erythrocyte breakdown in normal homeostasis or in hemolytic crisis is stored mostly in the spleen in the form of hemosiderin. Hemosiderosis is present in normal rats and females demonstrate more than males. A dose effect was noted in severity in males and may be treatment related. In females, severity of the lesion increased minimally at the highest dose group but does not appear significant when compared to controls and lower dose groups.

EMH is present in the normal spleen and is more common in young versus aged animals (Suttie 2006). A dose effect was noted in severity and prevalence in the 80-dose group females; males demonstrated comparable findings across all dose groups. Increases in EMH compared to corn oil control spleens may be the result of a hematoxic insult.

Table 2. Prevalence and Severity of Selected Splenic Findings										
Dose group	0		1.25		5		20		80	
Sex	M	F	M	F	M	F	M	F	M	F
Number examined	10[b]	10	10	10	10	10	10	10	10[c]	10[d]
Spleen: Hemosiderosis										
None										
Minimal	10		7		6	3	3			
Mild		8	3	7	4	5	7	10	2	5
Moderate		2		3		2			7	5
Severe									1	
Spleen: Extra-medullary hematopoeisis										
None	4	10	7	7	4	7	4	6	3	1
Minimal	2		2	2	5		5		5	

Mild	3		1	1	1	3	1	4		3
Moderate	1								2	5
Severe										1

[b]= pre-term death, animal 11-0097 (Minimal hemosiderosis; Mild EMH)
[c]= pre-term death, animals 11-0144 (Moderate hemosiderosis) and 11-0099 (Moderate hemosiderosis; minimal EMH), pre-term death, animal 11-0149 included (Moderate hemosiderosis; Minimal EMH)
[d]= pre-term death, animal11-0151(Mild hemosiderosis; Severe EMH)
NE= Not examined

A finding specific to females was renal mineralization at the corticomedullary junction. Compared to controls, the prevalence of mineralization was higher in DNAN treated females; however, there was not a clear dose related trend in either prevalence or severity of this lesion. Renal mineralization was not noted in control or DNAN treated males.

Basophilic tubules examined were often dilated, surrounded by thickened basement membranes and occasional mitotic figures, all changes specific to regeneration. Necrosis and degeneration were not noted. Comparable numbers of treated and control animals exhibited this lesion, it was, therefore, not considered treatment related.

Congenital hydronephrosis (pelvic dilatation) occurs at a low incidence in the F344 rat. A genetic basis for hydronephrosis has also been shown for other rat strains. Hydronephrosis is more often unilateral, affecting the right side (Montgomery 1990). Hydronephrosis was noted on the right side of ¾ Sprague Dawley rats. Since this lesion exhibited itself similarly in control versus treated animals, it was not considered treatment related.

Renal lymphocytic interstitial infiltrates were noted in the majority of male and female controls and all dose groups in minimal to mild amounts. These were considered background lesions and of minimal significance.

One control male (11-0147) demonstrated interstitial fibrosis and tubular atrophy. This is indicative of a prior insult with fibrotic repair. The cause is not evident in the section examined.

Table 3. Prevalence and Severity of Selected Renal Findings

Dose group	0		1.25		5		20		80	
Sex	M	F	M	F	M	F	M	F	M	F
Number examined	10[b]	10	NE	NE	10	10	10	10	10[c]	10[d]
Kidney: Mineral										
None	10	7			10	4	10	0	10	3
Minimal		1				2		7		5
Mild		2				4		2		2
Moderate								1		
Severe										
Kidney: Basophilic tubules										
None	7	10			6	10	7	10	9	9
Minimal	1				2		2		1	
Mild	2				1		1			
Moderate										1

Severe										
Kidney: Pelvic Dilatation										
None	9	10			9	10	9	10	9	10
Minimal					1					
Mild										
Moderate									1	
Severe	1						1			

[b]= pre-term death, animal 11-0097 included (No renal findings)
[c]= pre-term death, animals 11-0144(No renal findings) and 11-0099 (No renal findings), and 11-0149 (Moderate autolysis/none) included
[d]= pre-term death, animal11-0151 (Mild mineral; Moderate basophilic tubules) included
NE= Not examined

Hepatic lymphohistiocytic infiltrates were found in controls and all male and female dose groups. These focal accumulations are considered by some to be a background lesion, and for these aggregates using the cell type in the diagnosis, instead of inflammation or inflammatory cell infiltrate, may be preferable and less misleading. The frequency of these mononuclear cell aggregates may be exacerbated by treatment (Thoolen 2010). A mild increase in severity was noted in females with dosage increase and may be treatment related.

Minimal to mild, focal hepatic biliary hyperplasia was observed in 1/10 80-dose males and 1/10 5-dose females. Biliary hyperplasia can be a nonspecific response to hepatic injury. Due to the isolated nature and minimal severity of the lesions, they are not considered to be treatment related.

Table 4. Prevalence and Severity of Selected Hepatic Findings

Dose group	0		1.25		5		20		80	
Sex	M	F	M	F	M	F	M	F	M	F
Number examined	10[b]	10	1[e]	NE	10	10	10	10	10[c]	10[d]
Liver: Infiltrates, lymphohistiocytic										
None					1		4	1	2	1
Minimal	5	5	1		3		4	1	0	3
Mild	5	5			4	9	2	6	6	1
Moderate					1	1		2	2	1
Severe					1					4

[b]= pre-term death, animal 11-0097 (Minimal lymphohistiocytic infiltrates) included
[c]= pre-term death, animals 11-0144 (Moderate lymphohistiocytic infiltrates) and 11-0099 (No hepatic findings) and 11-0149 (None) included
[d]= pre-term death, animal 11-0151(Minimal lymphohistiocytic infiltrates) included
[e]=animal 11-0103, examined due to gross observation of pale liver
NE=Not examined

Plasmacytosis of the submandibular lymph node was a common finding. It can be a common finding in rodents (Stefanski 1990 and Ward 1999). It was noted in 2/9 control males (mild), 5/9 control females (3 mild; 2 moderate), 4/9 80-dose males (1 minimal, 2 mild, 1 moderate) and 8/10 80-dose females (1 minimal, 5 mild, 2 moderate). This finding is not considered significant and is likely a physiological response to common

environmental antigens and/or daily introduction of the foreign toxicant through daily dosing.

Pancreatic acinar atrophy or degeneration and fibrosis were rare, uncommon findings in the male rodents. It was described in males, 1/10 80-dose and 2/10 control group, one of these control males additionally noted islet cell hyperplasia. Focal or lobular atrophy is a common lesion in aged F344 rats and occasionally seen in young adults (Eustis 1990). Acinar atrophy was noted at 2 years and interim sacrifices of control vehicle Harlan Sprague Dawley females used by the National Toxicology Program to report spontaneous lesions in Harlan SD females. Similar reports in males could not be found at the time of this report. This lesion is not significant nor treatment related due to the equal distribution of this lesion between the control group and high dose groups.

Varied cardiac findings were noted in dose groups examined, control and 80- dose only. 3/10 control males noted lymphocytic infiltrates with myocardial necrosis with or without fibrosis and 2/10 noted mononuclear infiltrates with or without fibrosis. Histiocytic infiltrates were found in 1/10 control females; 1/10 80-dose male (pre-term death) and 1/10 80-dose female. These findings were isolated and limited in severity and not considered significant.

Cerebellar or brain stem gliosis was noted in 3/10 80-dose males and 1/10 80-dose females. 4/5 of these animals, combined, were pre-term deaths. These lesions are likely compound related.

Ultimobranchial cysts are congenital anomalies of the thyroid gland. These cysts are found in almost every lobe when serial sections are performed (Hardisty 1990). Thyroid glands were examined in the 0-dose and 80-dose groups only. No cysts were noted in the 0-dose groups (0/10 male; 0/10 female). 4/10 were noted in the male 80-dose group and 5/10 in the female 80-dose group. These cysts are not treatment related.

There were few lesions of note that occurred in individual animals. Alveolar bronchioloization was noted in animal 11-0162. Bronchiolization occurs rarely as a spontaneous lesion in aged rats but is a frequent feature of chronic inflammation induced in the centriacinar areas of the lungs of rats by repeated inhalation of toxicants (Renne 2009). Because of the route of agent administration and the focal nature of the lesion it is unlikely associated with exposure. Chronic exposure to an environmental inhalant irritant is a possible explanation for this lesion. Animal 11-0157 was grossly noted to have an enlarged irregular spleen with a fibrous adhesion extending from the spleen to the left abdominal wall, left ovary and pancreas. In this case, the fibrous tissue was interpreted as mesentery. The multiple adhesions may have been due to a twist or injury to the mesentery with fibrotic repair which would lead to adhesions to multiple organs. There was no evidence of a diffuse peritonitis causing this lesion. Animal 11-0137 was noted grossly with an adhesion of the left liver lobe. Microscopically, fibrosis, biliary hyperplasia and hemosiderin-laden macrophages were observed. All microscopic findings are associated with injury repair. The cause of the original insult is not evident in sections examined. Animal 11-0101 was observed, grossly, to have an enlarged heart. Microscopically, cardiac myofibers were enlarged and disorderly with degeneration and loss of cells. The lung contained hemosiderin-laden macrophages consistent with cardiac compromise. With cardiac failure there is

chronic passive congestion of the lungs due to poor blood flow, red blood cells pass through pulmonary alveoli and are then broken down to hemosiderin in macrophages.

Other findings that occurred infrequently or comparable to controls were considered to be background lesions or of minimal significance and not treatment related. These lesions were prostatic, epididymal and coagulating gland lymphocytic infiltrates, harderian gland lymphocytic infiltrates, rare lymphoid hyperplasia of submandibular or mesenteric lymph nodes, and adrenal gland vacuolation. Alveolar histiocytosis was noted in few male and female 0-dose and 80-dose groups. Foci of alveolar histiocytosis are commonly observed in untreated rats (Boorman 1990).

There were five pre-study deaths. Animal 11-0097 (0-dose, male) was sacrificed pre-study due to the report of a dosing error. Histologic and gross findings were consistent with this report. Subcutaneous edema extended grossly from the ventral, cervical neck to proximal of the forelimbs and an esophageal rupture was visualized. This was consistent with the inflammation and edema visualized histologically within the fibroadipose tissue of the larynx, pharynx, trachea and thyroid gland. Inflammation and necrosis were also noted in the muscles of the esophagus. The cause of death in animal 11-0099, 11-0149, 11-0144 (80-dose, males) and 11-0151 (80-dose female) could not be determined definitively by histological examination. There was mild decomposition (autolysis) of some tissues at time of examination which did not allow for complete examination of all tissues. The deaths of rats, 11-0099, 11-0144, 11-0149 and 11-0151 may have been associated with the neurological findings.

A number of gross lesions were noted at necropsy. The most common observations were pale livers, "mottled" livers, "mottled kidneys", renal hydronephrosis, enlarged and or dark spleens, enlarged mesenteric lymph nodes, small testes, enlarged submandibular lymph nodes, and hydrometra. None of the livers or kidneys examined histologically had substantial enough lesions to account for the gross observations of pale, "or mottled". A few of the grossly enlarged mesenteric and submandibular lymph nodes were noted to have microscopic changes of lymphoid or plasmacytic hyperplasia, which may have accounted for the gross observations. All gross observations of hydronephrosis and hydrometra, examined microscopically, corresponded with renal pelvic and uterine dilatation, respectively. The amount of fluid within the uterine lumen varies throughout the estrus cycle. During proestrus the uterus normally becomes distended with watery fluid, appearing grossly as a "hydrometra" (Leininger 1990). Hydrometra was not considered an adverse finding. The enlarged spleens are likely associated with the histologic finding of hemosiderosis often accompanied by vascular congestion. The dark coloration is likely due to a combination of brown hemosiderin pigment and vascular congestion.

Clinical chemistry values, generally, did not correlate to individual organ insults. For example, hepatic fibrosis or infiltrates did not result in significant hepatocellular damage to result in elevated hepatic enzymes. Hematologic values in the 80-dose females tended to correlate with a hemolytic anemia. Decreased hemoglobin, red blood cell count and hematocrit are indicative of a change in the circulating red blood cell mass. Increased mean cell volume (MCV) is due to the increased size of immature erythrocytes and increased red cell distribution width is an index of the variation of erythrocyte size both findings noted in regenerative anemia. The decreased mean

corpuscular hemoglobin concentration (MCHC) is likely due to a high number of immature ethrythrocytes which do not have their full component of hemoglobin.

DISCUSSION

In this study it is evident that DNAN is a testicular toxicant. Testicular toxicants can target multiple sites within the male reproductive system. Toxicants can act directly on testicular blood supply and cells or at extratesticular sites resulting in direct damage to those cells or those cells they physiologically support.

Repetitive and prolonged dosing, regardless of the mechanism of toxicity, will result in germ cell damage and loss. Germ cells are affected because they are dependent on the function and processes of other cell types within the testis; a disruption of the germ cell supporting environment often results in their death (Creasy 1997). Since progressive germ cell loss occurs throughout a repeat dose, long term study, the end result is often seminiferous tubules lined only by Sertoli cells. Even though Sertoli cells are sensitive to alterations in function, they are extremely resistant to cell death (Creasy 2001). The primary target cell cannot be determined in this study design. If it is necessary to elucidate the target cell, a time course study should be performed in order to identify the earliest stage of pathologic change. The most interesting time period then should be chosen for an in-depth analysis (Creasy 1997). Reversibility of these lesions cannot be determined in this current study design. A recovery study should be timed in multiples of the spermatogenic process allowing for suitable recovery time of all germ cell layers.

Rats in this study exhibited gross, microscopic and hematologic changes as rats orally exposed to other explosives, such as 2,4,6-Trinitrotoluene (TNT). With TNT, dose-dependent anemia was seen in treated rats with reductions in hemoglobin, hematocrit and red blood cell counts (Yinon 1990). Enlarged spleens with hemosiderin and evidence of hematopoeisis were also observed. In the current study, enlarged spleens, splenic hemosiderosis, extramedullary hematopoeisis and changes in hematologic values were observed. A dose effect was noted in severity and prevalence of extramedullary hematopoeisis in the 80-dose group females and was noted in severity of splenic hemosiderosis in 80-dose males.

Glial lesions within the cerebellum or brain stem were noted in four rats (1/10, 80-dose female and 3/10, 80-dose males). Two of the males and the female were pre-term deaths. Microscopically, lesions appeared as spongiotic grey or white matter with increased glial cells and astrocytes occasionally with macrophages (gitter cells). Astrocytes function to sequester potential neurotoxins, microglial cells function in reparative or phagocytic processes after damage and gitter cells additionally phagocytose cellular debris, evidence that injury has occurred. Rats, in the current study, demonstrated ataxia and difficulty walking after dosing. Exposure to 1,3-Dinitrobenzene results in the formation of gliovascular lesions in the brain stems of experimental animals, particularly the nuclei of the auditory pathway, vestibular system and deep cerebellar roof nuclei as well as neurolgic signs of walking on toes, hunched back, and partial disuse of rear legs (Philbert 2000). 1,3,5-Trinitrobenzene (TNB) and nitrobenzene (NB) produce similar histologic, neuroanatomic and clinical findings.

Although NB, DNB, and TNB share similar chemical structures, they differ in their metabolism possibly explaining the variability in lesion onset (Chandra 1995). A study with TNB suggested that dose may also play a role; only rats exposed to 71 mg/kg-d for 10 days demonstrated brain lesions while rats exposed to 35.5 mg/kg-d for 10 days, 35.5 mg/kg-d for 4 days and 71 mg/kg-d for 4 days did not show brain lesions (Chandra 1995).

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APPENDIX A
HISTOPATHOLOGY DATA

0 Dose group
Males

11-0097 (pre-term death)
1. Fibroadipose tissue larynx, pharynx, trachea and thyroid gland: Cellulitis, fibrinous and pyogranulomatous, diffuse, severe with edema, hemorrhage and plant material.
2. Skeletal muscle, sternohyoid and sternothyroid: Myositits, pyogranulomatous, multifocal, moderate with edema, fibrin and hemorrhage.
3. Esophagus: Edema, submucosa, diffuse, severe with fibrin, hemorrhage and myonecrosis.
4. Lymph node, tracheobronchial: Draining hemorrhage, diffuse, moderate.
5. Lung: Congestion, diffuse, moderate.
6. Lung, bronchoalveolar lymphoid tissue (BALT): Hyperplasia, multifocal, minimal.
7. Liver: Infiltrates, lymphohistiocytic, focal, minimal.
8. Fibroadipose tissue, thymus: Infiltrates, neutrophilic and histiocytic, multifocal, mild with minimal hemorrhage.
9. Fibroadipose tissue, submandibular salivary gland: Cellulitis, fibrinosuppurative, diffuse with edema.
10. Lymph node, submandibular: Histiocytosis, multifocal, minimal.
11. Prostate, interstitium: Infiltrates, lymphocytic, focal, minimal.
12. Harderian gland, alveoli: Dilatation, multifocal, moderate with moderate porphyrin.
13. Thymus: Apoptosis, cortical, multifocal, mild.
14. Spleen: Hemosiderosis, multifocal, minimal.
15. Spleen: Extramedullary hematopoeisis (EMH), multifocal, mild.
16. Pharynx; larynx; trachea; thyroid gland; tongue; submandibular salivary gland; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; ileum; haired skin; mammary gland; skeletal muscle; peripheral nerve; heart; kidney; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; epididymis; testes; joint; bone; bone marrow; harderian gland; eye; optic nerve: No significant findings.

