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MAMMALIAN TOXICITY OF MUNITION COMPOUNDS

PHASE II: Effects of Multiple Doses

PART I: Trinitroglycerin

Progress Report No. 2

by

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11 November 1977

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Washington, D.C. 20314

Contract No. DAMD-17-74-C-4073
MRI Project No. 3900-B

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Animal experimentation: Animal experiments were conducted according to the "Guide for Laboratory Animal Facilities and Care" (1965) prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences, National Research Council; the regulations and standards prepared by the Department of Agriculture; and Public Law 91-579, "Laboratory Animal Welfare Act," 1970.

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UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: Progress Report No. 2, 1 Mar 75-25 Feb 76	2. GOVT ACCESSION NO. / 3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle): MAMMALIAN TOXICITY OF MUNITION COMPOUNDS - PHASE II. Effects of Multiple Doses - PART I. Trinitroglycerin.	5. TYPE OF REPORT & PERIOD COVERED: Progress Report, 1 March 1975 to 25 February 1976
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): Cheng-Chun Lee, Harry V. Ellis, III, John J. Kowalski, John R. Hodgson, Shang W. Hwang, Robert D. Short, Jadgish C. Bhandari, Jaime L. Sanyer, Thomas W. Reddig, Jan L. Minor, Danny O. Helton	8. CONTRACT OR GRANT NUMBER(s): DAMD-17-74-C-4073
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Midwest Research Institute, 425 Volker Boulevard, Kansas City, MO 64110	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS	12. REPORT DATE: 25 February 1976
	13. NUMBER OF PAGES: 192
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office): Headquarters, U.S. Army Medical Research and Development Command, Washington, DC 20314 (185 p.)	15. SECURITY CLASS. (of this report): Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Subacute Toxicity	Disposition
Subchronic Toxicity	Metabolism
Trinitroglycerin	Methemoglobinemia
Glyceryl Trinitrate	

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The effects of oral doses of trinitroglycerin (TNG) after administration for up to 13 weeks, were investigated in dogs, rats and mice. A detailed study on the disposition and metabolism was performed in rats and the metabolic pathways compared in dogs, mice, rabbits, monkeys and human cadaver liver.

No adverse effects were seen in dogs given 1 mg/kg/day TNG for 4 weeks followed by 5 mg/kg/day for an additional 9 weeks. Larger doses (25 to →

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UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

230350

20. Abstract (concluded)

200 mg/kg/day for 5 days) produced methemoglobinemia, peaking about 4 hr after dosing, with the peak height and duration dose-related. Rats eating 1,400 mg/kg/day of TNG showed toxic effects: decreased feed consumption, weight loss, rough coat, testicular atrophy and degeneration and hemosiderosis. Some recovery occurred despite continued dosing, before the 13th week. Mice fed 11 or 100 mg/kg/day for 3 weeks followed by 60 or 580 mg/kg/day for 10 weeks had some extramedullary hematopoiesis, but no other effects.

Mice absorbed 50 to 70% of an oral dose of TNG within 24 hr; the other species, 72 to 95%. Most body tissues picked up the ^{14}C radiolabel. Rats and mice excreted ^{14}C compounds in air and urine, the other species primarily in urine. TNG was denitrated to form di- and mononitroglycerins, glycerol, other polar compounds and various glucuronides. Livers from all species tested denitrated TNG in vitro.

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PREFACE

This report was prepared at Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri 64110, under U.S. Department of the Army Contract No. DAMD-17-74-C-4073, MRI Project No. 3900-B, "Munition Compounds Mammalian Toxicity Study." The work was supported by the U.S. Army Medical Bioengineering Research and Development Laboratory, USAMRDC, Department of the Army. Cpt. John P. Glennon, Dr. Jack C. Dacre, Dr. David H. Rosenblatt and Cpt. Robert Rice, Environmental Research Requirement Branch, are the consecutive technical monitors for the project.

This work was conducted in the Biological Sciences Division, under the direction of Dr. William B. House, between 1 March 1975 and 29 February 1976. The experimental work was directed by Dr. Cheng Chun Lee, Head, Pharmacology and Toxicology with the assistance of Dr. Harry V. Ellis, III, Associate Pharmacologist, and Mr. John J. Kowalski, Assistant Biologist. Dr. John R. Hodgson, Senior Biochemist, supervised the studies on metabolism, cytogenesis and mutagenesis. Dr. Shang W. Hwang, Associate Pharmacologist, assisted the studies on metabolism. Dr. Robert D. Short, Associate Pharmacologist, supervised the *in vitro* study on metabolism and *in vivo* study on drug metabolizing enzymes. Dr. J. C. Bhandari and Dr. Jaime L. Sanyer, Associate Veterinary Pathologists, supervised the necropsy and the histology preparation and performed the microscopic examination. Mr. Thomas W. Reddig (ASCP certified M.T.), Laboratory Supervisor, supervised the hematology and clinical laboratory tests. Mr. Jan L. Minor, Assistant Toxicologist, supervised the computer program and analysis of experimental data. Dr. Danny O. Helton, Associate Chemist, performed the TNG assay in feed. Technical personnel included Robert C. Byrne, Bruce S. Andersen, Mary A. Kowalski, Francis H. Brown, Ellen R. Ellis, Ernesto A. Castillo, Judith D. Girvin, Patricia L. Wilkerson, Bhanu S. Gosalia, Laurel M. Halfpap and William M. Bracken.

Approved for:

MIDWEST RESEARCH INSTITUTE

W. B. House, Director
Biological Sciences Division

20 February 1976

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ABSTRACT

The effects of TNG after administration up to 13 weeks were investigated in dogs, rats and mice. A detailed study on the disposition and metabolism was performed in rats and the metabolic pathways were compared in various species including liver of human cadavers.

In dogs, oral administration of 0.01, 0.1 or 1 mg/kg/day of TNG consecutively for 4 weeks and 0.05, 0.5 or 5 mg/kg/day for 9 additional weeks did not cause any adverse effects, any changes in peripheral blood elements or clinical laboratory tests, any numerical or morphological aberrations of chromosomes in the peripheral lymphocytes or kidney cultures, any change in serum concentration of IgE, or any lesions. Oral administration of 25 or 50 mg/kg/day of TNG for 5 consecutive days did not cause any apparent adverse signs; 100 or 200 mg/kg/day caused cyanosis lasting a few hours 2 to 3 hours after dosing. In addition, 200 mg/kg/day decreased the activity without any serious effects. All these dose levels caused formation of methemoglobin which reached peak concentration in 1 to 2 hours. The height of the peaks and the duration of the methemoglobin concentrations were dose-related. Methemoglobin disappeared from the blood in 8 to 24 hours depending upon the dose. Small amounts of methemoglobin remained in some dogs 24 hours after larger doses. Administration of 3 mg/kg/day of methylene blue 2 hours after administration of 200 mg/kg/day apparently prevented the methemoglobin formation to reach the high peak. However, the concentration of methemoglobin persisted for 40 hours or longer after the administration of TNG.

In rats, the TNG intake of the males fed the low, middle and high levels of TNG in feed averaged 0.8, 6.0 or 59.0 mg/kg/day, respectively, during the first 5 weeks; and 2.6, 24.5 or 229.5 mg/kg/day, respectively, during the additional 8 weeks. The TNG intake of the females averaged 0.9, 6.4 or 59.3 mg/kg/day during the first 5 weeks and averaged 3.1, 26.5 or 233.8 mg/kg/day during the additional 8 weeks. Both the male and female rats fed the low or the middle level of TNG did not show any adverse signs, any changes in peripheral blood elements or clinical laboratory tests, or lesions in any tissues related to TNG. The high level of TNG in feed decreased the feed consumption and retarded the weight gain of rats, the effects were more profound in the males than in the females. In addition, the high level of TNG caused elevation of SGOT in some rats without any apparent lesions. These effects on feed consumption, growth and SGOT were reversible after discontinuation of TNG feeding for 4 weeks.

In another study, the rats were fed 2.5% TNG or 25% lactose in feed for 13 weeks. The TNG intake of the males and females averaged 1,176 or 1,076 mg/kg/day, respectively, during the first week and increased to

1,588 or 1,773 mg/kg/day, respectively, during the last 5 weeks. Both the male and the female rats fed TNG consumed less feed, lost weight quickly, were slightly less active, and had rough hair coat through the 8th week. Thereafter, they started to gain weight and to recover from these effects. TNG feeding caused testicular atrophy and degeneration and aspermatogenesis in the males, and caused some changes in organ weights in both the males and the females. In addition, both the male and female rats fed TNG had hemosiderosis in the spleen and/or the liver. Feeding of 25% lactose did not cause any adverse signs, any changes in peripheral blood elements or clinical blood chemistry tests, any effects on calcium concentration of the blood or iron content of the liver, or any lesions related to lactose. However, lactose feeding slightly increased the weights of caecum of both the male and the female rats.

