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Subacute toxic effects of TNB in F344 male and female rats were evaluated by feeding powdered certified laboratory chow diet supplemented with varied concentrations of TNB (0, 50, 200, 400, 800 and 1200 mg/kg diet) so as to achieve a final target dose of (0, 5, 20, 40, 80 and 120 mg TNB/kg body weight) for fourteen days. The calculated average daily TNB doses (50, 200, 400, 800 and 1200 mg/kg) for males was 4, 16, 34, 55 and 94 mg/kg b.w. and for females 4, 17, 34, 58 and 79 mg/kg b.w. Food intake by male and female rats consuming 1200 mg TNB/kg diet was reduced and resulted in a significant decrease in absolute body weights. A decrease in testicular weight in males and an increase in spleen weight of both sexes fed 800 or 1200 mg TNB/kg diet were noted. Histopathological changes for TNB toxicity (200-1200 mg/kg) were evident in the kidneys (hyaline droplets), spleen (extramedullary hematopoiesis) and testes (seminiferous tubular degeneration). Hematology and clinical chemistry studies indicated a decrease in red blood cell count and hematocrit (400-1200 mg/kg), a decrease in alkaline phosphatase (400-1200 mg/kg) and an increase in Heinz bodies (800-1200 mg/kg) as compared to controls in both sexes. Methemoglobin levels were also significantly increased (400-1200 mg/kg).

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QUALITY ASSURANCE STATEMENT

The portions of this toxicology project performed and reported by Pathology Associates, Inc. has been inspected and audited by the quality assurance unit as required by the Good Laboratory Practice (GLP) standards promulgated by the U.S. Environmental Protection Agency. Results of these activities indicate that the portions of the study performed and reported by PAI conformed with GLP standards and applicable Standard Operating Procedures. The following table is a record of the inspections/audits performed and reported by the QAU.

Date of Inspection	Phase Inspected	Date Findings Reported to Management and Study Director
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06-22-94	Final	06-22-94
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10-09-92	Data	10-09-92
09-08-92	Processing	09-09-92
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09-02-92	Necropsy	09-04-92
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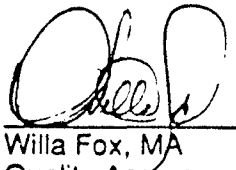

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PAI-Cin

7-14-94
Date

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Compliance Statement

This study was conducted in compliance with the Good Laboratory Practice Regulations as set forth in Title 21 of the U.S. Code of Federal Regulations Part 792 issued August 17, 1989. All deviations from the protocol and/or GLPs are listed in Appendix K. There were no deviations from the aforementioned regulations which affected the quality or integrity of the study or the interpretation of the results in the report.

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Study Timetable:

Study Initiation: July 28, 1992

Initiation of Dosing: August 18, 1992

Completion of Necropsy: September 2, 1992

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INTRODUCTION

Nitroaromatics, such as 1,3-dinitrobenzene (DNB), 1,3,5-trinitrobenzene (TNB), and N-methyl-N,2,4,6-tetranitroaniline (tetryl), have been detected as environmental contaminants of groundwater and soil near production sites and in some instances at military test grounds. TNB is formed during the nitration step of TNT synthesis as a result of oxidation of methyl groups. Although the complete mechanism of TNB formation during TNT photolysis is unknown, it has been suggested that it is produced by decarboxylation of 2,4,6-trinitrobenzaldehyde, a major TNT photoproduct (Burlinson, 1980). It is also found in aquatic systems and surface soils as a by-product of photolysis of TNT. DNB and TNB are not easily biodegradable, persist in the environment, eventually leach out, and contaminate groundwater near waste disposal sites. Tetryl is an explosive that has been in use, largely for military purposes, since 1906. Wastewaters and soil at the original production sites and other plants devoted to munitions assembly, contain large quantities of these compounds (Walsh and Jenkins, 1992).

Toxicity data on these compounds are limited. The oral LD_{50} of DNB, TNB and tetryl were 59 mg/kg, 284 mg/kg and greater than 5 g/kg, respectively, in rats for combined sexes. TNB and tetryl were not toxic at 2 g/kg when applied to rabbit skin for 24 hours. However, the dermal LD_{50} of DNB was 1.99 g/kg for combined sexes of rabbits. None of these compounds produced skin irritation but positive (DNB) and severe (TNB, tetryl) eye irritation potentials in rabbits were noted. The sensitization tests showed that DNB and tetryl are not skin sensitizers while TNB caused mild allergic reaction in guinea pigs (Fitzgerald et. al., 1992 a,b,c). Some of the toxicological effects of DNB are: formation of methemoglobin, testicular degeneration and reproductive failure, weight loss and anemia in hamsters, rats and mice. Neurological and hematological disorders have also been reported in dogs. DNB is toxic to humans; the estimated lethal dose range is 5-50 mg/kg. It is readily absorbed through the skin (Von Burg, 1989). Tetryl was observed to be a powerful skin sensitizer in ammunition plant workers. Dermatitis, liver atrophy, spleen effects, headaches, weight loss and respiratory irritation were reported following tetryl exposure (U.S. EPA, 1990). Atmospheric concentration of 1.5 mg/m^3 or below did not produce systemic poisoning in persons working with tetryl. DNB, TNB, and tetryl have been shown to be genotoxic in the Salmonella mutagenesis assay (McGregor et. al., 1989). TNB has also been shown to form adducts of blood proteins and tissue DNA in rats (Reddy et. al., 1991).

Objective of the Study

This study was conducted in order to evaluate the toxicity of TNB when administered in the diet for 14 days and to provide data to select doses for a 90 day subchronic study.

MATERIALS AND METHODS

Test Material Preparation

1,3,5-Trinitrobenzene powder (CAS #99-35-4) was prepared by Dr. W. Koppes at the Naval Surface Warfare Center and determined to be 99.83% pure which was

confirmed by the U.S. Army Biomedical Research and Development Laboratory and the U.S. EPA. Certified powdered Purina Laboratory Chow 5002 was purchased (Ralston-Purina Co., St. Louis, MO) and stored at 4°C until used. TNB diets were prepared weekly. First, 1.2 g of TNB was added to 25 g of powdered diet in a mortar and thoroughly ground with a pestle. Afterwards 225 g of the diet was added and mixed for 15 minutes followed by an additional 250 g of diet which was mixed for another 15 minutes. Finally, the remaining diet (500 g) was added and mixed for 30 minutes in a mechanical mixer (Kitchen Aid, St. Joseph, MI)) for uniform distribution of TNB in the diet. This was verified by determining the TNB concentration in the diet, taken from each of the 1 kg mixtures, by quantitative analysis done by HPLC. The premixed diet (1.2 g/kg) was further diluted with fresh powdered diet to obtain the desired TNB concentration in the lower dose groups. The diet feeders were refilled twice a week and changed weekly.

Analyses of the TNB-feed mixtures were carried out on acetone extracts of the mixtures, utilizing a Waters 600E chromatography system (Waters, Milford, MA), equipped with a 490E programmable multiwavelength detector, operating at 254 nm. The entire chromatography system was interfaced with a Berthold HPLC computer program, Version 1.65 (Berthold, Nashua, NH). The TNB was eluted from a Zorbax C-8 column (9.4 mm x 25 cm) (MAC-DOD Analytical, Chadds Ford, PA) with a water-methanol gradient, at a flow rate of 3 ml/min. The gradient had an initial condition of 20% methanol which was increased in a linear fashion from 20% to 50% in 15 minutes and then to 65% in 25 minutes, and finally to 100% in 10 minutes. The column was washed for an additional 5 minutes and brought back to 20% methanol by reverse gradient and equilibrated for an additional 10 minutes at initial conditions before the next sample was injected. Working standards were prepared in Burdick and Jackson HPLC grade high purity methanol (Baxter, Obetz, OH). Analytical data of these mixtures is presented in Appendix I.

Animals and Maintenance

Male and female Fischer 344 rats, confirmed free of viral antibodies, bacteria and parasites, were obtained from Charles River Laboratories (Kingston, New York). The animals, 38 days old and weighing approximately 100-125 g when delivered, were held for 1 week in quarantine prior to initiation of treatment. The animals were housed in a temperature (20-22°C) and humidity (40-60%) controlled room on a 12:12 hour light:dark cycle. For the study, they were housed individually in elevated wire mesh cages and water was administered ad libitum. Animal identification was via ear tags with the rats assigned to control and treatment groups according to a computer-generated set of random numbers. The weight variation of the animals of each sex used did not exceed ± 2 s.d. of the mean weight at the time of delivery. The cages were identified with a color-coded identification card indicating the animal and treatment group. All aspects of the study were conducted in compliance with the guidelines of the American Association for Accreditation of Laboratory Animal Care.

All rats were observed twice daily for physiological and behavioral responses as well as for mortality or morbidity. Food and water consumption were recorded

twice weekly. Body weights were taken prior to the start of the study, once weekly during the study and at the final sacrifice.

A pilot palatability study was conducted at three dose levels; 1400, 700 and 350 mg/kg diet, for three weeks in order to establish dose levels for the 14 day study. The data are presented in Appendix H. Rats fed 1400 mg/kg consumed less food therefore lower doses were selected for the 14 day study.

Experiment Design

Group	No. of Animals	Animal Nos.	Sex	Diet Concentration mg/kg	Target Dose mg/kg b. w./day
1	5	1-5	F	0	0
2	5	6-10	F	1200	120
3	5	11-15	F	800	80
4	5	16-20	F	400	40
5	5	21-25	F	200	20
6	5	26-30	F	50	5
7	5	31-35	M	0	0
8	5	36-40	M	1200	120
9	5	41-45	M	800	80
10	5	46-50	M	400	40
11	5	51-55	M	200	20
12	5	56-60	M	50	5

Hematology and Clinical Chemistry

Hematology parameters were assessed using a Serono-Baker Hematology Analyzer, Model 9000, coupled to a computer running Labcat® software (Innovation Programming, Inc., Princeton, NJ). Total red and white blood cell counts, platelet count, differential leukocyte count, hemoglobin, and packed cell volume were measured and computed. Methemoglobin samples were analyzed on a IL 482 Co-Oximeter. Heinz bodies were determined using a crystal violet procedure (Lee et. al., 1993) with microscopic examination for positive cells (>5 Heinz bodies).

Clinical chemistry was performed using a Cobas Fara II centrifugal analyzer with a non-selective electrode (ISE) module. This system was also interfaced with a personal computer and the Labcat software system. Clinical chemistry analytes included sodium, potassium, total protein, albumin, calcium, total bilirubin, blood urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase, glucose and alkaline phosphatase.

Statistical Evaluation

Males and females were considered separately in all statistical analyses. A one-factor (dose) analysis of variance (ANOVA) was used to analyze normally-distributed measures: body weights, organ weights, organ weight ratios, food and water consumption, hematology and clinical chemistry. When a treatment effect was noted ($p \leq 0.05$, F-test) the difference between the control and the treatment groups was probed using a multiple comparison procedure (Dunnett's t-test).

Necropsy and Histopathology

Prior to necropsy, the animals were anesthetized with pentobarbital (60 mg/kg bw, i. p.) and blood samples were collected via cardiac puncture after the body weight was recorded. Following euthanasia via exsanguination, all external surfaces, orifices, external surface of the brain, cervical tissues, all organs, and the thoracic, abdominal and pelvic cavities were examined for gross lesions.

During necropsy the following tissues were weighed: brain, liver, spleen, kidneys, adrenals, lungs, thymus, testes w/epididymides, ovaries, and heart.

The following tissues were harvested from each animal and preserved in 10% neutral buffered formalin:

skin
mandibular and
mesenteric lymph nodes
mammary glands
thigh muscle
sciatic nerve
sternum
femur with marrow
thymus
trachea
lungs with bronchi
heart and aorta
thyroid
parathyroids
esophagus
stomach
duodenum
jejunum
tongue
salivary gland
ileum
colon
cecum
rectum
liver
pancreas
spleen
kidneys
adrenals
urinary bladder
seminal vesicles
prostate
testes, including epididymides
ovaries
uterus
nasal cavity with turbinates
brain
pituitary
preputial or clitoral glands
Zymbal's gland
spinal cord

Subsequently, these tissues were trimmed, processed and embedded in paraffin. Blocks were sectioned at 5µ and slides were prepared and stained with hematoxylin and eosin. All tissues were examined in the high dose and control groups of both sexes. The spleen, testes and kidneys (males only) were identified as target organs and examined in the appropriate groups.

The inflammatory and degenerative lesions were graded according to severity using a scale of one to four (minimal, mild, moderate or marked). Data were tabulated according to individual animal and summarized by group. Labcat histopathology software was used for data management.

Specimen, Raw data, and Final Report Storage

All tissue specimens, blocks and slides, raw data and final report will be placed in the U.S. EPA storage facility.

RESULTS

Food and Water Consumption

Mean weekly food and water consumption data are listed in Table 1 while individual data is presented in Appendix A. A significant decrease ($p \leq 0.05$) in food consumption was evident in females in the 1200 and 800 mg TNB dose groups throughout the study but only during the first week in males. Females receiving 400 and 200 mg TNB also had decreased values but only during the second week of the study. The 50 mg TNB dose group showed no changes. Water consumption was significantly decreased ($p \leq 0.05$) in the two high dose male groups during the first week only.

Using the food consumption data, the average daily TNB dose levels received by different groups (see Experimental Design) are presented in Table 2.

Body Weights, Organ Weights and Weight Ratios

The mean group values for terminal body weights are listed in Table 3 while organ weights (heart, brain, spleen, adrenals, thymus, ovaries/testes, kidneys, lungs, and liver) are given in Tables 4 (females) and 5 (males). Mean group values for organ to body weight ratios are present in Tables 6 (females) and 7 (males). Individual body weights are found in Appendix B with individual organ weights present in Appendix C.

A significant decrease ($p \leq 0.05$) from control terminal body weights was noted in both sexes in the 1200 mg TNB dose group. This decrease was evident at end of weeks 1 and 2 in males and week 2 in females. Organ weights as a percent of the total body weight were significantly ($p \leq 0.05$) different from controls for the following organs:

Brain - The 1200 mg TNB dose group of both sexes had increased values.
Thymus - The 1200 mg TNB dose group (males) had a decreased value.
Spleen - The 1200, 800 and 400 mg TNB dose groups of both sexes had increased values.
Testes - The 1200 and 800 mg TNB dose groups (males) had decreased values.
Liver - The 400 mg TNB dose group (males) had an increased value while the 50 mg TNB dose group (females) was decreased.
Kidneys - The 1200, 800, 400 and 200 mg TNB dose groups (males) had increased values.

Hematology

Hematology analyses performed were total white blood cell count (WBC), platelet count, red blood cell count (RBC), hemoglobin (HGB), packed cell volume (HCT), Heinz bodies, methemoglobin, and differential leukocyte count. Group data are summarized in Tables 8 (females) and 9 (males). Individual data are listed in Appendix D.

Significant findings were noted for red blood cell counts, hemoglobin, hematocrit, heinz bodies and methemoglobin.

1. WBC and Differential:

   There were no significant differences in total white cell count amongst the groups in either sex. A relative shift to an increased lymphocyte percentage was evident in all male treatment groups and in females receiving 1200 and 800 mg TNB diet. There was no change in monocyte percentage.

   Note: Several groups had high mean WBC values with large standard deviations usually due to a single aberrant animal within the group.

2. RBC:

   A significant decrease ($p \leq 0.05$) in total red cell count was present in all female groups and in males receiving 1200 and 800 mg TNB diet.

3. Hemoglobin:

   A significant decrease ($p \leq 0.05$) was noted in hemoglobin levels in females receiving 1200 and 800 mg TNB diet but not in male groups.

4. Hematocrit:

   A significant decrease ($p \leq 0.05$) was exhibited in all female groups and in males receiving 1200, 800 and 400 mg TNB diet.

5. Platelets:

   A significant decrease ($p \leq 0.05$) was present in females receiving 400 mg TNB diet.

6. Heinz Bodies:

   A significant increase ($p \leq 0.05$) in the percent of Heinz bodies was associated with the 1200 and 800 mg TNB dose groups of both sexes.

7. Methemoglobin:

   A significant increase ($p \leq 0.05$) in methemoglobin levels was evident in both sexes in the 1200, 800, and 400 mg TNB dose groups. Note: This data was obtained from different animals - See Appendix J.

Clinical Chemistry

The mean group values for each analyte are compiled in Tables 10 (females) and 11 (males). Individual data are present in Appendix E. The only finding which was biologically significant was decreased levels of alkaline phosphatase.

1. Total Protein

   The mean values for females ranged from 6.2 to 6.7 g/dl while in males the range was 6.4 to 7.4. The only significant change occurred in the 200 and 50 mg TNB dose groups (males). These slight increases are within normal biological range for the rat.

2. Albumin

   The mean values for females ranged from 3.4 to 3.8 g/dl while in males the range was 3.8 to 4.1. There were no significant differences amongst the groups.

3. Calcium

   The mean values for females ranged from 10.0 to 11.0 mg/dl while in males the range was 11.1 to 12.1. There were no significant differences amongst the groups.

4. Total Bilirubin

   The mean values for females and males ranged 0.0 to 0.2 mg/dl. The high dose males were the only group to show a significant change ($p \leq 0.05$). This minimal increase was within normal biological range for the rat.

5. Blood Urea Nitrogen (BUN)

   The mean values for females ranged from 20 to 27 mg/dl while in males the range was 19 to 22. There were no significant differences amongst the groups.

6. Creatinine

   The mean values in females ranged from 0.2 to 0.6 mg/dl while in males the range was 0.4 to 0.5. The low dose (50 mg TNB diet) females were the only group which showed significant change ($p \leq 0.05$). This mild decrease is not biologically significant.

7. Aspartate Aminotransferase (AST)

   The mean values for females ranged from 84 to 110 IU/L while in males the range was 110 to 142. There were no significant differences amongst the groups.

8. Alanine Aminotransferase (ALT)

   The mean values for females ranged from 14 to 32 IU/L while in males the range was 29 to 41. There were no significant differences amongst the groups except in the low dose females (50 mg TNB diet) which had a decreased level. This mild decrease is within normal biological range for the rat.

9. Alkaline Phosphatase

   The mean values for females ranged from 83 to 131 IU/L while in males the range was 93 to 155. All female treated groups had significantly lower values ($p \leq 0.05$) while males demonstrated this same decrease in the 1200, 800 and 400 mg TNB dose groups. This reduction was dose related.

10. Sodium (Na)

    The mean values for females ranged from 140 to 145 mmol/L while in males the range was 145 to 147. There were no significant differences amongst the groups.

11. Potassium (K)

    The mean values for females ranged from 5.3 to 8.5 mmol/L while in males the range was 5.8 to 6.6. Significant differences ($p \leq 0.05$) were noted in treated females in the 1200, 400 and 50 mg TNB dose groups. The mild decrease present in these groups is not biologically significant.

12. Glucose

    The mean values in females ranged from 129 to 187 mg/dL while in males the range was 183 to 265 Only one group (1200 mg TNB diet; males) displayed a significant change ($p \leq 0.05$). The mild decrease in this group was within normal biological range for the rat.

Clinical Observations

Clinical observations are listed in Appendix F. There were no clinical observations that were meaningful except the weight loss noted in one animal in each of groups 2 and 8.

Mortality

There were no early deaths in any of the groups.

Gross Pathology

Gross lesions or changes noted at the terminal sacrifice were mainly confined to 1200 and 800 mg TNB dose groups (males). The prominent change was a mild to moderate reduction in testicular size.

Histopathology (Appendix G)

All tissues were histopathologically examined in all control and high dose animals of both sexes. The spleen was examined in all groups while the kidneys and testes in male rats only. Prominent changes were noted in the testes, spleen, bone marrow and kidneys.

The testes were characterized in the 1200 and 800 mg TNB dose groups (males) by moderate to severe seminiferous tubular degeneration. The affected tubules were lined by fewer spermatogenic cells and contained a reduced number of mature spermatides. Cell debris and some multinucleated cells were also present in the tubules as well as in the ducts of the epididymis. The diameter of the affected tubules was decreased with the interstitium being more condensed and prominent.

The kidneys of male rats in the 1200, 800, 400 and 200 mg TNB dose groups exhibited an increased incidence of cortical tubular hyaline droplet deposition. Many of these droplets were large and irregular resulting in early tubular degeneration.

The spleen and bone marrow both featured minimal to moderate erythroid cell hyperplasia. This was evident in both sexes in the 1200, 800 and 400 mg TNB dose groups. Only the spleen was examined in all the animals since this same regenerative compensatory change can be noted in multiple organs. Mild anemia, as noted by the hematology results, was the probable initiating factor for this response.

Two female rats in the 1200 mg TNB dose group had the following cerebellar lesions; hemorrhage, microgliosis, vacuolization and necrosis. The severity of these changes ranged from minimal to moderate.

The remaining diagnoses as listed in the tables should be considered spontaneous since their incidence levels were low except for the inflammatory changes noted in the clitoral/preputial glands. Considering the lesion incidence level in these glands was no different than the control group, the remaining animals were not examined.

SUMMARY

The administration to Fischer 344 rats of 1,3,5-trinitrobenzene at various doses in the diet for fourteen days resulted in the following significant findings:

1. Smaller testicular size and seminiferous tubular degeneration in the 1200 and 800 mg TNB dose groups.

2. Increased splenic weight and regenerative anemia with a compensatory erythroid cell hyperplasia in the 1200, 800 and 400 mg TNB dose groups of both sexes.

3. Excessive hyaline droplet formation in cortical kidney tubules of male rats at all dose levels except the 50 mg TNB dose group.

4. Body weight gain was reduced in both sexes administered 1200 mg TNB.

5. The male 1200, 800, 400 and 200 mg TNB dose groups exhibited significant increases in relative kidney weights.

6. The 1200 mg TNB dose group of both sexes had significant increased relative brain weights while this same group in males had a significant decrease in relative thymus weight.

7. Food consumption was significantly decreased in the female 1200 and 800 mg TNB dose groups at all weighing intervals and in males in these same groups during the first week.

8. Cerebellar inflammation, hemorrhage and vacuolization were prominent in several females in the 1200 mg TNB dose group.