No: parathyroid gland; jejunum; cecum; colon; rectum

Gross necropsy findings: Diffuse hepatic reticular pattern; enlarged, edematous thymus; enlarged heart; Subcutaneous edema extending from ventral, cervical neck to just proximal of forelimbs; edematous adipose surrounding salivary glands; esophageal rupture proximal to thoracic inlet

11-0105
1. Lung: Alveolar histiocytosis, focal, minimal with extravasated red blood cells.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
3. Heart: Infiltrates, lymphohistiocytic, multifocal, moderate with myocardial necrosis and fibrosis.
4. Kidney, left: Infiltrates, lymphocytic, interstitial, multifocal, mild.
5. Adrenal gland, cortex, fasiculata: Vacuolation, diffuse, moderate.

6. Epididymis, interstitium: Infiltrates, lymphoid, multifocal, minimal.
7. Prostate: Infiltrates, lymphocytic, interstitial and glandular, multifocal, mild.
8. Kidney, left: Basophilic tubules, multifocal, minimal.
9. Spleen: Hemosiderosis, multifocal, minimal.
10. Spleen: EMH, multifocal, minimal.
11. Esophagus; trachea; larynx; thyroid gland; tongue; submandibular lymph node; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; skeletal muscle; peripheral nerve; urinary bladder; seminal vesicle; coagulating gland; epididymis; testes; prostate; spinal cord; nasal cavity; joint; bone; bone marrow; harderian gland: No significant findings.
No: parathyroid gland; submandibular salivary gland; extraorbital lacrimal gland; mammary gland; eyes

Gross necropsy findings: Overall body condition obese; Pale, friable liver; enlarged spleen

11-0112
1. Lung, BALT: Hyperplasia, multifocal, mild.
2. Liver: Infiltrates, lymphohistiocytic, multifocal, minimal.
3. Heart, myocardium: Infiltrates, histiocytic, focal, mild.
4. Kidney, left: Infiltrates, lymphocytic, interstitial, multifocal, minimal.
5. Kidney, left: Basophilic tubules, focal, mild.
6. Adrenal gland, cortex, fasiculata: Vacuolation, diffuse, moderate
7. Epididymis: Infiltrates, lymphocytic, interstitial, multifocal, minimal.
8. Prostate: Glandular dilatation, diffuse, moderate with rare interstitial lymphocytes.
9. Harderian gland: Infiltrates, lymphocytic, multifocal, mild.
10. Lymph node, site not specified: Hyperplasia, lymphocytic, multifocal, mild with minimal plasmacytosis and draining hemorrhage.
11. Spleen: Hemosiderosis, multifocal, minimal.
12. Spleen: EMH, multifocal, mild.
13. Esophagus; trachea; larynx; thyroid gland; tracheobronchial lymph node; tongue; submandibular salivary gland; submandibular lymph nodes; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle peripheral nerve; urinary bladder; seminal vesicle; epididymis; testes; spinal cord; joint; bone; bone marrow; eye; optic nerve: No significant findings.
No: parathyroid gland; coagulating gland; extraorbital lacrimal gland

Gross necropsy findings: Overall body condition obese; pale liver; cage bedding in stomach, small intestine and colon

11-0116
1. Lung, BALT: Hyperplasia, lymphocytic, multifocal, mild.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
3. Heart, myocardium: Infiltrates, histiocytic, multifocal, mild with fibrosis.
4. Kidney, right and left: Basophilic tubules, multifocal, mild.
5. Kidney, right: Infiltrates, lymphocytic, interstitial, multifocal, minimal.

6. Prostate: Infiltrates, lymphocytic, interstitial and glandular, multifocal, mild with glandular dilatation.
7. Spleen: Hemosiderosis, multifocal, minimal.
8. Spleen: EMH, multifocal, mild.
9. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tracheobronchial lymph node; tongue; submandibular salivary gland; submandibular lymph nodes; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle peripheral nerve; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; epididymis; testes; spinal cord; joint; bone; bone marrow; harderian gland; eye; optic nerve: No significant findings.
No: parathyroid gland; 1 lung section, slide 2; extraorbital lacrimal gland; jejunum; optic nerve

Gross necropsy findings: Overall body condition obese; pale liver

11-0150
1. Thyroid gland: Ultimobranchial cyst.
2. Lung: Alveolar histiocytosis, focal, mild with eosiphilic crystals and rare neutrophils.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
4. Lymph node, submandibular: Plasmacytosis, multifocal, moderate.
5. Pancreas: Fibrosis, islet cells, multifocal mild with hemorrhage, islet hyperplasia and acinar degeneration.
6. Heart: Infiltrates, histiocytic, multifocal, mild with myocardial necrosis.
7. Kidney, right: Infiltrates, mononuclear, focal, minimal with glomerulosclerosis.
8. Kidney, left: Infiltrates, mononuclear, interstitial, focal, minimal.
9. Spleen: Hemosiderosis, multifocal, minimal.
10. Spleen: EMH, multifocal, moderate.
11. Stomach, glands: Dilatation, multifocal, moderate.
12. Esophagus; trachea; larynx; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle peripheral nerve; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; epididymis; testes; prostate; spinal cord; joint; bone; bone marrow; harderian gland; eye; extraorbital lacrimal gland: No significant findings.
No: parathyroid gland; tracheobronchial lymph node; skeletal mm/peripheral nerve; prostate; optic nerve.

Gross necropsy findings: Overall body condition obese; diffusely mottled liver

11-0107
1. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
2. Coagulating gland: Infiltrates, mononuclear, focal, minimal.
3. Prostate: Infiltrates, lymphohistiocytic, interstitial, multifocal, mild.
4. Harderian gland: Infiltrates, lymphocytic, focal, minimal.
5. Spleen: Hemosiderosis, multifocal, minimal.
6. Spleen: Hyperplasia, lymphoid, multifocal, minimal.

7. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tracheobronchial lymph node; lung; tongue; submandibular salivary gland; submandibular lymph node; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle; peripheral nerve; heart; kidney; adrenal gland; urinary bladder; seminal vesicle; epididymis; testes; spinal cord; nasal cavity; joint; bone; bone marrow; extraorbital lacrimal gland; eye; optic nerve: No significant findings.

Gross necropsy findings: Right and left mottled kidneys

11-0108

1. Lung: Alveolar histiocytosis, multifocal, minimal.
2. Lung: BALT: Hyperplasia, lymphoid, multifocal, mild.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
4. Submandibular salivary gland: Infiltrates, lymphocytic, focal, minimal.
5. Pancreas, islet cell: Hyperplasia, multifocal, moderate with hemosiderin laden macrophages.
6. Pancreas, acinar cells: Atrophy, focal, mild.
7. Adrenal gland, zona fasiculata: Vacuolation, multifocal, mild.
8. Prostate: Prostatitis, suppurative, multifocal, moderate with minimal epithelial hyperplasia.
9. Spleen: Hemosiderosis, multifocal, minimal.
10. Spleen: EMH, multifocal, minimal.
12. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tongue; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle peripheral nerve; heart; kidney; urinary bladder; seminal vesicle; coagulating gland; epididymis; testes; spinal cord; joint; bone; bone marrow; harderian gland; eye; optic nerve; extraorbital lacrimal gland: No significant findings

No: submandibular lymph node; rectum

Gross necropsy findings: None

11-0118

1. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
2. Lymph node, submandibular: Sinus plasmacytosis, multifocal, mild.
3. Pancreas: Infiltrates, mononuclear, multifocal, minimal.
4. Spleen: Hemosiderosis, multifocal, minimal.
5. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; lung; tracheobronchial lymph node; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; jejunum; ileum; cecum; colon; haired skin; skeletal muscle peripheral nerve; heart; kidney; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; epididymis; testes; prostate; spinal cord; joint; bone; bone marrow; harderian gland; eye: No significant findings

No: rectum; mammary gland; optic nerve; extraorbital lacrimal gland

Gross necropsy findings: None

11-0140

1. Lung: Alveolar histiocytosis, multifocal, mild with few hemosiderin-laden macrophages and eosinophilic crystals.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
3. Lymph node, submandibular: Sinus plasmacytosis, multifocal, mild.
4. Heart: Fibrosis, focally extensive, moderate with lymphocytic infiltrates and myodegeneration.
5. Epididymis: Infiltrates, lymphocytic, multifocal, minimal.
6. Prostate: Infiltrates, lymphocytic, interstitial, multifocal, moderate with intraglandular neutrophils.
7. Harderian gland: Infiltrates, lymphocytic, multifocal, minimal.
8. Spleen: Hemosiderosis, multifocal, multifocal, minimal.
9. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; skeletal muscle peripheral nerve; kidney; adrenal gland; seminal vesicle; coagulating gland; testes; spinal cord; joint; bone; bone marrow; eye; optic nerve: No significant findings

No: mammary gland; urinary bladder, extraorbital lacrimal gland

Gross necropsy findings: Left lung: mottled red 2mm lesions; right and left kidneys mottled; cage bedding in stomach and cecum

11-0147

1. Trachea, submucosa: Glandular dilatation, multifocal, moderate with rare luminal fluid and neutrophils.
2. Trachea, submucosa: Infiltrates, lymphocytic, focally extensive, mild.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
4. Lymph node, submandibular: Sinus plasmacytosis, diffuse, moderate with lymphoid hyperplasia.
5. Peyers patch, colon: Hyperplasia, lymphoid, focal, moderate.
6. Kidney, right: Fibrosis, multifocal, moderate with tubular atrophy and hemosiderin.
7. Kidney, pelvis, right: Dilatation, diffuse, severe.
8. Prostate: Infiltrates, mononuclear, interstitial, focal, minimal.
9. Spleen: Hemosiderosis, multifocal, minimal.
10. Esophagus; larynx; thyroid gland; parathyroid gland; lung; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; rectum; haired skin; mammary gland; skeletal muscle peripheral nerve; heart; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; epididymis; testes; spinal cord; joint; bone; bone marrow; harderian gland; eye; optic nerve; extraorbital lacrimal gland: No significant findings.

No: rectum; only one eye in plane of section

Gross necropsy findings: Enlarged mesenteric lymph nodes; right kidney hydronephrosis; right and left kidneys slightly mottled

0 Dose group
Females

11-0154
1. Esophagus, skeletal muscle: Infiltrates, histiocytic, multifocal, mild with myodegeneration.
2. Lung: Alveolar histiocytosis, multifocal, mild with rare neutrophils and bronchiolar epithelial hyperplasia,
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
4. Kidney, right and left: Mineral, corticomedullary, multifocal, mild.
5. Spleen: Hemosiderosis, multifocal, mild.
6. Trachea; larynx; thyroid gland; parathyroid gland; tongue; submandibular salivary gland; submandibular lymph nodes; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle peripheral nerve; heart; adrenal gland; urinary bladder; uterus; ovary; spinal cord; joint; bone; bone marrow; harderian gland; eye; optic nerve: No significant findings

Gross necropsy findings: Cage bedding in intestine.

11-0162
1. Lung: Bronchiolization, focal, mild with lymphocytic infiltrates.
2. Lung: BALT hyperplasia, multifocal, mild.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
4. Kidney, left and right: Infiltrates, mononuclear, multifocal, mild.
5. Spleen: Hemosiderosis, multifocal, moderate.
6. Spleen: Hyperplasia, lymphoid, multifocal, minimal.
7. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle peripheral nerve; heart; adrenal gland; urinary bladder; uterus; ovary; spinal cord; joint; bone; bone marrow; harderian gland; eye; optic nerve: No significant findings.

No: submandibular lymph nodes

Gross necropsy findings: Right liver lobe 4mm pale area; right and left kidney mildly mottled; cage bedding in intestinal tract

11-0168
1. Lung, BALT: Hyperplasia, multifocal, minimal.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
3. Pancreas, acinar cells: Atrophy, focal, minimal.
4. Lymph node, site not specified: Sinus plasmacytosis, diffuse, mild.
5. Spleen: Hemosiderosis, multifocal, mild.
6. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tongue; submandibular salivary gland; submandibular lymph nodes; thymus; cerebrum; cerebellum; pituitary gland; stomach; duodenum; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle peripheral nerve; heart; kidney; adrenal gland; urinary

bladder; uterus; ovary; spinal cord; joint; bone; bone marrow; harderian gland; eye; optic nerve: No significant findings
No: mammary gland

Gross necropsy findings: None

11-0171

1. Lung: Alveolar histiocytosis, focal, minimal.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
3. Lymph node, submandibular: Sinus plasmacytosis, diffuse, mild.
4. Heart: Hemosiderin, focal, minimal.
5. Kidney, right and left: Infiltrates, mononuclear, focal, minimal with rare mineral.
6. Lymph node, site not specified: Sinus plasmacytosis, diffuse, moderate.
7. Spleen: Hemosiderosis, multifocal, mild.
8. Spleen: Hyperplasia, lymphoid, multifocal, minimal.
9. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle peripheral nerve; adrenal gland; urinary bladder; uterus; ovary; spinal cord; joint; bone; bone marrow; harderian gland; eye; optic nerve: No significant findings.

Gross necropsy findings: Enlarged right and left submandibular lymph nodes.

11-0173

1. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
2. Lymph node, submandibular: Sinus plasmacytosis, diffuse, mild.
3. Spleen: Hyperplasia, lymphoid, multifocal, minimal.
4. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; lung; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle; peripheral nerve; heart; kidney; adrenal gland; urinary bladder; uterus; ovary; spinal cord; nasal cavity; joint; bone; bone marrow; intraorbital lacrimal gland; harderian gland; eye; optic nerve: No significant findings.

Gross necropsy findings: Cage bedding in cecum.

11-0175

1. Lung, BALT: Hyperplasia, lymphoid, multifocal, minimal.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
3. Uterus: Dilatation, diffuse, moderate.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tracheobronchial lymph node; tongue; submandibular salivary gland; submandibular lymph node; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle; peripheral nerve; heart; kidney; adrenal gland; urinary bladder; ovary; spinal

cord; nasal cavity; joint; bone; bone marrow; extraorbital lacrimal gland; harderian gland; eye; optic nerve: No significant findings.

Gross necropsy findings: None.

11-0190

1. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild with rare hepatocellular necrosis.
2. Lymph node, submandibular: Sinus plasmacytosis, diffuse, moderate.
3. Heart: Infiltrates, lymphohistiocytic, focal, minimal.
4. Harderian gland: Infiltrates, lymphocytic, focal, minimal.
5. Spleen: Hemosiderosis, multifocal, mild.
6. Esophagus; trachea; larynx; thyroid gland; tracheobronchial lymph node; lung; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle peripheral nerve; kidney; adrenal gland; urinary bladder; uterus; ovary; spinal cord; joint; bone; bone marrow; eye; optic nerve: No significant findings.

No: parathyroid gland

Gross necropsy findings: Enlarged mesenteric lymph nodes

11-0191

1. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
2. Lymph node, submandibular: Sinus plasmacytosis, diffuse, moderate.
3. Pancreas, acinar cells: Atrophy, focal, minimal.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; lung; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle peripheral nerve; heart; kidney; adrenal gland; urinary bladder; uterus; ovary; spinal cord; joint; bone; bone marrow; harderian gland; eye; optic nerve: No significant findings.

Note: splenic hemosiderosis

Gross necropsy findings: Enlarged mesenteric lymph nodes; pale adrenal glands; cage bedding in cecum.

11-0197

1. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
2. Kidney, right and left: Mineralization, corticomedullary, multifocal, mild.
3. Spleen: Hemosiderosis, multifocal, moderate.
4. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; lung; tongue; submandibular salivary gland; submandibular lymph nodes; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle peripheral nerve; heart; kidney; adrenal gland; urinary bladder; uterus; ovary; spinal cord; joint; bone; bone marrow; harderian gland; eye; optic nerve; extraorbital lacrimal gland: No significant findings

Note: Minimum parathyroid gland.
No: mammary gland

Gross necropsy findings: Distal 40mm segment of ileal mucosa red; cage bedding in stomach.