An average of 59.0 to 59.3 mg/kg/day of TNG in feed for 5 weeks and 202.4 to 206.2 mg/kg/day for additional 8 weeks did not cause any numerical or morphological aberrations of the chromosomes in the peripheral lymphocytes or kidney cultures. Treatment of Chinese hamster ovary cells with TNG at concentrations which killed 65% or 99% of the population did not appear to induce any mutations. An average of 1,406 to 1,416 mg/kg/day of TNG in feed for 13 weeks did not alter the serum concentration of IgE.

In mice, the TNG intake of the males fed the low, middle and high levels of TNG in feed averaged 1.3, 11.5 or 106.7 mg/kg/day, respectively, during the first 3 weeks; and 6.4, 60.2 or 607.2 mg/kg/day, respectively, during the subsequent 10 weeks. The TNG intake of the females averaged 1.3, 10.9 or 94.9 mg/kg/day during the first 3 weeks and averaged 6.9, 58.7 or 561.2 mg/kg/day during the subsequent 10 weeks. Both the male and the female mice fed the low, middle or high level of TNG did not show any adverse signs, any changes in peripheral blood elements or clinical blood chemistry tests. Extramedullary hematopoiesis was seen in the liver and spleen of mice fed TNG for 13 weeks and for 13 weeks plus recovery for 4 weeks.

The metabolic pathways of TNG were similar in rats, mice, rabbits, dogs and monkeys. About 50 to 70% of an oral dose of TNG-1,3-^{14}C was absorbed in 24 hours in mice, whereas 75 to 95% was absorbed in rats, rabbits, dogs and monkeys. In rats and mice, the majority of the absorbed radioactivity was excreted in the urine and expired air. In rabbits, dogs and monkeys, most of the absorbed radioactivity was excreted in the urine with only small amounts in the expired air. The radioactivity was highly concentrated in the liver of all species. Other tissues, including kidney, spleen, lung and/or skeletal muscle, also contained significant amounts of radioactivity.

TNG and free DNGs were excreted only in small amounts in the urine of mice, rats and rabbits, and in slightly greater amounts in the urine of dogs and monkeys. Large amounts of unidentified polar compounds and glycerol were found in all species. Mice excreted only small amounts of free MNG, MNG-glucuronides and DNG-glucuronides. Most of the urinary metabolites in rats and rabbits were free MNG and DNG-glucuronides. Dogs and monkeys excreted mostly free MNGs and MNG-glucuronides.

After oral administration of TNG-1,3-^{14}C to rats, the radioactivity appeared in the bile in 15 minutes. The rate of biliary excretion increased with time and reached a peak in 3 hours. The blood concentration of radioactivity continued to increase through the 5th to 6th hour. The biliary excretion of radioactivity was high for 2-MNG, TNG and 1,2-MNG and low for 1-MNG and 1,3-DNG. The amount of radioactivity remaining the gastrointestinal tract plus contents and in the feces was small.

TNG was primarily metabolized to DNGs *in vitro* by livers of various species. Livers from rats and mice produced more 1,3-DNG than 1,2-DNG; whereas, livers from rabbits, dogs, monkeys and humans produced more 1,2-DNG than 1,3-DNG. Livers from mice and humans had a low ability to metabolize TNG. The rat liver quickly metabolized TNG *in vitro* to DNGs in 15 minutes. Then the amount of DNGs decreased when the incubation was continued for up to 2 hours. MNG was not appreciably metabolized by liver *in vitro*. The addition of other tissue homogenates, including stomach and intestines, or pancreas, spleen and kidneys, did not modify the liver to metabolize TNG or MNGs *in vitro*. Placentas from mice, rats or humans and mouse embryo, liver or carcass during late gestation had a poor ability, relative to liver, to metabolize TNG *in vitro*. TNG metabolism in rat livers increased with time after birth up to 7 days. The metabolism did not change between 7 and 21 days. The ability of rat liver at 21 days after birth to metabolize TNG was lower than that of the adult liver.

Feeding of 0.5% TNG for 2 weeks did not affect the liver enzyme(s) to metabolize zoxazolamine *in vivo*, nor affected the O-demethylase activity in the liver *in vitro*.

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INTRODUCTION

Under Contract No. DAMD-17-74-C-4073, entitled "Munition Compounds Mammalian Toxicity Study," we conducted Phase I studies on the effects of acute exposure of various munition compounds.[1] During Phase II, we studied the effects of multiple exposure of selected compounds including trinitroglycerin (TNG), 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT) and nitrocellulose (NC). Subacute and subchronic toxicities were performed in dogs, rats and mice to determine the maximum tolerated dose and to define the biological nature and target organ(s) of the toxic effects. Reversibility of any adverse effects was determined. Mutagenicity of the compound was assessed. Immunologic response was studied by the detection of the serum IgE antibodies. Effects of the test compound on drug metabolizing enzymes were investigated in in vivo and/or in vitro studies. A detailed study on the disposition and metabolism of the radiolabeled compound was performed in rats; the metabolites were isolated and identified. Comparison in the pathways of biotransformation was also studied in vivo in mice, rabbits, dogs and monkeys and in vitro system using tissues of the various species including the liver of human cadavers. Special studies were also performed to elucidate certain adverse effects. This report summarizes the results of Phase II studies on TNG.

I. DOGS

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I. DOGS

A. Subacute and Subchronic Toxicities and Reversibility

1. Introduction

These studies were performed to define the nature and extent of effects of TNG on the biological system at the biochemical and cellular levels and to elucidate the dose-response relationship in the dogs after administration for 4 weeks and 13 weeks. The reversibility of any adverse effects was studied after the treatment of TNG was discontinued for 4 weeks.

2. Material and Methods

a. Number of Dogs, Sex and Treatment

A total of 32 young healthy beagle dogs (Hazelton Research Animals, Cumberland, VA) weighing between 6.6 and 11.8 kg were used for these experiments. The dogs were conditioned and observed carefully in our animal quarters for 3 weeks after their arrival from the supplier. They were divided into four groups, each consisting of four males and four females. The average weights of all groups were kept close.

Three groups of dogs were given 0.01, 0.1 or 1 mg/kg/day of TNG in capsules. TNG was desensitized in lactose as 10% mixture (SDM No. 17, Atlas Chemical Division, ICI America Inc., Wilmington, DE) and the lactose mixture was weighed twice weekly for individual dogs throughout the study. At the end of 4 weeks, adverse effects were not observed in any dogs. Starting the 5th week TNG doses for all groups were increased 5-fold to 0.05, 0.5 or 5.0 mg/kg/day, respectively. The 4th group served as the controls and was given empty capsules daily thoughout the experiments. Purina dog chow and water were available ad libitum, except wherever specified.

b. Experimental Procedures

All dogs were observed daily for behavioral changes and toxic signs. Body weights of all dogs were recorded weekly. Blood, urine and fecal samples were collected for laboratory tests three times before treatment at weekly intervals and at 4, 8, 13 and/or 17 weeks during experiment. The tests included hematology, clinical blood chemistry tests, serum electrolytes, urinalysis, and presence of occult blood. For fasting blood glucose, the dogs were fasted overnight for 16 hours. At termination, the dogs were euthanized

with an overdose of pentobarbital sodium and examined for gross lesions. Weights of liver, spleen, kidneys, adrenals and gonads were recorded, and organ weight to body weight ratios were calculated. Various tissues were removed, fixed, processed, sectioned and stained for microscopic examination of lesions. The procedures for hematology, clinical blood chemistry tests, urinalysis and histopathology, and the normal values are given in Appendix I.

The concentrations of Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} in serum were determined with the atomic absorption spectrophotometer, according to the procedure used by Pybus,[27] originally described for Ca and Mg. The resonance lines used for the analysis of each of the elements are: Ca, 4227 Å; Mg, 2852 Å; Na, 3302 Å; and K, 7665 Å. Sodium was determined by using the 3302 Å line rather than the more sensitive 5890 Å line to avoid large dilutions of the serum. In this way, Ca, Mg, and Na were determined after a 50-fold dilution (0.2 mg serum to 10 ml) of the serum with 1,000 ppm strontium in 0.1 N perchloric acid. Potassium was determined after a second dilution (1:1) or a total of 100-fold dilution. Phosphate interference and the interference of sodium on potassium was eliminated by the addition of strontium. The perchloric acid was used to remove protein interference. The serum chloride concentration was determined with a Buchler-Cotlove chloridometer.

Bromosulfophthalein (BSP) retention test was performed at termination. A single dose of 5 mg/kg of the sterile test dye (Dade, Miami, FL) was injected intravenously following fasting for 16 hours. Serum level of the dye at 15 minutes was determined and the percent of retention in the plasma was calculated.[37]

The results of the various parameters were compared with the respective baseline levels and/or with those of the control groups at the respective time interval according to the Dunnett's Multiple Comparison Procedure.[47]

c. Experimental Design

At the end of 4 and again at 13 weeks of continuous treatment, one male and one female dog from each group were to be euthanized for necropsy. The treatment for one other male and female dog from each group was to be discontinued at the end of 4 and 13 weeks and they were to be euthanized at the end of 8 and 17 weeks, respectively, to study the reversibility of any adverse effects.