9. Increased methemoglobin levels were present in the 1200, 800 and 400 mg TNB dose groups of both sexes.

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Table 1: Food and Water Consumption

Dose (mg TNB/kg diet)	Food (g/kg b.w./day) Week 1	Food (g/kg b.w./day) Week 2	Water (g/kg b.w./day) Week 1	Water (g/kg b.w./day) Week 2
		Females		
0	96.0 ±2.0	89.4 ±1.0	159.2 ±5.7	146.8 ±6.0
1200	56.8 * ±3.8	70.4 * ±2.4	128.0 ±7.8	141.9 ±15.3
800	75.6 * ±3.6	77.7 * ±1.1	158.8 ±6.0	166.6 ±3.9
400	85.6 ±4.1	80.7 * ±0.8	159.0 ±11.0	153.6 ±2.6
200	91.4 ±0.8	84.3 * ±0.7	159.2 ±10.7	144.3 ±3.4
50	96.8 ±1.6	84.6 ±0.8	146.9 ±20.3	132.9 ±7.8
		Males		
0	97.1 ±1.2	78.0 ±0.6	157.1 ±5.1	121.8 ±2.9
1200	72.4 * ±5.9	75.7 ±3.0	100.0 * ±9.2	117.8 ±3.9
800	71.7 * ±2.8	73.0 ±1.0	119.8 * ±3.8	117.2 ±2.1
400	88.6 ±3.1	77.9 ±0.8	137.7 ±6.8	116.1 ±2.8
200	94.4 ±3.1	74.8 ±2.4	144.1 ±6.9	108.8 ±7.6
50	102.2 ±3.8	78.5 ±1.5	153.3 ±7.9	116.4 ±3.7

Mean ± Standard Error.
* Significantly different from the control group ($p \leq 0.05$) by Dunnett's test.

Table 2: Calculated Daily TNB Consumption

Group	Sex	TNB (mg/kg diet)	Expected Target Dose (mg/kg)	(mg/kg b.w.) Week 1	Week 2
1	F	0	0		
2	F	1200	120	65.83 ± 8.72 *	92.87 ± 6.33
3	F	800	80	56.69 ± 5.46	61.46 ± 1.44
4	F	400	40	35.11 ± 3.40	33.16 ± 0.67
5	F	200	20	18.29 ± 0.33	16.70 ± 0.29
6	F	50	5	4.84 ±0.16	4.23 ± 0.08
7	M	0	0		
8	M	1200	120	83.96 ± 6.84	99.90 ± 6.80
9	M	800	80	53.81 ± 4.17	57.71 ± 1.54
10	M	400	40	36.34 ± 2.56	31.00 ± 0.65
11	M	200	20	18.88 ± 1.24	14.82 ± 0.93
12	M	50	5	5.11 ± 0.38	3.93 ± 0.15

* Mean ± Standard Error

Table 3: Body Weights (grams)

Dose (mg TNB/kg diet)	Study Day 8/11	08/18	08/25	09/01	Necropsy**
			Females		
0	115±3	128±3	134±5	144±3	125±4
1200	116±2	130±1	123±2	116±5 *	103±3 *
800	115±2	128±3	129±2	137±2	120±3
400	115±2	133±4	139±3	147±3	129±2
200	118±2	131±3	138±4	148±4	129±4
50	116±1	130±2	138±2	146±3	127±2
			Males		
0	147±4	175±4	197±3	213±3	188±2
1200	151±8	179±9	166±15 *	173±14 *	154±13 *
800	149±5	179±7	183±6	196±7	172±6
400	150±4	179±5	193±6	211±9	185±8
200	150±3	178±4	200±3	208±8	185±6
50	147±7	176±8	201±8	221±8	196±7

Mean ±Standard Error.

* Signifcantly different from the control group ($p \leq 0.05$) by Dunnett's test.

**All rats fasted for 16-18 hours.

Table 4: Organ Weights (grams)/Females

	Dose Groups (mg TNB/kg diet)					
	0	1200	800	400	200	50
Liver	4.59 ±0.33	3.66* ±0.16	4.35 ±0.06	4.67 ±0.15	4.53 ±0.20	4.11 ±0.11
Kidneys	1.20 ±0.04	1.02* ±0.02	1.15 ±0.01	1.28 ±0.03	1.20 ±0.03	1.22 ±0.04
Heart	0.52 ±0.01	0.44* ±0.03	0.49 ±0.02	0.53 ±0.02	0.52 ±0.02	0.54 ±0.01
Ovaries	0.13 ±0.02	0.09* ±0.002	0.12 ±0.005	0.13 ±0.01	0.14 ±0.02	0.13 ±0.01
Brain	1.65 ±0.04	1.57 ±0.03	1.61 ±0.04	1.61 ±0.03	1.59 ±0.05	1.59 ±0.04
Spleen	0.36 ±0.01	0.55* ±0.05	0.62* ±0.02	0.51* ±0.02	0.39 ±0.02	0.39 ±0.02
Adrenals	0.10 ±0.03	0.06 ±0.002	0.06 ±0.01	0.06 ±0.003	0.07 ±0.005	0.07 ±0.003
Lungs	0.84 ±0.06	0.66 ±0.02	0.73 ±0.02	0.85 ±0.07	0.80 ±0.03	1.14 ±0.22
Thymus	0.29 ±0.01	0.18* ±0.03	0.25 ±0.02	0.29 ±0.03	0.27 ±0.01	0.28 ±0.02

Mean ±Standard Error.

* Signifcantly different from the control group ($p \leq 0.05$) by Dunnett's test.

Table 5: Organ Weights (grams)/Males

	Dose Groups (mg TNB/kg diet)					
	0	1200	800	400	200	50
Liver	6.21 ±0.16	5.54 ±0.49	6.19 ±0.19	6.70 ±0.35	6.48 ±0.38	6.66 ±0.26
Kidneys	1.57 ±0.05	1.52 ±0.10	1.65 ±0.06	1.68 ±0.05	1.69 ±0.03	1.69 ±0.07
Heart	0.71 ±0.03	0.58* ±0.05	0.69 ±0.02	0.69 ±0.03	0.70 ±0.03	0.72 ±0.04
Testes	3.60 ±0.11	1.89* ±0.23	1.77* ±0.06	3.55 ±0.08	3.46 ±0.07	3.56 ±0.14
Brain	1.70 ±0.03	1.70 ±0.04	1.71 ±0.06	1.70 ±0.05	1.69 ±0.03	1.67 ±0.08
Spleen	0.47 ±0.01	0.75* ±0.07	0.79* ±0.04	0.57 ±0.03	0.45 ±0.03	0.48 ±0.02
Adrenals	0.08 ±0.01	0.07 ±0.004	0.07 ±0.005	0.07 ±0.004	0.07 ±0.01	0.08 ±0.005
Lungs	1.04 ±0.04	1.05 ±0.16	0.88 ±0.04	0.96 ±0.03	0.96 ±0.01	0.99 ±0.04
Thymus	0.32 ±0.02	0.21* ±0.03	0.25 ±0.01	0.31 ±0.02	0.27 ±0.01	0.31 ±0.01

Mean ±Standard Error.

* Signifcantly different from the control group ($p \leq 0.05$) by Dunnett's test.

Table 6: Organ -to-Body Weight Ratios/Females

	Dose Groups (mg TNB/kg diet)					
	0	1200	800	400	200	50
Liver (%)	3.65 ±0.17	3.54 ±0.07	3.63 ±0.05	3.62 ±0.10	3.52 ±0.13	3.22* ±0.05
Kidneys (%)	0.96 ±0.02	0.99 ±0.04	0.96 ±0.02	0.99 ±0.01	0.93 ±0.02	0.96 ±0.03
Heart (%)	0.41 ±0.01	0.42 ±0.02	0.41 ±0.02	0.41 ±0.01	0.40 ±0.01	0.43 ±0.01
Ovaries (%)	0.10 ±0.01	0.08 ±0.002	0.10 ±0.004	0.10 ±0.01	0.11 ±0.01	0.10 ±0.01
Brain (%)	1.33 ±0.05	1.52* ±0.03	1.35 ±0.05	1.25 ±0.02	1.23 ±0.03	1.25 ±0.03
Spleen (%)	0.29 ±0.003	0.53* ±0.04	0.52* ±0.02	0.39* ±0.01	0.31 ±0.01	0.31 ±0.01
Adrenals (%)	0.08 ±0.02	0.06 ±0.001	0.05 ±0.004	0.05 ±0.002	0.06 ±0.003	0.06 ±0.002
Lungs (%)	0.67 ±0.05	0.64 ±0.02	0.61 ±0.01	0.66 ±0.05	0.63 ±0.02	0.90 ±0.19
Thymus (%)	0.23 ±0.003	0.17 ±0.03	0.21 ±0.01	0.22 ±0.02	0.21 ±0.005	0.22 ±0.01

Mean ±Standard Error.

* Signifcantly different from the control group ($p \leq 0.05$) by Dunnett's test.

Table 7: Organ -to-Body Weight Ratios/Males

	Dose Groups (mg TNB/kg diet)					
	0	1200	800	400	200	50
Liver (%)	3.31 ±0.05	3.60 ±0.07	3.61 ±0.12	3.62* ±0.12	3.49 ±0.12	3.41 ±0.05
Kidneys (%)	0.84 ±0.02	1.00* ±0.02	0.96* ±0.01	0.91* ±0.02	0.92* ±0.02	0.86 ±0.02
Heart (%)	0.38 ±0.01	0.38 ±0.005	0.40 ±0.01	0.38 ±0.01	0.38 ±0.01	0.37 ±0.01
Testes (%)	1.91 ±0.04	1.22* ±0.06	1.04* ±0.04	1.93 ±0.05	1.88 ±0.05	1.82 ±0.01
Brain (%)	0.91 ±0.01	1.13* ±0.10	1.00 ±0.04	0.92 ±0.03	0.92 ±0.04	0.85 ±0.02
Spleen (%)	0.25 ±0.01	0.49* ±0.03	0.46* ±0.02	0.31* ±0.004	0.24 ±0.01	0.25 ±0.01
Adrenals (%)	0.04 ±0.003	0.05 ±0.003	0.04 ±0.002	0.04 ±0.003	0.04 ±0.003	0.04 ±0.003
Lungs (%)	0.55 ±0.02	0.73 ±0.19	0.51 ±0.01	0.52 ±0.02	0.52 ±0.02	0.51 ±0.01
Thymus (%)	0.17 ±0.01	0.13* ±0.02	0.15 ±0.01	0.17 ±0.01	0.14 ±0.01	0.16 ±0.01

Mean ±Standard Error.

* Signifcantly different from the control group ($p \leq 0.05$) by Dunnett's test.

Table 8: Hematology Values/Females

	Dose Groups (mg TNB/kg diet)					
	0	1200	800	400	200	50
RBC ($x10^6/\mu l$)	8.47 ±1.00	5.62* ±0.17	5.71* ±0.36	6.58* ±0.14	7.46* ±0.16	7.51* ±0.23
Hemoglobin (g/DL)	17.5 ±2.04	13.3* ±0.61	13.8* ±0.96	15.4 ±2.57	15.5 ±0.80	15.3 ±0.10
Hematocrit (%)	48.1 ±5.69	35.4* ±2.00	35.7* ±2.35	37.2* ±0.41	42.0* ±1.45	42.1* ±1.14
WBC ($x10^3/\mu L$)	6.0 ±1.75	6.3 ±2.90	13.2 ±6.69	37.9 ±48.58	7.0 ±4.20	5.0 ±0.33
Platelets ($x10^3/\mu L$)	899 ±23.7	1047 ±94.6	1337 ±317.7	1408* ±542.8	918 ±27.9	887 ±43.0
Segmented Leukocytes (%)	29 ±3.0	16* ±3.6	19* ±2.7	22 ±6.0	22 ±5.4	29 ±2.8
Lymphocytes (%)	71 ±3.0	84* ±3.6	81* ±2.7	78 ±6.0	78 ±5.4	71 ±2.8
Heinz Bodies (%)	0.0 ±0.00	6.1* ±2.35	3.5* ±1.44	0.7 ±0.21	0.0 ±0.00	0.0 ±0.00

Mean ± Standard Deviation
* Significantly different from the control group ($P \leq 0.05$) by the Dunnett's test.

Table 9: Hematology Values/Males

	Dose Groups (mg TNB/kg diet)					
	0	1200	800	400	200	50
RBC ($x10^6/\mu l$)	7.82 ±0.43	6.36* ±0.55	6.59* ±0.27	7.17 ±0.49	7.95 ±0.42	7.88 ±0.23
Hemoglobin (g/DL)	15.9 ±0.83	14.4 ±2.49	13.7 ±0.59	14.8 ±0.63	17.2 ±2.83	18.6 ±3.92
Hematocrit (%)	42.8 ±1.60	36.5* ±1.84	37.3* ±1.86	38.7* ±3.71	43.6 ±1.48	44.3 ±2.24
WBC ($x10^3/\mu L$)	7.5 ±4.20	31.3 ±52.69	6.7 ±0.99	11.9 ±13.44	21.3 ±34.40	39.2 ±46.00
Platelets ($x10^3/\mu L$)	1007 ±98.9	1433 ±493.7	1180 ±48.8	1663 ±751.1	1004 ±98.3	1055 ±149.1
Segmented Leukocytes (%)	30 ±4.2	16* ±5.9	19* ±2.6	15* ±2.7	13* ±3.4	16* ±5.5
Lymphocytes (%)	70 ±4.2	84* ±5.9	82* ±2.6	85* ±2.7	87* ±3.4	84* ±5.5
Heinz Bodies (%)	0.0 ±0.00	4.4* ±1.09	3.1* ±1.15	0.8 ±0.34	0.0 ±0.00	0.0 ±0.00

Mean ± Standard Deviation
* Significantly different from the control group ($P \leq 0.05$) by the Dunnett's test.

Table 10: Clinical Chemistry Measurements/Females

	Dose Groups (mg TNB/kg diet)					
	0	1200	800	400	200	50
Total Protein (g/dl)	6.2 ±0.29	6.4 ±0.51	6.7 ±0.16	6.4 ±0.38	6.5 ±0.27	6.4 ±0.62
Albumin (g/dl)	3.5 ±0.26	3.8 ±0.22	3.8 ±0.13	3.8 ±0.19	3.7 ±0.05	3.4 ±0.12
Calcium (mg/dl)	10.8 ±0.47	10.2 ±0.70	11.0 ±0.36	10.9 ±0.28	10.8 ±0.41	10.0 ±0.72
Total Bilirubin (mg/dl)	0.1 ±0.00	0.2 ±0.05	0.1 ±0.05	0.1 ±0.05	0.0 ±0.05	0.1 ±0.06
BUN (mg/dl)	21 ±0.6	27 ±9.4	20 ±2.9	20 ±0.9	21 ±2.5	20 ±1.2
Creatinine (mg/dl)	0.5 ±0.12	0.6 ±0.09	0.4 ±0.11	0.4 ±0.08	0.5 ±0.05	0.2* ±0.17
AST (U/L)	95 ±9.2	107 ±18.9	100 ±18.6	90 ±11.1	110 ±33.6	84 ±17.0
ALT (U/L)	32 ±14.1	25 ±8.1	25 ±5.1	27 ±2.6	27 ±2.6	14* ±7.8
ALK Phos. (U/L)	131 ±1.4	83* ±9.2	98* ±10.1	96* ±6.9	106* ±7.6	107* ±4.9
Glucose (mg/dl)	129 ±14.8	187 ±38.7	163 ±32.9	169 ±24.0	176 ±5.0	186 ±24.7
Na (mmol/L)	144 ±0.8	145 ±1.9	145 ±1.8	142 ±7.1	144 ±0.8	140 ±5.7
K (mmol/L)	8.5 ±2.67	5.6* ±0.63	6.2 ±0.59	6.1* ±0.49	6 4 ±0.94	5.3* ±1.49

Mean ± Standard Deviation
* Significantly different from the control group (P≤ 0.05) by the Dunnett's test.

Table 11: Clinical Chemistry Measurements/Males

	Dose Groups (mg TNB/kg diet)					
	0	1200	800	400	200	50
Total Protein (g/dl)	6.4 ±0.30	6.4 ±0.46	6.9 ±0.16	6.6 ±0.19	6.9* ±0.39	7.4* ±0.25
Albumin (g/dl)	3.9 ±0.19	3.8 ±0.25	4.1 ±0.14	4.0 ±0.27	4.0 ±0.21	3.9 ±0.11
Calcium (mg/dl)	11.4 ±0.28	11.1 ±0.56	11.4 ±0.27	11.8 ±0.46	12.1 ±1.67	11.3 ±0.41
Total Bilirubin (mg/dl)	0.1 ±0.05	0.2* ±0.05	0.1 ±0.00	0.1 ±0.04	0.1 ±0.05	0.0 ±0.04
BUN (mg/dl)	20 ±2.2	22 ±0.9	21 ±0.9	20 ±1.7	19 ±1.7	19 ±1.0
Creatinine (mg/dl)	0.5 ±0.07	0.4 ±0.05	0.5 ±0.05	0.5 ±0.07	0.5 ±0.05	0.5 ±0.08
AST (U/L)	121 ±29.2	142 ±15.0	110 ±26.8	131 ±61.2	121 ±36.7	123 ±47.5
ALT (U/L)	41 ±6.8	34 ±11.4	29 ±4.6	36 ±17.5	37 ±17.5	34 ±3.7
ALK Phos. (U/L)	153 ±7.6	93* ±10.2	113* ±13.5	125* ±6.8	140 ±12.0	155 ±12.2
Glucose (mg/dl)	265 ±27.5	183* ±6.8	212 ±15.0	221 ±29.6	224 ±13.1	225 ±57.9
Na (mmol/L)	145 ±1.3	145 ±2.6	146 ±0.4	146 ±2.0	145 ±4.9	147 ±0.7
K (mmol/L)	6.2 ±0.45	6.6 ±0.35	6.7 ±0.97	6.6 ±0.26	6.0 ±0.70	5.8 ±0.40

Mean ± Standard Deviation
* Significantly different from the control group ($P \leq 0.05$) by the Dunnett's test.

APPENDIX A

FOOD AND WATER CONSUMPTION

GROUP FEED AND WATER DATA

Group	Sex	Diet Concentration (mg TNB/kg diet)	Feed (g/wk) Week 1	Feed (g/wk) Week 2	Water (g/wk) Week 1	Water (g/wk) Week 2
1	F	0	86.0 ± 6.6	89.8 ± 3.0	142.6 ± 12.8	147.5 ± 12.1
2	F	1200	51.6 ± 6.7	57.3 ± 7.7	116.5 ± 13.8	117.0 ± 30.8
3	F	800	67.7 ± 4.8	74.2 ± 2.5	142.4 ± 10.0	157.2 ± 11.4
4	F	400	79.4 ± 3.8	83.3 ± 3.5	147.0 ± 13.0	158.6 ± 6.7
5	F	200	83.9 ± 4.1	86.6 ± 4.6	146.2 ± 21.9	148.3 ± 12.2
6	F	50	88.1 ± 4.6	86.5 ± 4.1	133.7 ± 37.9	135.8 ± 17.2
7	M	0	118.7 ± 3.9	116.4 ± 3.6	191.9 ± 13.0	181.7 ± 4.6
8	M	1200	88.7 ± 3.9	96.6 ± 22.8	135.1 ± 18.8	143.2 ± 24.4
9	M	800	89.6 ± 4.2	99.9 ± 4.7	149.9 ± 9.8	160.6 ± 10.2
10	M	400	110.6 ± 6.6	114.9 ± 11.7	172.4 ± 21.2	171.8 ± 22.5
11	M	200	117.1 ± 5.1	109.3 ± 14.3	178.5 ± 11.7	159.8 ± 31.3
12	M	50	125.1 ± 5.3	121.0 ± 5.6	187.3 ± 12.2	179.1 ± 5.4

Mean ± Standard Deviation

INDIVIDUAL FEED AND WATER DATA

FEMALES

GP-ANI NUMBER	FEED (G/WK) WEEK 1	FEED (G/WK) WEEK 2	WATER (G/WK) WEEK 1	WATER (G/WK) WEEK 2
1-1	96.90	94.50	163.30	156.00
1-2	81.20	85.90	123.50	136.00
1-3	88.90	90.00	141.60	138.20
1-4	77.90	91.10	146.10	167.10
1-5	85.30	87.40	138.30	140.00
2-6	46.00	43.20	106.20	57.80
2-7	58.30	58.70	129.30	133.60
2-8	41.50	56.40	94.40	121.90
2-9	54.70	64.90	127.80	146.50
2-10	57.70	63.50	124.60	125.10
3-11	63.30	78.00	135.80	170.10
3-12	64.40	73.30	130.10	147.00
3-13	65.20	74.20	159.50	172.30
3-14	76.40	*	146.00	148.90
3-15	69.10	71.20	140.70	147.90
4-16	81.00	83.40	149.60	159.40
4-17	83.30	83.40	146.80	147.90
4-18	76.30	79.20	143.20	156.50
4-19	82.70	81.10	168.00	160.50
4-20	73.50	89.50	127.60	168.50
5-21	91.90	93.50	164.00	171.50
5-22	82.20	87.70	175.20	148.90
5-23	82.40	80.20	139.10	138.30
5-24	82.00	83.90	112.30	138.60
5-25	80.80	79.80	140.30	144.20
6-26	93.30	86.20	160.90	110.00
6-27	93.50	91.90	174.90	158.10
6-28	83.20	79.20	138.40	122.80
6-29	83.00	88.20	129.00	141.10
6-30	87.40	86.80	65.10	146.90

* EXCESSIVE SPILLAGE

INDIVIDUAL FEED AND WATER DATA

MALES

GP-ANI NUMBER	FEED (G/WK) WEEK 1	FEED (G/WK) WEEK 2	WATER (G/WK) WEEK 1	WATER (G/WK) WEEK 2
7-31	119.20	113.50	216.20	187.60
7-32	125.40	119.30	187.20	175.80
7-33	119.20	121.90	190.60	179.50
7-34	115.20	115.20	177.00	178.90
7-35	114.60	112.30	188.70	186.50
8-36	*	134.20	166.30	174.10
8-37	84.80	103.70	115.90	132.40
8-38	*	90.50	129.00	157.70
8-39	92.60	90.50	129.20	149.10
8-40	*	63.90	*	102.60
9-41	93.00	107.90	159.80	177.50
9-42	83.60	94.10	137.70	153.20
9-43	91.20	102.00	162.60	161.60
9-44	94.50	97.40	147.60	147.40
9-45	85.60	98.20	141.70	163.10
10-46	120.30	122.40	208.40	197.60
10-47	103.40	130.30	165.60	191.80
10-48	112.80	109.40	157.50	167.00
10-49	113.70	116.30	182.30	168.50
10-50	103.00	96.00	148.30	134.10
11-51	108.80	81.60	172.00	98.50
11-52	122.50	118.60	177.30	171.30
11-53	114.40	112.90	166.70	169.40
11-54	122.00	121.40	200.60	187.10
11-55	117.70	112.00	175.80	172.70
12-56	132.50	127.30	206.80	187.20
12-57	129.50	127.80	184.00	181.00
12-58	122.10	117.90	178.20	172.20
12-59	117.80	118.30	172.70	174.10
12-60	123.40	113.80	194.90	180.80