11-0206
1. Lung: Alveolar histiocytosis, multifocal, mild.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
3. Lymph node, submandibular: Sinus plasmacytosis, diffuse, mild.
4. Haired skin, mammary gland: Infiltrates, lymphohistiocytic, multifocal, mild.
5. Kidney, right: Mineral, multifocal, minimal.
6. Spleen: Hemosiderosis, multifocal, mild.
7. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tongue; submandibular salivary gland; submandibular lymph nodes; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle peripheral nerve; heart; kidney; adrenal gland; urinary bladder; uterus; ovary; spinal cord; joint; bone; bone marrow; harderian gland; eye; optic nerve; extraorbital lacrimal gland: No significant findings

No: rectum

Gross necropsy findings: None

80 dose group
Male

11-0109
1. Lung: Alveolar histiocytosis, multifocal, moderate.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild with rare hepatocellular necrosis.
3. Liver: Bile-laden kupffer cells, multifocal, mnimal.
4. Spleen: Hemosiderosis, diffuse, mild.
5. Spleen: EMH, multifocal, moderate.
6. Lymph node, mesenteric: Hyperplasia, follicular, diffuse, moderate.
7. Lymph node, submandibular: Sinus plasmacytosis, multifocal, mild.
8. Cerebellum: Gliosis and astrocytosis, bilaterally symmetrical, mild with rarefaction.
9. Epididymis: Aspermia, multifocal, moderate with degenerate and necrotic cells, eosinophilic material.
11. Testes: Degeneration, diffuse, moderate.
12. Spleen: Congestion, diffuse, mild.
13. Esophagus; trachea; larynx; thyroid gland; tongue; thymus; cerebrum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle; peripheral nerve; heart; kidney; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; prostate spinal cord; nasal cavity; joint; bone; bone marrow; harderian gland; eye; optic nerve; extraorbital lacrimal gland: No significant findings.

No: parathyroid gland; rectum;

Gross necropsy findings: Overall body condition thin; dark spleen; enlarged mesenteric lymph nodes.

11-0123

1. Skeletal muscle, esophagus: Infiltrates, mononuclear, focally extensive, mild with myodegeneration, regeneration and fibrosis.
2. Lung: Alveolar histiocytosis, multifocal, mild with few hemosiderin-laden macrophages.
3. Lung, BALT: Hyperplasia, multifocal, mild.
4. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
5. Liver: Biliary hyperplasia, focal, mild with lymphocytic infiltrates.
6. Spleen: Hemosiderosis, diffuse, severe.
7. Spleen: EMH, multifocal, minimal.
8. Cerebellum: Lipofuscinosis, multifocal, minimal.
9. Pancreas, acinar cells: Atrophy and loss, multifocal, mild with mononuclear infiltrates.
10. Epididymis: Aspermia, multifocal, moderate with degenerate and necrotic cells, eosinophilic material.
11. Testes: Degeneration, multifocal, severe with atrophy.
12. Harderian gland: Infiltrates, lymphoid, multifocal, minimal.
13. Thyroid gland: Ultimobranchial cyst, focal.
14. Lymph node, submandibular: Sinus plasmacytosis, multifocal, mild.
15. Trachea; larynx; parathyroid gland; tracheobronchial lymph node; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; pituitary gland; stomach; duodenum; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle; peripheral nerve; heart; kidney; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; prostate; spinal cord; joint; bone; bone marrow; extraorbital lacrimal gland; harderian gland; eye; optic nerve: No significant findings.

Gross necropsy findings: Overall thin body condition; small testes; dark spleen; bright yellow stomach contents.

11-0129

1. Liver: Infiltrates, lymphohistiocytic, multifocal and random, moderate with rare hepatocellular necrosis.
2. Liver: Bile-laden kupffer cells, mild.
3. Spleen: Hemosiderosis, multifocal, moderate.
4. Spleen: Hyperplasia, lymphoid, multifocal, minimal.
5. Lymph node, submandibular: Sinus plasmacytosis, multifocal, moderate.
6. Cecum, submucosa: Hemosiderin-laden macrophages, multifocal, mild.
7. Epididymis: Aspermia, multifocal, moderate with degenerate and necrotic cells, eosinophilic material.
8. Testes: Degeneration, multifocal, moderate with few multinucleated giant cells and rare atrophy.
9. Kidney, tubular epithelium: Brown globular pigment, multifocal, mild.

10. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; lung; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; colon; rectum; haired skin; mammary gland; skeletal muscle; peripheral nerve; heart; kidney; adrenal gland; urinary bladder; seminal vesicle; prostate; spinal cord; nasal cavity; joint; bone; bone marrow; intraorbital lacrimal gland; harderian gland; eye; optic nerve: No significant findings.
No: parathyroid gland, adrenal medulla in section; mammary gland; Coagulating Gland

Gross necropsy findings: Overall thin body condition; small testes; dark spleen

11-0111

1. Lung: Alveolar histiocytosis, multifocal, mild with cholesterol clefts.
2. Lung, BALT: Hyperplasia, lymphoid, multifocal, mild.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild with rare hepatocellular necrosis.
4. Liver: Bile-laden kupffer cells, minimal.
5. Spleen: Hemosiderosis, diffuse, moderate.
6. Spleen: EMH, multifocal, moderate.
7. Spleen: Congestion, diffuse, mild.
8. Epididymis: Aspermia, multifocal, severe with intratubular cellular debris.
9. Testes: Degeneration, diffuse, moderate with few atrophic tubules.
10. Prostate, gland: Cellular debris, intraglandular, mild.
11. Esophagus; trachea; tracheobronchial lymph node; larynx; thyroid gland; parathyroid gland; tongue; submandibular salivary gland; submandibular lymph node; mesenteric lymph node; thymus; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle peripheral nerve; heart; kidney; adrenal gland; urinary bladder; seminal vesicle; spinal cord; joint; bone; harderian gland; eye; optic nerve; bone marrow: No significant findings.
No: Coagulating Gland

Gross necropsy findings: Small testes; dark spleen

11-0132

1. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
2. Liver: Bile-laden kupffer cells, multifocal, mild.
3. Spleen: Hemosiderosis, diffuse, moderate.
4. Spleen: EMH, multifocal, minimal.
5. Spleen: Congestion, diffuse, mild.
6. Lymph node, submandibular: Sinus plasmacytosis, multifocal, minimal.
7. Kidney, pelvis, right: Dilatation, diffuse, moderate.
8. Epididymis: Aspermia, diffuse, severe with intratubular cellular debris.
9. Testes: Degeneration, diffuse, severe with moderate atrophic tubules.
10. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; lung; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle peripheral nerve; heart; adrenal gland;

urinary bladder; seminal vesicle; coagulating gland; prostate; spinal cord; joint; bone; bone marrow; harderian gland; eye; optic nerve: No significant findings
No: mammary gland; rectum; extraorbital lacrimal gland.

Gross necropsy findings: Small testes; dark spleen; cage bedding throughout intestinal lumen; enlarged mesenteric lymph nodes

11-0144 (Pre-term death)
1. Thyroid gland: Ultimobranchial cyst.
2. Lung: Congestion, diffuse, moderate.
3. Lung, BALT: Hyperplasia, lymphoid, multifocal, mild.
4. Liver: Infiltrates, lymphohistiocytic, multifocal and random, moderate.
5. Spleen: Lymphoid hyperplasia, multifocal, mild.
6. Spleen: Hemosiderosis, multifocal, moderate.
7. Instestine: Autolysis, diffuse, moderate.
8. Epididymis: Aspermia, multifocal, severe with intratubular cellular debris.
9. Testes: Degeneration, diffuse, severe with few atrophic tubules.
10. Esophagus; trachea; larynx; parathyroid gland; lung; tongue; submandibular salivary gland; submandibular lymph nodes; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; rectum; haired skin; skeletal muscle peripheral nerve; heart; kidney; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; prostate; spinal cord; joint; bone; bone marrow; harderian gland; eye; optic nerve: No significant findings.
No: mammary gland; bladder mucosa not in plane of section.

Gross necropsy findings: 5mm dark red focus right kidney; red focal area jejunal serosa; dark red lungs

11-0099 (pre-term death)
1. Thyroid gland: Ultimobranchial cyst.
2. Lung: Congestion, diffuse, moderate.
3. Lung: Alveolar histiocytosis, multifocal, minimal with foreign material.
4. Spleen: Hemosiderosis, multifocal, moderate.
5. Spleen: Hyperplasia, lymphoid, multifocal, mild.
6. Spleen: EMH, multifocal, minimal.
7. Thymus: Congestion, diffuse, moderate with severe medullary erythrocyte extravasation.
8. Kidney: Autolysis, multifocal, moderate.
9. Brain stem, grey matter: Gliosis, focal, moderate.
10. Heart: Infiltrates, histiocytic, focal, mild with rare myocardial degeneration.
11. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tongue; thymus; cerebrum; cerebellum; pituitary gland; skeletal muscle peripheral nerve; heart; liver; adrenal gland; spinal cord; joint; harderian gland; eye; optic nerve: No significant findings.
No: haired skin, intestine, mammary gland; urinary bladder; no repro; bone; bone marrow; lymph nodes; salivary gland; salivary lymph nodes

Gross necropsy findings: Enlarged kidneys; generalized autolysis.

11-0110
1. Thyroid gland: Ultimobranchial cyst.
2. Spleen: Hemosiderosis, diffuse, moderate.
3. Spleen: EMH, multifocal, minimal.
4. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
5. Lymph node, submandibular: Sinus plasmacytosis, diffuse, mild.
6. Kidney, left: Infiltrates, mononuclear, interstitial, focal, minimal.
7. Epididymes: Aspermia, multifocal, severe with eosinophilic and cellular debris.
8. Epididymes, interstitium: Granuloma, focal.
9. Testes: Degeneration and atrophy, diffuse, severe.
10. Esophagus; trachea; larynx; parathyroid gland; lung; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; haired skin; mammary gland; skeletal muscle peripheral nerve; heart; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; prostate; spinal cord; joint; bone; bone marrow; harderian gland; eye; optic nerve; extraorbital lacrimal gland: No significant findings.
No: rectum; mammary gland

Gross necropsy findings: Dark spleen; small testes

11-0102
1. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
3. Liver, kupffer cells: Pigment-laden kupffer cells, multifocal, mild.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Spleen: Congestion, diffuse, moderate.
6. Skeletal muscle, hindlimb: Infiltrates, histiocytic, multifocal with myonecrosis.
7. Kidney, right: Basophilic tubules, focal, minimal.
8. Epididymis: Aspermia, multifocal, severe with eosinophilic and cellular debris.
9. Testes: Degeneration and atrophy, diffuse, severe with rare multinucleated giant cells.
10. Extraorbital lacrimal gland: Infiltrates, mononuclear, multifocal, mild.
11. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; lung; tongue; submandibular salivary gland; submandibular lymph nodes; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; peripheral nerve; heart; adrenal gland; urinary bladder; seminal vesicle; coagulating gland; prostate; spinal cord; joint; bone; bone marrow; harderian gland; eye; optic nerve: No significant findings.

Gross necropsy findings: Overall body condition thin; pale mucous membranes; thin body condition; dark spleen; bedding material throughout intestinal lumen

11-0149 (Found dead)
1. Trachea, thyroid gland, lung, kidney : Autolysis, multifocal, moderate.
2. Lung: Congestion, diffuse, moderate.
3. Thymus: Congestion diffuse, moderate with extravasated red blood cells.
4. Liver: Infiltrates, neutrophilic, multifocal, minimal.
5. Spleen: Hemosiderosis, diffuse, moderate.
6. Spleen: EMH, multifocal, minimal.

7. Cerebellum: Gliosis, multifocal and symmetrical, minimal with lipofuscin pigment.
8. Heart: Infiltrates, histiocytic, focal, minimal.
9. Epididymis: Aspermia, multifocal, severe with eosinophilic and cellular debris.
11. Testes: Degeneration and atrophy, diffuse, moderate with multinucleated giant cells.
12. Esophagus; larynx; parathyroid gland; tongue; cerebrum; pituitary gland; haired skin; skeletal muscle peripheral nerve; adrenal gland; spinal cord; joint; bone; bone marrow; harderian gland; eye; optic nerve: No significant findings.
No: mammary gland; prostate; intestine; lymph node; salivary gland; accessory sex organs; urinary bladder

Gross necropsy findings: Bilaterally enlarged kidneys; small testes; dark autolytic spleen; dark liver edges

80 dose group
Female

11-0151 (pre-term death)
1. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
2. Liver: Bile-laden kupffer cells, multifocal, minimal.
3. Liver: EMH, multifocal, minimal.
4. Spleen: EMH, diffuse, severe.
5. Spleen: Hemosiderosis, diffuse, mild.
6. Lymph node, submandibular: Sinus plasmacytosis, diffuse, mild.
7. Cerebellum: Gliosis, focal, mild with rarefaction and rare gitter cells.
8. Stomach, submucosa: Infiltrates, mononuclear, focally extensive, mild with edema.
9. Kidney, left: Basophilic tubules, focally extensive, moderate with lymphoid infiltrates.
10. Kidney, right and left: Mineral, corticomedullary, multifocal, mild.
11. Bulbar conjunctiva, eye: Infiltrates, neutrophilic, multifocal, mild.
12. Thymus: Cyst, focal.
13. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; lung; tongue; submandibular salivary gland; thymus; cerebrum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle; peripheral nerve; heart; kidney; adrenal gland; uterus; ovary; spinal cord; joint; bone; bone marrow; harderian gland; eye; optic nerve: No significant findings.
No: urinary bladder; extraorbital lacrimal gland; tracheobronchial lymph node

Gross necropsy findings: Overall thin body condition; enlarged, dark spleen; enlarged submandibular lymph nodes; right and left kidney dark

11-0182
1. Thyroid gland: Ultimobranchial cyst with few lymphoid infiltrates.
2. Spleen: Hemosiderosis, diffuse, moderate.
3. Spleen: EMH, multifocal, mild.
4. Lymph node, submandibular: Sinus plasmacytosis, multifocal, mild.
5. Kidney, left: Infiltrates, mononuclear, focal, minimal.
6. Esophagus; trachea; larynx; parathyroid gland; tracheobronchial lymph node; lung; liver; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum;

colon; rectum; haired skin; mammary gland; skeletal muscle; peripheral nerve; heart; adrenal gland; urinary bladder; uterus; ovary; spinal cord; joint; bone; bone marrow; extraorbital lacrimal gland; harderian gland; eye; optic nerve: No significant findings.
No: thymus; mammary gland;

Gross necropsy findings: Dark spleen

11-0188
1. Liver: Infiltrates, lymphohistiocytic, multifocal and random, severe with minimal hepatocellular necrosis.
2. Liver: Bile-laden kupffer cells, multifocal, mild.
3. Spleen: Hemosiderosis, diffuse, moderate.
4. Spleen: Hyperplasia, lymphoid, multifocal, mild.
5. Lymph node, submandibular: Sinus plasmacytosis, diffuse, moderate.
6. Lymph node, submandibular: Hyperplasia, lymphoid, diffuse, moderate.
7. Harderian gland: Infiltrates, lymphocytic, focal, minimal.
8. Lacrimal gland, extraorbital: Infiltrates, lymphohistiocytic, multifocal, mild.
9. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tracheobronchial lymph node; lung; tongue; submandibular salivary gland; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; skeletal muscle; thymus; peripheral nerve; heart; kidney; adrenal gland; urinary bladder; uterus; spinal cord; joint; bone; bone marrow; eye; optic nerve: No significant findings.
No: ovaries; mammary gland;

Gross necropsy findings: Dark spleen

11-0180
1. Thyroid gland: Ultimobranchial cyst.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
3. Liver: Bile plugs, few.
4. Kidney, right: Mineral, corticomedually junction, multifocal, minimal.
5. Kidney, left: Infiltrates, mononuclear, multifocal, mild.
6. Uterus: Dilatation, diffuse, severe with mild submucosal lymphocytes.
7. Harderian gland: Infiltrates, mononuclear, multifocal, minimal.
8. Lacrimal gland, extraorbital: Infiltrates, lymphocytic, multifocal, mild.
9. Spleen: Hemosiderosis, multifocal, mild.
10. Spleen: EMH, multifocal, moderate.
11. Esophagus; trachea; larynx; parathyroid gland;lung; tongue; submandibular salivary gland; submandibular lymph node; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle; peripheral nerve; heart; adrenal gland; urinary bladder; ovary; spinal cord; nasal cavity; joint; bone; bone marrow; eye; optic nerve: No significant findings.
No: tracheobronchial lymph node; mammary gland;

Gross necropsy findings: Dark spleen; right and left kidneys pale; hydrouterus (hydrometra)

11-0200

1. Larynx, submucosa: Hyperplasia, lymphoid, multifocal, minimal.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, severe with mild hepatocellular necrosis.
3. Liver: Bile plugs, bile and hemosiderin laden kupffer cells, multifocal, mild.
4. Spleen: Hemosiderosis, diffuse, moderate.
5. Spleen: EMH, diffuse, moderate.
6. Spleen: Congestion, diffuse, mild.
7. Lymph node, submandibular: Sinus plasmacytosis, multifocal, mild.
8. Kidney, right and left: Mineral, coritcomedullary junction, multifocal, minimal.
9. Ovary, corpus luteum: Necrosis, multifocal, moderate.
10. Uterus: Dilatation, unilateral, minimal.
11. Esophagus; trachea; thyroid gland; parathyroid gland; lung; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle; heart; adrenal gland; urinary bladder; spinal cord; joint; bone; bone marrow; harderian gland; eye; optic nerve; extraorbital lacrimal gland: No significant findings.