Since adverse effects were not observed in any dogs and TNG-related lesions were not found in the dogs that were euthanized for necropsy at the end of 4 weeks, the treatment for one male and one female dog from only the high dose group was discontinued to study reversibility. These two dogs

were euthanized at the end of 8 weeks. All other dogs were continued on treatment. At the end of 13 weeks, one male and one female dog from each group were euthanized and the treatment for all other dogs was discontinued. Since TNG treatment for 13 weeks did not cause any adverse effects or lesions, the dogs that were kept for the study of reversibility were not euthanized for necropsy at the end of 17 weeks.

3. Results

a. General Observations and Weight Gain

The control dogs and the dogs receiving daily various doses of TNG were healthy throughout the treatment periods of 4 or 13 weeks, respectively. Their body weights before, during and after treatment are summarized in Table 1. These dogs consistently gained weight.

b. Blood Analysis

Since the hematology and clinical blood chemistry results of the male dogs and the female dogs were not significantly different, the data of both sexes from the control group and the groups treated with low, middle and high doses of TNG were combined for discussion and are summarized in Tables 2 through 5, respectively. The peripheral blood elements and various clinical chemistry value were not apparently altered by TNG. However, when compared with the baseline levels within the groups, or when compared with control dogs at respective time intervals, there were a number of changes. These changes were slight and inconsistent and occurred in both the control dogs and the dogs treated with TNG.

The treatment for one male and one female dog from only the high dose group was discontinued after 4 weeks to study reversibility of any adverse effects. The peripheral blood elements and clinical chemistry values of these dogs treated with high dose of TNG for 4 weeks (Table 6) and of dogs treated with low, middle and high doses of TNG for 13 weeks (Table 7) after allowed to recover for 4 weeks were not apparently different from those of the control dogs.

Methemoglobin concentration and Heinz bodies in the erythrocytes of these dogs were negative at the end of 4, 8 and 13 weeks of treatment. All blood samples were taken 24 hours after the preceding doses of TNG. Serum electrolytes including Na^{+}, K^{+}, Ca^{++}, Mg^{++} and Cl^{-} were determined at the end of 4 weeks and again at 13 weeks. As shown in Table 8, the various electrolytes were not apparently altered during treatment.

c. BSP Retention

BSP retention was determined for the dogs terminated at the end of 4 and 13 weeks. The results are summarized in Table 9. TNG did not cause any apparent retention of BSP in these dogs.

d. Urinalysis and Fecal Examination

The results of urinalysis of the control dogs and dogs treated with various doses of TNG are summarized in Tables 10 through 13. Small amounts of protein, erythrocytes, leukocytes, and/or epithelial cells were occasionally present in the urine of both the control and the treated dogs before and during treatment.

The results of fecal examination are summarized in Table 14. Occult blood was occasionally found in the feces of the control and the treated dogs before and/or during treatment.

e. Organ Weights

The absolute and relative organ weights of all dogs terminated during the experiment are summarized in 15 and 16, respectively. Treatment of TNG did not cause any apparent change in the organ weights. The difference in the weights of testes was related to the age and maturity of these dogs.

f. Gross and Microscopic Examination of Tissues

The dogs terminated for necropsy at various times were in good nutritional condition without any apparent gross changes. At the end of 4 weeks, one dog (No. 31) treated with 1.0 mg/kg/day had a mild subacute inflammation in the liver and a mild thyroiditis (Table 17). These lesions were not considered to be related to TNG. The other dogs were healthy without any lesions. The bone marrows and the myeloid/erythroid (M/E) ratios of all dogs were normal.

At the end of 13 weeks, a number of spontaneous lesions occasionally occurred in both the control dogs and dogs treated with TNG. One control dog (No. 5) had severe pneumonia, a moderate tonsilitis and a mild hyperplasia of the prescapular lymph node (Table 18). The other control dog (No. 6) had a mild subacute inflammation of the lung. One dog (No. 11) treated with the low dose of TNG had a mild hyperplasia of the mesenteric lymph node, one dog (No. 19) treated with the middle dose had a

mild tonsillitis, and one dog (No. 28) treated with the high dose had a mild pneumonia and a congenital hypoplasia of one testis. The bone marrows and the M/E ratios of these dogs were normal.

4. Discussion and Conclusions

In dogs, daily treatment of 0.01, 0.1 or 1.0 mg/kg/day of TNG for 4 weeks and 0.05, 0.5 or 5.0 mg/kg/day for 9 additional weeks did not cause any adverse effects. All dogs continued to gain weight during the treatment period. Peripheral blood elements and various clinical chemistry tests of the blood, including BSP retention, did not show any apparent changes. Urinalysis and fecal examination for occult blood did not reveal any abnormality. TNG did not cause any gross or microscopic changes in tissues after treatment for 4 or 13 weeks.

B. Effect of TNG on Methemoglobin Formation

1. Introduction

It is known that TNG causes methemoglobin formation in man. This experiment was designed to administer larger doses of TNG for 5 consecutive days. Blood samples were collected at various intervals after each dose for the measurement of methemoglobin concentration to determine: first, the dose-response relationship if methemoglobin is formed; second, the disappearance rate of methemoglobin from the blood; third, the effects after repeated administrations. In addition, the protective effect of methylene blue on TNG induced methemoglobinemia was investigated.

2. Material and Methods

A total of 16 young adult beagle dogs (Hazelton Research Animals, Cumberland, VA) weighing between 8.8 and 14.2 kg were used and conditioned in our animal quarters for 3 weeks. They were divided into four groups, each consisting of two males and two females. Each group was given 25, 50, 100 or 200 mg/kg/day of TNG for 5 consecutive days. TNG was desensitized as 10% mixture in lactose and was given in capsules. Purina dog chow and water were available *ad libitum*.

After each dose, blood samples from all dogs were taken at 0.5, 1, 2, 4, 8, 16 and 24 hours for the determination of methemoglobin and total hemoglobin concentrations. To study any protective effect on methemoglobin formation, 3 mg/kg of methylene blue (USP water solution for injection) was

injected intravenously to the dogs 2 hours after administration of 200 mg/kg/day of TNG on the third day. Collection of samples was continued for the determination of methemoglobin and total hemoglobin concentrations and treatment with TNG for these dogs was discontinued on the 4th and 5th days.

3. Results

After treatment of 25 mg/kg/day of TNG, methemoglobin appeared 1 to 2 hours after dosing each day and reached peak concentrations of 2.8 to 8.7% of total hemoglobin at 4 hours (Table 19). Methemoglobin disappeared from the blood 8 to 16 hours after daily TNG dosing. Small amounts of methemoglobin appeared again in some dogs 24 hours after the third dose.

After treatment of 50 mg/kg/day of TNG, methemoglobin also appeared 1 to 2 hours after dosing each day and also reached peak concentrations at 4 hours (Table 20). The daily peak concentrations averaged 9.2 to 15.0% of total hemoglobin. Similarly, methemoglobin disappeared from the blood 8 to 16 hours after administration of TNG.

After treatment of 100 mg/kg/day of TNG, these dogs became slightly cyanotic 2 to 3 hours after dosing each day and the cyanosis lasted for a couple of hours. Methemoglobin appeared in the blood earlier, i.e. 0.5 to 1 hour after dosing (Table 21). Methemoglobin concentrations progressively increased with time and reached peaks of 24.7 to 42.6% of total hemoglobin at 4 hours. The concentrations of methemoglobin remained high at 8 hours and decreased thereafter. Small amounts of methemoglobin remained in some dogs 24 hours after administration of TNG.

After treatment of 200 mg/kg/day of TNG, these dogs exhibited cyanosis and inactivity 2 to 3 hours after dosing each day without any serious effects. They returned to normal in several hours. Methemoglobin appeared within 1 hour on the first day and reached peak concentration of 28.3% of total hemoglobin at 4 hours (Table 22). Methemoglobin disappeared from the blood 8 to 16 hours after dosing. On the second day, methemoglobin concentration quickly increased to 25.9% of total hemoglobin at 2 hours after dosing and reached 50.7% at 4 hours. The methemoglobin decreased slowly and disappeared from the blood by 24 hours. On the third day, methemoglobin concentration increased to 3.0% at 2 hours. At this time, 3 mg/kg of methylene blue was injected intravenously to each dog. Instead of increasing quickly to a peak, the methemoglobin concentration decreased slightly and remained relatively high through 16 hours after administration of TNG. Thereafter, it decreased to 3.6% and did not disappear from the blood until 40 hours after last TNG dosing.

Methemoglobin concentrations of these dogs after daily treatment of various doses of TNG on the first through the 4th days are better illustrated in Figure 1.