* EXCESSIVE SPILLAGE

APPENDIX B

BODY WEIGHTS

INDIVIDUAL BODY WEIGHTS (GRAMS)

	08/11	08/18	08/25	09/01	NECROPSY
1-1	125.4	138.7	151.9	155.4	138.63
1-2	117.3	127.3	129.4	137.6	119.55
1-3	111.9	129.1	134.4	145.2	125.12
1-4	112.9	122.1	121.4	140.3	121.95
1-5	106.9	122.3	131.1	139.4	120.39
2-6	121.6	133.7	123.9	97.9	95.16
2-7	120.4	133.2	128.6	124.2	108.54
2-8	115.4	128.2	116.3	112.5	98.58
2-9	115.6	127.3	121.4	120.0	105.72
2-10	108.3	127.9	122.8	125.4	108.95
3-11	120.9	137.1	131.4	140.4	122.44
3-12	118.5	125.4	126.9	132.7	114.15
3-13	114.6	130.9	131.6	141.9	130.64
3-14	112.8	126.2	132.1	138.9	118.74
3-15	108.4	121.9	123.9	130.7	114.04
4-16	121.9	137.6	143.5	149.3	129.73
4-17	117.4	128.2	137.2	144.1	127.91
4-18	113.2	128.2	134.1	144.7	127.91
4-19	113.4	124.5	132.0	140.9	122.20
4-20	109.7	147.1	148.6	158.9	137.19
5-21	126.7	140.8	152.2	161.4	141.05
5-22	118.3	130.9	137.6	145.3	131.24
5-23	116.6	131.5	134.8	146.6	126.49
5-24	117.0	128.1	134.6	143.7	127.37
5-25	112.2	123.8	129.9	136.7	117.66
6-26	116.2	132.6	140.3	149.8	128.14
6-27	120.7	133.4	146.6	150.2	133.87
6-28	113.2	124.1	133.4	134.8	121.28
6-29	115.5	128.8	134.4	148.7	126.36
6-30	112.9	130.5	136.8	146.3	127.13

INDIVIDUAL BODY WEIGHTS (GRAMS)

	08/11	08/18	08/25	09/01	NECROPSY
7-31	154.9	181.0	199.1	211.7	188.24
7-32	156.1	185.5	204.9	219.4	194.61
7-33	146.7	176.4	199.5	217.6	188.00
7-34	141.5	169.7	195.5	213.6	185.75
7-35	134.7	161.3	185.6	204.6	181.99
8-36	179.4	212.5	209.5	210.1	192.90
8-37	154.6	182.2	172.2	175.1	155.84
8-38	145.3	172.8	166.5	177.3	152.04
8-39	141.3	169.0	167.3	178.4	156.90
8-40	134.8	156.7	114.9	125.5	111.09
9-41	164.3	200.7	204.9	222.1	193.89
9-42	152.9	182.3	178.2	185.5	165.59
9-43	154.6	182.8	186.0	197.3	175.90
9-44	137.9	168.6	178.5	187.7	161.94
9-45	136.3	161.6	168.6	187.2	162.39
10-46	155.9	187.9	211.8	228.7	200.54
10-47	162.4	193.3	200.3	230.3	202.99
10-48	145.7	172.9	187.9	202.3	176.72
10-49	147.2	175.5	192.3	212.8	184.59
10-50	138.5	164.3	173.4	178.9	158.96
11-51	157.2	181.2	200.3	176.2	162.87
11-52	157.9	188.2	209.1	220.2	196.58
11-53	150.7	180.6	201.3	217.6	191.22
11-54	144.8	169.6	197.2	216.9	190.87
11-55	139.9	168.2	189.7	207.9	182.17
12-56	166.4	197.6	225.9	248.0	219.40
12-57	154.1	183.9	206.8	226.2	198.65
12-58	148.1	175.8	198.6	218.0	193.04
12-59	139.9	170.6	192.4	212.3	190.29
12-60	126.6	150.7	179.6	198.9	176.60

APPENDIX C

ORGAN WEIGHTS

INDIVIDUAL ORGAN WEIGHTS

GP-ANI NUMBER		BODY WEIGHT	ADRENAL WEIGHT	THYMUS WEIGHT	OVARIES WEIGHT	% ADRENAL	% THYMUS	% OVARIES
1	1	138.63	0.089	0.320	0.198	0.064	0.231	0.143
1	2	119.55	0.197	0.287	0.129	0.165	0.240	0.108
1	3	125.12	0.069	0.278	0.122	0.055	0.222	0.098
1	4	121.95	0.068	0.272	0.110	0.056	0.223	0.090
1	5	120.39	0.067	0.277	0.100	0.056	0.230	0.083
2	6	95.16	0.049	0.065	0.079	0.051	0.068	0.083
2	7	108.54	0.060	0.196	0.089	0.055	0.181	0.082
2	8	98.58	0.058	0.174	0.090	0.059	0.177	0.091
2	9	105.72	0.059	0.228	0.086	0.056	0.216	0.081
2	10	108.95	0.057	0.228	0.083	0.052	0.209	0.076
3	11	122.44	0.061	0.257	0.121	0.050	0.210	0.099
3	12	114.15	0.048	0.212	0.131	0.042	0.186	0.115
3	13	130.64	0.078	0.227	0.132	0.060	0.174	0.101
3	14	118.78	0.064	0.301	0.118	0.054	0.253	0.099
3	15	114.04	0.047	0.252	0.106	0.041	0.221	0.093
4	16	129.73	0.059	0.283	0.100	0.045	0.218	0.077
4	17	127.91	0.066	0.267	0.138	0.052	0.209	0.108
4	18	127.91	0.067	0.239	0.106	0.052	0.187	0.083
4	19	122.20	0.054	0.237	0.141	0.044	0.194	0.115
4	20	137.19	0.071	0.413	0.140	0.052	0.301	0.102
5	21	141.05	0.082	0.322	0.193	0.058	0.228	0.137
5	22	131.24	0.068	0.269	0.107	0.052	0.205	0.082
5	23	126.49	0.082	0.257	0.150	0.065	0.203	0.119
5	24	127.37	0.079	0.250	0.172	0.062	0.196	0.135
5	25	117.66	0.057	0.246	0.097	0.048	0.209	0.082
6	26	128.14	0.067	0.280	0.142	0.052	0.219	0.111
6	27	133.87	0.082	0.286	0.147	0.061	0.214	0.110
6	28	121.28	0.065	0.237	0.106	0.054	0.195	0.087
6	29	126.36	0.069	0.281	0.091	0.055	0.222	0.072
6	30	127.13	0.076	0.335	0.147	0.060	0.264	0.116

WEIGHTS IN GRAMS

INDIVIDUAL ORGAN WEIGHTS

GP-ANI NUMBER		BODY WEIGHT	ADRENAL WEIGHT	THYMUS WEIGHT	TESTES WEIGHT	% ADRENAL	% THYMUS	% TESTES
7	31	188.24	0.056	0.293	3.708	0.030	0.156	1.970
7	32	194.61	0.084	0.290	3.898	0.043	0.149	2.003
7	33	188.00	0.092	0.287	3.627	0.049	0.153	1.929
7	34	185.75	0.086	0.346	3.473	0.046	0.186	1.870
7	35	181.99	0.073	0.400	3.268	0.040	0.220	1.796
8	36	192.90	0.076	0.291	2.715	0.039	0.151	1.407
8	37	155.84	0.062	0.193	1.922	0.040	0.124	1.233
8	38	152.04	0.070	0.252	1.791	0.046	0.166	1.178
8	39	156.90	0.079	0.212	1.615	0.050	0.135	1.029
8	40	111.09	0.057	0.087	1.386	0.051	0.078	1.248
9	41	193.89	0.073	0.275	1.865	0.038	0.142	0.962
9	42	165.59	0.059	0.245	1.897	0.036	0.148	1.146
9	43	175.90	0.082	0.210	1.721	0.047	0.119	0.978
9	44	161.94	0.062	0.250	1.841	0.038	0.154	1.137
9	45	162.39	0.054	0.271	1.548	0.033	0.167	0.953
10	46	200.54	0.064	0.393	3.704	0.032	0.196	1.847
10	47	202.99	0.071	0.310	3.745	0.035	0.153	1.845
10	48	176.72	0.072	0.298	3.410	0.041	0.169	1.930
10	49	184.59	0.055	0.277	3.529	0.030	0.150	1.912
10	50	158.96	0.076	0.272	3.373	0.048	0.171	2.122
11	51	162.87	0.067	0.253	3.272	0.041	0.155	2.009
11	52	196.58	0.077	0.289	3.458	0.039	0.147	1.759
11	53	191.22	0.056	0.284	3.641	0.029	0.149	1.904
11	54	190.87	0.091	0.237	3.345	0.048	0.124	1.753
11	55	182.17	0.077	0.260	3.569	0.042	0.143	1.959
12	56	219.40	0.060	0.327	4.031	0.027	0.149	1.837
12	57	198.95	0.089	0.303	3.650	0.045	0.152	1.835
12	58	193.04	0.076	0.281	3.490	0.039	0.146	1.808
12	59	190.29	0.085	0.326	3.378	0.045	0.171	1.775
12	60	176.60	0.069	0.319	3.247	0.039	0.181	1.839

INDIVIDUAL ORGAN WEIGHTS

GP-ANI NUMBER		BODY WEIGHT	HEART WEIGHT	BRAIN WEIGHT	SPLEEN WEIGHT	% HEART	% BRAIN	% SPLEEN
1	1	138.63	0.513	1.678	0.391	0.370	1.210	0.282
1	2	119.55	0.485	1.747	0.355	0.406	1.461	0.297
1	3	125.12	0.513	1.502	0.352	0.410	1.200	0.281
1	4	121.95	0.541	1.646	0.352	0.444	1.350	0.289
1	5	120.39	0.523	1.698	0.353	0.434	1.410	0.293
2	6	95.16	0.340	1.503	0.359	0.357	1.579	0.377
2	7	108.54	0.457	1.632	0.592	0.421	1.504	0.545
2	8	98.58	0.409	1.520	0.537	0.415	1.542	0.545
2	9	105.72	0.511	1.656	0.653	0.483	1.566	0.618
2	10	108.95	0.459	1.549	0.602	0.421	1.422	0.553
3	11	122.44	0.523	1.700	0.664	0.427	1.388	0.542
3	12	114.15	0.451	1.537	0.639	0.395	1.346	0.560
3	13	130.64	0.445	1.575	0.626	0.341	1.206	0.479
3	14	118.78	0.540	1.531	0.536	0.455	1.289	0.451
3	15	114.04	0.489	1.727	0.647	0.429	1.514	0.567
4	16	129.73	0.516	1.591	0.494	0.398	1.226	0.381
4	17	127.91	0.520	1.557	0.540	0.407	1.217	0.422
4	18	127.91	0.534	1.553	0.460	0.417	1.214	0.360
4	19	122.20	0.463	1.641	0.481	0.379	1.343	0.394
4	20	137.19	0.590	1.694	0.554	0.430	1.235	0.404
5	21	141.05	0.567	1.635	0.418	0.402	1.159	0.296
5	22	131.24	0.542	1.643	0.368	0.413	1.252	0.280
5	23	126.49	0.479	1.490	0.380	0.379	1.178	0.300
5	24	127.37	0.541	1.703	0.440	0.425	1.337	0.345
5	25	117.66	0.468	1.462	0.358	0.398	1.243	0.304
6	26	128.14	0.538	1.605	0.445	0.420	1.253	0.347
6	27	133.87	0.499	1.638	0.430	0.373	1.224	0.321
6	28	121.28	0.530	1.597	0.327	0.437	1.317	0.270
6	29	126.36	0.560	1.669	0.363	0.443	1.321	0.287
6	30	127.13	0.575	1.450	0.382	0.452	1.141	0.300

INDIVIDUAL ORGAN WEIGHTS

GP-ANI NUMBER		BODY WEIGHT	HEART WEIGHT	BRAIN WEIGHT	SPLEEN WEIGHT	% HEART	% BRAIN	% SPLEEN
7	31	188.24	0.720	1.626	0.441	0.382	0.864	0.234
7	32	194.61	0.812	1.815	0.472	0.417	0.933	0.243
7	33	188.00	0.670	1.689	0.449	0.356	0.898	0.239
7	34	185.75	0.703	1.666	0.485	0.378	0.897	0.261
7	35	181.99	0.666	1.694	0.490	0.366	0.931	0.269
8	36	192.90	0.699	1.783	0.784	0.362	0.924	0.406
8	37	155.84	0.618	1.729	0.797	0.397	1.109	0.511
8	38	152.04	0.578	1.750	0.809	0.380	1.151	0.532
8	39	156.90	0.601	1.569	0.872	0.383	1.000	0.556
8	40	111.09	0.422	1.652	0.497	0.380	1.487	0.447
9	41	193.89	0.728	1.863	0.876	0.375	0.961	0.452
9	42	165.59	0.694	1.820	0.775	0.419	1.099	0.468
9	43	175.90	0.701	1.532	0.835	0.399	0.871	0.475
9	44	161.94	0.688	1.720	0.657	0.425	1.062	0.406
9	45	162.39	0.622	1.630	0.806	0.383	1.004	0.496
10	46	200.54	0.775	1.771	0.619	0.386	0.883	0.309
10	47	202.99	0.735	1.753	0.629	0.362	0.864	0.310
10	48	176.72	0.708	1.573	0.565	0.401	0.890	0.320
10	49	184.59	0.665	1.778	0.550	0.360	0.963	0.298
10	50	158.96	0.585	1.601	0.473	0.368	1.007	0.298
11	51	162.87	0.616	1.735	0.357	0.378	1.065	0.219
11	52	196.58	0.797	1.677	0.480	0.405	0.853	0.244
11	53	191.22	0.710	1.737	0.453	0.371	0.908	0.237
11	54	190.87	0.721	1.564	0.505	0.378	0.819	0.265
11	55	182.17	0.661	1.715	0.458	0.363	0.941	0.251
12	56	219.40	0.825	1.942	0.510	0.376	0.885	0.232
12	57	198.95	0.794	1.657	0.521	0.399	0.833	0.262
12	58	193.04	0.694	1.496	0.467	0.360	0.775	0.242
12	59	190.29	0.662	1.670	0.504	0.348	0.878	0.265
12	60	176.60	0.602	1.578	0.419	0.341	0.894	0.237

INDIVIDUAL ORGAN WEIGHTS

GP-ANI NUMBER		BODY WEIGHT	KIDNEY WEIGHT	LUNGS WEIGHT	LIVER WEIGHT	% KIDNEY	% LUNGS	% LIVER
1	1	138.63	1.296	0.907	5.789	0.935	0.654	4.176
1	2	119.55	1.203	1.035	4.475	1.006	0.866	3.743
1	3	125.12	1.250	0.784	4.697	0.999	0.627	3.754
1	4	121.95	1.151	0.803	4.035	0.944	0.658	3.309
1	5	120.39	1.085	0.670	3.942	0.901	0.557	3.274
2	6	95.16	1.057	0.655	3.135	1.111	0.688	3.294
2	7	108.54	1.005	0.619	3.858	0.926	0.570	3.554
2	8	98.58	1.025	0.642	3.476	1.040	0.651	3.526
2	9	105.72	1.057	0.733	3.937	1.000	0.693	3.724
2	10	108.95	0.964	0.658	3.912	0.885	0.604	3.591
3	11	122.44	1.116	0.701	4.344	0.911	0.573	3.548
3	12	114.15	1.151	0.746	4.276	1.008	0.654	3.746
3	13	130.64	1.187	0.808	4.566	0.909	0.618	3.495
3	14	118.78	1.154	0.729	4.248	0.972	0.614	3.576
3	15	114.04	1.159	0.684	4.293	1.016	0.600	3.764
4	16	129.73	1.334	0.844	4.270	1.028	0.651	3.291
4	17	127.91	1.239	0.724	4.530	0.969	0.566	3.542
4	18	127.91	1.238	0.722	4.669	0.968	0.564	3.650
4	19	122.20	1.213	0.884	4.717	0.993	0.723	3.860
4	20	137.19	1.368	1.090	5.161	0.997	0.795	3.762
5	21	141.05	1.269	0.891	4.781	0.900	0.632	3.390
5	22	131.24	1.166	0.737	4.329	0.888	0.562	3.299
5	23	126.49	1.191	0.848	4.442	0.942	0.670	3.512
5	24	127.37	1.253	0.816	5.135	0.984	0.641	4.032
5	25	117.66	1.094	0.728	3.953	0.930	0.619	3.360
6	26	128.14	1.334	1.471	3.969	1.041	1.148	3.097
6	27	133.87	1.231	0.814	4.468	0.920	0.608	3.338
6	28	121.28	1.129	1.862	3.902	0.931	1.535	3.217
6	29	126.36	1.121	0.739	3.944	0.887	0.585	3.121
6	30	127.13	1.270	0.815	4.244	0.999	0.641	3.338

INDIVIDUAL ORGAN WEIGHTS

GP-ANI NUMBER		BODY WEIGHT	KIDNEY WEIGHT	LUNGS WEIGHT	LIVER WEIGHT	% KIDNEY	% LUNGS	% LIVER
7	31	188.24	1.499	0.981	6.225	0.796	0.521	3.307
7	32	194.61	1.740	1.166	6.763	0.894	0.599	3.475
7	33	188.00	1.607	1.094	6.094	0.855	0.582	3.241
7	34	185.75	1.530	0.982	6.163	0.824	0.529	3.318
7	35	181.99	1.478	0.961	5.808	0.812	0.528	3.191
8	36	192.90	1.822	1.048	6.907	0.945	0.543	3.581
8	37	155.84	1.586	0.821	5.270	1.018	0.527	3.382
8	38	152.04	1.501	0.829	5.716	0.987	0.545	3.760
8	39	156.90	1.509	0.875	5.913	0.962	0.558	3.769
8	40	111.09	1.186	1.665	3.902	1.068	1.499	3.512
9	41	193.89	1.850	0.969	6.228	0.954	0.500	3.212
9	42	165.59	1.637	0.839	6.126	0.989	0.507	3.699
9	43	175.90	1.689	0.976	6.906	0.960	0.555	3.926
9	44	161.94	1.542	0.829	5.866	0.952	0.512	3.622
9	45	162.39	1.537	0.787	5.828	0.946	0.485	3.589
10	46	200.54	1.772	1.019	7.586	0.884	0.508	3.783
10	47	202.99	1.764	0.995	7.236	0.869	0.490	3.565
10	48	176.72	1.657	0.882	6.478	0.938	0.499	3.666
10	49	184.59	1.663	0.911	6.680	0.901	0.494	3.619
10	50	158.96	1.518	0.976	5.522	0.955	0.614	3.474
11	51	162.87	1.572	0.973	5.009	0.965	0.597	3.075
11	52	196.58	1.710	0.964	6.870	0.870	0.490	3.495
11	53	191.22	1.678	0.936	6.590	0.878	0.489	3.446
11	54	190.87	1.724	0.923	7.192	0.903	0.484	3.768
11	55	182.17	1.761	0.995	6.712	0.967	0.546	3.684
12	56	219.40	1.892	1.115	7.581	0.862	0.508	3.455
12	57	198.95	1.757	0.998	6.754	0.883	0.502	3.395
12	58	193.04	1.736	0.934	6.667	0.899	0.484	3.454
12	59	190.29	1.543	1.006	6.109	0.811	0.529	3.210
12	60	176.60	1.510	0.888	6.196	0.855	0.503	3.508

APPENDIX D

HEMATOLOGY DATA

Hematology Data/Females

DOSE	ANIMAL	RBC COUNT	HGB	HCT	PLATELETS	WBC COUNT	NEUTRO-PHILS	LYMPHO-CYTES	HEINZ BODIES
TNB mg/kg diet	#	mIII/ cu mm	g/dl	%	thsn/ cu mm	thsn/ cu mm	%	%	%
0	1	9.87	20.2	55.9	913	6.3	29	71	0.0
	2	8.99	18.9	51.0	898	7.3	32	68	0.0
	3	8.37	17.0	48.0	916	3.0	26	74	0.0
	4	7.79	16.0	44.2	858	6.2	31	69	0.0
	5	7.32	15.3	41.4	908	7.2	25	75	0.0
1200	6	5.80	12.7	34.1	942	1.7	13	87	9.6
	7	5.41	12.6	33.2	1023	6.1	20	80	6.7
	8	5.75	13.3	35.2	979	8.3	16	84	5.8
	9	5.47	13.7	36.4	1154	9.2	11	89	5.1
	10	5.66	14.0	38.3	1137	6.2	18	82	3.2
800	11	5.65	14.2	37.5	1224	16.0	16	84	5.6
	12	5.36	12.3	33.4	1154	6.2	18	82	2.4
	13	6.31	13.8	38.7	1139	7.3	21	79	4.3
	14	5.68	14.9	33.6	1897	22.5	19	81	2.9
	15	5.57	14.0	35.4	1269	14.0	23	77	2.2
400	16	6.78	14.0	37.8	1097	6.2	22	78	0.9
	17	6.48	13.1	36.9	993	5.5	28	72	0.5
	18	6.53	18.9	36.8	2249	114.4	*	*	*
	19	6.43	13.6	37.4	1038	4.8	16	84	0.6
	20	6.66	17.3	37.1	1664	58.8	*	*	*
200	21	7.32	14.9	41.1	884	5.0	26	74	0.0
	22	7.39	15.0	41.1	912	5.3	22	78	0.0
	23	7.37	15.0	40.6	905	5.4	20	80	0.0
	24	7.47	16.8	43.9	930	14.5	15	85	0.0
	25	7.73	15.6	43.1	958	4.8	29	71	0.0
50	26	7.54	15.4	41.9	889	5.2	28	72	0.0
	27	7.73	15.3	43.3	891	4.8	31	69	0.0
	28	*	*	*	*	*	*	*	*
	29	7.59	15.4	42.5	936	4.6	26	74	0.0
	30	7.19	15.2	40.6	831	5.3	32	68	0.0