No peripheral nerve;

Gross necropsy findings: Dark, enlarged spleen; hydrouterus; cage bedding in intestinal tract

11-0201

1. Lung: Alveolar histiocytosis, multifocal, mild.
2. Spleen: Hemosiderosis, diffuse, mild.
3. Spleen: Congestion, diffuse, mild.
4. Spleen: EMH, diffuse, moderate.
5. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
6. Lymph node, submandibular: Sinus plasmacytosis, diffuse, moderate.
7. Kidney, right: Mineral, focal, minimal.
8. Harderian gland: Infiltrates, lymphocytic, multifocal, minimal.
9. Ovary, corpus luteum: Necrosis, multifocal, mild.
10. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle peripheral nerve; heart; adrenal gland; urinary bladder; uterus; spinal cord; joint; bone; bone marrow; eye; optic nerve: No significant findings.

No: pituitary gland

Gross necropsy findings: Enlarged, dark spleen; enlarged submandibular lymph nodes; cage bedding in intestinal tract.

11-0158

1. Esophagus, skeletal muscle: Infiltrates, histiocytic, focally extensive, mild.
2. Lung: Alveolar histiocytosis, multifocal, mild.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, severe.

4. Liver: Kupffer cells, moderate with hemosiderin and bile.
5. Spleen: Hemosiderosis, diffuse, moderate.
6. Spleen: EMH, multifocal, mild.
7. Spleen: Hyperplasia, lymphoid, multifocal, minimal.
8. Lymph node, submandibular: Sinus plasmacytosis, multifocal, minimal.
9. Kidney, right and left: Mineral, corticomedullary, multifocal, minimal.
10. Kidney, left: Infiltrates, lymphocytic, focal, minimal.
11. Harderian gland: Infiltrates, mononuclear, multifocal, minimal.
12. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tongue; submandibular salivary gland; thymus; mesenteric lymph node; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle peripheral nerve; heart; adrenal gland; urinary bladder; uterus; ovary; spinal cord; joint; bone; bone marrow; harderian gland; eye; optic nerve: No significant findings.

Gross necropsy findings: Dark spleen

11-0195

1. Thyroid gland: Ultimobranchial cyst.
2. Esophagus, skeletal muscle: Infiltrates, histiocytic, focal, minimal with rare myonecrosis.
3. Lung: Alveolar histiocytosis, focal, minimal.
4. Liver: Infiltrates, lymphohistiocytic, multifocal and random, severe.
5. Liver: Bile-laden kupffer cells, moderate.
6. Liver: EMH, multifocal, minimal.
7. Spleen: Hemosiderosis, diffuse, mild.
8. Spleen: Congestion, diffuse, moderate.
9. Spleen: EMH, diffuse, moderate.
10. Lymph node, submandibular: Hyperplasia, lymphoid, diffuse, mild.
11. Lymph node, submandibular: Sinus plasmacytosis, diffuse, mild.
12. Esophagus; trachea; larynx; thyroid gland; parathyroid gland; tongue; submandibular salivary gland; mesenteric lymph node; thymus; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle peripheral nerve; heart; kidney; adrenal gland; urinary bladder; uterus; ovary; spinal cord; joint; bone; bone marrow; harderian gland; eye; optic nerve: No significant findings.

Gross necropsy findings: Enlarged dark spleen; cage bedding throughout intestinal lumen

11-0177

1. Thyroid gland: Ultimobranchial cyst.
2. Skeletal muscle, esophagus and oropharynx: Regeneration, myocyte, multifocal, mild with minimal histiocytic infiltrates.
3. Lung: Alveolar histiocytosis, multifocal, minimal with rare neutrophils.
4. Liver: Infiltrates, lymphohistiocytic, multifocal and random, moderate with rare hepatocellular necrosis.
5. Liver: EMH, multifocal, minimal.

6. Spleen: Hemosiderosis, diffuse, moderate.
7. Spleen: EMH, multifocal, mild.
8. Spleen: Hyperplasia, lymphoid, multifocal, minimal.
9. Pituitary gland: Infiltrates, lymphocytic, focal, minimal.
10. Adrenal gland: Hemosidersosis, minimal.
11. Kidney, left: Mineral, focal, minimal.
12. Esophagus; trachea; larynx; parathyroid gland; tongue; submandibular salivary gland; submandibular lymph nodes; thymus; mesenteric lymph node; cerebrum; cerebellum; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle peripheral nerve; heart; kidney; urinary bladder; uterus; ovary; spinal cord; joint; bone; bone marrow; harderian gland; eye; optic nerve; extraorbital lacrimal gland: No significant findings.

Gross necropsy findings: Enlarged dark spleen; pale mottled kidneys; mildly enlarged left kidney

11-0186

1. Thyroid gland: Ultimobranchial cyst.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
3. Liver: Bile-laden kupffer cells, multifocal, mild.
4. Spleen: Hemosiderosis, diffuse, mild.
5. Spleen: EMH, multifocal, moderate.
6. Lymph node, mesenteric: Sinus histiocytosis, diffuse, moderate.
7. Lymph node, mesenteric: Hyperplasia, lymphoid, diffuse, moderate.
8. Lymph node, submandibular: Sinus plasmacytosis, diffuse, mild.
9. Heart: Infiltrates, lymphocytic, focal, minimal.
10. Kidney, right and left: Mineral, corticomedullary, multifocal, mild.
12. Esophagus; trachea; larynx; parathyroid gland; lung; tongue; submandibular salivary gland; thymus; cerebrum; cerebellum; pituitary gland; stomach; duodenum; pancreas; jejunum; ileum; cecum; colon; rectum; haired skin; mammary gland; skeletal muscle peripheral nerve; adrenal gland; urinary bladder; uterus; ovary; spinal cord; joint; bone; bone marrow; harderian gland; eye; optic nerve; extraorbital lacrimal gland: No significant findings.

Gross necropsy findings: Dark spleen with constriction; right and left kidney pale and mottled; cage bedding in intestinal tract.

20 dose group
Male

11-0106

1. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
2. Kidney, left: Basophilic tubules, focally extensive, mild.
3. Spleen: Hemosiderosis, multifocal, mil.
4. Adrenal gland; thymus; testes; epididymis: No significant findings.

Gross necropsy findings: None

11-0120

1. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
2. Kidney, right and left: Infiltrates, lymphocytic, interstitial, multifocal, minimal.
3. Spleen: Hemosiderosis, multifocal, mild.
4. Spleen: EMH, multifocal, minimal.
5. Spleen: Hyperplasia, multifocal, minimal.
6. Adrenal gland; thymus; testes; epididymis: No significant findings.

Gross necropsy findings: Friable liver.

11-0121

1. Kidney, left: Infiltrates, lymphocytic, interstitial, focal, minimal.
2. Liver: Cellular alteration, focal, minimal.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
4. Spleen: Hemosiderosis, multifocal, minimal.
5. Spleen: EMH: multifocal, minimal.
6. Adrenal gland; thymus; testes; epididymis: No significant findings.

Gross necropsy findings: None

11-0125

1. Kidney, left: Infiltrates, lymphocytic, interstitial, focal, minimal.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
3. Adrenal gland; spleen; thymus; testes; epididymis: No significant findings.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Spleen: EMH, multifocal mild.
6. Testes, seminiferous tubules: Multinucleated giant cells, multifocal, minimal.

Gross necropsy findings: Overall body condition obese.

11-0127

1. Kidney, right: Basophilic tubules, focal, minimal.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
3. Epididymis: Infiltrates, lymphocytic, interstitial, multifocal, minimal.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Spleen: EMH, multifocal, minimum.
6. Adrenal gland; thymus; testes: No significant findings.

Gross necropsy findings: None

11-0130

1. Adrenal gland, right and left: Infiltrates, lymphohistiocytic, multifocal, moderate with lipofuscin and cortical cell hypertrophy.
2. Kidney, right and left: Infiltrates, lymphocytic, interstitial, multifocal, minimal.
3. Kidney, left: Basophilic tubule, focal, minimal.
4. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
5. Spleen: Hemosiderosis, multifocal, mild.
6. Spleen: EMH, multifocal, minimum.
7. Epididymis: Infiltrates, lymphocytic, multifocal, minimal.
8. Thymus; testes: No significant findings.

Gross necropsy findings: Overall body condition obese.

11-0133
1. Kidney, right and left: Infiltrates, lymphocytic, interstitial, multifocal, minimal.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, moderate.
3. Spleen: Hemosiderosis, multifocal, mild.
4. Adrenal gland; thymus; testes; epididymis: No significant findings.

Gross necropsy findings: None

11-0137
1. Kidney, right: Infiltrates, lymphocytic, interstitial, multifocal, minimal.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
3. Liver: Fibrosis, capsular and subcapsular, focally extensive, severe with hepatocellular hypertrophy, biliary hyperplasia, neovascularization, hemosiderin-laden macrophages and lymphocytes.
4. Testes: Infiltrates, lymphocytic, interstitial, focal, minimal.
5. Spleen: Hemosiderosis, multifocal, mild.
6. Adrenal gland; thymus; epididymis: No significant findings.

Gross necropsy findings: Adhesion left liver lobe.

11-0139
1. Kidney, right: Pelvic dilatation, diffuse, severe with medullary tubular atrophy and loss.
2. Liver: infiltrates, lymphohistiocytic, multifocal and random, moderate.
3. Artery, liver: Fibroblast proliferation, tunica adventitia, mild with lymphocytic infiltrates.
4. Spleen: Hemosiderosis, multifocal, minimum.
5. Spleen: EMH, multifocal, minimum.
6. Adrenal gland; thymus; epididymis: No significant findings.

Gross necropsy findings: Right kidney, hydronephrosis.

11-0148
1. Kidney, left: Infiltrates, lymphocytic, interstitial, multifocal, mild.
2. Kidney, left: Cyst, focal.
3. Liver: Infiltrates. Lymphohistiocytic, multifocal and random, minimal.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Kidney; adrenal gland; thymus; testes; epididymis: No significant findings.

Gross necropsy findings: None.

20 dose group
Female

11-0153
1. Kidney, right: Mineral, corticomedullary, multifocal, minimal.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
3. Spleen: Hemosiderosis, multifocal, mild.
4. Spleen: EMH, multifocal, mild.

5. Thymus; adrenal gland: Significant findings.

Gross necropsy findings: None.

11-0155

1. Kidney, right and left: Mineral, corticomedullary, multifocal, minimal.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
3. Uterus: Dilatation, diffuse, moderate.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Adrenal gland; thymus; ovary: No significant findings.

Gross necropsy findings: Hydrouterus (Hydrometra)

11-0160

1. Kidney, left: Mineral, corticomedullary, multifocal, mild.
2. Kidney, left: Infiltrates, lymphohistiocytic, focal, minimal.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Adrenal gland; thymus: No significant findings.

Gross necropsy findings: Mild hydrouterus (hydrometra); cage bedding throughout intestinal tract.

11-0164

1. Kidney, right: Mineral, corticomedullary, multifocal, minimal.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
3. Spleen: Hemosiderosis, multifocal, mild.
4. Adrenal gland; thymus: No significant findings.

Gross necropsy findings: None

11-0167

1. Kidney, right and left: Mineral, corticomedullary, multifocal, mild.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
3. Spleen: Hemosiderosis, multifocal, mild.
4. Adrenal gland; thymus: No significant findings.

Gross necropsy findings: Cage bedding in stomach and intestines.

11-0179

1. Kidney, right: Infiltrates, lymphocytic, pelvic, focally extensive, mild.
2. Kidney, right and left: Mineral, corticomedullary, multifocal, mild.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Spleen: EMH, multifocal, mild.
6. Adrenal gland; thymus: No significant findings.

Gross necropsy findings: Hydrouterus. (Hydrometra)

11-0183

1. Kidney, right and left: Mineral, corticomedullary, multifocal, minimal.
2. Spleen: Hemosiderosis, multifocal, mild.

3. Spleen: EMH, multifocal, mild.
4. Adrenal gland; thymus: No significant findings.

Gross necropsy findings: Mottled right and left kidneys.

11-0184

1. Kidney, left: Infiltrates, lymphocytic, interstitial, focal, minimal.
2. Kidney, right and left: Mineral, corticomedullary, multifocal, minimal.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
4. Spleen: Hemosiderosis, multifocal, minimal.
5. Adrenal gland; thymus: No significant findings.

Gross necropsy findings: None

11-0194

1. Kidney, left: Mineral, corticomedullay, multifocal, minimal.
2. Lymph node, submandibular: Sinus plasmacytosis, diffuse, moderate.
3. Lymph node, submandibular: Hyperplasia, follicular, diffuse, moderate.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Spleen: EMH, multifocal, mild.
6. Adrenal gland; thymus; submandibular salivary gland: No significant findings.

Gross necropsy findngs: Right and left enlarged submandibular lymph nodes; cage bedding in intestine.

11-0198

1. Adrenal gland: Infiltrates, lymphohistiocytic, multifocal, minimal with lipofuscin pigment.
2. Kidney, right and left: Mineral, corticomedullary, multifocal, moderate.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Thymus: No significant findings.

Gross necropsy findings: Cage bedding in intestinal tract.

5 dose group
Male

11-0100

1. Liver: Microvacuolation, diffuse, moderate.
2. Spleen: Hemosiderosis, multifocal, minimal.
3. Spleen: EMH, multifocal, minimal.
4. Kidney: No significant findings.

Gross necropsy findings: Diffusely mottled liver.

11-0101

1. Kidney, left: Basophilic tubules, focal, with lymphocytic infiltrates.
2. Liver: Microvacuolation, portal and centrilobular, diffuse, moderate.
3. Heart: Fibrosis, multifocal, mild with myofiber hypertrophy, disarray, degeneration and loss.

4. Heart: Infiltrates, histiocytic, focal, mild.
5. Lung: Hemosiderin laden macrophages, multifocal, moderate with congestion, erythrophagocytosis and rare eosinophilic crystals.
6. Spleen: Hemosiderosis, multifocal, mild.
7. Spleen: EMH, multifocal, mild.

Gross necropsy findings: Overall body condition obese; enlarged heart; pale liver with 3mm dark area; right and left pale kidney;

11-0115

1. Kidney, right and left: Basophilic tubules, multifocal, mild.
2. Spleen: Hemosiderosis, multifocal, minimal.
3. Spleen: EMH, multifocal, minimal.
4. Liver: Infiltrates, lymphohisitocytic, multifocal and random, minimal.

Gross necropsy findings: Mildly pale liver; right and left mottled kidneys

11-0117

1. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
2. Spleen: Hemosiderosis, multifocal, mild.
3. Kidney: No significant findings.

Gross necropsy findings: Overall body condition obese; right and left pale kidneys with dark areas; cage bedding in stomach and cecum.

11-0124

1. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
2. Spleen: Hemosiderosis, multifocal, minimal.
3. Kidney: No significant findings.

Gross necropsy findings: Cage bedding in stomach

11-0131

1. Kidney, left: Infiltrates, lymphocytic, interstitial, focal, minimal.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
3. Liver: Vacuolation, macro-, centrilobular, multifocal, mild.
4. Spleen:Hemosiderosis, multifocal, minimal.

Gross necropsy findings: Generalized small liver.

11-0135

1. Kidney, left: Basophilic tubules, multifocal, minimal.
2. Kidney, left: Infiltrates, lymphocytic, interstitial, focal, minimal.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, severe with rare hepatocellular necrosis.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Spleen: EMH, multifocal, minimal.

Gross necropsy findings: Overall body condition obese.

11-0138

1. Kidney, left: Infiltrates, lymphocytic, interstitial, focal, minimal.

2. Kidney, left: Pelvic dilatation, minimal.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
4. Spleen: Hemosiderosis, multifocal, minimal.