4. Discussion and Conclusions

Oral administration of 25 or 50 mg/kg/day of TNG for 5 consecutive days did not cause any apparent adverse signs; 100 or 200 mg/kg/day caused cyanosis lasting a few hours 2 to 3 hours after dosing. In addition, 200 mg/kg/day decreased the activity without any serious effects. All these doses quickly caused formation of methemoglobin. Methemoglobin concentrations reached peaks in 1 to 4 hours. The height of the peaks and the duration of the methemoglobin concentrations were dose-related. Methemoglobin disappeared from the blood 8 to 24 hours depending upon the dose. Small amounts of methemoglobin remained in some dogs 24 hours after larger doses.

After 25, 50 or 100 mg/kg/day of TNG for 5 consecutive days, there appeared no tolerance in the formation of methemoglobin nor was there any accumulative effect on the formation of methemoglobin. However, after 200 mg/kg/day of TNG, the methemoglobin concentrations on the second day were considerably higher than those on the first day and the methemoglobin at 2 hours on the third day was higher than that on the second day. This probably indicates some accumulative effect of TNG on the formation of methemoglobin.

Administration of 3 mg/kg of methylene blue 2 hours after administration of 200 mg/kg of TNG apparently prevented the methemoglobin formation to reach the high peak. However, the concentration of methemoglobin persisted for 40 hours or longer after the administration of TNG.

C. Cytogenetic Effect of TNG

1. Introduction

The cytogenic effect of TNG on somatic cell chromosomes was studied. The lymphocyte and kidney cultures from dogs treated with repeated doses of TNG were obtained and examined for any damage.

2. Material and Methods

a. Animals

Dogs treated with the high dose of TNG from the subacute and subchronic toxicity studies were used. These dogs were administered 1 mg/kg/day of TNG for 4 weeks and 5 mg/kg/day for 9 additional weeks.

b. Lymphocyte and Kidney Cultures

At the end of 4 and 13 weeks, blood samples were aseptically drawn from the jugular vein of both the control and treated dogs. The lymphocytes were cultured by the method of Moorhead et al.[5] Kidney tissue samples were removed at necropsy and cultured by the trypsinization method of Fernandes.[6] All cultures were maintained in Eagle's medium as modified by Vogt and Dulbecco.[7]

c. Chromosome Analysis

Actively dividing kidney cultures and phytohemagglutin-stimulated lymphocytes were arrested in metaphase by short-term colchicine treatment. The cells were trypsinized, swollen in hypotonic solution, and processed for spreading on glass slides by the method of Moorhead and Nowell.[8] Slides were stained with giemsa and scanned under low power optics. Cell polyploidy was estimated by examination of 200 cells. Slides showing minimum scattering of cells in metaphase were selected for analysis under oil immersion optics. Chromosomes were counted and morphological aberrations were examined from photographic negatives of up to 50 metaphase cells.

3. Results

The results of numerical and morphological aberrations of chromosomes are shown in Tables 23 and 24, respectively. Dogs treated with TNG did not show any apparent changes in the chromosome frequency distribution or number of tetraploids or any apparent changes in the chromatid breaks or translocations, in the peripheral lymphocytes or kidney cultures.

4. Discussion and Conclusion

Administration of 1 mg/kg/day of TNG for 4 weeks and 5 mg/kg/day for 9 additional weeks in dogs did not appear to cause any numerical or morphological aberrations of chromosomes in the peripheral lymphocytes or kidney cultures. However, only a few dogs were terminated and studied at the end of 4 and 13 weeks.

D. Immunologic Response to TNG

1. Introduction

In human, anaphylactic reactions were associated to a high immunoglobin E (IgE) titer.[9] IgE, the allergic or hypersensitive antibody, of dogs treated with TNG was determined.

2. Material and Methods

The immunodiffusion technique of Mancini et al.[10/] was used for determination of serum IgE titer. Replicate 1 ml samples of serum from the control dogs and dogs treated with various doses of TNG at various intervals were placed in wells in an immunodiffusion chamber along with suitable standards. These dogs were used for subchronic and subchronic toxicity studies as described in Section I.A. The diffusion chamber was incubated at 37°C for 48 hours and the diameter of the precipitin ring was measured. Since the square root of the diameter is directly proportional to the concentration of the antibody, the IgE concentration was quantitated with the standard antibody reagent.

3. Results and Conclusion

The results of IgE concentration of control dogs and dogs treated with TNG are summarized in Table 25. Treatment of various doses of TNG for 4, 8, and 13 weeks did not cause any apparent changes on serum concentration of IgE.

TABLE 1

BODY WEIGHTS OF DOGS TREATED WITH TNG IN CAPSULES

Dose[a/] (mg/kg/day)	Dog No.	Sex	Body Weights (kg) Initial	4 Weeks	8 Weeks	13 Weeks	17 Weeks
0	1	F	6.6	7.3			
0	2	M	10.7	11.1			
0.01	15	F	7.2	8.4			
0.01	16	M	9.2	10.1			
0.1	23	F	8.6	9.5			
0.1	24	M	9.6	10.8			
1.0	31	F	9.4	10.4			
1.0	32	M	10.0	11.2			
1.0	29	F	9.0	10.2[b/]	10.4		
1.0	30	M	10.3	12.0[b/]	12.0		
0	5	F	7.0	8.1	8.4	9.5	
0	6	M	9.3	10.0	10.6	11.2	
0.01-0.05	11	F	7.3	8.4	8.4	9.3	
0.01-0.05	12	M	8.7	9.4	9.6	10.4	
0.1-0.5	19	F	8.0	8.2	8.2	9.0	
0.1-0.5	20	M	9.3	10.2	11.0	11.7	
1.0-5.0	27	F	8.0	8.8	9.0	10.1	
1.0-5.0	28	M	11.8	12.8	13.2	14.3	
0	3	F	9.3	10.1	9.8	11.0[b/]	11.0
0	4	M	8.8	10.0	11.0	11.1[b/]	10.8
0	7	F	8.3	9.2	9.8	10.8[b/]	10.6
0	8	M	9.0	10.2	10.8	11.6[b/]	9.4
0.01-0.05	9	F	7.8	8.9	8.0	10.9[b/]	10.4
0.01-0.05	10	M	10.4	12.0	12.8	14.1[b/]	14.0
0.01-0.05	13	F	9.8	10.3	10.2	10.9[b/]	10.8
0.01-0.05	14	M	10.0	11.2	12.2	13.4[b/]	13.4
0.1-0.5	17	F	7.0	7.7	8.0	9.3[b/]	8.8
0.1-0.5	18	M	9.8	11.4	11.8	12.4[b/]	12.6
0.1-0.5	21	F	9.1	10.2	10.0	11.9[b/]	12.4
0.1-0.5	22	M	9.2	10.7	11.4	11.8[b/]	11.4
1.0-5.0	25	F	7.2	9.0	8.3	8.8[b/]	8.4
1.0-5.0	26	M	10.6	12.0	12.2	13.5[b/]	13.4

a/ Doses were increased 5-fold starting the 5th week.

b/ Dosing discontinued thereafter.

TABLE 2

LABORATORY DATA OF CONTROL DOGS FOR TNG

(B,N) BASELINE
(C,N) CONTROL
N = NUMBER OF DOGS

	WKS -2-0(B, B)	WKS 4 (C, B)	WKS 8 (C, 4)	WKS 13 (C, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	4.98 ± .25	4.76 ± .25	5.44 ± .19	6.18 ± .11 a/
RETICULOCYTES, %	.78 ± .07	.64 ± .08	.49 ± .07	.81 ± .09
HEMATOCRIT, VOL. %	41.8 ± 1.3	41.1 ± 1.2	40.3 ± 1.0	44.0 ± 1.1
HEMOGLORIN, GM, %	13.9 ± .4	14.0 ± .5	14.0 ± .2	15.0 ± .4
MCV, CUBIC MICRONS	84.4 ± 2.1	87.9 ± 4.8	74.1 ± 2.5	71.2 ± 1.7
MCHB, MICPO MICROGMS.	28.2 ± .8	29.8 ± 1.5	25.8 ± .8	24.4 ± .6
MCHBC, GM %	33.4 ± .2	34.0 ± .2	34.9 ± .4 a/	34.2 ± .1
PLATELETS ($X10^5$ /MM^3)	2.5 ± .2	2.1 ± .2	2.2 ± .3	2.1 ± .3
LEUKOCYTES ($X10^3$ /MM^3)	13.1 ± 1.0	9.7 ± .2 a/	12.0 ± 1.0	12.1 ± .6
NEUTROPHILS, %	58.7 ± 3.0	58.9 ± 2.1	58.5 ± 4.5	55.5 ± 5.1
LYMPHOCYTES, %	35.9 ± 3.1	34.4 ± 2.1	32.0 ± 2.5	39.8 ± 5.0
BANDS, %	1.7 ± .6	0.0 ± 0.0 a/	.3 ± .3	0.0 ± 0.0 a/
MONOCYTES, %	1.6 ± .3	1.9 ± .5	2.3 ± 1.1	0.0 ± 0.0
EOSINOPHILS, %	2.0 ± .4	4.9 ± .6	7.0 ± 3.0 a/	4.8 ± 1.3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN.	4.3 ± .3	4.5 ± .4	4.5 ± .5	8.6 ± .9 a/
GLUCOSE (FASTING), MG %	85.7 ± 2.2	85.8 ± 2.8	89.3 ± 2.1	98.0 ± 1.8 a/
SGOT, IU/L	32.2 ± 1.8	29.5 ± 2.2	25.8 ± 1.8	21.0 ± 1.2 a/
SGPT, IU/L	30.9 ± 1.8	31.1 ± 2.9	26.0 ± 2.2	20.3 ± .8 a/
ALK. PHOS., IU/L	76 ± 3	68 ± 4	58 ± 4 a/	55 ± 5 a/
BUN, MG %	15.1 ± .9	13.1 ± .8	15.3 ± 1.3	14.5 ± 1.0

ENTRIES ARE MEAN ± STANDARD ERROR
WKS -2-0(B, B) IS THE AVERAGE OF WEEKS 2, 1, AND 0 PRIOR TO TREATMENT.