* = Quantity not sufficient

Hematology Data/Males

DOSE	ANIMAL	RBC COUNT	HGB	HCT	PLATELETS	WBC COUNT	NEUTRO-PHILS	LYMPHO-CYTES	HEINZ BODIES
TNB mg/kg diet	#	mlll/ cu mm	g/dl	%	thsn/ cu mm	thsn/ cu mm	%	%	%
0	31	8.17	15.8	43.5	965	5.6	36	64	0.0
	32	7.50	17.1	42.7	1151	15.0	29	71	0.0
	33	8.25	16.2	44.2	1046	5.8	31	69	0.0
	34	7.91	15.7	43.5	986	6.2	27	73	0.0
	35	7.25	14.8	40.1	886	5.0	25	75	0.0
1200	36	7.23	14.5	39.3	2220	10.2	19	81	3.4
	37	6.54	18.7	37.0	1614	125.5	8	92	6.1
	38	6.18	13.5	36.5	1190	7.3	11	89	4.8
	39	5.89	12.9	34.4	1061	7.3	21	79	4.1
	40	5.96	12.6	35.5	1079	6.1	20	80	3.6
800	41	6.38	14.3	39.2	1173	7.9	17	83	3.4
	42	6.44	12.9	35.0	1118	5.5	22	78	2.3
	43	6.55	13.6	36.6	1191	6.9	19	81	4.6
	44	6.97	13.9	38.3	1236	6.6	16	84	2.1
	45	*	*	*	*	*	*	*	*
400	46	6.31	15.6	32.4	2811	35.8	12	88	1.2
	47	7.28	14.3	38.5	1201	4.8	19	81	0.4
	48	7.29	15.3	40.8	2041	7.8	15	85	0.7
	49	7.50	14.5	39.8	1218	4.9	17	83	0.5
	50	7.49	14.2	41.8	1043	6.0	14	86	1.0
200	51	8.60	16.8	45.4	919	5.6	15	85	0.0
	52	7.67	22.1	44.1	1158	82.8	9	91	0.0
	53	8.00	16.2	44.1	983	7.5	18	82	0.0
	54	7.51	15.1	41.4	924	4.9	12	88	0.0
	55	7.95	15.6	43.2	1034	5.6	13	87	0.0
50	56	8.08	23.2	48.0	1077	87.4	10	90	0.0
	57	8.13	16.5	44.2	868	4.2	19	81	0.0
	58	7.85	15.7	42.8	1002	7.3	20	80	0.0
	59	7.72	22.6	44.0	1280	91.7	11	89	0.0
	60	7.61	15.2	42.3	1048	5.4	22	78	0.0

* = Quantity not sufficient

APPENDIX E

CLINICAL CHEMISTRY DATA

Clinical Chemistries/Females

DOSE TNB mg/kg diet	ANIMAL #	GLUCOSE mg/dl	CREATININE mg/dl	BUN mg/dl	Na mmol/l	TOTAL PROTEIN g/dl	TOTAL BILIRUBIN mg/dl	AST U/L
0	1	118	0.6	21	143	6.6	0.1	101
	2	*	*	*	144	6.0	*	*
	3	*	*	*	*	*	*	*
	4	*	0.6	20	144	6.1	0.1	*
	5	139	0.4	21	145	6.0	0.1	88
1200	6	179	0.6	44	142	5.6	0.2	134
	7	245	0.5	21	144	6.5	0.2	100
	8	167	0.5	24	146	6.6	0.1	100
	9	143	0.7	24	147	7.0	0.2	118
	10	202	0.5	24	145	6.5	0.1	85
800	11	149	0.6	21	147	6.8	0.2	98
	12	180	0.3	18	143	6.5	0.1	103
	13	146	0.5	24	144	6.9	0.2	118
	14	212	0.4	17	143	6.6	0.1	70
	15	129	0.4	22	146	6.7	0.1	112
400	16	157	0.5	20	145	6.4	0.1	100
	17	166	0.4	22	129	6.1	0.1	76
	18	148	0.3	20	145	6.0	0.1	88
	19	163	0.4	20	144	6.9	0.2	85
	20	210	0.5	20	145	6.7	0.2	103
200	21	181	0.5	19	143	6.5	0.1	100
	22	171	0.4	25	144	6.0	0.1	94
	23	175	0.4	19	145	6.6	0.0	85
	24	*	0.5	21	145	6.7	0.0	*
	25	*	0.5	20	144	6.5	0.0	159
50	26	*	*	*	135	6.2	*	*
	27	168	0.3	19	145	7.2	0.1	72
	28	*	0.0	19	133	6.9	0.2	*
	29	203	0.3	21	145	5.8	0.1	96
	30	*	*	*	142	5.9	*	*

* = Quantity not sufficient

Clinical Chemistries/Females

DOSE TNB mg/kg diet	ANIMAL #	ALT U/L	AP U/L	K mmol/l	Ca mg/dl	ALBUMIN g/dl
0	1	42	130	12.0	11.3	3.8
	2	*	*	8.9	*	*
	3	*	*	*	*	*
	4	*	*	7.0	10.4	3.4
	5	22	132	5.9	10.6	3.3
1200	6	38	72	4.7	9.0	3.4
	7	23	79	6.2	10.4	3.8
	8	26	97	5.1	10.5	3.8
	9	22	82	5.8	10.6	4.0
	10	16	85	6.0	10.7	3.8
800	11	28	91	6.4	11.3	3.9
	12	16	90	7.2	10.7	3.7
	13	28	105	5.9	10.6	4.0
	14	25	91	5.9	11.4	3.9
	15	27	112	5.8	11.1	3.7
400	16	27	95	5.6	10.9	3.8
	17	31	105	6.1	10.8	3.8
	18	25	94	6.1	10.7	3.5
	19	25	101	5.8	10.8	4.0
	20	29	87	6.9	11.4	3.9
200	21	29	95	5.8	10.8	3.7
	22	23	110	5.7	10.8	3.6
	23	27	105	6.6	11.5	3.7
	24	*	*	8.0	10.6	3.7
	25	28	112	6.1	10.4	3.6
50	26	*	*	5.5	*	*
	27	19	103	5.8	10.6	3.5
	28	*	*	2.7	9.2	3.3
	29	8	110	6.1	10.2	3.3
	30	*	*	5.4	*	*

* = Quantity not sufficient

Clinical Chemistries/Males

DOSE TNB mg/kg diet	ANIMAL #	GLUCOSE mg/dl	CREATININE mg/dl	BUN mg/dl	Na mmol/l	TOTAL PROTEIN g/dl	TOTAL BILIRUBIN mg/dl	AST U/L
0	31	289	0.4	20	146	6.2	0.1	115
	32	261	0.5	16	146	6.4	0.0	91
	33	298	0.6	22	146	6.8	0.1	132
	34	244	0.5	20	145	6.4	0.0	166
	35	235	0.5	20	143	6.0	0.1	103
1200	36	191	0.5	22	148	6.6	0.1	137
	37	179	0.4	21	146	6.5	0.2	148
	38	186	0.5	21	144	6.8	0.1	123
	39	*	0.4	21	145	6.3	0.2	*
	40	176	0.4	23	141	5.6	0.2	158
800	41	224	0.5	21	146	7.1	0.1	95
	42	228	0.5	19	145	6.8	0.1	80
	43	190	0.5	21	146	6.8	0.1	141
	44	212	0.4	21	146	7.0	0.1	136
	45	208	0.4	21	146	6.7	0.1	99
400	46	209	0.6	20	143	6.9	0.1	76
	47	216	0.5	22	148	6.6	0.1	99
	48	180	0.5	18	146	6.4	0.1	174
	49	253	0.5	22	148	6.7	0.1	88
	50	246	0.4	20	146	6.5	0.0	216
200	51	*	*	*	136	6.3	*	*
	52	233	0.5	20	147	6.8	0.1	93
	53	214	0.5	21	147	6.9	0.1	167
	54	211	0.5	19	147	7.3	0.1	134
	55	237	0.4	17	147	7.2	0.0	90
50	56	185	0.6	19	148	7.6	0.0	202
	57	197	0.5	18	147	7.5	0.1	129
	58	327	0.4	18	147	7.0	0.0	81
	59	208	0.6	20	147	7.3	0.0	101
	60	207	0.5	20	146	7.6	0.0	101

* = Quantity not sufficient

Clinical Chemistries/Males

DOSE TNB mg/kg diet	ANIMAL #	ALT U/L	AP U/L	K mmol/l	Ca mg/dl	ALBUMIN g/dl
0	31	38	160	6.5	11.7	4.0
	32	35	140	5.4	11.1	4.0
	33	42	156	6.4	11.7	4.0
	34	52	154	6.2	11.4	3.6
	35	37	154	6.4	11.2	3.7
1200	36	36	94	7.1	11.5	3.8
	37	17	89	6.8	11.1	3.9
	38	40	107	6.3	11.6	4.1
	39	*	*	6.5	10.9	3.8
	40	42	83	6.3	10.2	3.4
800	41	25	112	5.3	11.3	4.3
	42	27	92	7.8	11.6	4.2
	43	28	129	6.7	11.2	4.0
	44	37	114	6.4	11.2	4.0
	45	28	118	7.4	11.8	4.0
400	46	30	114	6.4	12.3	4.3
	47	30	131	6.6	12.0	4.2
	48	21	130	6.4	11.1	3.7
	49	31	125	6.8	12.0	4.2
	50	66	127	7.0	11.6	3.8
200	51	*	*	5.8	*	*
	52	24	128	7.1	14.4	4.0
	53	62	152	6.3	12.1	4.2
	54	36	131	5.4	10.8	3.9
	55	26	148	5.5	10.9	3.7
50	56	34	142	6.1	11.3	3.9
	57	28	153	5.4	10.8	3.9
	58	34	146	5.6	11.7	3.9
	59	37	162	5.7	11.7	4.1
	60	37	172	6.4	11.0	3.8

* = Quantity not sufficient

APPENDIX F

CLINICAL OBSERVATIONS

CLINICAL OBSERVATIONS

DATE	GROUP 1
08/18/92	Study started. All animals look normal.
08/19/92	All animals look normal.
08/20/92	All animals look normal.
08/21/92	Food & Water changed. All animals look normal.
08/24/92	All animals look normal.
08/25/92	Food & Water changed. All animals look normal. Animals weighed.
08/26/92	All animals look normal.
08/27/92	All animals look normal.
08/28/92	Food & Water changed. All animals look normal.
08/31/92	All animals look normal.
09/01/92	Food & Water changed. All animals look normal. Animals weighed. Animals fasted at 2:30 PM.
09/02/92	Animals necropsied today.

DATE	GROUP 2
08/18/92	Study started. All animals look normal.
08/19/92	All animals look normal.
08/20/92	All animals look normal.
08/21/92	Food & Water changed. All animals look normal.
08/24/92	All animals look normal.
08/25/92	Food & Water changed. All animals look normal. Animals weighed.
08/26/92	All animals look normal.
08/27/92	All animals look normal.
08/28/92	Food & Water changed. #6 losing weight. Others look normal.
08/31/92	All animals look normal.
09/01/92	Food & Water changed. #6 still weak;others look normal Animals weighed. Animals fasted at 2:30 PM.
09/02/92	Animals necropsied today.

DATE	GROUP 3
08/18/92	Study started. All animals look normal.
08/19/92	All animals look normal.
08/20/92	All animals look normal.
08/21/92	Food & Water changed. All animals are normal.
08/24/92	All animals look normal.
08/25/92	Food & Water changed. All animals look normal. Animals weighed.
08/26/92	All animals look normal.
08/27/92	All animals look normal.
08/28/92	Food & Water changed. All animals look normal.
08/31/92	All animals look normal.
09/01/92	Food & Water changed. All animals look normal. Animals weighed. Animals fasted at 2:30 PM.
09/02/92	Animals necropsied today.

CLINICAL OBSERVATIONS

DATE	GROUP 4
08/18/92	Study started. #20 was replaced by an extra rat. All others look normal.
08/19/92	All animals look normal.
08/20/92	All animals look normal.
08/21/92	Food & Water changed. Bottle for #20 had defective stopper. All animals look normal.
08/24/92	All animals look normal.
08/25/92	Food & Water changed. All animals look normal. Animals weighed.
08/26/92	All animals look normal.
08/27/92	All animals look normal.
08/28/92	Food & Water changed. All animals look normal.
08/31/92	All animals look normal.
09/01/92	Food & Water changed. All animals look normal. Animals weighed. Animals fasted at 2:30 PM.
09/02/92	Animals necropsied today.

DATE	GROUP 5
08/18/92	Study started. All animals look normal.
08/19/92	All animals look normal.
08/20/92	All animals look normal.
08/21/92	Food & Water changed. All animals look normal.
08/24/92	All animals look normal.
08/25/92	Food & Water changed. All animals look normal. Animals weighed.
08/26/92	All animals look normal.
08/27/92	All animals look normal.
08/28/92	Food & Water changed. All animals look normal.
08/31/92	All animals look normal.
09/01/92	Food & Water changed. All animals look normal. Animals weighed. Animals fasted at 2:30 PM.
09/02/92	Animals necropsied today.

DATE	GROUP 6
08/18/92	Study started. All animals look normal.
08/19/92	All animals look normal.
08/20/92	All animals look normal.
08/21/92	Food & Water changed. All animals look normal.
08/24/92	All animals look normal.
08/25/92	Food & Water changed. #6-30 had a bad bottle stopper. It was replaced. Animals weighed. All animals look normal.
08/26/92	All animals look normal.
08/27/92	All animals look normal.
08/28/92	Food & Water changed. All animals look normal.
08/31/92	All animals look normal.
09/01/92	Food & Water changed. All animals look normal. Animals weighed. Animals fasted at 2:30 PM.
09/02/92	Animals necropsied today.

CLINICAL OBSERVATIONS

DATE	GROUP 7
08/18/92	Study started. All animals look normal.
08/19/92	All animals look normal.
08/20/92	All animals look normal.
08/21/92	Food & Water changed. All animals look normal.
08/24/92	All animals look normal.
08/25/92	Food & Water changed. All animals look normal. Animals weighed.
08/26/92	All animals look normal.
08/27/92	All animals look normal.
08/28/92	Food & Water changed. All animals look normal.
08/31/92	All animals look normal.
09/01/92	Food & Water changed. All animals look normal. Animals weighed. Animals fasted at 2:30 PM.
09/02/92	Animals necropsied today.

DATE	GROUP 8
08/18/92	Study started. All animals look normal.
08/19/92	All animals look normal.
08/20/92	All animals look normal.
08/21/92	Food & Water changed. All animals look normal.
08/24/92	All animals look normal.
08/25/92	Food & Water changed. #8-40 had a bad bottle stopper. It was replaced. Animals weighed. #8-40 losing weight-114.9g.
08/26/92	All animals look normal.
08/27/92	All animals look normal.
08/28/92	Food & Water changed. #8-40 getting back some weight. Others animals look normal.
08/31/92	All animals look normal.
09/01/92	Food & Water changed. All animals look normal. Animals weighed. #8-40 up to 125.5g. Animals fasted at 2:30 PM.
09/02/92	Animals necropsied today.

DATE	GROUP 9
08/18/92	Study started. All animals look normal.
08/19/92	All animals look normal.
08/20/92	All animals look normal.
08/21/92	Food & Water changed. All animals look normal.
08/24/92	All animals look normal.
08/25/92	Food & Water changed. All animals look normal. Animals weighed.
08/26/92	All animals look normal.
08/27/92	All animals look normal.
08/28/92	Food & Water changed. All animals look normal.
08/31/92	All animals look normal.
09/01/92	Food & Water changed. All animals look normal. Animals weighed. Animals fasted at 2:30 PM.
09/02/92	Animals necropsied today.

CLINICAL OBSERVATIONS

DATE	GROUP 10
08/18/92	Study started. All animals look normal.
08/19/92	All animals look normal.
08/20/92	All animals look normal.
08/21/92	Food & Water changed. All animals look normal.
08/24/92	All animals look normal.
08/25/92	Food & Water changed. All animals look normal. Animals weighed.
08/26/92	All animals look normal.
08/27/92	All animals look normal.
08/28/92	Food & Water changed. All animals look normal.
08/31/92	All animals look normal.
09/01/92	Food & Water changed. All animals look normal. Animals weighed. Animals fasted at 2:30 PM.
09/02/92	Animals necropsied today.

DATE	GROUP 11
08/18/92	Study started. All animals look normal.
08/19/92	All animals look normal.
08/20/92	All animals look normal.
08/21/92	Food & Water changed. All animals look normal.
08/24/92	All animals look normal.
08/25/92	Food & Water changed. All animals look normal. Animals weighed.
08/26/92	All animals look normal.
08/27/92	All animals look normal.
08/28/92	Food & Water changed. All animals look normal.
08/31/92	All animals look normal.
09/01/92	Food & Water changed. All animals look normal. Animals weighed. Animals fasted at 2:30 PM.
09/02/92	Animals necropsied today.

DATE	GROUP 12
08/18/92	Study started. All animals look normal.
08/19/92	All animals look normal.
08/20/92	All animals look normal.
08/21/92	Food & Water changed. All animals look normal.
08/24/92	All animals look normal.
08/25/92	Food & Water changed. All animals look normal. Animals weighed.
08/26/92	All animals look normal.
08/27/92	All animals look normal.
08/28/92	Food & Water changed. All animals look normal.
08/31/92	All animals look normal.
09/01/92	Food & Water changed. All animals look normal. Animals weighed. Animals fasted at 2:30 PM.
09/02/92	Animals necropsied today.

APPENDIX G

HISTOPATHOLOGY DATA

HISTOPATHOLOGY DATA

REPORTS CODE TABLE

N	Tissues within normal histological limits
A	Autolysis precluding adequate evaluation
U	Tissues unavailable for evaluation
*	Tissues not examined/not required by protocol
1	Minimal
2	Mild
3	Moderate
4	Marked

Abbreviations

Inflam.	Inflammation
Degen.	Degeneration

(End of Report)

PATHOLOGY ASSOCIATES, INC.
14 DAY TOXICITY EVALUATION OF 1,3,5-TRINITROBENZENE (TNB)
IN FISCHER (F344) RATS

Project Summary Table
SUMMARY: Incidence of NEOPLASTIC and NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: 92-002 FATES: ALL
DAYS : ALL SEX: FEMALE

GROUP:		1		2		3		4		5		6	
NUMBER OF ANIMALS:		5		5		5		5		5		5	
		#	%	#	%	#	%	#	%	#	%	#	%
BRAIN	# Ex	5		5		0		0		0		0	
Hemorrhage		0	(0)	2	(40)	0		0		0		0	
Microgliosis		0	(0)	2	(40)	0		0		0		0	
Necrosis		0	(0)	1	(20)	0		0		0		0	
Vacuolization		0	(0)	2	(40)	0		0		0		0	
SCIATIC NERVE	# Ex	5		5		0		0		0		0	
SPINAL CORD	# Ex	5		5		0		0		0		0	
SALIVARY GLAND	# Ex	5		5		0		0		0		0	
PANCREAS	# Ex	5		5		0		0		0		0	
MANDIBULAR LYMPH NODE	# Ex	5		5		0		0		0		0	
ZYMBAL'S GLAND	# Ex	5		5		0		0		0		0	
PITUITARY	# Ex	5		5		0		0		0		0	
ADRENALS	# Ex	5		5		0		0		0		0	
Accessory Cortical Nodule		1	(20)	0	(0)	0		0		0		0	
THYROID	# Ex	4		5		0		0		0		0	
PARATHYROID	# Ex	4		5		0		0		0		0	
TRACHEA	# Ex	5		5		0		0		0		0	

(Report Continued)

PATHOLOGY ASSOCIATES, INC.
14 DAY TOXICITY EVALUATION OF 1,3,5-TRINITROBENZENE (TNB)
IN FISCHER (F344) RATS

Project Summary Table
SUMMARY: Incidence of NEOPLASTIC and NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: 92-002 FATES: ALL
DAYS : ALL SEX: FEMALE

GROUP:		1		2		3		4		5		6	
NUMBER OF ANIMALS:		5		5		5		5		5		5	
		#	%	#	%	#	%	#	%	#	%	#	%
ESOPHAGUS	# Ex	5		5		0		0		0		0	
THYMUS	# Ex	5		5		0		0		0		0	
Necrosis, Cortical		0	(0)	1	(20)	0		0		0		0	
Depletion, Lymphoid		0	(0)	1	(20)	0		0		0		0	
HEART	# Ex	5		5		0		0		0		0	
Inflammation, Chronic		1	(20)	1	(20)	0		0		0		0	
COLON	# Ex	5		5		0		0		0		0	
JEJUNUM	# Ex	5		5		0		0		0		0	
AORTA	# Ex	5		5		0		0		0		0	
LIVER	# Ex	5		5		0		0		0		0	
SPLEEN	# Ex	5		5		5		5		5		5	
Hyperplasia, Erythroid Cell		0	(0)	5	(100)	5	(100)	5	(100)	0	(0)	0	(0)
Depletion, Lymphoid		0	(0)	3	(60)	0	(0)	0	(0)	0	(0)	0	(0)
Fibrosis		0	(0)	1	(20)	0	(0)	1	(20)	0	(0)	0	(0)
TONGUE	# Ex	5		5		0		0		0		0	
SKELETAL MUSCLE	# Ex	5		5		0		0		0		0	
LUNG	# Ex	5		5		0		0		0		0	
KIDNEYS	# Ex	5		5		0		0		0		0	
Mineralization, NOS		3	(60)	1	(20)	0		0		0		0	

(Report Continued)

PATHOLOGY ASSOCIATES, INC.
14 DAY TOXICITY EVALUATION OF 1,3,5-TRINITROBENZENE (TNB)
IN FISCHER (F344) RATS

Project Summary Table
SUMMARY: Incidence of NEOPLASTIC and NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: 92-002 FATES: ALL
DAYS : ALL SEX: FEMALE

GROUP:		1		2		3		4		5		6	
NUMBER OF ANIMALS:		5		5		5		5		5		5	
		#	%	#	%	#	%	#	%	#	%	#	%
KIDNEYS	# Ex	5		5		0		0		0		0	
Inflammation, Chronic		0	(0)	1	(20)	0		0		0		0	
URINARY BLADDER	# Ex	5		5		0		0		0		0	
STOMACH	# Ex	5		5		0		0		0		0	
DUODENUM	# Ex	5		5		0		0		0		0	
ILEUM	# Ex	5		5		0		0		0		0	
CECUM	# Ex	5		5		0		0		0		0	
RECTUM	# Ex	5		5		0		0		0		0	
MESENTERIC LYMPH NODE	# Ex	5		5		0		0		0		0	
OVARIES	# Ex	5		5		0		0		0		0	
UTERUS	# Ex	5		5		0		0		0		0	
SKIN	# Ex	5		5		0		0		0		0	
MAMMARY GLAND	# Ex	5		5		0		0		0		0	
CLITORAL GLANDS	# Ex	5		5		0		0		0		0	
Inflammation, Chronic		3	(60)	2	(40)	0		0		0		0	
Inflammation, Chronic/Active		1	(20)	2	(40)	0		0		0		0	
Dilatation, Ductal		2	(40)	3	(60)	0		0		0		0	