Gross necropsy findings: Right and left kidneys mottled

11-0141

1. Liver: Infiltrates, lymphohistiocytic, focal, minimal.
2. Spleen: Hemosiderosis, multifocal, mild.
3. Spleen: EMH, multifocal, minimal.
4. Kidney: No significant findings.

Gross necropsy findings: Overall body condition obese.

11-0146

1. Kidney, right and left: Basophilic tubules, multifocal, minimal.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
3. Spleen: Hemosiderosis, multifocal, minimal.
4. Spleen: EMH, multifocal, minimal.

Gross necropsy findings: None

5 dose group
Female

11-0156

1. Kidney, right and left: Mineral, corticomedullary, multifocal, minimal.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
3. Spleen: Hyperplasia, lymphoid, multifocal, minimal.
4. Spleen: Hemosiderosis, multifocal, mild.

Gross necropsy findings: None

11-0157

1. Kidney, right and left: Mineral, multifocal, mild.
2. Kidney, left: Infiltrates, lymphocytic, interstitial, focal, minimal.
3. Spleen: Hyperplasia, follicular, lymphoid, diffuse, moderate.
4. Spleen: Fibrosis, focal with hemosiderin laden macrophages and lymphocytic infiltrates.
5. Spleen: Hemosiderosis, multifocal, mild.
6. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
7. Liver: Kupffer cell proliferation, multifocal, moderate.
8. Liver: Bile duct hyperplasia, multifocal, minimal.
9. Liver: Infiltrates, lymphocytic, portal, diffuse, mild.
10. Adrenal gland; thymus; ovary; pancreas: No significant findings.

Gross necropsy findings: Enlarged irregular spleen; abdominal adhesion from spleen to left abdominal wall; adhesion from spleen to left ovary; left liver lobe 4mm pale area; adhesion from pancreas to spleen; cage bedding in cecum.

11-0163

1. Kidney, right and left: Mineral, multifocal, mild.
2. Kidney, right and left: Infiltrates, lymphocytic, focal, minimal.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, moderate.
4. Spleen: Hemosiderosis, multifocal, moderate.
5. Spleen: EMH, multifocal, mild.

Gross necropsy findings: Right and left kidneys mottled; cage bedding in intestine.

11-0166

1. Kidney, left: Infiltrates, lymphocytic, interstitial, multifocal, mild.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
3. Lung: Alveolar histiocytosis, multifocal, minimal.
4. Ovary: Atrophy, diffuse, moderate.
5. Spleen: Hemosiderosis, multifocal,mild.
6. Spleen: Hyperplasia, lymphoid, multifocal, minimal.
7. Uterus: No significant findings.

Gross necropsy findings: Lungs mottled red; right ovary hemorrhagic cyst.

11-0174

1. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
2. Kidney, right and left: Mineral, corticomedullary, multifocal, mild.
3. Kidney, right: Infiltrates, lymphocytic, focal, minimal.
4. Spleen: Hemosiderosis, multifocal, minimal.
5. Spleen: EMH, multifocal, mild.

Gross necropsy findings: Cage bedding in stomach and cecum.

11-0187

1. Kidney, left: Mineral, focal, minimal.
2. Kidney, right and left: Infiltrates, lymphocytic, focal, minimal.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
4. Liver: Biliary hyperplasia, focally extensive, mild.
5. Spleen: Hyperplasia, lymphoid, multifocal, mild.
6. Spleen: Hemosiderosis, multifocal, moderate.

Gross necropsy findings: Cage bedding in stomach and cecum.

11-0189

1. Kidney, right and left: Mineral, corticomedullary, multifocal, mild.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
3. Uterus: Dilatation, unilateral, moderate.
4. Spleen: Hemosiderosis, multifocal, minimal.
5. Ovary: No significant findings.

Gross necropsy findings: Right and left kidney mildly pale; right side-hydrouterus (hydrometra)

11-0193

1. Lymph node, submandibular: Sinus plasmacytosis, diffuse, mild.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.

3. Spleen: Hemosiderosis, multifocal, minimal.
4. Submandibular salivary gland; kidney: No significant findings.

Gross necropsy findings: Right and left enlarged submandibular lymph nodes; right and left pale kidneys.

11-0202
1. Kidney, right and left: Mineral, corticomedullary, multifocal, mild.
2. Kidney, right and left: Infiltrates, lymphohistiocytic, interstitial, multifocal, mild.
3. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Spleen: EMH, multifocal, mild.

Gross necropsy findings: None.

11-0204
1. Kidney, right and left: Mineral, corticomedullary, multifocal, mild.
2. Liver: Infiltrates, lymphohistiocytic, multifocal and random, mild.
3. Spleen: Hemosiderosis, multifocal, mild.

Gross necropsy findings: None

1.25 group
Male

11-0103
1. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
2. Spleen: Hemosiderosis, multifocal, mild.

Gross necropsy findings: Enlarged spleen; enlarged left liver lobe

11-0095
1. Spleen: Hemosiderosis, multifocal, minimal.

Gross necropsy findings: Enlarged left liver lobe; obese.

11-0113
1. Spleen: Hemosiderosis, multifocal, minimal.
2. Spleen: EMH, multifocal, minimal.

Gross necropsy findings: Mildly mottled liver; obese.

11-0114
1. Liver: Microvacuolation, diffuse, mild.
2. Spleen: Hemosiderosis, multifocal, mild.
3. Spleen: EMH, multifocal, mild.

Gross necropsy findings: Pale liver.

11-0134
1. Kidney, left: Tubular loss, multifocal, mild with tubular regeneration, dilatation, fibrosis and lymphocytic infiltrates.
2. Kidney, right: Basophilic tubules, focal, minimal.

3. Spleen: Hemosiderosis, multifocal, minimal.

Gross necropsy findings: Overall body condition obese; mottled kidneys.

11-0142
1. Liver: Infiltrates, lymphohistiocytic, multifocal and random, minimal.
2. Spleen: Hemosiderosis, multifocal, minimal.

Gross necropsy findings: Liver: raised nodule.

11-0122
1. Spleen: Hemosiderosis, multifocal, mild.

Gross necropsy findings: Obese

11-0104
1. Spleen: Hemosiderosis, multifocal, minimal.

Gross necropsy findings: Small amount of cage bedding in stomach.

11-0126
1. Spleen: Hemosiderosis, multifocal, minimal.
2. Spleen: EMH, multifocal, minimal.

Gross necropsy findings: Cage bedding in cecum.

11-0145
1. Spleen: Hemosiderosis, multifocal, minimal.

Gross necropsy findings: None

1.25 group
Female

11-0152
1. Spleen: Hemosiderosis, multifocal, mild.
2. Spleen: EMH, multifocal, minimal.

Gross necropsy findings: Mild staining of hair around eyes.

11-0159
1. Spleen: Hemosiderosis, multifocal, mild.

Gross necropsy findings: Cage bedding in stomach and cecum.

11-0165
1. Spleen: Hemosiderosis, multifocal, mild.

Gross necropsy findings: Right lobe of liver 4mm pale area

11-0170
1. Spleen: Hemosiderosis, multifocal, moderate.
2. Spleen: EMH, multifocal, minimal.

Gross necropsy findings: None

11-0172
1. Spleen: Hemosiderosis, multifocal, moderate.
2. Spleen: EMH, multifocal, mild.

Gross necropsy findings: Right and left kidneys mottled and pale; cage bedding in stomach and cecum.

11-0176
1. Spleen: Hemosiderosis, multifocal, mild.

Gross necropsy findings: Cage bedding in stomach.

11-0178
1. Hemosiderosis, multifocal, moderate.

Gross necropsy findings: Dark spleen; cage bedding in stomach.

11-0181
1. Hemosiderosis, multifocal, mild.

Gross necropsy findings: None

11-0192
1. Spleen: Hemosiderosis, multifocal, mild.

Gross necropsy findings: Cage bedding throughout intestinal tract.

11-0196
1. Spleen: Hemosiderosis, multifocal, mild.

Gross necropsy findings: None

APPENDIX B
SEVERITY GRADES

Lung, BALT: Hyperplasia, lymphoid
Minimal = minimal elevation of mucosa, mainstem bronchi; affecting one lung lobe
Mild = mild elevation of mucosa, mainstem bronchi with 1-2 increased bronchiolar lymphocytes; affecting more than one lung lobe.
Moderate = moderate elevation of mucosa, mainstem bronchi with increased bronchiolar lymphocytes, greater than mild criteria; affecting majority of lung lobes.
Severe = moderate to severe elevation of mucosa, mainstem bronchi and bronchiolar lymphocytes with or without follicle formation and generally affecting all lung lobes.

Liver: Infiltrates, lymphohistiocytic (microgranulomas)
Minimal = 0-4 foci
Mild = 5-14 foci
Moderate = 15-25 foci
Severe = > 25 foci

Lymph node: Plasmacytosis
Minimal = few plasma cells within sinuses with majority of cells lymphocytes and macrophages
Mild = increased numbers of plasma cells than minimal with resident lymphocytes and macrophages outnumbering plasma cells
Moderate = plasma cells filling sinuses, outnumbering resident lymphocytes and macrophages
Severe = plasma cells diffusely expanding sinuses with few resident lymphocytes and macrophages, with or without expansion into cortex

Lung: Alveolar histiocytosis
Minimal = minimal increase of alveolar macrophages from resident cells, affecting 1 or more lung lobes
Mild = mild increase of alveolar macrophages, multifocal distribution, affecting 25-50% of lung lobe, few lobes involved
Moderate = moderate increase of alveolar macrophages, multifocal distribution, affecting 50-75% of lung lobe, majority of lobes involved
Severe = severe increase of alveolar macrophages, multifocal to diffuse distribution, affecting > 75% of lung lobe, majority of lobes involved

Heart: Infiltrates, mononuclear
Minimal = focal, few cells
Mild = multifocal, few cells
Moderate = focally extensive or multifocal with moderate number of cells, affecting some surrounding tissue architecture
Severe = focally extensive or multifocal with numerous cells, significantly affecting surrounding tissue architecture

Kidney: Mineral, corticomedullary
Minimal = focal area, unilateral or bilateral
Mild = multifocal, few areas, unilateral or bilateral
Moderate= multifocal, moderate, bilateral
Severe = diffuse corticomedullary, bilateral

Spleen: Hemosiderosis
(Minimal = multifocal throughout red pulp, few hemosiderin laden cells, acceptable background in male or female and not coded)
(Mild = multifocal throughout red pulp, increased hemosiderin laden cells from minimal, acceptable background in females, not coded)
Moderate= Diffusely throughout red pulp
Severe = Diffusely throughout red pulp, possibly filling red pulp area.

Testis: Degeneration and atrophy
Minimal = Less than five tubules demonstrate partial (degeneration) or complete (atrophy) loss of germ cells.
Mild = More than five tubules but less than 25% of tubules demonstrate partial (degeneration) or complete (atrophy) loss of germ cells.
Moderate = Between 25 – 75% of tubules demonstrate partial (degeneration) or complete (atrophy) loss of germ cells.
Severe = Greater than 75% of tubules demonstrate partial (degeneration) or complete (atrophy) loss of germ cells.

Appendix X

Summary of Benchmark Dose Modeling

Table X-1
Protocol No. 0DBP-38-10-07-01
Subchronic Oral Toxicity of 2,4-Dinitroanisole in Rats

Summary of Benchmark Dose Modeling of Extramedullary Hematopoeisis in Female Rats

Model	p value	AIC	Scaled residual at 0	Scaled residual at 1.25	BMD	$BMDL_{10}$
Gamma	0.3204	52.9455	-1.229	1.221	4.084	2.307
Logistic	0.3434	53.4892	-1.522	0.816	9.757	6.087
Log-logistic	0.2145	53.2526	-0.927	1.463	1.972	0.736
Multistage 2	0.1761	54.9430	-1.243	1.205	4.251	2.307
Multistage 3	0.1839	54.9207	-1.266	1.175	4.524	2.312
Probit	0.3432	53.4180	-1.518	0.821	9.687	6.510
Log-Probit	0.1499	55.8901	-1.520	0.908	10.062	4.195
Quantal-linear	0.3204	52.9455	-1.229	1.221	4.084	2.307
Weibull	0.3204	52.9455	-1.229	1.221	4.084	2.307

Appendix Y

Study Protocol with Modifications

ANIMAL USE PROTOCOL
TOXICOLOGY DIRECTORATE
U.S. ARMY PUBLIC HEALTH COMMAND
ABERDEEN PROVING GROUND, MD 21010-5403

PROTOCOL TITLE: The Subchronic Oral Toxicity of 2,4-Dinitroanisole (DNAN) in Rats (*Rattus norvegicus*)

PROTOCOL NUMBER: ØDBP-38-10-07-01

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I. NON-TECHNICAL SYNOPSIS

The oral toxicity of 2,4-Dinitroanisole (DNAN), an insensitive, energetic material used in explosive formulations, will be determined using a series of three laboratory studies in rats. The first study will be the approximate lethal dose (ALD) procedure. Based upon the results of the ALD, a 14-day oral toxicity study will be performed in order to learn the effects and tolerance of repeated daily dosing with DNAN. This study will serve as a range finding tool for the more definitive 90-day subchronic oral toxicity study. Since the data generated from these studies will be used to generate occupational exposure guidelines, the 14- and 90-day studies will be performed regardless of the outcome of the acute study. Rats will be dosed with DNAN via oral gavage and monitored throughout the study observation periods for body weight and

clinical signs. At the conclusion of the exposure/observation period for each portion of the study, the rats will be anesthetized, blood samples collected from animals in the 14-day and 90-day studies, and a necropsy will be performed. The EPA Health Effects Test Guidelines (OPPTS 870.1100 Acute Oral Toxicity and OPPTS 870.3100 90-Day Oral Toxicity Study in Rodents) state the rat is the preferred species for these studies. Rats have been historically used for oral toxicity studies and are therefore the recommended species due to the extensive historical database.

II. BACKGROUND

II.1. Background: DNAN (CAS # 119-27-7) is a tan powder with a wax-like consistency that is practically insoluble in water (reference 1). It is classified as a flammable solid and is being investigated as a less-sensitive replacement for 2,4,6-Trinitrotoluene (TNT) in melt-cast insensitive munition formulations. DNAN is used industrially in the synthesis of dyes and has been used as an insecticide in the past by the US Military. The use of DNAN as an energetic material in explosive formulations dates back to World War II when it was used as the main ingredient in Amatol 40 for various warheads. At the time, DNAN's use as an ingredient in explosive formulations was based primarily on the scarcity of higher performance materials, such as TNT. Renewed interest in the energetic properties of DNAN has been fueled by the need to develop munitions that are less prone to inadvertent initiation during transport and routine handling. The reduced sensitivity to environmental stimuli and nearly equal performance during testing make DNAN-based formulations desirable replacements for currently fielded munitions. [Reference 2] To ensure its safe use by military personnel and production employees handling the material on a daily basis, the repeated-dose toxicity of DNAN must be investigated.

II.2. Literature Search for Duplication:

II.2.1. Literature Source(s) Searched: Medline, TOXFILE, FEDRIP, BIOSIS, EMBASE, CA SEARCH, DTIC, BRD

II.2.2. Date of Search: 09 June 2010

II.2.3. Period of Search: 1926 - 2010

II.2.4. Key Words of Search: dinitroanisole, DNAN, toxic, rats

II.2.5. Results of Search: The literature search revealed four articles pertaining to the toxicity of DNAN, however, no repeated-dose oral toxicity studies on DNAN were found that would suggest that this study would be a duplicate effort. The majority of the toxicity studies on DNAN were performed for an occupational exposure level assessment on Picatinnay Arsenal Explosive-21 (PAX-21), an explosive mixture containing DNAN, RDX, ammonium perchlorate, and MNA. The table below provides an overview of the toxicity information available on DNAN. Although an oral LD_{50} value was reported as part of the PAX-21 assessment, this value was from a single acute

study and has not been verified. The ALD procedure is being performed to confirm the reported LD_{50} prior to initiation of the repeated dose studies to prevent gross errors in selection of doses for the 14-day study leading to deaths or a need to repeat the study. Confirming the LD_{50} using the ALD procedure will provide data that are more accurate and potentially minimize animal use. None of the remaining studies provides information relevant to a repeated dose exposure via the oral route. As such, the present study is not a duplication of the information available in the literature.