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure [4]).

TABLE 3

LABORATORY DATA OF DOGS BEFORE AND DURING ADMINISTRATION OF TNG

DOSE 0.01-0.05 MG/KG/DAY [a/]

(B,N) BASELINE
(T,N) TREATMENT
N = NUMBER OF DOGS

	WKS -2-0(B, A)	WKS 4 (T, B)	WKS 9 (T, 4)	WKS 13 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	4.58 ± .08	4.50 ± .16	5.31 ± .35 [b/]	6.00 ± .24 [b/]
RETICULOCYTES, %	.68 ± .06	.57 ± .10	.51 ± .21	.67 ± .15
HEMATOCRIT, VOL, %	38.1 ± .6 [c/]	38.9 ± .5	42.8 ± 1.4 [b/]	43.8 ± .8 [b/]
HEMOGLOBIN, GM, %	12.7 ± .2 [c/]	13.2 ± .3	14.9 ± .3 [b/]	15.3 ± .3 [b/]
MCV, CUBIC MICRONS	83.2 ± 2.2	87.1 ± 2.9	81.1 ± 3.1	73.2 ± 2.9
MCHB, MICRO MICROGMS.	27.8 ± .7	29.5 ± 1.1	28.3 ± 1.3	25.6 ± 1.1
MCHBC, GM %	33.4 ± .2	33.9 ± .3	34.9 ± .4 [b/]	34.9 ± .1 [b/]
PLATELETS ($X10^5$ /MM^3)	2.4 ± .2	2.1 ± .1	2.1 ± .2	1.8 ± .2 [b/]
LEUKOCYTES ($X10^3$ /MM^3)	12.7 ± .6	9.8 ± .9 [b/]	10.5 ± 1.2	13.3 ± .7
NEUTROPHILS, %	57.3 ± 2.0	50.9 ± 3.6	54.0 ± 3.7	58.0 ± 5.9
LYMPHOCYTES, %	39.0 ± 1.8	42.8 ± 3.7	41.5 ± 3.3	35.8 ± 5.8
BANDS, %	1.3 ± .2	0.0 ± 0.0 [b/]	.3 ± .3 [b/]	0.0 ± 0.0 [b/]
MONOCYTES, %	1.3 ± .2	1.8 ± .4	.3 ± .3	.3 ± .3
EOSINOPHILS, %	1.1 ± .4	3.4 ± .7 [b/]	4.0 ± .9 [b/]	5.5 ± .3 [b/]
BASOPHILS, %	.2 ± .1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	.1 ± .1	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3
CLOTTING TIME, MIN.	4.4 ± .1	5.3 ± .5	6.9 ± .6 [b/]	8.6 ± .9 [b/]
GLUCOSE (FASTING), MG %	89.0 ± 2.4	80.1 ± 2.0 [b/]	89.5 ± 4.1	97.5 ± 3.6
SGOT, IU/L	35.3 ± 1.5	30.0 ± 2.8	26.8 ± 1.7	23.5 ± 2.1 [b/]
SGPT, IU/L	31.0 ± 2.1	25.9 ± 3.0	29.8 ± 5.2	30.8 ± 5.8
ALK, PHOS., IU/L	87 ± 4	71 ± 2 [b/]	61 ± 4 [b/]	59 ± 5 [b/]
BUN, MG %	15.7 ± 1.3	12.4 ± .6	14.0 ± 1.8	13.8 ± .8

ENTRIES ARE MEAN ± STANDARD ERROR
WKS -2-0(B, A) IS THE AVERAGE OF WEEKS 2, 1, AND 0 PRIOR TO TREATMENT.

a/ Dose was increased 5-fold starting the 5th. week.
b/ Significantly different from the baseline level (Dunnett's multiple comparison procedure [4/]).
c/ Significantly different from the control dogs at the respective time interval (Dunnett's multiple comparison procedure [4/]).

TABLE 4

LABORATORY DATA OF DOGS BEFORE AND DURING ADMINISTRATION OF TNG

DOSE 0.1-0.5 MG/KG/DAY [a/]

(B.N) BASELINE
(T.N) TREATMENT
N = NUMBER OF DOGS

	WKS -2-0(B, 8)	WKS 4 (T, 8)	WKS 8 (T, 4)	WKS 13 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	4.97 ± .10	4.41 ± .12 [b/]	5.73 ± .26 [b/]	6.41 ± .24 [b/]
RETICULOCYTES, %	.70 ± .06	.47 ± .09	.41 ± .07	.44 ± .18
HEMATOCRIT, VOL. %	40.9 ± 1.0	40.1 ± .8	42.8 ± 1.8	48.5 ± 2.6 [b/]
HEMOGLOBIN, GM. %	13.4 ± .3	13.6 ± .3	15.1 ± .5 [b/]	16.4 ± .7 [b/]
MCV, CUBIC MICRONS	82.3 ± 1.5	91.1 ± 1.7 [b/]	74.7 ± 1.7 [b/]	75.5 ± 1.5 [b/]
MCHB, MICRO MICROGMS.	27.1 ± .6	31.0 ± .6 [b/]	26.4 ± .5	25.5 ± .4
MCHBC, GM %	32.9 ± .2	34.0 ± .2 [b/]	35.4 ± .4 [b/]	33.8 ± .5
PLATELETS ($X10^5$ /MM^3)	2.4 ± .1	2.2 ± .1	2.2 ± .3	2.1 ± .1
LEUKOCYTES ($X10^3$ /MM^3)	12.7 ± .8	10.1 ± .9	9.7 ± .5	12.7 ± .4
NEUTROPHILS, %	61.4 ± 2.3	60.8 ± 3.7	56.5 ± 4.0	64.5 ± 3.9
LYMPHOCYTES, %	35.4 ± 2.5	34.6 ± 3.7	35.3 ± 3.5	32.3 ± 3.3
BANDS, %	.7 ± .3	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0
MONOCYTES, %	1.1 ± .3	.9 ± .4	3.5 ± .6 [b/]	0.0 ± 0.0
EOSINOPHILS, %	1.5 ± .6	3.9 ± 1.0	2.0 ± .9	3.3 ± 1.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN.	4.6 ± .2	4.4 ± .3	6.1 ± .9	6.6 ± .9 [b/]
GLUCOSE (FASTING), MG %	93.6 ± 3.8	87.9 ± 2.2	96.5 ± 5.3	100.8 ± 1.9
SGOT, IU/L	32.0 ± 2.9	31.9 ± 2.7	28.5 ± 2.8	29.8 ± 4.5
SGPT, IU/L	32.1 ± 2.3	30.5 ± 2.1	49.5 ± 17.6	26.8 ± 1.7
ALK, PHOS., IU/L	54 ± 5	58 ± 6	50 ± 5	46 ± 7
BUN, MG %	15.5 ± 1.1	11.0 ± .6 [b/]	13.5 ± .3	12.8 ± .9

ENTRIES ARE MEAN ± STANDARD ERROR
WKS -2-0(B, 8) IS THE AVERAGE OF WEEKS 2, 1, AND 0 PRIOR TO TREATMENT.

a/ Dose was increased 5-fold starting the 5th week.
b/ Significantly different from the baseline level (Dunnett's multiple comparison procedure [4/]).