(Report Continued)

PATHOLOGY ASSOCIATES, INC.
14 DAY TOXICITY EVALUATION OF 1,3,5-TRINITROBENZENE (TNB)
IN FISCHER (F344) RATS

Project Summary Table
SUMMARY: Incidence of NEOPLASTIC and NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: 92-002 FATES: ALL PAGE 4
DAYS : ALL SEX: FEMALE

GROUP:		1		2		3		4		5		6	
NUMBER OF ANIMALS:		5		5		5		5		5		5	
		#	%	#	%	#	%	#	%	#	%	#	%
EYES	# Ex	5		5		0		0		0		0	
HARDERIAN GLAND	# Ex	5		5		0		0		0		0	
FEMUR/BONE MARROW	# Ex	5		5		0		0		0		0	
Hyperplasia, Erythroid Cell		0	(0)	5	(100)	0		0		0		0	
Pigment, NOS		0	(0)	2	(40)	0		0		0		0	
STERNUM	# Ex	5		5		0		0		0		0	
NASAL CAVITY	# Ex	5		5		0		0		0		0	

(Report Continued)

PATHOLOGY ASSOCIATES, INC.
14 DAY TOXICITY EVALUATION OF 1,3,5-TRINITROBENZENE (TNB)
IN FISCHER (F344) RATS

Project Summary Table
SUMMARY: Incidence of NEOPLASTIC and NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: 92-002 FATES: ALL
DAYS : ALL SEX: MALE

GROUP:		7		8		9		10		11		12	
NUMBER OF ANIMALS:		5		5		5		5		5		5	
		#	%	#	%	#	%	#	%	#	%	#	%
BRAIN	# Ex	5		5		0		0		0		0	
SCIATIC NERVE	# Ex	5		5		0		0		0		0	
SPINAL CORD	# Ex	5		5		0		0		0		0	
SALIVARY GLAND	# Ex	5		5		0		0		0		0	
PANCREAS	# Ex	5		5		0		0		0		0	
MANDIBULAR LYMPH NODE	# Ex	5		5		0		0		0		0	
Plasmacytosis		0	(0)	1	(20)	0		0		0		0	
ZYMBAL'S GLAND	# Ex	5		5		0		0		0		0	
PITUITARY	# Ex	5		5		0		0		0		0	
ADRENALS	# Ex	5		5		0		0		0		0	
THYROID	# Ex	5		5		0		0		0		0	
PARATHYROID	# Ex	4		5		0		0		0		0	
TRACHEA	# Ex	5		5		0		0		0		0	
ESOPHAGUS	# Ex	5		5		0		0		0		0	

(Report Continued)

PATHOLOGY ASSOCIATES, INC.
14 DAY TOXICITY EVALUATION OF 1,3,5-TRINITROBENZENE (TNB)
IN FISCHER (F344) RATS

Project Summary Table
SUMMARY: Incidence of NEOPLASTIC and NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: 92-002 FATES: ALL
DAYS : ALL SEX: MALE

GROUP:		7		8		9		10		11		12	
NUMBER OF ANIMALS:		5		5		5		5		5		5	
		#	%	#	%	#	%	#	%	#	%	#	%
THYMUS	# Ex	5		5		0		0		0		0	
HEART	# Ex	5		5		0		0		0		0	
Inflammation, Chronic		1	(20)	0	(0)	0		0		0		0	
COLON	# Ex	5		5		0		0		0		0	
JEJUNUM	# Ex	5		5		0		0		0		0	
AORTA	# Ex	5		5		0		0		0		0	
LIVER	# Ex	5		5		0		0		0		0	
SPLEEN	# Ex	5		5		5		5		5		5	
Hyperplasia, Erythroid Cell		0	(0)	5	(100)	5	(100)	4	(80)	0	(0)	0	(0)
Depletion, Lymphoid		0	(0)	1	(20)	0	(0)	0	(0)	0	(0)	0	(0)
TONGUE	# Ex	5		5		0		0		0		0	
SKELETAL MUSCLE	# Ex	5		5		0		0		0		0	
LUNG	# Ex	5		5		0		0		0		0	
KIDNEYS	# Ex	5		5		5		5		5		5	
Hyaline Droplets		0	(0)	5	(100)	5	(100)	5	(100)	5	(100)	1	(20)
Mineralization, NOS		4	(80)	4	(80)	5	(100)	5	(100)	5	(100)	5	(100)
Degeneration, Tubular		3	(60)	4	(80)	5	(100)	5	(100)	4	(80)	5	(100)
URINARY BLADDER	# Ex	5		5		0		0		0		0	

(Report Continued)

PATHOLOGY ASSOCIATES, INC.
14 DAY TOXICITY EVALUATION OF 1,3,5-TRINITROBENZENE (TNB)
IN FISCHER (F344) RATS

Project Summary Table
SUMMARY: Incidence of NEOPLASTIC and NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: 92-002 FATES: ALL
DAYS : ALL SEX: MALE

GROUP:		7		8		9		10		11		12	
NUMBER OF ANIMALS:		5		5		5		5		5		5	
		#	%	#	%	#	%	#	%	#	%	#	%
PROSTATE	# Ex	5		5		0		0		0		0	
STOMACH	# Ex	5		5		0		0		0		0	
DUODENUM	# Ex	5		5		0		0		0		0	
Ectopic Pancreas		1	(20)	0	(0)	0		0		0		0	
ILEUM	# Ex	5		5		0		0		0		0	
CECUM	# Ex	5		5		0		0		0		0	
RECTUM	# Ex	5		5		0		0		0		0	
MESENTERIC LYMPH NODE	# Ex	5		5		0		0		0		0	
TESTES	# Ex	5		5		5		5		5		5	
Degen., Seminiferous Tubule		0	(0)	5	(100)	5	(100)	0	(0)	0	(0)	0	(0)
EPIDIDYMIS	# Ex	5		5		0		0		0		0	
Hypospermia		0	(0)	5	(100)	0		0		0		0	
SEMINAL VESICLES	# Ex	5		5		0		0		0		0	
Atrophy, NOS		1	(20)	1	(20)	0		0		0		0	
SKIN	# Ex	5		5		0		0		0		0	
MAMMARY GLAND	# Ex	5		5		0		0		0		0	

(Report Continued)

PATHOLOGY ASSOCIATES, INC.
14 DAY TOXICITY EVALUATION OF 1,3,5-TRINITROBENZENE (TNB)
IN FISCHER (F344) RATS

Project Summary Table
SUMMARY: Incidence of NEOPLASTIC and NON-NEOPLASTIC Microscopic Findings

PROJECT ID. NO: 92-002 FATES: ALL
DAYS : ALL SEX: MALE

GROUP:		7		8		9		10		11		12	
NUMBER OF ANIMALS:		5		5		5		5		5		5	
		#	%	#	%	#	%	#	%	#	%	#	%
PREPUTIAL GLANDS	# Ex	5		5		0		0		0		0	
Inflammation, Chronic		4	(80)	2	(40)	0		0		0		0	
Inflammation, Chronic/Active		1	(20)	3	(60)	0		0		0		0	
Dilatation, Ductal		3	(60)	2	(40)	0		0		0		0	
EYES	# Ex	5		5		0		0		0		0	
Inflammation, Chronic, Cornea		2	(40)	1	(20)	0		0		0		0	
HARDERIAN GLAND	# Ex	5		5		0		0		0		0	
FEMUR/BONE MARROW	# Ex	5		5		0		0		0		0	
Hyperplasia, Erythroid Cell		0	(0)	5	(100)	0		0		0		0	
STERNUM	# Ex	5		5		0		0		0		0	
NASAL CAVITY	# Ex	5		5		0		0		0		0	

(End of Report)

PATHOLOGY ASSOCIATES, INC.
14 DAY TOXICITY EVALUATION OF 1,3,5-TRINITROBENZENE (TNB)
IN FISCHER (F344) RATS

Severity Summary Table

PROJECT ID. NO: 92-002 FATES: ALL
DAYS: ALL SEX: FEMALE

GROUP:		1		2		3		4		5		6	
NUMBER OF ANIMALS:		5		5		5		5		5		5	
		#	SEV	#	SEV	#	SEV	#	SEV	#	SEV	#	SEV
BRAIN	# Ex	5		5		0		0		0		0	
Hemorrhage		0		2	0.80	0		0		0		0	
Microgliosis		0		2	1.00	0		0		0		0	
Necrosis		0		1	0.40	0		0		0		0	
Vacuolization		0		2	1.00	0		0		0		0	
SCIATIC NERVE	# Ex	5		5		0		0		0		0	
SPINAL CORD	# Ex	5		5		0		0		0		0	
SALIVARY GLAND	# Ex	5		5		0		0		0		0	
PANCREAS	# Ex	5		5		0		0		0		0	
MANDIBULAR LYMPH NODE	# Ex	5		5		0		0		0		0	
ZYMBAL'S GLAND	# Ex	5		5		0		0		0		0	
PITUITARY	# Ex	5		5		0		0		0		0	
ADRENALS	# Ex	5		5		0		0		0		0	
THYROID	# Ex	4		5		0		0		0		0	
PARATHYROID	# Ex	4		5		0		0		0		0	
TRACHEA	# Ex	5		5		0		0		0		0	

(Report Continued)

Severity Summary Table

PROJECT ID. NO: 92-002
DAYS: ALL
FATES: ALL
SEX: FEMALE

GROUP:	1		2		3		4		5		6	
NUMBER OF ANIMALS:	5		5		5		5		5		5	
	#	SEV	#	SEV	#	SEV	#	SEV	#	SEV	#	SEV
ESOPHAGUS # Ex	5		5		0		0		0		0	
THYMUS # Ex	5		5		0		0		0		0	
Necrosis, Cortical	0		1	0.60	0		0		0		0	
Depletion, Lymphoid	0		1	0.60	0		0		0		0	
HEART # Ex	5		5		0		0		0		0	
Inflammation, Chronic	1	0.20	1	0.20	0		0		0		0	
COLON # Ex	5		5		0		0		0		0	
JEJUNUM # Ex	5		5		0		0		0		0	
AORTA # Ex	5		5		0		0		0		0	
LIVER # Ex	5		5		0		0		0		0	
SPLEEN # Ex	5		5		5		5		5		5	
Hyperplasia, Erythroid Cell	0		5	2.40	5	2.60	5	1.80	0		0	
Depletion, Lymphoid	0		3	0.80	0		0		0		0	
Fibrosis	0		1	0.40	0		1	0.20	0		0	
TONGUE # Ex	5		5		0		0		0		0	
SKELETAL MUSCLE # Ex	5		5		0		0		0		0	
LUNG # Ex	5		5		0		0		0		0	
KIDNEYS # Ex	5		5		0		0		0		0	

(Report Continued)

PATHOLOGY ASSOCIATES, INC.
14 DAY TOXICITY EVALUATION OF 1,3,5-TRINITROBENZENE (TNB)
IN FISCHER (F344) RATS

Severity Summary Table

PROJECT ID. NO: 92-002 FATES: ALL
DAYS: ALL SEX: FEMALE

GROUP:		1		2		3		4		5		6	
NUMBER OF ANIMALS:		5		5		5		5		5		5	
		#	SEV	#	SEV	#	SEV	#	SEV	#	SEV	#	SEV
Mineralization, NOS		3	0.60	1	0.20	0		0		0		0	
Inflammation, Chronic		0		1	0.20	0		0		0		0	
URINARY BLADDER	# Ex	5		5		0		0		0		0	
STOMACH	# Ex	5		5		0		0		0		0	
DUODENUM	# Ex	5		5		0		0		0		0	
ILEUM	# Ex	5		5		0		0		0		0	
CECUM	# Ex	5		5		0		0		0		0	
RECTUM	# Ex	5		5		0		0		0		0	
MESENTERIC LYMPH NODE	# Ex	5		5		0		0		0		0	
OVARIES	# Ex	5		5		0		0		0		0	
UTERUS	# Ex	5		5		0		0		0		0	
SKIN	# Ex	5		5		0		0		0		0	
MAMMARY GLAND	# Ex	5		5		0		0		0		0	
CLITORAL GLANDS	# Ex	5		5		0		0		0		0	
Inflammation, Chronic		3	0.80	2	1.00	0		0		0		0	
Inflammation, Chronic/Active		1	0.60	2	1.00	0		0		0		0	

(Report Continued)

Severity Summary Table

PROJECT ID. NO: 92-002 FATES: ALL
DAYS: ALL SEX: FEMALE

GROUP:		1	2	3	4	5	6
NUMBER OF ANIMALS:		5	5	5	5	5	5
		# SEV	# SEV	# SEV	# SEV	# SEV	# SEV
Dilatation, Ductal		2 0.80	3 1.40	0	0	0	0
EYES	# Ex	5	5	0	0	0	0
HARDERIAN GLAND	# Ex	5	5	0	0	0	0
FEMUR/BONE MARROW	# Ex	5	5	0	0	0	0
Hyperplasia, Erythroid Cell		0	5 2.00	0	0	0	0
Pigment, NOS		0	2 0.60	0	0	0	0
STERNUM	# Ex	5	5	0	0	0	0
NASAL CAVITY	# Ex	5	5	0	0	0	0

* Severity calculated by the number of tissues examined.

(Report Continued)

PATHOLOGY ASSOCIATES, INC.
14 DAY TOXICITY EVALUATION OF 1,3,5-TRINITROBENZENE (TNB)
IN FISCHER (F344) RATS

Severity Summary Table

PROJECT ID. NO: 92-002 FATES: ALL
DAYS: ALL SEX: MALE

GROUP:		7		8		9		10		11		12	
NUMBER OF ANIMALS:		5		5		5		5		5		5	
		#	SEV	#	SEV	#	SEV	#	SEV	#	SEV	#	SEV
BRAIN	# Ex	5		5		0		0		0		0	
SCIATIC NERVE	# Ex	5		5		0		0		0		0	
SPINAL CORD	# Ex	5		5		0		0		0		0	
SALIVARY GLAND	# Ex	5		5		0		0		0		0	
PANCREAS	# Ex	5		5		0		0		0		0	
MANDIBULAR LYMPH NODE	# Ex	5		5		0		0		0		0	
Plasmacytosis		0		1	0.40	0		0		0		0	
ZYMBAL'S GLAND	# Ex	5		5		0		0		0		0	
PITUITARY	# Ex	5		5		0		0		0		0	
ADRENALS	# Ex	5		5		0		0		0		0	
THYROID	# Ex	5		5		0		0		0		0	
PARATHYROID	# Ex	4		5		0		0		0		0	
TRACHEA	# Ex	5		5		0		0		0		0	
ESOPHAGUS	# Ex	5		5		0		0		0		0	

(Report Continued)

Severity Summary Table

PROJECT ID. NO: 92-002 FATES: ALL
DAYS: ALL SEX: MALE

GROUP:		7		8		9		10		11		12	
NUMBER OF ANIMALS:		5		5		5		5		5		5	
		#	SEV	#	SEV	#	SEV	#	SEV	#	SEV	#	SEV
THYMUS	# Ex	5		5		0		0		0		0	
HEART	# Ex	5		5		0		0		0		0	
Inflammation, Chronic		1	0.20	0		0		0		0		0	
COLON	# Ex	5		5		0		0		0		0	
JEJUNUM	# Ex	5		5		0		0		0		0	
AORTA	# Ex	5		5		0		0		0		0	
LIVER	# Ex	5		5		0		0		0		0	
SPLEEN	# Ex	5		5		5		5		5		5	
Hyperplasia, Erythroid Cell		0		5	2.80	5	3.00	4	1.40	0		0	
Depletion, Lymphoid		0		1	0.20	0		0		0		0	
TONGUE	# Ex	5		5		0		0		0		0	
SKELETAL MUSCLE	# Ex	5		5		0		0		0		0	
LUNG	# Ex	5		5		0		0		0		0	
KIDNEYS	# Ex	5		5		5		5		5		5	
Hyaline Droplets		0		5	1.80	5	2.20	5	1.80	5	1.80	1	0.20
Mineralization, NOS		4	0.80	4	1.00	5	1.40	5	1.60	5	1.20	5	1.00
Degeneration, Tubular		3	0.60	4	0.80	5	1.40	5	1.20	4	1.20	5	1.20
URINARY BLADDER	# Ex	5		5		0		0		0		0	

(Report Continued)

PATHOLOGY ASSOCIATES, INC.
14 DAY TOXICITY EVALUATION OF 1,3,5-TRINITROBENZENE (TNB)
IN FISCHER (F344) RATS

Severity Summary Table

PROJECT ID. NO: 92-002 FATES: ALL
DAYS: ALL SEX: MALE

GROUP:		7		8		9		10		11		12	
NUMBER OF ANIMALS:		5		5		5		5		5		5	
		#	SEV	#	SEV	#	SEV	#	SEV	#	SEV	#	SEV
PROSTATE	# Ex	5		5		0		0		0		0	
STOMACH	# Ex	5		5		0		0		0		0	
DUODENUM	# Ex	5		5		0		0		0		0	
ILEUM	# Ex	5		5		0		0		0		0	
CECUM	# Ex	5		5		0		0		0		0	
RECTUM	# Ex	5		5		0		0		0		0	
MESENTERIC LYMPH NODE	# Ex	5		5		0		0		0		0	
TESTES	# Ex	5		5		5		5		5		5	
Degen., Seminiferous Tubule		0		5	4.00	5	4.00	0		0		0	
EPIDIDYMIS	# Ex	5		5		0		0		0		0	
Hypospermia		0		5	3.00	0		0		0		0	
SEMINAL VESICLES	# Ex	5		5		0		0		0		0	
Atrophy, NOS		1	0.40	1	0.60	0		0		0		0	
SKIN	# Ex	5		5		0		0		0		0	
MAMMARY GLAND	# Ex	5		5		0		0		0		0	
PREPUTIAL GLANDS	# Ex	5		5		0		0		0		0	

(Report Continued)

PATHOLOGY ASSOCIATES, INC.
14 DAY TOXICITY EVALUATION OF 1,3,5-TRINITROBENZENE (TNB)
IN FISCHER (F344) RATS

Severity Summary Table

PROJECT ID. NO: 92-002 FATES: ALL
DAYS: ALL SEX: MALE

GROUP:	7		8		9		10		11		12	
NUMBER OF ANIMALS:	5		5		5		5		5		5	
	#	SEV	#	SEV	#	SEV	#	SEV	#	SEV	#	SEV
Inflammation, Chronic	4	1.40	2	0.40	0		0		0		0	
Inflammation, Chronic/Active	1	0.20	3	1.40	0		0		0		0	
Dilacation, Ductal	3	1.20	2	0.80	0		0		0		0	
EYES # Ex	5		5		0		0		0		0	
Inflammation, Chronic, Cornea	2	0.40	1	0.20	0		0		0		0	
HARDERIAN GLAND # Ex	5		5		0		0		0		0	
FEMUR/BONE MARROW # Ex	5		5		0		0		0		0	
Hyperplasia, Erythroid Cell	0		5	1.60	0		0		0		0	
STERNUM # Ex	5		5		0		0		0		0	
NASAL CAVITY # Ex	5		5		0		0		0		0	

* Severity calculated by the number of tissues examined.