STUDY	RESULT
Acute Oral LD_{50} (rats)	199 mg/kg
Primary skin irritation (rabbits)	Slight irritation w/reversibility in 24-48 hrs
Primary eye irritation (rabbits)	Mild irritation w/reversibility in 48 hrs
Dermal sensitization (guinea pigs)	Not a sensitizer
Ames Assay	Mutagenic to bacteria; Not mutagenic to mammalian cells
In vivo mouse bone marrow micronucleus assay	Not genotoxic
In vitro dermal penetration	0.74 ug/cm^2-hr steady state flux
Acute inhalation (rats)	Non-toxic at 3 mg/m^3 (highest achievable concentration)
Subacute inhalation (rats) w/DNAN dissolved in acetone	Mortality and clinical effects at 500 and 1500 mg/m^3 w/microscopic findings at 150 mg/m^3. Clinical effects also observed in acetone controls.

III. OBJECTIVE/HYPOTHESIS

The objective of this study is to determine the oral LD_{50} resulting from the acute oral administration of DNAN, and to determine if adverse effects occur from a subacute (14-day) and subchronic (90-day) repetitive oral exposure regime of DNAN to male and female laboratory rats.

IV. MILITARY RELEVANCE

As a result of the Department of Defense (DOD)-wide initiative to improve munitions safety, the US Army is developing insensitive munitions (IM) for incorporation into its inventory of conventional ammunition and missiles. The Army's IM Program is dedicated to developing munitions that reliably perform as they are intended but are less prone to inadvertent initiation from external stimuli such as bullet/fragment impact, heat from fire, and shock from neighboring explosions (reference 3). The production of insensitive munitions requires the use of intrinsically insensitive explosives that contribute to lower order responses to inadvertent external stimuli. Despite the slightly lower performance of DNAN compared to TNT, there has been a renewed interest in its use in explosive formulations based on the lower sensitivity as a melt-cast medium observed during testing and the less stringent shipping requirements. This has lead to the development of a range of DNAN-based melt-castable explosives at Picatinny Arsenal (collectively known as "PAX" explosives) (reference 2). To support possible fielding of these PAX explosives, a Toxicity Clearance would have to be granted and

occupational exposure guidelines developed. Consequently, repeated-dose toxicity data in a mammalian system need to be generated to assess any health hazards associated with the use of this material.

V. MATERIALS AND METHODS

V.1. Experimental Design and General Procedures: This study consists of three experiments: an acute test (ALD), a 14-day, and a 90-day repeated dose test to test the oral toxicity of DNAN in the rat. Rats will be dosed with DNAN via oral gavage and monitored throughout the study observation periods for body weight and clinical signs. At the conclusion of the exposure/observation period for each portion of the study, the rats will be anesthetized, blood samples collected from animals in the 14-day and 90-day study, and a necropsy will be performed. The 14-day and 90-day tests will both include a vehicle control group.

V.1.a. Administration of Test Substance: Oral dosing will be performed using a stainless steel 16 ga x 2 inch gavage needle. As per EPA Health Effects Test Guidelines, the volume given will not exceed 10 ml/kilogram of body weight (reference 4). The test material will be analyzed for purity prior to study initiation. All concentration verification analysis of the dosing solutions and stability analyses will be performed by USAPHC, Directorate of Laboratory Sciences (DLS), Method Development Team (MDT) IAW DLS SOP 801.

V.1.b. Study Conduct: The study described will be conducted in a manner consistent with the principles of the Good Laboratory Practice (GLP) regulations in the Toxic Substances Control Act (TSCA): 40 CFR (Code of Federal Regulations) 792, plus amendments (reference 5). The investigators and technicians will adhere to The Guide for Care and Use of Laboratory Animals (reference 6).

V.1.c. Study Timeframe: July 2010 - January 2011

V.1.1. Experiment 1: Acute Test

The objective(s) of this portion of the study is to determine the acute oral LD_{50} and slope of DNAN to the Sprague-Dawley rat and to set dosage levels for the subacute (14-day) study. The general procedures of this acute study will follow the Directorate of Toxicology (DTOX) Standing Operating Procedure (SOP) for the ALD Procedure (SOP #017) as well as the EPA Health Effects Test Guidelines for Acute Oral Toxicity (OPPTS 870.1100) (references 7&4). All oral dosing will be administered using a gavage needle (16 ga. x 2 inches). The DNAN will be dissolved/suspended in an appropriate diluent (i.e., corn oil) to facilitate the oral gavage procedure. This phase of the study will require 12 male and 12 female young adult Sprague-Dawley rats with one rat/sex receiving a graduated dose. Dose intervals will be set at approximately 1.5x the previous dose up to a maximum of 2000 mg/kg. Based on the solubility of DNAN in the diluent, it may be necessary to give the higher dose animals multiple doses within a 24-hour period to stay within the 10 ml/kg maximum dosage volume. All dosed animals will

be held for a 14-day observation period during which time clinical observations will be taken daily (5 days/week) and body weights will be taken at least weekly. Following the 14-day observation period, all animals will be humanely euthanized with CO_2 and submitted for gross necropsy. The total number of animals necessary for this test, as described, is 24.

V.1.2. Experiment 2: 14-Day Repeated Dose Test

The purpose of the 14-day range finding study is to determine if there are any adverse effects from short-term repetitive oral exposures and to set dosage levels for the subchronic study. This test will follow the procedures outlined in TOX SOP #037 (reference 8). Briefly, 7 dose groups, consisting of 6 males and 6 females for each dose group, along with a vehicle control group [N=(6+6)x8=96], will be orally dosed with DNAN in an appropriate vehicle, as determined by the ALD, via oral gavage for 14-days (7 days per week). Dose selection will depend on the results of the ALD (e.g., 1x, 0.75x, 0.5x, 0.25x, 0.125x, 0.0625x, 0.03125x of the LD_{50}). The vehicle control group will receive a volume equivalent to the highest exposure group. Based on previous animal shipments involving underweight/young rats, 2 additional rats of each sex will be ordered to ensure the study is initiated using the required 6 animals/sex/dose group. The rats used for weight matching but not placed on study will either be transferred to another protocol or humanely euthanized per study guidance. On the day following the administration of the last dose of the test substance, animals will be anesthetized, bled, euthanized, and necropsied. The following tissues will be harvested and weighed: adrenals, brain, heart, kidneys, epididymides, liver, ovaries, spleen, testes, thymus, and uterus. This tissue list may be altered at the discretion of the study staff based on observed toxicity and gross pathology findings. All gross pathology changes will be recorded on PHC form 333. If a necropsy cannot be performed immediately after a deceased animal is discovered, death will be ensured by a thoracotomy and the animal will be refrigerated at temperatures low enough to minimize autolysis. The total number of animals necessary for this test, as described, is 100.

Blood will be collected and evaluated per TOX SOP #053 (reference 9). Briefly, 2-3 ml of blood will be collected via cardiac puncture following anesthesia (isoflurane or CO_2 gas). A portion of blood will be transferred to a 1.3 ml EDTA microtube and evaluated for total red blood cell and white blood cell counts, packed cell volume, hemoglobin, and five-part differential. A portion will be transferred to a 1.3 ml serum-gel microtube and evaluated for the following chemistries: BUN, CREA, GLU, TP, ALB, ALT, ALK P, AST, GLOB, CHOL, LDH, TBIL, CA, PHOS, and electrolytes. The remainder will be transferred to a 1.3 ml microtube for analysis of prothrombin time.

V.1.3. Experiment 3: 90-Day Subchronic Study

The main element will be the 90-day subchronic oral toxicity study, which can be found in TOX SOP #037 (reference 8). Since the study must be conducted in such a manner that it can be submitted to the EPA, this procedure shall closely adhere to the EPA Health Effects Testing Guidelines for 90-Day Oral Toxicity Study in Rodents

(reference 10). The route of administration will be by oral gavage with pure test compound suspended/dissolved in an appropriate vehicle, dosed through a 16 ga. x 2 inch gavage needle 7 days per week for a period of 90 days. Fifty rats of each sex (N=100) will be distributed into 4 dose groups and a vehicle control group (10 rats of each sex per dose group). Dosages will be based on the results of the 14-day repeated dose study. Based on previous animal shipments involving underweight/young rats, 2 additional rats of each sex will be ordered to ensure the study is initiated using the required 10 animals/sex/dose group. The rats used for weight matching but not placed on study will either be transferred to another protocol or humanely euthanized per study guidance.

In addition to the main study, 15 rats of each sex (N=30) may be added to serve as satellite groups for the two highest test dose groups and the control group (5 rats/sex/group). These animals will be dosed concurrently with the main study animals for 90-days (7 days/week) and held for a period of approximately one month following dosing. The purpose of the satellite group is to evaluate the reversibility, persistence, or delayed occurrence of toxic effects associated with subchronic exposure to DNAN. The use of satellite groups will be determined based on the results of the 14-day study (i.e. gross necropsy observations and organ weight effects).

Four additional animals of each sex (N=8) will be ordered for health monitoring purposes. Two animals of each sex will be sent to an approved vendor to determine health status at the end of the acclimation period and again near the end of the study per TOX SOP #028 (reference 11). The total number of animals necessary to perform this test, as described, is 142.

All animals in the main study (not the satellite groups) will have an ophthalmological examination as per TOX SOP #096, prior to DNAN administration (reference 12). Surviving rats in the high dose and control groups will have an ophthalmological examination at the termination of the study. If changes in the eyes are detected in the high dose or control groups, all animals in the other dose groups will be examined also.

Urinalysis (using timed urine collection) will be performed using metabolism cages on at least 8 animals per dose group during weeks 11-13 of the study. All urinalysis procedures will follow those outlined in TOX SOP #100 (reference 13).

In addition to the general clinical observations taken daily by the study director or co-investigators, functional observation battery (FOB) procedures will also be conducted. Once prior to initiation of treatment and once weekly during treatment of the animals a careful clinical examination will be performed, at similar times of the day, outside the home cage (preferably in a standard arena). Once near the end of the exposure, but not prior to week 11, sensory reactivity to stimuli of different types (i.e., elicited responses for visual, auditory, and proprioceptive stimuli), grip strength, and motor activity tests will be conducted. These FOB procedures are outlined in TOX SOP #138 (reference 14)

At the termination of the study, the day following the administration of the last dose of the test substance, each animal will be anesthetized via isoflurane or CO_2 gas, bled, euthanized, and submitted for a full gross necropsy. All blood drawing procedures will follow TOX SOP #053 (reference 9). Briefly, 2-3 ml of blood will be collected via cardiac puncture following anesthesia (isoflurane or CO_2 gas). A portion of blood will be transferred to a 1.3 ml EDTA microtube and evaluated for total red blood cell and white blood cell counts, packed cell volume, hemoglobin, and five-part differential. A portion will be transferred to a 1.3 ml serum-gel microtube and evaluated for the following chemistries: BUN, CREA, GLU, TP, ALB, ALT, ALK P, AST, GLOB, CHOL, LDH, TBIL, CA, PHOS, and electrolytes. The remainder will be transferred to a 1.3 ml microtube for analysis of prothrombin time. All gross pathology changes will be recorded on PHC form 333. If a necropsy cannot be performed immediately after a deceased animal is discovered, appropriate measures will be taken to ensure the animal is dead,and the animal will be refrigerated at temperatures low enough to minimize autolysis. The following organs and tissues, or representative samples, will be preserved in a suitable medium for future histopathological examination: all gross lesions; brain (including sections of medulla/pons, cerebellar cortex and cerebral cortex); pituitary; thyroid/parathyroid; thymus; lungs and trachea; pharynx; larynx; nose; heart; bone marrow (either femur, sternum, or rib at the costochondral junction); salivary glands; liver; spleen; kidney; adrenals; pancreas; testes; uterus; aorta; esophagus; stomach; duodenum; jejunum; ileum; caecum; colon; rectum; urinary bladder; representative lymph node; peripheral nerve; trachea; mammary gland; thigh musculature; eyes; femur (including articular surface); spinal cord at three levels (cervical, midthoracic, and lumbar) and exorbital lachrymal glands. In addition, the following organs will be weighed: liver, kidneys, adrenals, gonads, spleen, brain, epididymides, uterus, thymus and heart. This tissue list may be altered at the discretion of the study staff based on observed toxicity and gross pathology findings. Prior to being weighed, organs will be carefully dissected and trimmed to remove fat and other tissue in a uniform manner. Full histopathological examinations will be performed on organs and tissues of all animals in the control and high dosage groups. Further histopathology in other dosage groups will be carried out on organs which show lesions similar to those observed in the high dosage group or for which clinical observations indicate such a need. Animals in the satellite groups, if used, will undergo a full necropsy using the procedures described above approximately one month after the 90-day study period.

Treatment Group	Test Animals		
ACUTE STUDY:	Males	Females	
LD50	12	12	
Sub-Total	**12**	**12**	**24**
14-DAY STUDY:	Males	Females	
Vehicle Control	6	6	
Dose TBD	6	6	
Dose TBD	6	6	
Dose TBD	6	6	
Dose TBD	6	6	
Dose TBD	6	6	
Dose TBD	6	6	
Dose TBD	6	6	
Weight Matching Animals	2	2	
Sub-Total	**50**	**50**	**100**
90-DAY STUDY:	Males	Females	
Vehicle Control	10	10	
Satellite Group*	5	5	
Dose TBD	10	10	
Dose TBD	10	10	
Dose TBD	10	10	
Satellite Group*	5	5	
Dose TBD	10	10	
Satellite Group*	5	5	
Weight Matching Animals	2	2	
Health Monitoring	4	4	
Sub-Total	**71**	**71**	**142**
Total	**133**	**133**	
Grand Total			**266**

* Satellite groups are optional and will be based upon the toxicological effects observed during the 14-day study.

V.2. Data Analysis: For variables that are measured only at the end of the study, the dose groups will be compared using a one-factor analysis of variance (ANOVA). Organ to brain and organ to body weight ratios will be calculated and analyzed similarly to the other parameters measured at the end of the study. If the dose group effect is significant, post hoc tests will be used to compare pairs of dose groups and dose groups to the control group; a Tukey's multiple comparison test if the variance of the groups is similar and a Dunnett's T3 test if the variances are unequal. Variance equality will be determined by a Levene's test.

For absolute organ weights, comparison of the dose groups will be made using an analysis of covariance (ANCOVA), with body weight at the end of the study being the covariate used. Even though the dose groups will be assigned at Day 0 to keep the average weight for each dose group similar, the weights can change during the study

dependent on the dose group. The ANCOVA will adjust for any differences in body weights among the dose groups at the end of the study, because heavier animals would tend to have heavier organs. If the dose group effect is significant, a least significant differences post hoc test will be used to compare pairs of dose groups and dose groups to the control group.

Dose groups will also be compared with respect to absolute body weights, as well as weekly changes in body weight and net weight changes using a one-factor ANOVA. Dose groups will also be compared with respect to net food consumption for the study using a one-factor ANOVA. If the ANOVA is significant, the post hoc tests will be used to compare pairs of dose groups; a Tukey's multiple comparison test if the variance of the groups are similar and a Dunnett's T3 test if the variance are unequal. Variance equality will be determined by a Levene's test.

For FOB data, a Chi-square test will be used to compare treatment groups for categorical data. If significant effects are observed, either a Fisher's exact test or a Kruskal-Wallis test will be used to compare pairs of treatment groups. Interval data will be analyzed for differences between treatment groups using an ANOVA followed by a Tukey's multiple comparison test if the variance of the groups is similar and a Dunnett's T3 test if the variances are unequal. Variance equality will be determined by a Levene's test. Responses for each week and sex will be analyzed separately.

Other observational data including gross necropsy observations and histopathology data may be converted to categorical data and analyzed using a Chi-square test. If significant effects are observed, either a Fisher's exact test or a Kruskal-Wallis test will be used to compare pairs of treatment groups.

SPSS 16.0 will be used to perform all analyses and statistical significance will be defined as $p \leq .05$ for all tests.

Sample sizes were selected in accordance with the EPA Health Effects Testing Guidelines for 90-Day Oral Toxicity Studies in Rodents (reference 10). These samples sizes have been widely used and have been demonstrated to provide adequate statistical power in this test.

Records will be kept in standard USAPHC laboratory notebooks and/or three ring binders. 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. These records will be made available to oversight organizations such as the US EPA, AAALACi, 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 USAPHC.

V.3. Laboratory Animals Required and Justification

V.3.1. Non-animal Alternatives Considered: The objective(s) addressed by this study are adverse health effects of oral exposures of DNAN to the laboratory rat. This data will aid in the development of occupational exposure guidelines and will be used to compare the toxicity of DNAN to that of currently fielded energetics. To date, there are no non-animal models, which would provide the necessary toxicological information on DNAN to allow for an accurate comparison with previously performed animal testing on other explosives. Therefore, it is necessary to perform these studies with DNAN in an animal model.

V.3.2. Animal Model and Species Justification: The EPA Health Effects Test Guidelines (OPPTS 870.1100 Acute Oral Toxicity and OPPTS 870.3100 90-Day Oral Toxicity Study in Rodents) state that the rat is the preferred species. Sprague-Dawley rats have been historically used for oral toxicity studies in the USAPHC DTOX and are therefore the recommended species due to the extensive historical database.