TABLE 5

LABORATORY DATA OF DOGS BEFORE AND DURING ADMINISTRATION OF TNG

DOSE 1-5 MG/KG/DAY [a/]

(B,N) BASELINE
(T,N) TREATMENT
N = NUMBER OF DOGS

	WKS -2-0(B, 8)	WKS 4 (T, 7)	WKS 8 (T, 4)	WKS 13 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	4.88 ± .09	3.91 ± .17 [b,c/]	5.37 ± .19	6.03 ± .24 [b/]
RETICULOCYTES, %	.82 ± .06	.63 ± .08	.52 ± .06	1.06 ± .17
HEMATOCRIT, VOL, %	40.3 ± .7	39.6 ± 1.0	41.3 ± .7	45.5 ± 1.7 [b/]
HEMOGLOBIN, GM, %	13.3 ± .3	13.4 ± .4	14.6 ± .5	15.6 ± .6 [b/]
MCV, CUBIC MICRONS	82.7 ± 1.1	102.0 ± 4.3 [b,c/]	76.9 ± 2.6	75.5 ± .8
MCHB, MICRO MICROGMS,	27.2 ± .4	34.6 ± 1.3 [b,c/]	27.3 ± .6	25.8 ± .2
MCHBC, GM %	32.9 ± .2	33.9 ± .5	35.5 ± .5 [b/]	34.2 ± .2
PLATELETS ($X10^5$ /MM^3)	2.8 ± .2	2.7 ± .1 [c/]	2.6 ± .2	2.1 ± .2 [b/]
LEUKOCYTES ($X10^3$ /MM^3)	13.5 ± .7	10.2 ± .3 [b/]	12.6 ± 1.2	12.8 ± .5
NEUTROPHILS, %	62.9 ± 2.5	54.3 ± 2.7	55.8 ± 3.8	55.5 ± 4.8
LYMPHOCYTES, %	32.9 ± 2.4	38.7 ± 2.8	38.5 ± 4.0	40.3 ± 4.8
BANDS, %	.2 ± .1 [c/]	0.0 ± 0.0	.5 ± .3	0.0 ± 0.0
MONOCYTES, %	1.4 ± .4	2.0 ± .9	2.0 ± .8	0.0 ± 0.0
EOSINOPHILS, %	2.6 ± .8	5.0 ± 2.1	3.3 ± 1.6	4.3 ± 1.4
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN,	4.5 ± .2	4.7 ± .6	5.3 ± .7	8.0 ± 1.3 [b/]
GLUCOSE (FASTING), MG %	86.3 ± 4.4	83.9 ± 2.6	92.0 ± 4.2	97.5 ± 1.0
SGOT, IU/L	30.8 ± 1.5	27.7 ± 1.1	25.0 ± 2.1	28.5 ± 1.7
SGPT, IU/L	33.8 ± 1.2	30.7 ± 2.1	30.0 ± 3.5	34.8 ± 3.8 [c/]
ALK, PHOS., IU/L	80 ± 6	67 ± 6	57 ± 4	49 ± 5 [b/]
BUN, MG %	15.6 ± .7	12.0 ± .6 [b/]	14.0 ± 1.2	12.3 ± .6 [b/]

ENTRIES ARE MEAN ± STANDARD ERROR
WKS -2-0(B, 8) IS THE AVERAGE OF WEEKS 2, 1, AND 0 PRIOR TO TREATMENT.

a/ Dose was increased 5-fold starting the 5th week.
b/ Significantly different from the baseline level (Dunnett's multiple comparison procedure [4/]).
c/ Significantly different from the control dogs at the respective time interval (Dunnett's multiple comparison procedure [4/]).

TABLE 6

LABORATORY DATA OF DOGS TREATED WITH TNG FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C, 2)	1.0 (T, 2)
ERYTHROCYTES ($X10^6$ /MM^3)	4.36	4.44
RETICULOCYTES, %	.45	.57
HEMATOCRIT, VOL. %	41.0	40.0
HEMOGLOBIN, GM. %	13.6	13.3
MCV, CUBIC MICRONS	94.0	90.0
MCHB, MICRO MICROGMS.	31.3	29.9
MCHBC, GM %	33.3	33.2
PLATELETS ($X10^5$ /MM^3)	2.4	2.5
LEUKOCYTES ($X10^3$ /MM^3)	9.8	8.2
NEUTROPHILS, %	54.0	57.0
LYMPHOCYTES, %	42.5	36.5
BANDS, %	0.0	0.0
MONOCYTES, %	0.0	1.5
EOSINOPHILS, %	3.5	5.0
BASOPHILS, %	0.0	0.0
ATYPICAL, %	0.0	0.0
NUCLEATED RBC, %	.5	.5
CLOTTING TIME, MIN.	8.3	6.3
GLUCOSE (FASTING), MG %	108.5	102.5
SGOT, IU/L	24.0	24.0
SGPT, IU/L	28	29
ALK. PHOS., IU/L	53	40
BUN, MG %	13.5	14.0

ENTRIES ARE MEAN

TABLE 7

LABORATORY DATA OF DOGS TREATED WITH TNG FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF DOGS

DOSE: MG/KG/DAY[a/]	0 (C. 2)	0.01-0.05 (T. 2)	0.1-0.5 (T. 2)	1.0-5.0 (T. 2)
ERYTHROCYTES ($X10^5$ /MM ^{3})	6.2.	6.54	6.44	6.50
RETICULOCYTES. %	.63	.65	.40	.42
HEMATOCRIT. VOL. %	44.0	45.5	46.0	48.0
HEMOGLOBIN. GM. %	15.1	15.7	16.3	16.5
MCV. CUBIC MICRONS	70.9	69.6	71.5	74.0
MCH. MICRO MICROGMS.	24.4	24.1	25.3	25.4
MCHBC. GM %	34.5	34.5	35.3	34.4
PLATELETS ($X10^5$ /MM ^{3})	2.3	1.7	2.5	2.6
LEUKOCYTES ($X10^3$ /MM ^{3})	15.0	14.9	11.1	11.4
NEUTROPHILS. %	69.0	58.0	61.0	52.5
LYMPHOCYTES. %	24.5	32.5	34.0	41.0
BANDS. %	0.0	0.0	0.0	0.0
MONOCYTES. %	3.0	3.0	3.0	1.5
EOSINOPHILS. %	1.5	7.0	2.0	5.0
BASOPHILS. %	0.0	0.0	0.0	0.0
ATYPICAL. %	0.0	0.0	0.0	0.0
NUCLEATED RBC. %	0.0	0.0	0.0	0.0
CLOTTING TIME. MIN.	5.7	6.7	6.5	7.2
GLUCOSE (FASTING). MG %	103.0	99.0	95.5	101.5
SGOT. IU/L	19.5	29.0	19.5	24.0
SGPT. IU/L	28.0	35.5	67.5	35.5
ALK. PHOS.. IU/L	38	43	38	34
BUN. MG %	14.0	14.0	12.0	10.5

ENTRIES ARE MEAN

a/ Doses were increased 5-fold starting the 5th week.

TABLE 8

SERUM ELECTROLYTES OF DOGS TREATED WITH TNG

Dose[a] (mg/kg/day)	Serum Electrolytes (meq/ℓ)				
	Na	K	Ca	Mg	Cl
	Before Treatment (8 dogs/group)				
Control	153±1[b]	4.5±0.2	5.5±0.1	1.6±0.0	107±1
0.01-0.05	148±1	4.7±0.1	5.5±0.1	1.5±0.1	103±1
0.1-0.5	151±1	4.4±0.1	5.5±0.1	1.5±0.1	105±1
1.0-5.0	150±1	4.5±0.1	5.7±0.1	1.5±0.0	104±1
	Treatment for 4 Weeks (2 dogs/group)				
Control	150[c]	5.1	5.7	1.5	103
0.01-0.05	151	5.2	5.6	1.6	105
0.1-0.5	151	4.9	5.5	1.4	103
1.0-5.0	145	5.1	5.4	1.5	105
	Treatment for 13 Weeks (2 dogs/group)				
Control	145[c]	4.9	5.4	1.7	105
0.01-0.05	148	4.9	5.4	1.5	107
0.1-0.5	146	4.9	5.6	1.7	109
1.0-5.0	146	4.8	5.6	1.6	108

a/ Doses were increased 5-fold starting the 5th week.
b/ Mean ± S.E.
c/ Mean.

TABLE 9

BSP RETENTION OF DOGS TREATED WITH TNG

Dose[a/] (mg/kg/day)	Dog No.	% Retention
	At the end of 4 weeks	
Control	1	4
Control	2	7
0.01-0.05	15	7
0.01-0.05	16	4
0.1-0.5	23	10
0.1-0.5	24	5
1.0-5.0	31	4
1.0-5.0	32	5
	At the end of 13 weeks	
Control	5	3
Control	6	5
0.01-0.05	11	3
0.01-0.05	12	4
0.1-0.5	19	4
0.1-0.5	20	3
1.0-5.0	27	4
1.0-5.0	28	5

a/ Doses were increased 5-fold starting the 5th week.