(End of Report)

PATHOLOGY ASSOCIATES, INC.
14 DAY TOXICITY EVALUATION OF 1,3,5-TRINITROBENZENE (TNB)
IN FISCHER (F344) RATS

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 1 SEX: FEMALE
DAYS: ALL FATES: ALL

ANIMAL ID:	1	2	3	4	5
BRAIN	N	N	N	N	N
SCIATIC NERVE	N	N	N	N	N
SPINAL CORD	N	N	N	N	N
SALIVARY GLAND	N	N	N	N	N
PANCREAS	N	N	N	N	N
MANDIBULAR LYMPH NODE	N	N	N	N	N
ZYMBAL'S GLAND	N	N	N	N	N
PITUITARY	N	N	N	N	N
ADRENALS		N	N	N	N
Accessory Cortical Nodule	P	-	-	-	-
THYROID	N	U	N	N	N
PARATHYROID	N	U	N	N	N

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 1 SEX: FEMALE
DAYS: ALL FATES: ALL

ANIMAL ID:	1	2	3	4	5
TRACHEA	N	N	N	N	N
ESOPHAGUS	N	N	N	N	N
THYMUS	N	N	N	N	N
HEART	N	N		N	N
Inflammation, Chronic	-	-	1	-	-
COLON	N	N	N	N	N
JEJUNUM	N	N	N	N	N
AORTA	N	N	N	N	N
LIVER	N	N	N	N	N
SPLEEN	N	N	N	N	N
TONGUE	N	N	N	N	N
SKELETAL MUSCLE	N	N	N	N	N

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 1 SEX: FEMALE
DAYS: ALL FATES: ALL

ANIMAL ID:	1	2	3	4	5
LUNG	N	N	N	N	N
KIDNEYS			N	N	
Mineralization, NOS	1	1	-	-	1
URINARY BLADDER	N	N	N	N	N
STOMACH	N	N	N	N	N
DUODENUM	N	N	N	N	N
ILEUM	N	N	N	N	N
CECUM	N	N	N	N	N
RECTUM	N	N	N	N	N
MESENTERIC LYMPH NODE	N	N	N	N	N
OVARIES	N	N	N	N	N
UTERUS	N	N	N	N	N

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 1 SEX: FEMALE
DAYS: ALL FATES: ALL

ANIMAL ID:	1	2	3	4	5
SKIN	N	N	N	N	N
MAMMARY GLAND	N	N	N	N	N
CLITORAL GLANDS	N				
Inflammation, Chronic	-	1	2	1	-
Inflammation, Chronic/Active	-	-	-	-	3
Dilatation, Ductal	-	-	2	-	2
EYES	N	N	N	N	N
HARDERIAN GLAND	N	N	N	N	N
FEMUR/BONE MARROW	N	N	N	N	N
STERNUM	N	N	N	N	N
NASAL CAVITY	N	N	N	N	N

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 2 SEX: FEMALE
DAYS: ALL FATES: ALL

ANIMAL ID:	6	7	8	9	10
BRAIN	N	N		N	
Hemorrhage	-	-	1	-	3
Microgliosis	-	-	2	-	3
Necrosis	-	-	-	-	2
Vacuolization	-	-	2	-	3
SCIATIC NERVE	N	N	N	N	N
SPINAL CORD	N	N	N	N	N
SALIVARY GLAND	N	N	N	N	N
PANCREAS	N	N	N	N	N
MANDIBULAR LYMPH NODE	N	N	N	N	N
ZYMBAL'S GLAND	N	N	N	N	N
PITUITARY	N	N	N	N	N
ADRENALS	N	N	N	N	N
THYROID	N	N	N	N	N

(Report Continued)

PATHOLOGY ASSOCIATES, INC.
14 DAY TOXICITY EVALUATION OF 1,3,5-TRINITROBENZENE (TNB)
IN FISCHER (F344) RATS

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 2 SEX: FEMALE
DAYS: ALL FATES: ALL

ANIMAL ID:	6	7	8	9	10
PARATHYROID	N	N	N	N	N
TRACHEA	N	N	N	N	N
ESOPHAGUS	N	N	N	N	N
THYMUS		N	N	N	N
Necrosis, Cortical	3	-	-	-	-
Depletion, Lymphoid	3	-	-	-	-
HEART	N		N	N	N
Inflammation, Chronic	-	1	-	-	-
COLON	N	N	N	N	N
JEJUNUM	N	N	N	N	N
AORTA	N	N	N	N	N
LIVER	N	N	N	N	N
SPLEEN					
Hyperplasia, Erythroid Cell	2	2	3	2	3
Depletion, Lymphoid	2	1	-	1	-
Fibrosis	-	2	-	-	-

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 2 SEX: FEMALE
DAYS: ALL FATES: ALL

ANIMAL ID:	6	7	8	9	10
TONGUE	N	N	N	N	N
SKELETAL MUSCLE	N	N	N	N	N
LUNG	N	N	N	N	N
KIDNEYS	N	N	N		
Mineralization, NOS	-	-	-	-	1
Inflammation, Chronic	-	-	-	1	-
URINARY BLADDER	N	N	N	N	N
STOMACH	N	N	N	N	N
DUODENUM	N	N	N	N	N
ILEUM	N	N	N	N	N
CECUM	N	N	N	N	N
RECTUM	N	N	N	N	N
MESENTERIC LYMPH NODE	N	N	N	N	N

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 2 SEX: FEMALE
DAYS: ALL FATES: ALL

ANIMAL ID:	6	7	8	9	10
OVARIES	N	N	N	N	N
UTERUS	N	N	N	N	N
SKIN	N	N	N	N	N
MAMMARY GLAND	N	N	N	N	N
CLITORAL GLANDS					N
Inflammation, Chronic	3	-	-	2	-
Inflammation, Chronic/Active	-	3	2	-	-
Dilatation, Ductal	3	2	2	-	-
EYES	N	N	N	N	N
HARDERIAN GLAND	N	N	N	N	N
FEMUR/BONE MARROW					
Hyperplasia, Erythroid Cell	2	2	1	2	3
Pigment, NOS	2	1	-	-	-
STERNUM	N	N	N	N	N
NASAL CAVITY	N	N	N	N	N

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 3 SEX: FEMALE
DAYS: ALL FATES: ALL

ANIMAL ID:	11	12	13	14	15
BRAIN	•	•	•	•	•
SCIATIC NERVE	•	•	•	•	•
SPINAL CORD	•	•	•	•	•
SALIVARY GLAND	•	•	•	•	•
PANCREAS	•	•	•	•	•
MANDIBULAR LYMPH NODE	•	•	•	•	•
ZYMBAL'S GLAND	•	•	•	•	•
PITUITARY	•	•	•	•	•
ADRENALS	•	•	•	•	•
THYROID	•	•	•	•	•
PARATHYROID	•	•	•	•	•
TRACHEA	•	•	•	•	•

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 3 SEX: FEMALE
DAYS: ALL FATES: ALL

ANIMAL ID:	11	12	13	14	15
ESOPHAGUS	•	•	•	•	•
THYMUS	•	•	•	•	•
HEART	•	•	•	•	•
COLON	•	•	•	•	•
JEJUNUM	•	•	•	•	•
AORTA	•	•	•	•	•
LIVER	•	•	•	•	•
SPLEEN					
Hyperplasia, Erythroid Cell	3	2	3	2	3
TONGUE	•	•	•	•	•
SKELETAL MUSCLE	•	•	•	•	•
LUNG	•	•	•	•	•

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 3 SEX: FEMALE
DAYS: ALL FATES: ALL

ANIMAL ID:	11	12	13	14	15
KIDNEYS	•	•	•	•	•
URINARY BLADDER	•	•	•	•	•
STOMACH	•	•	•	•	•
DUODENUM	•	•	•	•	•
ILEUM	•	•	•	•	•
CECUM	•	•	•	•	•
RECTUM	•	•	•	•	•
MESENTERIC LYMPH NODE	•	•	•	•	•
OVARIES	•	•	•	•	•
UTERUS	•	•	•	•	•
SKIN	•	•	•	•	•
MAMMARY GLAND	•	•	•	•	•

(Report Continued)

PATHOLOGY ASSOCIATES, INC.
14 DAY TOXICITY EVALUATION OF 1,3,5-TRINITROBENZENE (TNB)
IN FISCHER (F344) RATS

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 3 SEX: FEMALE
DAYS: ALL FATES: ALL

ANIMAL ID:	11	12	13	14	15
CLITORAL GLANDS	•	•	•	•	•
EYES	•	•	•	•	•
HARDERIAN GLAND	•	•	•	•	•
FEMUR/BONE MARROW	•	•	•	•	•
STERNUM	•	•	•	•	•
NASAL CAVITY	•	•	•	•	•

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 4 SEX: FEMALE
DAYS: ALL FATES: ALL

ANIMAL ID:	16	17	18	19	20
BRAIN	•	•	•	•	•
SCIATIC NERVE	•	•	•	•	•
SPINAL CORD	•	•	•	•	•
SALIVARY GLAND	•	•	•	•	•
PANCREAS	•	•	•	•	•
MANDIBULAR LYMPH NODE	•	•	•	•	•
ZYMBAL'S GLAND	•	•	•	•	•
PITUITARY	•	•	•	•	•
ADRENALS	•	•	•	•	•
THYROID	•	•	•	•	•
PARATHYROID	•	•	•	•	•
TRACHEA	•	•	•	•	•

(Report Continued)

PATHOLOGY ASSOCIATES, INC.
14 DAY TOXICITY EVALUATION OF 1,3,5-TRINITROBENZENE (TNB)
IN FISCHER (F344) RATS

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 4 SEX: FEMALE
DAYS: ALL FATES: ALL

ANIMAL ID:	16	17	18	19	20
ESOPHAGUS	*	*	*	*	*
THYMUS	*	*	*	*	*
HEART	*	*	*	*	*
COLON	*	*	*	*	*
JEJUNUM	*	*	*	*	*
AORTA	*	*	*	*	*
LIVER	*	*	*	*	*
SPLEEN					
Hyperplasia, Erythroid Cell	2	2	2	1	2
Fibrosis	-	-	-	1	-
TONGUE	*	*	*	*	*
SKELETAL MUSCLE	*	*	*	*	*
LUNG	*	*	*	*	*

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 4 SEX: FEMALE
DAYS: ALL FATES: ALL

ANIMAL ID:	16	17	18	19	20
KIDNEYS	•	•	•	•	•
URINARY BLADDER	•	•	•	•	•
STOMACH	•	•	•	•	•
DUODENUM	•	•	•	•	•
ILEUM	•	•	•	•	•
CECUM	•	•	•	•	•
RECTUM	•	•	•	•	•
MESENTERIC LYMPH NODE	•	•	•	•	•
OVARIES	•	•	•	•	•
UTERUS	•	•	•	•	•
SKIN	•	•	•	•	•
MAMMARY GLAND	•	•	•	•	•

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 4 SEX: FEMALE
DAYS: ALL FATES: ALL

ANIMAL ID:	16	17	18	19	20
CLITORAL GLANDS	*	*	*	*	*
EYES	*	*	*	*	*
HARDERIAN GLAND	*	*	*	*	*
FEMUR/BONE MARROW	*	*	*	*	*
STERNUM	*	*	*	*	*
NASAL CAVITY	*	*	*	*	*

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 5 SEX: FEMALE
DAYS: ALL FATES: ALL

ANIMAL ID:	21	22	23	24	25
BRAIN	*	*	*	*	*
SCIATIC NERVE	*	*	*	*	*
SPINAL CORD	*	*	*	*	*
SALIVARY GLAND	*	*	*	*	*
PANCREAS	*	*	*	*	*
MANDIBULAR LYMPH NODE	*	*	*	*	*
ZYMBAL'S GLAND	*	*	*	*	*
PITUITARY	*	*	*	*	*
ADRENALS	*	*	*	*	*
THYROID	*	*	*	*	*
PARATHYROID	*	*	*	*	*
TRACHEA	*	*	*	*	*

(Report Continued)

Tabulated Animal Data

ANIMAL ID:	21	22	23	24	25
ESOPHAGUS	*	*	*	*	*
THYMUS	*	*	*	*	*
HEART	*	*	*	*	*
COLON	*	*	*	*	*
JEJUNUM	*	*	*	*	*
AORTA	*	*	*	*	*
LIVER	*	*	*	*	*
SPLEEN	N	N	N	N	N
TONGUE	*	*	*	*	*
SKELETAL MUSCLE	*	*	*	*	*
LUNG	*	*	*	*	*
KIDNEYS	*	*	*	*	*

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 5 SEX: FEMALE
DAYS: ALL FATES: ALL

ANIMAL ID:	21	22	23	24	25
URINARY BLADDER	*	*	*	*	*
STOMACH	*	*	*	*	*
DUODENUM	*	*	*	*	*
ILEUM	*	*	*	*	*
CECUM	*	*	*	*	*
RECTUM	*	*	*	*	*
MESENTERIC LYMPH NODE	*	*	*	*	*
OVARIES	*	*	*	*	*
UTERUS	*	*	*	*	*
SKIN	*	*	*	*	*
MAMMARY GLAND	*	*	*	*	*
CLITORAL GLANDS	*	*	*	*	*

(Report Continued)

PATHOLOGY ASSOCIATES, INC.
14 DAY TOXICITY EVALUATION OF 1,3,5-TRINITROBENZENE (TNB)
IN FISCHER (F344) RATS

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 5 SEX: FEMALE
DAYS: ALL FATES: ALL

ANIMAL ID:	21	22	23	24	25
EYES	•	•	•	•	•
HARDERIAN GLAND	•	•	•	•	•
FEMUR/BONE MARROW	•	•	•	•	•
STERNUM	•	•	•	•	•
NASAL CAVITY	•	•	•	•	•

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 6 SEX: FEMALE
DAYS: ALL FATES: ALL

ANIMAL ID:	26	27	28	29	30
BRAIN	•	•	•	•	•
SCIATIC NERVE	•	•	•	•	•
SPINAL CORD	•	•	•	•	•
SALIVARY GLAND	•	•	•	•	•
PANCREAS	•	•	•	•	•
MANDIBULAR LYMPH NODE	•	•	•	•	•
ZYMBAL'S GLAND	•	•	•	•	•
PITUITARY	•	•	•	•	•
ADRENALS	•	•	•	•	•
THYROID	•	•	•	•	•
PARATHYROID	•	•	•	•	•
TRACHEA	•	•	•	•	•

(Report Continued)

PATHOLOGY ASSOCIATES, INC.
14 DAY TOXICITY EVALUATION OF 1,3,5-TRINITROBENZENE (TNB)
IN FISCHER (F344) RATS

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 6 SEX: FEMALE
DAYS: ALL FATES: ALL

ANIMAL ID:	26	27	28	29	30
ESOPHAGUS	•	•	•	•	•
THYMUS	•	•	•	•	•
HEART	•	•	•	•	•
COLON	•	•	•	•	•
JEJUNUM	•	•	•	•	•
AORTA	•	•	•	•	•
LIVER	•	•	•	•	•
SPLEEN	N	N	N	N	N
TONGUE	•	•	•	•	•
SKELETAL MUSCLE	•	•	•	•	•
LUNG	•	•	•	•	•
KIDNEYS	•	•	•	•	•

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 6 SEX: FEMALE
DAYS: ALL FATES: ALL

ANIMAL ID:	26	27	28	29	30
URINARY BLADDER	•	•	•	•	•
STOMACH	•	•	•	•	•
DUODENUM	•	•	•	•	•
ILEUM	•	•	•	•	•
CECUM	•	•	•	•	•
RECTUM	•	•	•	•	•
MESENTERIC LYMPH NODE	•	•	•	•	•
OVARIES	•	•	•	•	•
UTERUS	•	•	•	•	•
SKIN	•	•	•	•	•
MAMMARY GLAND	•	•	•	•	•
CLITORAL GLANDS	•	•	•	•	•

(Report Continued)

Tabulated Animal Data

ANIMAL ID:	26	27	28	29	30
EYES	*	*	*	*	*
HARDERIAN GLAND	*	*	*	*	*
FEMUR/BONE MARROW	*	*	*	*	*
STERNUM	*	*	*	*	*
NASAL CAVITY	*	*	*	*	*

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 7 SEX: MALE
DAYS: ALL FATES: ALL

ANIMAL ID:	31	32	33	34	35
BRAIN	N	N	N	N	N
SCIATIC NERVE	N	N	N	N	N
SPINAL CORD	N	N	N	N	N
SALIVARY GLAND	N	N	N	N	N
PANCREAS	N	N	N	N	N
MANDIBULAR LYMPH NODE	N	N	N	N	N
ZYMBAL'S GLAND	N	N	N	N	N
PITUITARY	N	N	N	N	N
ADRENALS	N	N	N	N	N
THYROID	N	N	N	N	N
PARATHYROID	N	U	N	N	N
TRACHEA	N	N	N	N	N

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 7 SEX: MALE
DAYS: ALL FATES: ALL

ANIMAL ID:	31	32	33	34	35
ESOPHAGUS	M	M	M	M	M
THYMUS	M	M	M	M	M
HEART		M	M	M	M
Inflammation, Chronic	1	-	-	-	-
COLON	M	M	M	M	M
JEJUNUM	M	M	M	M	M
AORTA	M	M	M	M	M
LIVER	M	M	M	M	M
SPLEEN	M	M	M	M	M
TONGUE	M	M	M	M	M
SKELETAL MUSCLE	M	M	M	M	M
LUNG	M	M	M	M	M

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 7 SEX: MALE
DAYS: ALL FATES: ALL

ANIMAL ID:	31	32	33	34	35
KIDNEYS	M				
Mineralization, NOS	-	1	1	1	1
Degeneration, Tubular	-	1	-	1	1
URINARY BLADDER	M	M	M	M	M
PROSTATE	M	M	M	M	M
STOMACH	M	M	M	M	M
DUODENUM	M		M	M	M
Ectopic Pancreas	-	P	-	-	-
ILEUM	M	M	M	M	M
CECUM	M	M	M	M	M
RECTUM	M	M	M	M	M
MESENTERIC LYMPH NODE	M	M	M	M	M
TESTES	M	M	M	M	M
EPIDIDYMIS	M	M	M	M	M

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 7 SEX: MALE
DAYS: ALL FATES: ALL

ANIMAL ID:	31	32	33	34	35
SEMINAL VESICLES	N	N	N		N
Atrophy, NOS	-	-	-	2	-
SKIN	N	N	N	N	N
MAMMARY GLAND	N	N	N	N	N
PREPUTIAL GLANDS					
Inflammation, Chronic	2	2	2	-	1
Inflammation, Chronic/Active	-	-	-	1	-
Dilatation, Ductal	-	2	2	2	-
EYES	N		N		N
Inflammation, Chronic, Cornea	-	1	-	1	-
HARDERIAN GLAND	N	N	N	N	N
FEMUR/BONE MARROW	N	N	N	N	N
STERNUM	N	N	N	N	N
NASAL CAVITY	N	N	N	N	N

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 8 SEX: MALE
DAYS: ALL FATES: ALL

ANIMAL ID:	36	37	38	39	40
BRAIN	N	N	N	N	N
SCIATIC NERVE	N	N	N	N	N
SPINAL CORD	N	N	N	N	N
SALIVARY GLAND	N	N	N	N	N
PANCREAS	N	N	N	N	N
MANDIBULAR LYMPH NODE		N	N	N	N
Plasmacytosis	2	-	-	-	-
ZYMBAL'S GLAND	N	N	N	N	N
PITUITARY	N	N	N	N	N
ADRENALS	N	N	N	N	N
THYROID	N	N	N	N	N
PARATHYROID	N	N	N	N	N

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 8 SEX: MALE
DAYS: ALL FATES: ALL

ANIMAL ID:	36	37	38	39	40
TRACHEA	N	N	N	N	N
ESOPHAGUS	N	N	N	N	N
THYMUS	N	N	N	N	N
HEART	N	N	N	N	N
COLON	N	N	N	N	N
JEJUNUM	N	N	N	N	N
AORTA	N	N	N	N	N
LIVER	N	N	N	N	N
SPLEEN					
Hyperplasia, Erythroid Cell	3	3	3	3	2
Depletion, Lymphoid	-	1	-	-	-
TONGUE	N	N	N	N	N
SKELETAL MUSCLE	N	N	N	N	N

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 8 SEX: MALE
DAYS: ALL FATES: ALL

ANIMAL ID:	36	37	38	39	40
LUNG	N	N	N	N	N
KIDNEYS					
Hyaline Droplets	2	2	2	2	1
Mineralization, NOS	2	1	1	1	-
Degeneration, Tubular	1	1	1	1	-
URINARY BLADDER	N	N	N	N	N
PROSTATE	N	N	N	N	N
STOMACH	N	N	N	N	N
DUODENUM	N	N	N	N	N
ILEUM	N	N	N	N	N
CECUM	N	N	N	N	N
RECTUM	N	N	N	N	N
MESENTERIC LYMPH NODE	N	N	N	N	N
TESTES					
Degen., Seminiferous Tubule	4	4	4	4	4

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 8 SEX: MALE
DAYS: ALL FATES: ALL

ANIMAL ID:	36	37	38	39	40
EPIDIDYMIS					
Hypospermia	3	2	3	3	4
SEMINAL VESICLES	M	M	M	M	
Atrophy, NOS	-	-	-	-	3
SKIN	M	M	M	M	M
MAMMARY GLAND	M	M	M	M	M
PREPUTIAL GLANDS					
Inflammation, Chronic	1	-	-	-	1
Inflammation, Chronic/Active	-	2	2	3	-
Dilatation, Ductal	-	2	-	2	-
EYES	M	M		M	M
Inflammation, Chronic, Cornea	-	-	1	-	-
HARDERIAN GLAND	M	M	M	M	M
FEMUR/BONE MARROW					
Hyperplasia, Erythroid Cell	1	2	2	2	1
STERNUM	M	M	M	M	M

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 8 SEX: MALE
DAYS: ALL FATES: ALL

ANIMAL ID:	36	37	38	39	40
NASAL CAVITY	N	N	N	N	N

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 9 SEX: MALE
DAYS: ALL FATES: ALL

ANIMAL ID:	41	42	43	44	45
BRAIN	*	*	*	*	*
SCIATIC NERVE	*	*	*	*	*
SPINAL CORD	*	*	*	*	*
SALIVARY GLAND	*	*	*	*	*
PANCREAS	*	*	*	*	*
MANDIBULAR LYMPH NODE	*	*	*	*	*
ZYMBAL'S GLAND	*	*	*	*	*
PITUITARY	*	*	*	*	*
ADRENALS	*	*	*	*	*
THYROID	*	*	*	*	*
PARATHYROID	*	*	*	*	*
TRACHEA	*	*	*	*	*

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 9 SEX: MALE
DAYS: ALL FATES: ALL

ANIMAL ID:	41	42	43	44	45
ESOPHAGUS	•	•	•	•	•
THYMUS	•	•	•	•	•
HEART	•	•	•	•	•
COLON	•	•	•	•	•
JEJUNUM	•	•	•	•	•
AORTA	•	•	•	•	•
LIVER	•	•	•	•	•
SPLEEN					
Hyperplasia, Erythroid Cell	3	3	3	3	3
TONGUE	•	•	•	•	•
SKELETAL MUSCLE	•	•	•	•	•
LUNG	•	•	•	•	•

(Report Continued)

Tabulated Animal Data

ANIMAL ID:	41	42	43	44	45
KIDNEYS					
Hyaline Droplets	3	2	2	2	2
Mineralization, NOS	1	1	2	2	1
Degeneration, Tubular	2	1	2	1	1
URINARY BLADDER	*	*	*	*	*
PROSTATE	*	*	*	*	*
STOMACH	*	*	*	*	*
DUODENUM	*	*	*	*	*
ILEUM	*	*	*	*	*
CECUM	*	*	*	*	*
RECTUM	*	*	*	*	*
MESENTERIC LYMPH NODE	*	*	*	*	*
TESTES					
Degen., Seminiferous Tubule	4	4	4	4	4

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 9 SEX: MALE
DAYS: ALL FATES: ALL

ANIMAL ID:	41	42	43	44	45
EPIDIDYMIS	•	•	•	•	•
SEMINAL VESICLES	•	•	•	•	•
SKIN	•	•	•	•	•
MAMMARY GLAND	•	•	•	•	•
PREPUTIAL GLANDS	•	•	•	•	•
EYES	•	•	•	•	•
HARDERIAN GLAND	•	•	•	•	•
FEMUR/BONE MARROW	•	•	•	•	•
STERNUM	•	•	•	•	•
NASAL CAVITY	•	•	•	•	•

(Report Continued)

Tabulated Animal Data

ANIMAL ID:	46	47	48	49	50
BRAIN	*	*	*	*	*
SCIATIC NERVE	*	*	*	*	*
SPINAL CORD	*	*	*	*	*
SALIVARY GLAND	*	*	*	*	*
PANCREAS	*	*	*	*	*
MANDIBULAR LYMPH NODE	*	*	*	*	*
ZYMBAL'S GLAND	*	*	*	*	*
PITUITARY	*	*	*	*	*
ADRENALS	*	*	*	*	*
THYROID	*	*	*	*	*
PARATHYROID	*	*	*	*	*
TRACHEA	*	*	*	*	*