V.3.3. Laboratory Animals

V.3.3.1. Genus and Species: *Rattus norvegicus*

V.3.3.2. Strain/Stock: Sprague-Dawley

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

V.3.3.4. Age: Acute – 7-9 weeks
14-Day – 6-8 weeks
90-Day – 6-8 weeks

V.3.3.5. Weight: Age appropriate

V.3.3.6. Sex: Male and female

V.3.3.7. Special Considerations: None

V.3.4. Number of Animals Required (By Species): 266 Rats

V.3.5. Refinement, Reduction, Replacement

V.3.5.1. Refinement:
No additional refinements will be employed other than the environmental enrichment strategy.

V.3.5.2. Reduction:
The ALD procedure will use fewer animals than other traditional methods of LD_{50}

determination. This method was selected based on the unverified acute test performed on DNAN for the PAX-21 assessment.

Tissue sharing may be allowed. Tissues will only be shared after all samples for the study have been collected and only if doing so will not affect the outcome of the study.

V.3.5.3. Replacement: There is no acceptable methodology available to replace these studies.

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: 16

V.4.1.1.1.2. Column C: 12

V.4.1.1.1.3. Column D: 160

V.4.1.1.1.4. Column E: 78

V.4.1.2. Pain Relief/Prevention

V.4.1.2.1. Anesthesia/Analgesia/Tranquilization: Anesthesia will be administered prior to cardiac blood collection and euthanasia for the 14- and 90-day studies. Anesthesia will consist of isoflurane or CO_2 gas. For isoflurane anesthesia, study staff will ensure the oxygen tank and isoflurane levels are sufficiently full and scavenger canisters are connected to both exhaust lines. The stopcock to the box will be turned to the open position and the stopcock to the nosecone to the off position. The oxygen tank will be turned on, the flow meter set to 1 L/min, the rat placed in the plastic box, and the lock latched. The isoflurane valve will be turned to approximately 3%. Once the rat is sufficiently anesthetized (immobile and not responsive to tapping on the box), the stopcock to the nosecone will be switched to on and the stopcock to the box to off. The rat will be transferred to the nosecone and it will be ensured that the rat is still sufficiently anesthetized, based on lack of responsiveness to toe-pinch, before performing terminal blood sampling. For CO_2 anesthesia, study staff will ensure that the CO_2 tank is sufficiently full and connected to the CO_2 chamber. The rat will be placed in the CO_2 chamber, the lid put on the chamber, and the CO_2 valve turned on at a low flow (approx, ¼ turn on the tank valve). When the rat is sufficiently anesthetized (faint breathing pattern) it will be removed from the chamber, quickly placed on a necropsy board and it will be ensured that the rat is sufficiently anesthetized, based on lack of responsiveness to toe-pinch, before performing terminal blood sampling.

V.4.1.2.2. Pre- and Post-procedural Provisions: Rats will be fasted overnight prior to dosing for the acute study as per EPA Acute Oral Guidelines (reference 4). Food may be withheld for an additional 3-4 hours post-dosing, but the total fasting period will not exceed 16 hours. Food may also be withheld overnight (no more than 16 hours) prior to necropsy for the 14- and 90-day studies. Food withheld prior to necropsy will be removed from the cage, weighed and processed, and will not be left hanging on the outside of the cage. In addition to signs of aspiration, which animals will be monitored for immediately following the oral gavage procedure and while being returned to their cages, a careful clinical examination will be made at least once each day during the observation period. Appropriate actions will be taken to minimize loss of animals to the study (e.g., necropsy or refrigeration of those animals found dead). Observations will be detailed and carefully recorded in LABCAT, the laboratory notebook or an appropriate spreadsheet. Observations will include, but not be limited to, evaluation of skin and fur, eyes and mucous membranes, respiratory and circulatory effects, autonomic effects such as salivation, central nervous system effects, including tremors and convulsions, changes in the level of activity, gait and posture, reactivity to handling or sensory stimuli, altered strength, and stereotypes or bizarre behavior (e.g., self mutilation, walking backwards). Observations, body weight, and food consumption frequency is described in detail for each study phase in section V.5.2.1.

V.4.1.2.3. Paralytics: None

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

V.4.1.3.1. Sources Searched: Medline, TOXFILE, AGRICOLA, BIOSIS, EMBASE, CA SEARCH

V.4.1.3.2. Date of Search: 09 June 2010

V.4.1.3.3. Period of Search: 1926-2010

V.4.1.3.4. Key Words of Search: dinitroanisole, DNAN, toxic, alternative, welfare, method, model, in vitro, pain, distress, simulate, video, computer, replacement, refinement, reduction

V.4.1.3.5. Results of Search: The literature search identified 20 references pertaining to alternatives to painful procedures. However, no alternatives to the painful procedures (i.e. illness and cardiac bleed) in this protocol or methods to relieve pain or distress without altering the outcome of the study were found. Since the goal of this investigation is to determine the effects from oral exposure (lethality from a one-time administration for the LD50, and sublethal effects from repetitive exposures in the 14- and 90-day studies), the observation of illness associated with toxicity is necessary. However, moribund animals or animals in overt pain unlikely to recover will be humanely euthanized as described in section V.4.6. (Euthanasia). Alternative methods of blood collection are available, however, due to the volume of blood required for the

hematology and clinical chemistry tests being performed, the intracardiac bleed method is necessary. For this procedure, the rats will be anesthetized to minimize pain. Because no validated in vitro tests are currently available to replace in vivo oral toxicity studies, this protocol must be conducted in vivo, necessitating possible painful procedures or illness.

V.4.1.4. Unalleviated Painful/Distressful Procedure Justification:

The nature of these studies precludes the use of totally painless procedures. An attempt to alleviate pain or distress by the administration of anesthetics, analgesics, or drugs may alter the manifestation of the toxic responses. Typical pain relievers such as opiates and non-steroidal anti-inflammatories as well as anesthetics have the ability to mask certain toxic signs that may be observed due to the administration of the test compound, especially those signs resulting from pain or distress. In addition, certain side effects such as alterations in blood chemistry and hematology may arise from the use of these drugs and could be misinterpreted by the investigator as clinical signs caused by the test material. The observation of the onset, duration and/or reversibility of toxic signs is critical to mechanistic interpretation, especially since the acute study is being used to set dosages for a longer-term study. "Toxic signs" are defined in TOX SOP #063 (reference 15). See V.4.6. for criteria for early removal from testing.

To prevent undue suffering, moribund animals will be euthanized as described in section V.4.6. Discussions were held with the Attending Veterinarian regarding the painful procedures. The use of analgesics in this model is untested and may alter the response to the compound, thus compromising the results of the experiments. Moribund animals will be euthanized to minimize distress. The minimal number of animals needed for statistical significance will be used. The final number of rats in each pain category will be reported to the IACUC annually and at the completion of the in-life portion of the protocol.

V.4.2. Prolonged Restraint: Not applicable

V.4.3. Surgery: None

V.4.3.1. Pre-surgical Provisions: Not applicable

V.4.3.2. Procedure: Not applicable

V.4.3.3. Post-surgical Provisions: Not applicable

V.4.3.4. Location: Not applicable

V.4.3.5. Surgeon: Not applicable

V.4.3.6. Multiple Major Survival Operative Procedures: None

V.4.3.6.1. Procedures: Not applicable

V.4.3.6.2. Scientific Justification: Not applicable

V.4.4. Animal Manipulations:

V.4.4.1. Injections: None

V.4.4.2. Biosamples: Approximately 2-3 ml of blood will be taken just prior to euthanasia for the 14- and 90-day studies. All blood sampling will occur under isoflurane or CO_2 gas anesthesia via cardiac puncture using an 18-21 gauge, 1-1.5 inch needle, as outlined in TOX SOP #053 (reference 9). Biosampling will be promptly followed by euthanasia via CO_2.

Urine will be collected from at least 8 rats for urinalysis during weeks 11-13 of the 90-day study. Rats will be placed in metabolism cages overnight for approximately 15-16 hours and free-catch urine will be collected. All urinalysis procedures will follow those outlined in TOX SOP #100 (reference 13).

V.4.4.3. Adjuvants: Not applicable

V.4.4.4. Monoclonal Antibody (MAbs) Production: Not applicable

V.4.4.5. Animal Identification: Individual animals will be identified by cage card and/or tail marking (number written on the tail with water-insoluble/permanent marker) for the acute and 14-day studies by cage card and/or tail marking or subcutaneous transponder for the 90-day study, according to TOX SOP #003 (reference 16). LABCAT may be used for the 90-day phase of the study in which case rats will be identified using subcutaneous transponders. Appropriately trained study staff will insert subcutaneous transponders. All animals will be assigned a unique identification number as per TOX SOP #003 (reference 16).

V.4.4.6. Behavioral Studies: A neurotoxicity screen and a functional observation battery test will be performed on all rats in the 90-day study as per TOX SOP #138 (reference 14). Once prior to initiation of treatment and once weekly during treatment of the animals a careful clinical examination will be performed, at similar times of the day, outside the home cage (preferably in a standard arena). Once near the end of the exposure, but not prior to week 11, sensory reactivity to stimuli of different types (i.e., elicited responses for visual, auditory, and proprioceptive stimuli), grip strength, and motor activity tests will be conducted.

V.4.4.7. Other Procedures: The method of test substance administration will be oral gavage. Each rat will be gently restrained by placing the index and middle finger on either side of the animal's neck with the remainder of the hand used to support the body. Just prior to dosing, the index and middle finger can be used to tilt the animal's head back and the gavage needle is inserted into either the side or the top of the mouth.

The gavage needle is then gently slid down the animal's esophagus until the hub of the gavage needle is at the opening of the animal's mouth. The 16 gauge x 2 inch gavage needle is the correct length to allow for the proper placement of the test material in rats. If any resistance is felt during the gavage procedure, the gavage needle is removed and the animal is briefly released before the procedure is attempted again. Once the material has been dispensed, the animal is briefly observed for any signs of aspiration.

V.4.4.8. Tissue Sharing: Tissue sharing may be allowed. Tissues will only be shared after all samples for the study have been collected and only if doing so will not affect the outcome of the study and if the requestor has an approved protocol.

V.4.5. Study Endpoint: The study endpoint is intervention euthanasia of moribund animals or euthanasia at the conclusion of the observation or dosing periods. The duration of the observational period for the acute test will not exceed 14 days. The study endpoint for the 14-day and 90-day study will be euthanasia on the day following the final administration of the test substance. Although some form of euthanasia is the projected study endpoint, the possibility still exists that a compound-related death may occur during an unobserved period (i.e., overnight). The novelty of the compound being tested prevents the assurance that a compound-related death may not occur. Any animal exhibiting the criteria for moribundity will be humanely euthanized. Intervention euthanasia will be conducted on moribund animals. Animals will be assessed for moribundity based on a weight of evidence of the following signs: impaired ambulation, which prevents animals from reaching food or water; excessive weight loss and extreme emaciation ($\geq$ 20% body weight); lack of physical or mental alertness; prolonged difficult/labored breathing; or a prolonged inability to remain upright. The Attending Veterinarian may be consulted, if needed, to evaluate potentially moribund animals, unless the PI/SD plans to immediately euthanize the animal.

The time at which signs of toxicity appear, their duration, and the time to death are important, especially if there is a tendency for deaths or morbidity to be delayed or if the signs of toxicity are reversible or recovery is possible. This is particularly important in the acute study when the type, onset and duration of toxic signs are still unknown. As such, potentially moribund animals will be monitored, in consultation with the Attending Veterinarian, during the acute study for possible reversal and recovery of toxic signs.

At the end of the observation or dosing period, all surviving animals will be anesthetized for cardiac blood sampling (14- and 90-day studies), euthanized by CO_2, and necropsied.

V.4.6. Euthanasia: Euthanasia will be accomplished by asphyxiation from CO_2 exposure according to TOX SOP #066 (reference 17) and death will be ensured with a thoracotomy prior to necropsy. Study staff will euthanize the animals.

V.5. Veterinary Care

V.5.1. Husbandry Considerations: The animals may be pair-housed (same sex) in

solid bottom shoebox cages and given water *ad libitum* throughout the acclimation period, but will be single-housed during all phases of the study. Animals will be fasted overnight for no more than 16 hours prior to dosing for the acute study; otherwise, they will be given certified rodent feed *ad libitum* as per TOX SOP #017 (reference 7). Although food will be given *ad libitum*, food intake will be monitored during the 14-day and 90-day studies. As such, feeders must be weighed by the study staff before and after providing additional food to the animals. Animals may be fasted overnight, for no longer than 16 hours, prior to necropsy for the 14- and 90-day studies. Food withheld prior to necropsy will be removed from the cage, weighed and processed, and will not be left hanging on the outside of the cage. Animal rooms will be maintained at the conditions specified in TOX SOP #004 (reference 18). Animals will undergo an acclimation period of no less than 5 days after their arrival in the animal facility.

V.5.1.1. Study Room: Studies will be conducted at the USAPHC Toxicology Directorate animal facility, Bldg E-2100 or Bldg E-2101, study room as assigned.

V.5.1.2. Special Husbandry Provisions: Animals may be pair-housed (same sex) during the acclimation period for all tests. Animals will be singly housed during study conduct for all tests due to the unknown toxicity of the test substance and food consumption monitoring on the 14- and 90-day studies. Certified enrichment (i.e., nylabones) may be provided throughout all phases of the study. Enrichment may be removed for observation of animals, but will be replaced following observation. Food enrichment may not be used for the 14- and 90-day studies due to food consumption monitoring. Due to food consumption monitoring, feeders must be weighed by the study staff before and after providing additional food to the animals. Additionally, if feeders are to be replaced for cleaning, the old and new feeders must be weighed and the difference in the weight of the feeders accounted for in the food consumption. As such, feeders should only be replaced by study staff during weekly feed weighing. The SD/PI will coordinate with the animal care staff prior to scheduled feeder replacement and cleaning. During weeks 11-13 of the 90-day study, rats will be placed in metabolism cages overnight for approximately 15-16 hours and free-catch urine will be collected.

V.5.1.3. Exceptions: Animals may be weighed several times throughout the acclimation period by study personnel to ensure they are gaining weight and in good health. Animals will not be weighed within the first 24 hours after arrival and will be weighed no more than once every other day. Food enrichment may not be used for the 14- and 90-day studies due to food consumption monitoring. Enrichment may be removed for observation of animals, but will be replaced following observation.

V.5.2. Veterinary Medical Care

V.5.2.1. Routine Veterinary Medical Care: All animals will be observed daily by assigned Veterinary Medicine personnel for husbandry conditions, humane care, and general health. Animals will be observed at least twice daily by assigned Veterinary Medicine personnel (once daily on weekends and holidays). The study staff will take observations at the time of dosing and at least one additional time throughout the day

on weekdays to adequately monitor during the light cycle. Observations will include, but not be limited to: evaluation of skin and fur, eyes and mucous membranes, respiratory and circulatory effects, autonomic effects such as salivation, central nervous system effects, including tremors and convulsions, changes in the level of activity, gait and posture, reactivity to handling or sensory stimuli, altered strength, and stereotypes or bizarre behavior (e.g., self mutilation, walking backwards). Observations by study personnel will be documented in LABCAT and/or in the study notebook or appropriate data entry sheet and a notation stating that the animals were observed and indicating any animals with marked weight loss or overt signs of toxicity will be entered into the animal room logbook. Appropriate actions will be taken to minimize loss of animals to the study (e.g., necropsy or refrigeration of those animals found dead). If the observed toxicity indicates a need for more frequent observations, the Attending Veterinarian will consult with the PI/SD.

For the acute study, individual weights of animals will be determined shortly before the test substance is administered, at least weekly thereafter, and at the end of the study. Changes in weights will be calculated and recorded when survival exceeds one day. The time of death will be recorded as precisely as possible. For the 14-day study, body weights will be recorded in the study notebook or appropriate data entry table on days -3, -1, 0, 1, 3, 7, and 14. Feeder weights will be recorded in the study notebook or appropriate data entry table on days 0, 7, and 14. For the 90-day study, body weights will be recorded in LABCAT or the study notebook or appropriate data entry table on days -3, -1, 0, and weekly thereafter and feeder weights will be recorded on day 0 and weekly thereafter. Weekly food consumption data may not be obtained for the week that urinalysis is being performed because the animals are fasted overnight while in the metabolism cages.

V.5.2.2. Emergency Veterinary Medical Care: All emergency animal health care will be provided by the Veterinary Medical staff in consultation with the PI whenever possible.