TABLE 10

URINALYSIS OF CONTROL DOGS FOR TNG

Dose: Empty Capsules

		Treatment Weeks			
		0	4	8	13
Glucose:	Negative	8	8	6	4
	< 250 mg %	0	0	0	0
	≥ 250 mg %	0	0	0	0
Protein:	Negative	6	8	6	4
	< 100 mg %	(4,8)	0	0	0
	≥ 100 mg %	0	0	0	0
Microscopic Examination					
RBC[a]:	Normal	8	8	6	4
	Moderate	0	0	0	0
	Excessive	0	0	0	0
WBC[a]:	Normal	7	6	6	3
	Moderate	(5)	(6,8)	0	(7)
	Excessive	0	0	0	0
Epithelium[b]:	Normal	3	4	5	1
	Moderate	(5,6)	(1,2,8)	(3)	(5,6,7)
	Excessive	(4,7,8)	(6)	0	0
Crystals[c]:	Normal	8	8	6	4
	Moderate	0	0	0	0
	Excessive	0	0	0	0
Casts:	Negative	8	8	6	4
	Positive	0	0	0	0

Numbers indicate number of dogs or dog numbers (in parenthesis).

a/ Normal, 10 or less cells; moderate, 10-100 cells; excessive, > 100 cells/ field (x 440).

b/ Normal, 5 or less cells; moderate, 5-25 cells; excessive, > 25 cells/field (x 100).

c/ Normal, none; moderate, 1-5 crystals; excessive, >5 crystals/ field (x 100).

TABLE 11

URINALYSIS OF DOGS BEFORE AND DURING ADMINISTRATION OF TNG

DOSE: 0.01-0.05 mg/kg/day

(Dose was increased 5-fold starting the 5th week)

		Treatment Weeks			
		0	4	8	13
Glucose:	Negative	8	8	6	4
	< 250 mg %	0	0	0	0
	> 250 mg %	0	0	0	0
Protein:	Negative	8	8	6	4
	< 100 mg %	0	0	0	0
	> 100 mg %	0	0	0	0
Microscopic Examination					
RBC[a]:	Normal	7	8	6	3
	Moderate	(14)	0	0	(11)
	Excessive	0	0	0	0
WBC[a]:	Normal	7	7	5	1
	Moderate	(14)	(12)	0	(9,10,11)
	Excessive	0	0	(14)	0
Epithelium[b]:	Normal	4	5	5	1
	Moderate	(9,13,14)	(14,15,16)	(14)	(9,10,11)
	Excessive	(16)	0	0	0
Crystals[c]:	Normal	8	8	6	4
	Moderate	0	0	0	0
	Excessive	0	0	0	0
Casts:	Negative	8	8	6	4
	Positive	0	0	0	0

Numbers indicate number of dogs or dog numbers (in parenthesis).

a/ Normal, 10 or less cells; moderate, 10-100 cells; excessive, > 100 cells/field (x 440).

b/ Normal, 5 or less cells; moderate, 5-25 cells; excessive, > 25 cells /field (x 100).

c/ Normal, none; moderate, 1-5 crystals; excessive, > 5 crystals/field (x 100).

TABLE 12

URINALYSIS OF DOGS BEFORE AND DURING ADMINISTRATION OF TNG

DOSE: 0.1-0.5 mg/kg/day

(Dose was increased 5-fold starting the 5th week)

		Treatment Weeks			
		0	4	8	13
Glucose:	Negative	8	8	6	4
	< 250 mg %	0	0	0	0
	> 250 mg %	0	0	0	0
Protein:	Negative	8	8	6	4
	< 100 mg %	0	0	0	0
	> 100 mg %	0	0	0	0
Microscopic Examination					
RBC[a]:	Normal	8	8	6	4
	Moderate	0	0	0	0
	Excessive	0	0	0	0
WBC[a]	Normal	8	8	6	4
	Moderate	0	0	0	0
	Excessive	0	0	0	0
Epithelium[b]:	Normal	3	4	5	2
	Moderate	(18,20,22)	(18,20,23)	(21)	(19,20)
	Excessive	(17,24)	(24)	0	0
Crystals[c]:	Normal	8	8	6	4
	Moderate	0	0	0	0
	Excessive	0	0	0	0
Casts:	Negative	8	8	6	4
	Positive	0	0	0	0

Numbers indicate number of dogs or dog numbers (in parenthesis).

a/ Normal, 10 or less cells; moderate, 10-100 cells; excessive, > 100 cells/field (x 440).

b/ Normal, 5 or less cells; moderate, 5-25 cells; excessive, > 25 cells/field (x 100).

c/ Normal, none; moderate, 1-5 crystals; excessive, > 5 crystals/field (x 100).

TABLE 13

URINALYSIS OF DOGS BEFORE AND DURING ADMINISTRATION OF TNG

DOSE: 1.0-5.0 mg/kg/day

(Dose was increased 5-fold starting the 5th week)

		Treatment Weeks			
		0	4	8	13
Glucose:	Negative	8	8	6	4
	< 250 mg %	0	0	0	0
	≥ 250 mg %	0	0	0	0
Protein:	Negative	6	7	6	4
	< 100 mg %	(28,32)	(27)	0	0
	≥ 100 mg %	0	0	0	0
Microscopic Examination					
RBC[a]:	Normal	6	7	5	4
	Moderate	(28,31)	(31)	(30)	6
	Excessive	0	0	0	0
WBC[a]:	Normal	6	7	5	4
	Moderate	(25,31)	0	(30)	0
	Excessive	0	(31)	0	0
Epithelium[b]:	Normal	4	4	5	2
	Moderate	(28,32)	(28,29,31)	(30)	(25,27)
	Excessive	(26,30)	(32)	0	0
Crystals[c]:	Normal	8	8	6	4
	Moderate	0	0	0	0
	Excessive	0	0	0	0
Casts:	Negative	8	8	6	4
	Positive	0	0	0	0

Numbers indicate number of dogs or dog numbers (in parenthesis).

a/ Normal, 10 or less cells; moderate, 10-100 cells; excessive, > 100 cells/field (x 440).

b/ Normal, 5 or less cells; moderate, 5-25 cells; excessive, >25 cells/field (x 100).

c/ Normal, none; moderate, 1-5 crystals; excessive, >5 crystals/field (x 100).

TABLE 14

DETECTION OF FECAL BLOOD IN DOGS BEFORE AND DURING ADMINISTRATION OF TNG

Dose[a/] (mg/kg/day)	Presence of Fecal Food	Treatment Weeks 0	4	8	13
Control	Negative	6[b/]	8	4	2
	Positive	(2,4)	0	(6,8)	0
0.01 - 0.05	Negative	7	6	6	2
	Positive	(12)	(10,11)	0	0
0.1 - 0.5	Negative	5	8	6	2
	Positive	(18,22,23)	0	0	0
1.0 - 5.0	Negative	5	7	6	2
	Positive	(25,30,32)	(29)	0	0

a/ Doses were increased 5-fold starting the 5th week.

b/ Numbers indicate number of dogs or dog numbers (in parenthesis).

TABLE 15

ABSOLUTE ORGAN WEIGHTS OF DOGS TREATED WITH TNG

Dose[a/] (mg/kg/day)	Dog No.	Terminal Body Weight (kg)	Absolute Organ Weights (gm)					
			Liver	Spleen	Kidneys	Adrenals	Ovaries	Testes
Treated for 4 Weeks								
Control	1	7.3	209	54	35	0.98	0.52	
Control	2	11.1	291	42	46	0.83		3.67
0.01	15	8.4	170	31	41	0.99	0.61	
0.01	16	10.1	241	39	41	0.95		2.69
0.1	23	9.5	247	58	53	0.98	0.73	
0.1	24	10.8	325	69	45	1.01		11.00
1.0	31	10.4	275	68	46	0.98	0.73	
1.0	32	11.2	325	78	62	0.85		17.00
Treated for 4 Weeks and Allowed to Recover for 4 Weeks								
1.0	29	10.4	242	42	42	1.06	1.10	
1.0	30	12.0	278	36	50	0.71		16.00
Treated for 13 Weeks								
Control	5	9.0	248	60	44	1.22	0.82	
Control	6	11.0	294	84	44	0.85		18.0
0.01-0.05	11	8.6	218	68	42	1.00	0.67	
0.01-0.05	12	11.1	230	114	42	0.94		12.0
0.1-0.5	19	9.0	238	58	--	1.16	0.68	
0.1-0.5	20	11.6	282	98	50	1.10		22.0
1.0-5.0	27	9.6	258	30	46	0.96	0.81	
1.0-5.0	28	13.8	300	70	66	1.34		17.4

a/ Doses were increased 5-fold starting the 5th week.

TABLE 16

RELATIVE ORGAN WEIGHTS OF DOGS TREATED WITH TNG

Dose[a/] (mg/kg/day)	Dog No.	Relative Organ Weights (gm/kg Body Weight) Liver	Spleen	Kidneys	Adrenals	Ovaries	Testes
Treated for 4 Weeks							
Control	1	28.7	7.4	4.8	0.13	0.07	
Control	2	26.2	3.8	4.2	0.07		0.33
0.01	15	20.3	3.7	4.9	0.12	0.07	
0.01	16	23.9	3.9	4.1	0.10		0.27
0.1	23	26.0	6.1	5.6	0.10	0.08	
0.1	24	30.2	6.4	4.2	0.09		1.02
1.0	31	26.5	6.5	4.4	0.09	0.07	
1.0	32	29.1	7.0	5.5	0.08		1.52
Treated for 4 Weeks and Allowed to Recover 4 Weeks							
1.0	29	23.3	4.0	4.0	0.12	0.11	
1.0	30	23.2	3.0	4.2	0.06		1.33
Treated for 13 Weeks							
Control	5	27.6	6.6	4.9	0.14	0.09	
Control	6	26.7	7.7	4.0	0.07		1.64
0.01-0.05	11	25.1	7.9	4.9	0.12	0.08	
0.01-0.05	12	20.7	10.3	3.8	0.08		1.08
0.1-0.5	19	26.4	6.4	--	0.13	0.08	
0.1-0.5	20	24.3	8.5	4.3	0.09		1.90
1.0-5.0	27	26.9	3.1	4.8	0.10	0.08	
1.0-5.0	28	21.8	5.1	4.8	0.10		1.26

a/ Doses were increased 5-fold starting the 5th week.