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 10 SEX: MALE
DAYS: ALL FATES: ALL

ANIMAL ID:	46	47	48	49	50
ESOPHAGUS	•	•	•	•	•
THYMUS	•	•	•	•	•
HEART	•	•	•	•	•
COLON	•	•	•	•	•
JEJUNUM	•	•	•	•	•
AORTA	•	•	•	•	•
LIVER	•	•	•	•	•
SPLEEN					M
Hyperplasia, Erythroid Cell	3	2	1	1	-
TONGUE	•	•	•	•	•
SKELETAL MUSCLE	•	•	•	•	•
LUNG	•	•	•	•	•

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 10 SEX: MALE
DAYS: ALL FATES: ALL

ANIMAL ID:	46	47	48	49	50
KIDNEYS					
Hyaline Droplets	2	2	1	2	2
Mineralization, NOS	2	2	1	2	1
Degeneration, Tubular	1	2	1	1	1
URINARY BLADDER	*	*	*	*	*
PROSTATE	*	*	*	*	*
STOMACH	*	*	*	*	*
DUODENUM	*	*	*	*	*
ILEUM	*	*	*	*	*
CECUM	*	*	*	*	*
RECTUM	*	*	*	*	*
MESENTERIC LYMPH NODE	*	*	*	*	*
TESTES	N	N	N	N	N
EPIDIDYMIS	*	*	*	*	*

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 10 SEX: MALE
DAYS: ALL FATES: ALL

ANIMAL ID:	46	47	48	49	50
SEMINAL VESICLES	*	*	*	*	*
SKIN	*	*	*	*	*
MAMMARY GLAND	*	*	*	*	*
PREPUTIAL GLANDS	*	*	*	*	*
EYES	*	*	*	*	*
HARDERIAN GLAND	*	*	*	*	*
FEMUR/BONE MARROW	*	*	*	*	*
STERNUM	*	*	*	*	*
NASAL CAVITY	*	*	*	*	*

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 11 SEX: MALE
DAYS: ALL FATES: ALL

ANIMAL ID:	51	52	53	54	55
BRAIN	*	*	*	*	*
SCIATIC NERVE	*	*	*	*	*
SPINAL CORD	*	*	*	*	*
SALIVARY GLAND	*	*	*	*	*
PANCREAS	*	*	*	*	*
MANDIBULAR LYMPH NODE	*	*	*	*	*
ZYMBAL'S GLAND	*	*	*	*	*
PITUITARY	*	*	*	*	*
ADRENALS	*	*	*	*	*
THYROID	*	*	*	*	*
PARATHYROID	*	*	*	*	*
TRACHEA	*	*	*	*	*

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 11 SEX: MALE
DAYS: ALL FATES: ALL

ANIMAL ID:	51	52	53	54	55
ESOPHAGUS	*	*	*	*	*
THYMUS	*	*	*	*	*
HEART	*	*	*	*	*
COLON	*	*	*	*	*
JEJUNUM	*	*	*	*	*
AORTA	*	*	*	*	*
LIVER	*	*	*	*	*
SPLEEN	N	N	N	N	N
TONGUE	*	*	*	*	*
SKELETAL MUSCLE	*	*	*	*	*
LUNG	*	*	*	*	*

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 11 SEX: MALE
DAYS: ALL FATES: ALL

ANIMAL ID:	51	52	53	54	55
KIDNEYS					
Hyaline Droplets	1	2	2	2	2
Mineralization, NOS	1	1	1	1	2
Degeneration, Tubular	-	1	2	2	1
URINARY BLADDER	*	*	*	*	*
PROSTATE	*	*	*	*	*
STOMACH	*	*	*	*	*
DUODENUM	*	*	*	*	*
ILEUM	*	*	*	*	*
CECUM	*	*	*	*	*
RECTUM	*	*	*	*	*
MESENTERIC LYMPH NODE	*	*	*	*	*
TESTES	M	M	M	M	M
EPIDIDYMIS	*	*	*	*	*

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 11 SEX: MALE
DAYS: ALL FATES: ALL

ANIMAL ID:	51	52	53	54	55
SEMINAL VESICLES	•	•	•	•	•
SKIN	•	•	•	•	•
MAMMARY GLAND	•	•	•	•	•
PREPUTIAL GLANDS	•	•	•	•	•
EYES	•	•	•	•	•
HARDERIAN GLAND	•	•	•	•	•
FEMUR/BONE MARROW	•	•	•	•	•
STERNUM	•	•	•	•	•
NASAL CAVITY	•	•	•	•	•

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 12 SEX: MALE
DAYS: ALL FATES: ALL

ANIMAL ID:	56	57	58	59	60
BRAIN	•	•	•	•	•
SCIATIC NERVE	•	•	•	•	•
SPINAL CORD	•	•	•	•	•
SALIVARY GLAND	•	•	•	•	•
PANCREAS	•	•	•	•	•
MANDIBULAR LYMPH NODE	•	•	•	•	•
ZYMBAL'S GLAND	•	•	•	•	•
PITUITARY	•	•	•	•	•
ADRENALS	•	•	•	•	•
THYROID	•	•	•	•	•
PARATHYROID	•	•	•	•	•
TRACHEA	•	•	•	•	•

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 12 SEX: MALE
DAYS: ALL FATES: ALL

ANIMAL ID:	56	57	58	59	60
ESOPHAGUS	•	•	•	•	•
THYMUS	•	•	•	•	•
HEART	•	•	•	•	•
COLON	•	•	•	•	•
JEJUNUM	•	•	•	•	•
AORTA	•	•	•	•	•
LIVER	•	•	•	•	•
SPLEEN	M	M	M	M	M
TONGUE	•	•	•	•	•
SKELETAL MUSCLE	•	•	•	•	•
LUNG	•	•	•	•	•

(Report Continued)

Tabulated Animal Data

ANIMAL ID:	56	57	58	59	60
KIDNEYS					
Hyaline Droplets	1	-	-	-	-
Mineralization, NOS	1	1	1	1	1
Degeneration, Tubular	1	1	1	1	2
URINARY BLADDER	*	*	*	*	*
PROSTATE	*	*	*	*	*
STOMACH	*	*	*	*	*
DUODENUM	*	*	*	*	*
ILEUM	*	*	*	*	*
CECUM	*	*	*	*	*
RECTUM	*	*	*	*	*
MESENTERIC LYMPH NODE	*	*	*	*	*
TESTES	N	N	N	N	N
EPIDIDYMIS	*	*	*	*	*

(Report Continued)

Tabulated Animal Data

PROJECT ID: 92-002 GROUP: 12 SEX: MALE
DAYS: ALL FATES: ALL

ANIMAL ID:	56	57	58	59	60
SEMINAL VESICLES	*	*	*	*	*
SKIN	*	*	*	*	*
MAMMARY GLAND	*	*	*	*	*
PREPUTIAL GLANDS	*	*	*	*	*
EYES	*	*	*	*	*
HARDERIAN GLAND	*	*	*	*	*
FEMUR/BONE MARROW	*	*	*	*	*
STERNUM	*	*	*	*	*
NASAL CAVITY	*	*	*	*	*

(End of Report)

APPENDIX H

PALATABILITY PRETEST DATA

PALATABILITY PRETEST DATA

Sex No.	TNB mg/kg Diet	Average Body Weight Taken at Different Weeks				Average Daily Food Intake (gms/day/rat) Measured at Different Weeks				Average TNB Intake (mg/day/kg B.W.) Measured at Different Weeks			
		0	1	2	3	0	1	2	3	0	1	2	3
F	0	120.12	132.98	144.00	151.84	11.51	11.00	12.00	11.75	0.00	0.00	0.00	0.00
5	1400	123.40	103.72	100.34	99.54	12.14	4.10	7.80	7.60	0.00	55.34	109.28	106.89
5	700	121.60	113.26	132.26	141.70	11.12	6.45	11.10	11.45	0.00	39.86	58.75	56.76
5	350	125.10	129.72	144.36	145.38	11.32	8.30	11.30	11.12	0.00	23.39	27.39	29.39
M	0	159.02	189.92	212.90	230.78	13.20	15.50	17.05	18.00	0.00	0.00	0.00	0.00
5	1400	161.64	135.76	127.54	131.30	14.28	5.05	8.35	8.45	0.00	52.08	91.66	90.09
5	700	164.62	162.64	191.44	209.68	13.90	9.00	15.05	16.15	0.00	38.73	55.05	53.91
5	350	159.68	177.08	196.26	213.08	13.34	13.40	15.45	16.05	0.00	23.42	27.55	27.03

Determination of Homogeneity

Weeks 1,2

Target Diet Concentration (mg/kg)	Site of Sampling	Concentration by Analysis (mg/kg)	Mean Concentration (mg/kg)	Deviation from Mean (%)
1400	Top	940	1123	16.30
	Middle	1200		6.86
	Bottom	1230		9.53
700	Top	430	413	4.12
	Middle	390		5.57
	Bottom	420		1.70
350	Top	290	270	7.41
	Middle	270		0.00
	Bottom	250		7.41

FOOD AND WATER CONSUMPTION

Group	Sex	Diet Concentration (mg TNB/kg)	Food Consumed (g/wk) Week 1	Food Consumed (g/wk) Week 2	Water Consumed (g/wk) Week 1	Water Consumed (g/wk) Week 2
1	F	0	77.4 ± 3.4	84.9 ± 3.2	115.5 ± 9.9	131.7 ± 14.2
2	F	1400	29.8 ± 6.3	62.7 ± 33.8	70.0 ± 19.0	92.0 ± 19.0
3	F	700	45.7 ± 8.9	76.9 ± 1.9	92.2 ± 27.7	155.4 ± 6.0
4	F	350	59.1 ± 7.2	79.1 ± 3.6	112.0 ± 9.1	149.6 ± 9.6
5	M	0	109.2 ± 5.2	119.2 ± 6.1	157.6 ± 8.7	168.9 ± 6.1
6	M	1400	36.2 ± 7.2	60.3 ± 9.7	53.8 ± 15.5	101.7 ± 15.5
7	M	700	65.0 ± 24.8	105.2 ± 7.6	92.7 ± 49.7	181.9 ± 11.4
8	M	350	85.6 ± 6.7	107.9 ± 5.7	132.7 ± 14.0	171.4 ± 3.2

Mean ± Standard Deviation

APPENDIX I

CHEMICAL ANALYSES

Analysis of Feed Mixtures

Week	Diet Concentration (mg TNB/kg)	Date Prepared	Date Analyzed	Concentration by Analysis (mg TNB/kg)	% Error
1	1200	10 Aug 92	17 Aug 92	1169	2.58
1	800	11 Aug 92	17 Aug 92	753	5.88
1	400	11 Aug 92	17 Aug 92	413	3.25
1	200	11 Aug 92	17 Aug 92	205	2.50
1	50	11 Aug 92	17 Aug 92	48	4.00
2	1200	24 Aug 92	25 Aug 92	1322	10.20
2	800	24 Aug 92	25 Aug 92	791	1.13
2	400	24 Aug 92	25 Aug 92	411	2.75
2	200	24 Aug 92	25 Aug 92	198	1.00
2	50	24 Aug 92	25 Aug 92	50	0.00

Determination of TNB Homogeneity in the Diet

Week 1 (8/18/92 - 8/25/92)

Diet Concentration (mg/kg)	Site of Sampling	Concentration by Analysis (mg/kg)	Mean Concentration (mg/kg)	Deviation from Mean (%)
1200	Top	1107	1169	5.30
	Middle	1216		4.05
	Bottom	1185		2.16
800	Top	716	753	4.91
	Middle	752		4.00
	Bottom	790		5.33
400	Top	379	413	8.23
	Middle	410		0.00
	Bottom	451		9.20
200	Top	213	205	3.90
	Middle	207		0.98
	Bottom	195		4.88
50	Top	45	48	6.25
	Middle	48		0.00
	Bottom	53		10.40

Determination of TNB Homogeneity in the Diet

Week 2 (8/25/92 - 9/1/92)

Diet Concentration (mg/kg)	Site of Sampling	Concentration by Analysis (mg/kg)	Mean Concentration (mg/kg)	Deviation from Mean (%)
1200	Top	1304	1322	1.36
	Middle	1337		1.13
	Bottom	1323		0.08
800	Top	739	791	6.57
	Middle	813		2.78
	Bottom	821		3.79
400	Top	430	411	4.62
	Middle	396		3.65
	Bottom	406		1.22
200	Top	199	198	0.51
	Middle	203		2.53
	Bottom	192		3.03
50	Top	50	50	0.00
	Middle	51		2.00
	Bottom	48		4.00

APPENDIX J

METHEMOGLOBIN DATA

METHEMOGLOBIN DATA

This appendix provides data on methemoglobin levels obtained following 14 days exposure to TNB. Additional animals were needed following the initial study because blood samples of the original animals were not analyzed expediently enough (<4 hours) to avoid false negative results. All study methodology was consistent with the original 14 day TNB exposure study previously completed.

TNB mg/kg Diet	No. of Animals	Sex	Animal Numbers	Group Number
0	5	F	151-155	1
1200	5	F	156-160	2
800	5	F	161-165	3
400	5	F	166-170	4
0	5	M	171-175	5
1200	5	M	176-180	6
800	5	M	181-185	7
400	5	M	186-190	8

Note: Analyses were performed by Jewish Hospital Laboratory Services, Cincinnati, Ohio.

METHEMOGLOBIN RESULTS (%)
14 DAY TNB EXPOSURE

GROUP	ANIMAL NO.	RESULT	GROUP	ANIMAL NO.	RESULT
1	151	0.70	2	156	4.70
1	152	1.20	2	157	6.00
1	153	0.80	2	158	6.00
1	154	0.20	2	159	4.70
1	155	0.70	2	160	4.90
N		5.000	N		5.000
MEAN		0.720	MEAN		5.260*
S.E.M.		0.159	S.E.M.		0.304
3	161	5.40	4	166	4.10
3	162	5.30	4	167	3.10
3	163	6.20	4	168	2.40
3	164	4.30	4	169	4.60
3	165	5.10	4	170	2.80
N		5.000	N		5.000
MEAN		5.260*	MEAN		3.400*
S.E.M.		0.304	S.E.M.		0.411
5	171	1.10	6	176	6.60
5	172	0.00	6	177	5.30
5	173	0.70	6	178	4.70
5	174	1.30	6	179	6.30
5	175	0.20	6	180	6.20
N		5.000	N		5.000
MEAN		0.660	MEAN		5.820*
S.E.M.		0.250	S.E.M.		0.354
7	181	6.90	8	186	5.00
7	182	5.90	8	187	4.00
7	183	3.70	8	188	3.70
7	184	6.20	8	189	4.60
7	185	5.70	8	190	4.50
N		5.000	N		5.000
MEAN		5.680*	MEAN		4.360*
S.E.M.		0.535	S.E.M.		0.229

* = Significantly different from the control group ($p \leq 0.05$)

Daily TNB Consumption

Group	Sex	Diet Concentration (mg TNB/kg)	Week 1	Week 2
1	F	0		
2	F	1200	64.65 ± 4.55 *	85.20 ± 4.38
3	F	800	47.14 ± 2.62	54.54 ± 3.62
4	F	400	27.74 ± 0.90	29.52 ± 1.33
5	M	0		
6	M	1200	59.14 ± 6.55	80.46 ± 2.01
7	M	800	55.27 ± 1.20	56.89 ± 1.10
8	M	400	30.17 ± 6.55	29.79 ± 2.01

* Mean ± Standard Error, (mg TNB/kg b.w.)

Determination of TNB Homogeneity in the Diet

Weeks 1 and 2

Diet Concentration (mg TNB/kg)	Site of Sampling	Concentration by Analysis (mg TNB/kg)	Mean Concentration (mg TNB/kg)	Deviation from Mean (%)
1200	Top	1180	1189	0.76
	Middle	1161		2.35
	Bottom	1225		3.11
800	Top	792	795	0.38
	Middle	806		1.38
	Bottom	786		1.13
400	Top	392	395	0.76
	Middle	387		2.03
	Bottom	404		2.28

Analysis of Feed Mixtures

Weeks 1 and 2

Diet Concentration (mg TNB/kg)	Date Prepared	Date Analyzed	Concentration by Analysis (mg TNB/kg)	% Error
1200	10 Feb 93	11 Feb 93	1189	0.91
800	11 Feb 93	16 Feb 93	795	0.68
400	11 Feb 93	16 Feb 93	395	1.35

FOOD AND WATER CONSUMPTION

Group	Sex	Diet Concentration (mg TNB/kg)	Food Consumed (g/wk) Week 1	Food Consumed (g/wk) Week 2	Water Consumed (g/wk) Week 1	Water Consumed (g/wk) Week 2
1	F	0	73.8 ± 3.6	98.2 ± 4.4	108.7 ± 7.1	145.9 ± 11.4
2	F	1200	44.8 ± 3.4	82.8 ± 2.8	75.9 ± 9.7	134.1 ± 20.8
3	F	800	50.1 ± 3.1	81.7 ± 5.0	96.8 ± 26.8	158.8 ± 32.3
4	F	400	61.8 ± 3.4	90.9 ± 4.3	96.4 ± 12.0	143.2 ± 13.7
5	M	0	108.1 ± 2.8	134.4 ± 1.8	136.1 ± 7.3	178.8 ± 6.3
6	M	1200	51.3 ± 5.3	100.2 ± 3.0	87.0 ± 16.7	154.3 ± 11.5
7	M	800	75.5 ± 1.8	115.3 ± 2.4	102.8 ± 6.5	165.4 ± 10.8
8	M	400	86.4 ± 8.4	125.6 ± 4.6	116.5 ± 7.2	177.2 ± 5.0

Mean ± Standard Deviation

INDIVIDUAL FEED AND WATER DATA

FEMALES

GP-ANI NUMBER	FEED (G/WK) WEEK 1	FEED (G/WK) WEEK 2	WATER (G/WK) WEEK 1	WATER (G/WK) WEEK 2
1-151	77.20	102.30	110.10	141.20
1-152	78.00	100.00	108.30	146.90
1-153	71.70	101.20	119.30	165.70
1-154	73.80	97.30	109.10	145.00
1-155	68.10	90.00	96.90	130.50
2-156	43.60	78.90	60.30	100.50
2-157	41.90	86.80	75.70	126.50
2-158	46.40	*	84.90	163.20
2-159	41.50	83.00	71.30	135.00
2-160	50.60	82.50	87.20	145.50
3-161	51.10	76.00	96.00	161.60
3-162	51.00	85.50	147.40	216.80
3-163	52.00	88.40	91.20	157.70
3-164	43.90	76.10	70.60	123.40
3-165	52.40	82.40	78.80	134.70
4-166	63.50	89.80	87.90	136.60
4-167	65.70	92.50	102.80	145.90
4-168	60.20	93.50	92.50	143.30
4-169	63.80	95.40	116.40	166.20
4-170	55.90	83.10	82.40	124.10

* EXCESSIVE SPILLAGE

INDIVIDUAL FEED AND WATER DATA

MALES

GP-ANI NUMBER	FEED (G/WK) WEEK 1	FEED (G/WK) WEEK 2	WATER (G/WK) WEEK 1	WATER (G/WK) WEEK 2
5-171	107.50	134.50	145.60	186.60
5-172	111.20	132.40	141.30	184.90
5-173	107.70	135.70	138.40	174.50
5-174	110.70	137.00	128.70	169.70
5-175	103.30	132.40	126.50	178.30
6-176	42.50	95.40	76.60	156.30
6-177	56.10	99.80	69.00	137.70
6-178	54.90	102.20	89.20	156.30
6-179	*	*	117.60	172.90
6-180	51.50	103.30	82.60	148.50
7-181	76.50	112.90	110.10	181.70
7-182	76.80	119.50	110.90	173.90
7-183	77.10	113.80	96.60	154.80
7-184	72.20	116.20	100.20	162.60
7-185	74.80	114.20	96.00	154.00
8-186	91.20	123.50	121.80	179.10
8-187	80.50	121.80	112.40	171.20
8-188	78.20	126.40	106.50	174.30
8-189	81.30	122.10	114.60	185.80
8-190	100.60	134.20	127.00	175.70

* EXCESSIVE SPILLAGE

APPENDIX K

PROTOCOL AND AMENDMENTS

PROTOCOL

14 Day Range Finding and Toxicity Evaluation on

1,3,5-Trinitrobenzene (TNB) in F344 Male and Female Rats

This study will be conducted in agreement with Good Laboratory Practice Standards, Environmental Protection Agency, Toxic Substances Control Act (TSCA) 40 CFR Part 792 (Federal Register, Vol 54, No. 158, August 17, 1989, pp. 34034 - 34050). All aspects of the studies will be conducted in accordance with written Standard Operating Procedures (SOP) of the performing unit and all raw data and performance documents will be maintained in agreement with GLP. An administratively separate quality assurance unit (QAU from PAI) will monitor the studies to assure adherence to good laboratory practices and the approved SOPs. Any deviation from the protocol or GLP will be noted in the raw data and reflected in the final report.

Testing Facility
A.W. Breidenbach Environmental Research Center
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Prime Contractor (Sponsor)
U.S. Army Biomedical Research and
Development Laboratory, Fort Detrick
Frederick, Maryland 21701-5010

Principle Investigator — Date
T.V. Reddy, Ph.D.

G. Reddy, Ph.D., Sponsor — Date

Project Manager — Date
G.R. Olson, DVM, Ph.D.
Pathology Associates, Inc.

Quality Assurance — Date
W.R. Fox, MA
Pathology Associates, Inc.

TITLE: 14 Day Range Finding and Toxicity Evaluation on 1,3,5-Trinitrobenzene (TNB), 1,3-Dinitrobenzene (DNB) and N-Methyl-N-2,4,6-Tetranitroaniline (Tetryl) in F344 Rats.

BACKGROUND:

Nitroaromatics, such as 1,3-dinitrobenzene (DNB), 1,3,5-trinitrobenzene (TNB), and N-methyl-N,2,4,6-tetranitroaniline (tetryl), have been detected as environmental contaminants of groundwater and soil near production sites and in some instances at military test grounds. The wastewaters discharged from trinitrotoluene (TNT) manufacturing processes contain a variety of aromatic compounds, including DNB and TNB. TNB is formed during the nitration step of TNT synthesis as a result of oxidation of methyl groups. Although the complete mechanism of TNB formation during TNT photolysis is unknown, Burlinson (1980) suggested that it is produced by decarboxylation of 2,4,6-trinitrobenzaldehyde, a major TNT photoproduct. It is also found in aquatic systems and surface soils as a by-products of photolysis of TNT. DNB and TNB are not easily biodegradable, persist in the environment, eventually leach out, and contaminate groundwater near waste disposal sites. Tetryl is an explosive that has been in use, largely for military purposes, since 1906. Wastewaters and soil at the original production sites and other plants devoted to munitions assembly, contain large quantities of tetryl. A recent estimate of tetryl in wastewaters generated from the production of tetryl at Joliet Army Ammunition Plant was about 36 lb/per day of each production line.