V.5.3. Environmental Enrichment

V.5.3.1. Enrichment Strategy: All enrichment will be provided in accordance with TOX SOP #122 (reference 19). Animals will be handled on a frequent basis and provided a form of enrichment (e.g., nylabones) throughout the study.

V.5.3.2. Enrichment Restriction: Rodent chow blocks will not be placed on cage floors for animals during the acclimation period of the acute study due to overnight fasting or during the 14- and 90-day studies due to food consumption monitoring. Food enrichment will not be used for the 14- and 90-day studies due to food consumption monitoring.

VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING:

Staff Member	Procedure	Training	Experience	Qualifications
Lee Crouse	Handling, Weighing, Oral gavage, Observations, CO2 Euthanasia, Microchipping, Cardiac Bleeding, Urine Collection, Anesthesia Necropsy,	Rodent handling techniques, WRAIR (11/1996); Rat handling and oral gavage training (7/2007, 3/2008, 5/2008, 5/2009); Implanting microchip IDs (CHPPM 6/2000); Rat cardiac bleeding under isoflurane (12/2008, 5/2009); Rat necropsy training (10/2007, 12/2007);	16+ Yrs Animal Research	M.S., Environmental Science
Emily LaFiandra	Handling, Weighing, Oral gavage, Observations, CO2 Euthanasia, Microchipping, Cardiac Bleeding, Urine Collection, Anesthesia Necropsy	Rat handling, gavage, injections, blood collection, & euthanasia training (CHPPM, 7/2007); Rat oral gavage training (3/2008, 5/2009); Rat bleeding techniques & tissue collection (4/2008); Rat saphenous bleeding (CHPPM 3/2008, 3/2009); Necropsy training (7/2007, 10/2007, 4/2008)	11+ Yrs Animal Research	M.S., Wildlife Biology; Ph.D., Natural Resources and Environmental Studies
John Houpt	Handling, Weighing, Observations, Necropsy, CO2 Euthanasia, Anesthesia	Rodent Handling Workshop, USAMRICD (11/2003); Rat necropsy training- tissue collecting & separating (CHPPM 3/2008, 12/2008)	23+ Yrs Animal Research	B.S., Biology
Michael Quinn	Handling, Weighing, Observations Oral gavage CO2 Euthanasia, Necropsy	Rodent & Small Animal Handling workshop (MRICD 6/2005); Rat oral gavage (CHPPM 3/2008); Necropsy training- rats (5/2005, 10/2007, 12/2009)	13+ Yrs Animal Research	M.S., Biology; Ph.D., Animal Science
Art O'Neill	Handling, Weighing, Observations, Necropsy	Rat oral gavage training (3/2008); Rat necropsy training (12/2007)	20+ Yrs Animal Research	B.S., Biology; LATG
Wilfred McCain	Handling, Observations, Weighing, Necropsy	Necropsy training (12/2007, 2/2008, 12/2008, 2/2009)	30+ Yrs Animal Research	Ph.D., Toxicology

Terry Hanna	Handling, Weighing, Oral gavage, Observations, CO2 Euthanasia, Anesthesia Necropsy Functional Observation Battery (FOB),	Rodent Handling & Techniques (3/1992); Rodent & Small Animal Handling Workshop (MRICD 2004, 2005, 2006); Rat handling and gavage training (CHPPM 2007), Rat oral gavage training (10/2004, 3/2008, 5/2009); Rat euthanasia via CO2 with thoracotomy (3/2009); Rat isoflurane anesthesia, cardiac blood draw, & CO2 euthanasia training (5/2009); Necropsy training (2/2009, 1/2010); Functional observation battery (FOB) training (5/2007, 8/2008, 1/2009); Acoustic Startle Response (handheld clicker & startle chamber operations) (1/2009)	15+ Yrs Animal Research	LAT
Alicia Bonney	Handling, Observations, Weighing, Necropsy	Rat handling & techniques training: observations, handling/restraint, oral gavage, weighing, basic injections, basic bleeding (CHPPM, 11/2008); Rat CO2 euthanasia with thoracotomy (3/2009); Rat necropsy training- tissue collecting & separating (CHPPM 3/2008, 1/2010)	2 Yrs Animal Research	Associates Degree, Histology/Science

VII. BIOHAZARD/SAFETY: In accordance with PHC Regs. 385-1 and 385-5 and TOX SOP# 083, standard laboratory protection, e.g., glasses, gloves, and gown, may be used. Test substances shall 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 (references 20, 21, and 22).

VIII. ENCLOSURES:

A. Appendix A – References

IX. ASSURANCES:

IX.1. As the Study Director/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 coordinations 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: 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 and WILL NOT 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.

Emily May LaFiandra

Emily May LaFiandra — Signature

20100729 — Date (YYYYMMMDD

IX.2. As the Primary Co-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. 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.

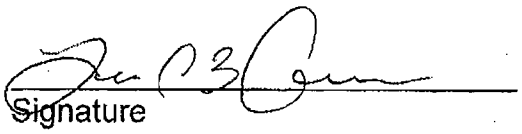
C. Training: I verify that I am technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

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/WILL NOT 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.

Lee C.B. Crouse

Primary Co-Investigator



Signature

20100729

Date (YYYYMMMDD

IX.3. ASSURANCES: 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. This potential pain and/or distress WILL/WILL NOT be relieved with the use of anesthetics, analgesics and/or tranquilizers. I will follow the direction of the SD/PI relative to potential pain and/or distress and relief by the use of anesthetics, analgesics and/or tranquilizers.

(PRINT)	(Signature)	(Date)
Theresa L. Hanna	Theresa L Hanna	7/29/10
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

1. Material Safety Data Sheet (MSDS), 2,4-Dinitroanisole, Ordnance Systems, Inc., 4509 West Stone Drive, Kingsport, TN 37660-9982, June 2, 2005.

2. Davies, P.J. and Provatas, A. Characterisation of 2,4-Dinitroanisole: An Ingredient for Use in Low Sensitivity Melt Cast Formulations. Defence Science and Technology Organisation, PO Box 1500, Edinburgh, South Australia 5111 Australia, DSTO-TR-1904, 2006.

3. Duncan, Kendal. 2002. Insensitive Munitions and the Army: Improving Safety and Survivability. Army Logistician PB700-02-1, Volume 34, Issue 1: 16-17.

4. Health Effects Test Guidelines. OPPTS 870.1100, Acute Oral Toxicity Study in Rodents. EPA 712–C–98–199, August 1998.

5. Title 40, Code of Federal Regulations (CFR), current revisions, Parts 160 and 792, Good Laboratory Practice Standards.

6. National Research Council. Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington, D.C., 1996.

7. USAPHC DTOX SOP No. OT017-T-001, Approximate Lethal Dose Procedures.

8. USAPHC DTOX SOP No. OT037-T-001, 14-Day Range Finding and 90-Day Oral Toxicity Study in Rodents.

9. USAPHC DTOX SOP No. SOP AP053-P-001, Animal Bleeding Technique.

10. Health Effects Test Guidelines. OPPTS 870.3100, 90-Day Oral Toxicity Study in Rodents. EPA 712–C–98–199, August 1998.

11. USAPHC DTOX SOP No. AP028-P-001, Animal Quality Control Procedures.

12. USAPHC DTOX SOP No. AP096-P-001, Ophthalmic Examinations.

13. USAPHC DTOX SOP No. AP100-P-001, Urinalysis.

14. USAPHC DTOX SOP No. AP138-T-001, Neurotoxicity Screen and Functional Observation Battery.

15. USAPHC DTOX SOP No. AP063-P-001, Test System Observations.

16. USAPHC DTOX SOP No. AP003-P-001, Individual Animal Identification.

17. USAPHC DTOX SOP No. AP066-P-001, Animal Euthanasia.

18. USAPHC DTOX SOP No. AP004-P-001, Animal Health Technician Duties within the Animal Facility.

19. USAPHC DTOX SOP No. AP122-P-001, Rodent and Rabbit Environmental Enrichment.

20. USAPHC Regulation 385-1, Safety and Occupational Health Program, May 01, 2001.

21. USAPHC Regulation 385-5, Occupational Health and Safety of Animal Users, June 2007.

22. USAPHC DTOX SOP No. GL083-P-001, Health and Safety of Laboratory Personnel.

PROTOCOL REVIEW, SUPPORT, APPROVAL SHEET

PROTOCOL NUMBER:	TITLE:
0DBP - 38 - 10-07-01 SUB-JONO TEST TYPE IACUC NUMBER	The Subchronic Oral Toxicity of 2,4-Dinitroanisole (DNAN) in Rats (*Rattus norvegicus*)

1. SCIENTIFIC MERIT (PEER REVIEW)			
1a. Printed Name (First, MI, Last) John Houpt	1b. Title Biologist	1c. Signature HOUPT.JOHN.TIMOTHY.1229302597	1d. Date (yyyy/mm/dd) 20100624
2. DIRECTOR			
2a. Printed Name (First, MI, Last) LTC Cindy A. Landgren	2b. Title Director, Toxicology, LTC, VC	2c. Signature LANDGREN.CINDY.ANNE.1163891359	2d. Date (yyyy/mm/dd) 20100624
3. PROGRAM MANAGER			
3a. Printed Name (First, MI, Last) Glenn J. Leach	3b. Title Program Manager, Toxicity Evaluation Program	3c. Signature	3d. Date (yyyy/mm/dd) 20100624
4. ATTENDING VETERINARIAN			
4a. Printed Name (First, MI, Last) MAJ Anne M. MacLarty, DVM, DACLAM	4b. Title Command Animal Programs Manager	4c. Signature MACLARTY.ANNE.MITCHELL.1094566530	4d. Date (yyyy/mm/dd) 20100628
5. ANALYTICAL CHEMISTRY (If Applicable)			
5a. Printed Name (First, MI, Last) David F. Morrow	5b. Title Consultant, DLS	5c. Signature	5d. Date (yyyy/mm/dd) 20100629
6. SAFETY MANAGER			
6a. Printed Name (First, MI, Last) Roy Valiant	6b. Title Safety Manager	6c. Signature VALIANT.ROY.A.1081780591	6d. Date (yyyy/mm/dd) 20100624
7. STATISTICIAN (If Applicable)			
7a. Printed Name (First, MI, Last) Robyn B. Lee	7b. Title Biostatistician	7c. Signature LEE.ROBYN.BELLEK.1267390289	7d. Date (yyyy/mm/dd) 20100707

PROTOCOL NUMBER: 0DBP - 38 - 10-07-01 (SUB-JONO / TEST TYPE / IACUC NUMBER)	TITLE: The Subchronic Oral Toxicity of 2,4-Dinitroanisole (DNAN) in Rats (Rattus norvegicus)		
8. SIO-QAT (GLP COMPLIANCE AND QA SUPPORT)			
8a. Printed Name (First, MI, Last) Michael P. Kefauver	8b. Title Quality Assessor, Quality Systems Office	8c. Signature KEFAUVER.MICHAEL.P.1229209678	8d. Date (yyyy/mm/dd) 20100624
9. CHAIRMAN, IACUC			
9a. Printed Name (First, MI, Last) Jesse J. Barkley	9b. Title Chairman, IACUC	9c. Signature Jesse Barkley	9d. Date (yyyy/mm/dd) 20100729
10. INSTITUTIONAL OFFICIAL			
10a. Printed Name (First, MI, Last) Stephen L. Kistner	10b. Title Deputy, Technical Services	10c. Signature KISTNER.STEPHEN.L.1228741481	10d. Date (yyyy/mm/dd) 20100729
11. STUDY DIRECTOR/PRI CIPAL INVESTIGATOR			
11a. Printed Name (First, MI, Last) Emily May LaFiandra	11b. Title Toxicologist	11c. Signature Emily May LaFiandra	11d. Date (yyyy/mm/dd) 20100803
12. OTHER ORGANIZATION(S) PROVIDING SUPPORT (AS NEEDED):			
12a. Printed Name (First, MI, Last)	12b. Title	12c. Signature	12d. Date (yyyy/mm/dd)
13. STUDY SPONSOR:			
13a. Printed Name (First, MI, Last)	13b. Title	13c. Signature	13d. Date (yyyy/mm/dd)

USACHPPM PROTOCOL MODIFICATION

For use of this form, see DTOX SOP 085

1. DATE: (YYYY/MM/DD) 2010/09/08	2. PROTOCOL NUMBER: 0DBP-38-10-07-01	3. MODIFICATION#: 1

4. PROTOCOL TITLE: The Subchronic Oral Toxicity of 2,4-Dinitroanisole (DNAN) in Rats (Rattus norvegicus)

5. STUDY DIRECTOR/PRINCIPAL INVESTIGATOR:	6. WORK PHONE:	7. OFFICE SYMBOL:
Emily LaFiandra	5-7749	MCHB-TS-TTE

SECTION I - PREVIOUSLY APPROVED AND CURRENTLY IN USE PROTOCOL MODIFICATIONS

1. MODIFICATION NUMBER	2. SHORT DESCRIPTION OF PRIOR APPROVED MODIFICATION(S)	3. NO. & SPECIES OF ANIMAL REQUESTED	4. APPROVED DATE (XX XXX XXXX)

SECTION II - CHANGE IN TOTAL # OF ANIMALS USED AND/OR CHANGE IN USDA PAIN CATEGORY

1a. CHANGE: INCREASE TOTAL APPROVED ANIMALS BY: 0 — 1b. N/A ☒

2. ORIGINAL PROTOCOL TOTAL: 266 — 3. PROTOCOL TOTAL AFTER MODIFICATION: 266

2a. USDA pain cat:	B: 16	C: 12	D: 160	E. 78	3a. USDA pain cat:	B: 16	C: 12	D: 160	E: 78

4. Yes	No	
☐	☒	Modification requires specific changes or additions to the experimental design of the protocol. (Section V.I. of the template.)
☐	☒	Modification requires changes to the technical methods, i.e., procedures, routes of administration, biosample collection, etc. (Section V.4. of the protocol template.) Indicate training of personnel for new methods, procedures being used.
☐	☒	Modification requires additions or changes in personnel performing procedures. (Section VI of the protocol template.) Include training and qualification information and tasks that each individual will be performing. If changing the Study Director/PI, a signed Assurance Statement needs to be submitted with the modifications.

SECTION III - MODIFICATION JUSTIFICATION

Explain the modification indicated above in the area below. Indicate any changes to the 3 R's (Refinement, Reduction, Replacement) resulting from changes in number of animals.

Protocol Page, Paragraph, Section	
V.5.1.3., pg 16, Exceptions and V.5.3.1., pg 17, Enrichment Strategy	1. MODIFICATION: Animals will be handled by the study staff and/or the veterinary staff up to twice per day during the acclimation period for socialization/conditioning.
	1a. JUSTIFICATION/REASON: This will help acclimate the animals to handling. This will reduce the stress associated with the handling and dosing procedure and will help facilitate dosing as the animals will be calmer and easier to handle.

Protocol Page, paragraph, section	Explain the modification selected above in the box below. Indicate any changes to the IRB, Refinement, Reduction, Replacement, resulting new changes in number of animals used.
V.5.1., pg 15, Husbandry Considerations and V.5.1.2., pg 16, Special Husbandry Provisions	2. MODIFICATION: When possible, animal husbandry procedures will be conducted after the completion of daily FOB and dosing. The SD and FOB technician will coordinate timing with the primary technician and/or the technician team leader.
	2a. JUSTIFICATION/REASON: This will provide for consistent timing of both dosing and FOB which will provide better quality data. Additionally, delaying animal husbandry activities until after FOB will prevent impacts of animal husbandry activities on FOB results.
	3. MODIFICATION:
	3a. JUSTIFICATION/REASON:
	4. MODIFICATION:
	4a. JUSTIFICATION/REASON:

Continued on next page YES ☐ NO ☒

SECTION IV: SIGNATURES AND DATES

1. STUDY DIRECTOR: (Printed Name) Emily LaFiandra	Signature Emily May LaFiandra	DATE: (yyyy/mm/dd) 20100908
2. PROGRAM MANAGER:: (Printed Name) Glenn Leach	Signature	DATE: (yyyy/mm/dd) 20100909
3. ATTENDING VETERINARIAN: (Printed Name) Anne M. MacLarty, MAJ, VC	Signature Anne M. MacLarty DVM	DATE: (yyyy/mm/dd) 20100909
4. CHPPM SAFETY OFFICER/OCC HEALTH REP: (IF APPLICABLE) Roy A. Valiant	Signature	DATE: (yyyy/mm/dd)
5. CHAIR, IACUC OR QA (If no animal related changes): (Printed Name) Jesse J. Barkley	APPROVED / REVIEWED YES ☑ NO ☐ Signature Jesse Barkley	DATE: (yyyy/mm/dd) 20100909