Toxicity data on these compound are limited. The oral LD50 of DNB, TNB and tetryl were 59 mg/kg, 284 mg/kg and greater than 5 g/kg, respectively, in rats for combined sexes. TNB and tetryl were not toxic at 2 g/kg when applied to rabbit skin for 24 hours. However, the dermal LD50 of DNB was 1.99 g/kg for combined sexes of rabbits. None of these compounds produced skin irritation potentials but positive (DNB) and severe (TNB, tetryl) eye irritation potentials in rabbits. The sensitization tests showed that DNB and tetryl are not skin sensitizers while TNB caused mild allergic reaction in guinea pigs. Some of the toxicological and behavioral effects of DNB are: formation of methemoglobin, testicular degeneration and reproductive failure, and weight loss and anemia in hamsters, rats and mice. Neurological and hematological disorders have also been reported in dogs. DNB is rather toxic to humans; the estimated lethal dose range is 5-50 mg/kg. It is readily absorbed through the skin. Fetal doses (amount and route of administration are not given) of tetryl produced toxic degeneration (necrosis) in the kidneys of dogs and rabbits and liver necrosis in dogs (not in rabbits). Tetryl was observed to be a powerful skin sensitizer in ammunition plant workers. Hardy and Maloof (1950) reported effects from accidental exposure of 11 people to tetryl: 2 died, 1 was disabled and 8 did not detect permanent disability. They also reported irreversible liver damage, dermatitis, and upper respiratory irritation following tetryl exposure. The effects of tetryl exposure include gastrointestinal symptoms and epidermal, respiratory, nervous system, hematopoietic and circulatory injury. Atmospheric concentration of 1.5 mg/m3 or below did not produce systemic poisoning in persons working with tetryl. DNB, TNB, and tetryl have been shown to be genotoxic in Salmonella mutagenesis assay. TNB has been shown to form adducts of blood proteins and tissue DNA in rats.

PROTOCOL

1. Study. 14 day range finding and toxicity evaluation with 1,3,5-trinitrobenzene (TNB) in F344 male and female rats.

2. Purpose. To evaluate subchronic toxicity of TNB when administered in the diet for 14 days and select the ideal doses for 90 days subchronic study.

3. Study Location. A.W. Breidenbach Environmental Research Center
U.S. Environmental Protection Agency
Cincinnati, OH 45268

4. Sponsor and Address. U.S. Army Biomedical Research and Development Laboratory, Fort Detrick
Frederick, Maryland 21701-5010

5. Principle Investigator. T.V. Reddy, Ph.D., Research Chemist
Environmental Monitoring Systems Laboratory
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

6. Study Coordinator. Barry Wiechman, MS.

7. Project Manager. G. R. Olson, DVM, Ph.D., Pathology Associates, Inc. (PAI)

8. Regulatory Compliance. This study is carried according to U.S. EPA Health Effects testing guidelines (40 CFR 798) in compliance with GLP (40 CFR 792).

9. Quality Assurance. The protocol in life phase and final report will be audited by the Quality Assurance Office in accordance with SOP's at Pathology Associates, West Chester, Ohio 45069.

10. Test Material. 1,3,5-Trinitrobenzene (TNB) Powder (CAS #99-35-4) is supplied by U.S. Army Biomedical research and Development Laboratory, Ft. Detrick, Frederick, Maryland 21702.

11. Experimental Design.

A. Selection of Dose: Toxikon Corporation, Woburn, MA 01801 has conducted acute toxicity studies on TNB. They administered TNB in corn oil to rats at a single oral (Bolus) dose and observed the clinical signs for 14 days, following dosing. Based on the results they have established 298 mg/kg BW, and 275 mg/kg BW, as the LD50 dose for male and female rats respectively. For combined sexes the reported LD50 dose was 284 mg/kg

concentrations to be tested in rats for the 14 day range finding study. (140, 70, 35, 17.5, 8.75 mg/kg BW). Control rats are fed only powdered chow diet.

B. Preparation of the Diet: Certified powdered Purina laboratory chow purchased from Purina labs and stored at 4°C until use. TNB diets are prepared once a week. Just before the diet preparation TNB is removed from the explosion proof storage shelves (kept in designated carcinogen room) weighed in the carcinogen room and mixed with the powdered diet (1.4 g/kg). First 1.4g TNB will be mixed with 250g powdered diet and mixed for 15 min. Then an additional 250 g powdered diet is added and mixed for an additional 15 min. Then the remaining diet will be added to bring the TNB concentration as 1.4 g/kg; and then mixed for an additional 1 hr. in a mechanical stirrer (Kitchen Aid heavy duty stand mixer, Model No. K5SS) for uniform distribution of TNB in the diet. This is also verified by determining the TNB concentration in the diet, taken from three different depths (top, middle and bottom layer) of the mixing chamber. Quantitative analysis of TNB is done by HPLC.

The premixed diet (1.4 g/kg) is further diluted to 2, 4, 8 and 16 times with fresh powdered diet to obtain the desired TNB concentration in the diet (70, 35, 17.5 and 8.75 mg/kg BW). Individual diet concentrations are determined as described before. The diet feeders are changed twice a week. TNB concentrations are manipulated in such a way that each rat (caged individually) will receive the desired amount of TNB. This is determined by calculating the daily average intake, followed by an adjustment of TNB content in the diet. Dietary intake and water consumption are measured twice a week. Body weights are recorded once a week.

C. Pilot Study: There is no information on the continuous feeding studies on TNB; therefore, a study will be conducted for 14 days with 3 dose levels (140, 70, and 35 mg/kg BW) with 5 male and female rats per group. From this study the chemical tolerance and food consumption will be evaluated. This palatability study is essential to determine the dose levels for the 14 day toxicity study.

Animals: Twenty male Fischer 344 rats (4W of age) with close body weight (±5 g) range will be obtained from Charles River Breeding Laboratory (Portage, MI) and are held for 1W for quarantine (by which time all the serological tests are evaluated). After quarantine 15 rats with similar body weights (±5g) are housed individually in clean polycarbonate shoe boxes with aspen bedding (rats are ear tagged and all cages are sequentially numbered for identification) 5 rats/each dose). Rats 1-5 are fed 140 mg/kg dose followed by 70 mg/kg (rats 6-10) and 35 mg/kg dose (rats 11-15) daily for 14 days. Food consumption and water consumption are recorded 2 times a week. Food and spoilage is taken into account while recording food consumption. Body weights are recorded once a week. During the 14 day period the rats are watched daily for possible physical changes and toxicity. All observations are recorded and used while designing 14 day toxicity study.

Range Finding Experiment: While the pilot study is in progress 40 male and 40 female F344 rats with close body weight range (±5g) will be purchased and held for 1 week for quarantine. After evaluation of the serological data and soon after release from quarantine 5 rats from each sex are sacrificed and used for necropsy quality controls or base line control animals to ensure the animals are healthy and within normal limits for all measurements at the time of arrival and after quarantine. Male and female rats after quarantine are also housed individually in clear polycarbonate show boxes in drawer rack cages with aspen bedding (San I Chips supplied by P.J. Murphy, Forest Products Corporation, NJ). Shoe boxes and bedding are changed along with food and water (2 times a week). Water is provided with 16 ounce bottles and stoppers and sipper tubes. At all times the animal rooms maintained on a 12 hour light/dark cycle at 22-23°C with relative humidity range 40-60%.

D. Randomization: Using computer-generated random numbers with assignment to groups. At the time of randomization, the weight variation of the animals of each sex used should not exceed ± 2 S±D of the mean weight, and the mean body weights for each group of each sex will not be statistically different.

E. Justification: Rats historically have been used in safety evaluation studies and are recommended by appropriate regulatory agencies.

F. Group designation and dose levels for pilot palatability study

Group	No. of rats (Male/Female)		Dose Level mg/kg/day	Termination time (days)
1	5	5	0	14
2	5	5	140	14
3	5	5	70	14
4	5	5	35	14

G. Group designation and dose levels for 14 day toxicity study.

Group	No. of Animals Male/Female		Dose Levels mg/kg/day	
1	5	5	0	
2	5	5	A	doses A to E will be
3	5	5	B	calculated from pilot
4	5	5	C	experiment
5	5	5	D	
6	5	5	E	

H. Analysis of the Diet: The stability and the homogeneity of TNB in the diet is determined by analyzing the TNB content (by HPLC) in the diet, soon after diet preparation and after each feeding intervals. If the TNB concentrations vary drastically then an alternate method of feeding (such as daily gavage) will be considered. This will be established during palatability pilot experiment so that 14 day and 90 day studies can be

carried without any interruption.

I. Observation of Animals:

(1) Clinical Observations: Twice daily - mortality and morbidity check. Once daily - cageside observation for obvious indications of a toxic effect; these effects will be recorded as they are observed.

Data for mortality and morbidity checks and cageside observations will be recorded on the same form. Because these are cageside animal checks, the observations will not be as specific as and may not necessarily duplicate those observations recorded on body weight days when thorough physical examinations are conducted.

(2) Physical Examinations: At each weighing interval. These observations will include, but not be limited to, changes in: skin and fur; eyes and mucous membranes; respiratory, circulatory, autonomic and central nervous systems; some motor activity and behavior.

(3) Body Weight: Prior to treatment, and weekly thereafter.

(4) Food Consumption: Weekly - twice.

(5) Water Consumption: Weekly - twice.

J. Clinical Pathology:

(1) Frequency: At termination.

(2) Number of Animals: All animals (samples per SOP following phenobarbital anesthetic.

K. Tests:

(1) Hematology

leukocyte count
erythrocyte count
heinz bodies
hemoglobin
methemoglobin
hematocrit
platelet count
differential leukocyte count

(2) Blood Chemistry

sodium
potassium
total protein
albumin
calcium
total bilirubin
urea nitrogen
creatinine
aspartate aminotransferase
alanine aminotransferase
lactic dehydrogenase
alkaline phosphatase

L. Termination:

(1) Unscheduled Sacrifices and Deaths

Necropsies, by trained personnel using procedures approved by board-certified pathologists, will be conducted on all moribund animals and on all animals that die.

(2) Sacrifice

After 14 days of treatment, all surviving animals will be weighed, anesthetized with sodium pentobarbital, and exsanguinated. Necropsies will be conducted on each animal by trained personnel using procedures approved by board-certified pathologists.

A pathologist will be readily available for consultation (further participation by a pathologist is available).

M. Postmortem Procedures:

(1) Gross Necropsy

The necropsy will include examination of :

The external surface
All orifices
Cranial cavity
Carcass
External surface o the brain (at necropsy); cut surfaces of the brain
The thoracic, abdominal and pelvic cavities and their viscera
The cervical tissues and organs

(2) Organ Weights

For each terminally sacrificed animal, the following organs (when present) will be weighed following careful dissection and trimming to remove fat and other contiguous tissue in a uniform manner:

brain	lungs
liver	thymus
spleen	testes with epididymides/ovaries
kidneys	heart
adrenals	

(3) Tissue Preservation

The following tissues (when present) from each animal will be preserved in 10% neutral buffered formalin:

skin	ileum
mandibular and	colon
mesenteric lymph nodes	cecum
mammary glands	rectum
thigh muscle	liver
sciatic nerve	pancreas
sternum with marrow	spleen
femur with marrow	kidneys
larynx	adrenals
thymus	urinary bladder
trachea	seminal vesicles
lungs and bronchi	prostate
heart and aorta	testes, including epididymis
thyroid	ovaries
parathyroids	uterus
esophagus	nasal cavity and nasal turbinates
stomach	brain
duodenum	pituitary
jejunum	preputial or clitoral glands
tongue	Zymbal's gland
salivary gland	thoracic spinal cord

N. Histopathology:

1. Following necropsy, a list of all gross lesions recorded will be submitted to the project officer at U.S. Army Biomedical Research and Development Laboratory for his evaluation and for any additional histopathology than those described below.

Histopathological evaluations are to be done on the following tissues from all the animals. Male an female from highest dose group and (140 mg/kg/day dose) and untreated controls. The tissues examined under light microscope are as follows:

cerebrum	pancreas
cerebellum	cecum
trachea	colon
thyroid	rectum
parathyroid	stomach
esophagus	skeletal muscle
salivary gland	sciatic nerve
harderian gland	tongue
exorbital gland	skin
heart	mammary gland
aorta	nasal region
lung	sternum
thymus	femur
spleen	vertebrae
mesenteric lymph node	spinal cord
liver	adrenals
kidney	pituitary
urinary bladder	eye(s)
duodenum	middle ear
jejunum	auditory sebaceous gland
ileum	
MALE	FEMALE
accessory sex glands	uterus
epididymis	ovaries
testes	

An average of 12 slides will be prepared for each rat covering all the tissues shown above (3 or 4 tissues are fixed on each slide). A total of 240 slides from 20 rats(5 male and 5 female from high dose 14 day study and 5 rats each from control group) from the 14 day study (highest dose, 140 mg/kg/day) will be examined. Based on the results from dose group tissues from other doses groups will be examined as needed. Following completion of each study all wet tissues, paraffin blocks and slides will be stored in PAI archives.

O. Final Report:

Four months after the termination of the in-life phase of the study, a final report which includes the following information (as appropriate) will be prepared and submitted to the Sponsor:

(1) Experimental Design and Methods

(2) Results

mortality	organ weights and organ/body
clinical observations	weight ratios
body weights	gross pathology
food and liquid consumption	histopathology
clinical pathology tests	

Statistical Evaluation:

Stat view computer software will be used for statistical analysis in 14-day and 90-day study for statistical analysis.

Dunnet's t-test will be used for comparing treatment group.

Kruskal-Wallis rank sums will be used to examine the differences among the treatment groups and Wilcoxon rank sum test was used to analyze pairwise differences between the control and each dose group.

Amendment 1
for
United States Army Study 92-002
14 DAY TOXICITY EVALUATION OF 1,3,5-TRINITROBENZENE (TNB) IN FISCHER (F344) RATS

For
United States Army
Biomedical Research and Development Laboratory
Fort Detrick
Frederick, MD 21701-5010

The purpose of Amendment 1 is to provide dose levels per group and correct the list of tissues to be examined microscopically.

1. Page 4, G - The middle table heading should be corrected by removing Male/Female, and two columns, one denoting the sex of the group and the other providing the target dose should be added. The target dose levels (mg/kg body weight/day) are as follows: Group 1, 0, Group 2, 120, Group 3, 80, Group 4, 40, Group 5, 20, Group 6, 5, Group 7, 0, Group 8, 120, Group 9, 40, Group 10, 40, Group 11, 20, Group 12, 5.

Reason: Each male and female group has been given an individual group number and the target dose column was omitted in the original protocol.

2. Page 4, G - The dose levels in diet are the following: Group 1, females, 0 mg/kg, Group 2, females, 1200 mg/kg, Group 3, females, 800 mg/kg, Group 4, females 400 mg/kg, Group 5, females, 200 mg/kg, Group 6, females, 50 mg/kg, Group 7, males, 0 mg/kg, Group 8, males, 1200 mg/kg, Group 9, males, 800 mg/kg, Group 10, males, 400 mg/kg, Group 11, 200 mg/kg, Group 12, males, 50 mg/kg.

Therefore, page 4, G will appear:

Group designation and dose levels for 14 day toxicity study.

Group	No. of Animals	Sex	Dose Levels mg/kg diet	Target Dose mg/kg body weight/day
1	5	Female	0	0
2	5	Female	1200	120
3	5	Female	800	80
4	5	Female	400	40
5	5	Female	200	20
6	5	Female	50	5
7	5	Male	0	0
8	5	Male	1200	120
9	5	Male	800	80
10	5	Male	400	40
11	5	Male	200	20
12	5	Male	50	5

Reason: Dose levels could not be determined until the completion of the palatibility test.

3. Page 8, N - remove middle ear and exorbital gland from the list.

Reason: These are not standard tissues to examine.

Amendment 1 Approval

U.S. Army Medical Research and
Development Laboratory
Fort Detrick
Frederick, Maryland 21701-5010

AW Breidenbach Environmental Research Ctr
US Environmental Protection Agency
Cincinnati, Ohio 45268

8-25-92
G. Reddy, Ph.D., Sponsor Date

T.V. Reddy
8-27-92
T.V. Reddy, Ph.D., PI Date

8-27-92
Willa Fox, MA, QA Date

Amendment 2
for
United States Army Study 92-002
14 DAY TOXICITY EVALUATION OF 1,3,5-TRINITROBENZENE (TNB) IN FISCHER (F344) RATS

For
United States Army
Biomedical Research and Development Laboratory
Fort Detrick
Frederick, MD 21701-5010

The purpose of Amendment 1 is to correct or provide additional information for the following: preparation of the diet, list of blood chemistry tests, sacrifice information, housing, animal fasting, study start and termination dates, animal body weight range, animal identification, test chemical purity, sacrifice order, and route justification.

1. Page 3, B - (Additional Information) Preparation of the Diet: Certified powdered Purina laboratory chow purchased from Purina Labs and stored at 4°C until use will be the diet used for the 14 day study. TNB diets are prepared as needed or at a minimum, biweekly. TNB will be stored and weighed in a designated carcinogen room. First, 1.2 g TNB will be added to 25 g of powdered diet in a mortar and thoroughly ground with a pestle. Afterwards 225 g of the diet will be added and mixed for 15 minutes. Then an additional 250 g powdered diet will be added and mixed for another 15 minutes. Finally, the remaining diet (500 g) will be added and mixed for 30 minutes in a mechanical stirrer (Kitchen Aid Heavy duty mixer) for uniform distribution of TNB in the diet. This is verified by determining the TNB concentration in the diet, taken from each of the 1 kg mixtures by quantitative analysis done by HPLC. The premixed diet (1.2 g/kg diet) is further diluted with fresh powdered diet to obtain the desired TNB concentration in the diet in the lower dose groups. The diet feeders are changed twice a week. Dietary intake and water consumption are measured twice a week. Body weights are recorded once a week.

Reason: a) The pilot study demonstrated that a reduced high dose level was required. b) HPLC analysis revealed that the chemical was stable in the food for at least two weeks. c) Mixing with a small amount (25 g) of diet first, allows for more complete uniform distribution of the chemical in the diet.

2. Page 4, C - Animals will be housed in suspended cages.

Reason: Prevent mixing of bedding with food.

3. Page 6, K. (2) - Eliminate lactic dehydrogenase from the list and add glucose.

Reason: American Association of Clinical Chemistry recommends that LDH not be routinely done due to its high variability and lack of specificity as an indicator of major organ toxicity in animal species. Glucose was inadvertently left off the list.

4. Page 6, L. (2) - (Additional Information) Animals will be fasted for 12 hours prior to the sacrifice.

Reason: More consistent serum chemistry values are obtained.

5. Page 2. (11.) - (Additional Information) Study Start Date: August 18, 1992
Termination Date: September 2, 1992

6. Page 4, C. - (Additional Information) Fischer 344 rats of close body weight (Males 120 g ± 5 g; Females 100 g ± 5 g) range will be obtained from Charles River Breeding Laboratory (Portage, MI).

Reason: Weights not specified in original protocol.

7. Page 4, C - (Additional Information) Animals will be individually identified by ear tags.

Reason: Unique identification not specified in original protocol.

8. Page 2, 10. - (Additional Information) Sponsor will be responsible for the purity of the test material (TNB)

Reason: Not specified in original protocol.

9. Page 6, 2. - (Additional Information) Animals will be sacrificed in random order.

Reason: To eliminate bias on the part of the prosector.

10. Page 2, A. - (Additional Information) The oral route of administration was chosen in this study since it was the most likely route for human exposure.

Reason: Justification needed.

Amendment 2 Approval

U.S. Army Medical Research and
Development Laboratory
Fort Detrick
Frederick, Maryland 21701-5010

AW Breidenbach Environmental Research Ctr
US Environmental Protection Agency
Cincinnati, Ohio 45268

8-28-92
G. Reddy, Ph.D., Sponsor — Date

T.V. Reddy
8.27.92
T.V. Reddy, Ph.D., PI — Date

8.27-92
Willa Fox, MA; QA — Date

Amendment 3
for
United States Army Study 92-002
14 DAY TOXICITY EVALUATION OF 1,3,5-TRINITROBENZENE (TNB) IN FISCHER (F344) RATS

For
United States Army
Biomedical Research and Development Laboratory
Fort Detrick
Frederick, MD 21701-5010

The purpose of Amendment 3 is to include an additional study to collect blood samples for hemotology analysis for methemoglobin.

1. Page 5, K. (1) - Add the following paragraph: An additional study will be conducted at the same dosage levels (under as similar conditions of the original 14-Day study as possible) to collect blood samples for metheoglobin analysis. The analysis will be performed by The Jewish Hospital, Cincinnati, Ohio.

Reason: The methemoglobin samples from the original study were not analyzed in time to produce credible results.

Amendment 3 Approval

U.S. Army Medical Research and
Development Laboratory
Fort Detrick
Frederick, Maryland 21701-5010

AW Breidenbach Environmental Research Ctr
US Environmental Protection Agency
Cincinnati, Ohio 45268

G. Reddy 4/12/93
G. Reddy, Ph.D., Sponsor Date

T.V. Reddy 4.6.93
T.V. Reddy, Ph.D., PI Date

4-6-93
Willa Fox, MA, QA Date

Deviations from the Protocol

1. The clinical pathology procedures were not performed under the auspices of a quality assurance unit. However, the laboratory was approved by an agent of the Food and Drug Administration in April, 1992. All raw data was checked and quality control standards evaluated from these procedures.

2. Methemoglobin values were obtained from additional animals that are reported in an addendum. The blood samples from the original animals were not analyzed fast enough in order to obtain accurate results. Only the three higher dose levels (1200, 800, 400 mg/kg) were used for this additional data.

3. The pilot palatability study was extended to three weeks to better assess taste aversion.

4. Diet preparation was slightly modified in order to obtain better homogeneity from smaller batches.

5. Clinical observations were made twice daily but recorded once daily.

Tirumuru V. Reddy, Ph.D.
Principal Investigator

DISTRIBUTION LIST

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Fort Detrick, Frederick, MD 21702-5010

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