TABLE 17

SUMMARY OF TISSUE LESIONS IN DOGS TREATED WITH TNG FOR 4 WEEKS

Lesions[a/]	Dose (mg/kg/day)							
	Controls		0.01		0.1		1.0	
Dog No:	1	2	15	16	23	24	31	32
Liver								
Subacute inflammation							±	
Thyroid								
Thyroiditis							±	
Bone Marrow								
M/E Ratio	1.1	1.2	1.1	1.0	1.2	1.2	1.3	1.1

Tissues not listed were normal.

a/ Severity of lesions: + = mild; ++ = moderate; +++ = severe; ++++ = very severe; ± = questionable.

TABLE 18

SUMMARY OF TISSUE LESIONS IN DOGS TREATED WITH TNG FOR 13 WEEKS

Lesions[a]	Dose (mg/kg/day)[b]							
	Controls		0.01-0.05		0.1-0.5		1.0-5.0	
Dog No:	5	6	11	12	19	20	27	28
Lung								
Pneumonia	+++							+
Subacute inflammation		±						
Tonsil								
Tonsilitis	++				±			
Lymph Node								
Hyperplasia	+		±					
Testes								
Hypoplasia (congenital)								+
Bone Marrow								
M/E Ratio	1.3	1.2	1.3	1.4	1.4	1.5	1.3	1.2

Tissues not listed were normal.

a/ Severity of lesions: + = mild; ++ = moderate; +++ = servere; ++++ = very severe; ± = questionable.

b/ Doses were increased 5-fold starting the 5th week.

TABLE 19

METHEMOGLOBIN CONCENTRATION (% OF TOTAL HEMOGLOBIN) IN DOGS TREATED WITH 25 MG/KG/DAY OF TNG FOR 5 DAYS

Day of Treatment	Hours After Daily Dose						
	1/2	1	2	4	8	16	24
1	C	0.6(0-2.5)[a/]	5.7(2.5-10.1)	7.3(5.2-10.1)	0	0	0
2	0	0	1.6(0-3.3)	2.8(0-5.1)	0	0	0
3	0	0	4.1(0-6.8)	8.7(3.1-19.1)	1.6(0-3.2)	0	0.8(0-3.0)
4	1.6(0-3.2)	0	3.1(0-6.5)	5.6(0-13.2)	0.9(0-3.7)	0	0
5	0	0	2.9(0-8.9)	2.8(0-5.0)	1.3(0-2.6)	0	0

a/ Average and range of four dogs.

TABLE 20

METHEMOGLOBIN CONCENTRATION (% OF TOTAL HEMOGLOBIN) IN DOGS TREATED WITH 50 MG/KG/DAY OF TNG FOR 5 DAYS

Day of Treatment	Hours After Daily Dose						
	1/2	1	2	4	8	16	24
1	0	4.4(0-13.0)[a/]	12.0(4.7-16.8)	15.7(12.4-17.9)	6.0(0-10.6)	0	0
2	0	0.7(0-3.1)	4.3(0-11.4)	9.2(3.2-16.6)	3.0(0-11.9)	0	0
3	0	0	8.7(3.2-12.2)	13.0(3.0-21.9)	5.8(0-13.5)	0	0.8(0-3.1)
4	0	0	5.5(0-12.7)	14.1(8.9-21.6)	4.1(3.2-6.0)	0	0
5	0	0	7.5(2.6-18.1)	12.0(2.8-18.3)	2.2(0-8.9)	0	0

a/ Average and range of four dogs.

TABLE 21

METHEMOGLOBIN CONCENTRATION (% OF TOTAL HEMOGLOBIN) IN DOGS TREATED WITH 100 MG/KG/DAY OF TNG FOR 5 DAYS

Day of Treatment	Hours After Daily Dose						
	1/2	1	2	4	8	16	24
1	0.6(0-2.3)[a]	2.4(0-7.1)	12.3(2.2-17.4)	25.6(11.7-34.3)	25.9(20.1-37.5)	4.6(0-15.0)	0.7(0-2.8)
2	0.8(0-3.0)	2.4(0-3.3)	10.7(5.2-14.6)	24.7(22.0-27.2)	20.9(5.4-31.7)	1.5(0-5.8)	0
3	0	1.6(0-6.2)	18.1(6.5-29.0)	42.6(27.2-74.1)	33.1(22.2-42.1)	0.8(0-3.3)	0.8(0-3.0)
4	1.8(0-3.6)	0.9(0-3.5)	11.5(9.4-12.7)	39.0(33.1-52.1)	30.2(5.0-52.8)	5.1(2.7-8.9)	0
5	0.7(0-2.9)	2.2(0-5.9)	22.6(17.6-33.5)	38.5(30.7-48.7)	22.6(5.5-38.2)	3.0(0-12.1)	0

a/ Average and range of four dogs.

TABLE 22

METHEMOGLOBIN CONCENTRATION (% OF TOTAL HEMOGLOBIN) IN DOGS TREATED WITH 200 MG/KG/DAY OF TNG FOR 3 DAYS

Day of Treatment[a]	Hours After Daily Dose						
	1/2	1	2	4	8	16	24
1[b]	0	2.9(2.8-2.9)[c]	11.2(2.7-19.6)	28.3(5.1-51.4)	16.6(0-33.1)	0	0
2	0	0	25.9(17.8-34.0)	50.7(34.0-67.3)	26.8(3.4-50.0)	7.3(3.4-11.2)	0
3	0	0	31.0(25.0-37.0)	16.3(10.7-21.9)	25.6(15.6-35.5)	16.5(15.3-17.6)	3.6(3.5-3.7)
4	4.0(3.6-4.3)	-	2.1(0-4.1)	5.5(0-11.1)	3.8(0-7.6)	0	0
5	0	0	0	0	0	0	0

a/ Treated with TNG for 3 days and with 3 mg/kg of methylene blue 2 hr after TNG on the 3rd day.
b/ One dog vomited after dosing.
c/ Average and range of four dogs.

TABLE 23

NUMERICAL ABERRATION OF CHROMOSOMES FROM DOGS TREATED WITH TNG[a/]

Treatment	Number of Dogs	Chromosome Frequency[b/] <76	77	78	79	> 90	Tetraploids Per 100 Cells[b/]
Control							
Lymphocyte	2	--	2	15	1	--	0.5[a/]
Kidney	2	1	1	17	1	--	0.5
TNG for 4 weeks							
Lymphocyte	1	2	4	38	--	--	0.0
Kidney	2	1	2	15	--	--	1.0
TNG for 13 weeks							
Lymphocyte	2	--	3	24	1	--	0.0
Kidney	1	1	3	24	1	--	0.75

a/ The dose was 1 mg/kg/day for 4 weeks and increased 5-fold starting the fifth week.

b/ Average or single value.

TABLE 24

MORPHOLOGICAL ABERRATIONS OF CHROMOSOMES FROM DOGS TREATED WITH TNG[a/]

Treatment	Number of Dogs	Chromatid Breaks and Gaps Per 50 Cells[b/]	Translocations Per 50 Cells[b/]	Total Aberrations Per 50 Cells[b/]
Control				
Lymphocyte	2	0.5	0.0	0.5
Kidney	2	0.0	0.0	0.0
TNG for 4 weeks				
Lymphocyte	1	0.0	1.0	1.0
Kidney	2	1.0	1.0	2.0
TNG for 13 weeks				
Lymphocyte	2	0.5	0.0	0.5
Kidney	1	0.0	0.0	0.0

a/ The dose was 1 mg/kg/day for 4 weeks and increased 5-fold starting the 5th week.

b/ Average or single value.

TABLE 25

SERUM IgE (IU/ml) OF DOGS TREATED WITH TNG

Dose (mg/kg/day)[a]	Treatment Weeks			
	0 Weeks	4 Weeks	8 Weeks	13 Weeks
Controls	1,673±149(8)[b]	2,129±239(8)	1,250±50(4)	1,300±119(4)
0.01-0.05	1,457±135(8)	1,607±57(8)		
0.1-0.5	1,354±147(8)	1,588±46(8)		
1.0-5.0	1,365±103(8)	1,583±38(8)	1,651±175(4)	1,068±169(4)

a/ Doses were increased 5-fold starting the 5th week.
b/ Mean ± S.E. (number of dogs).

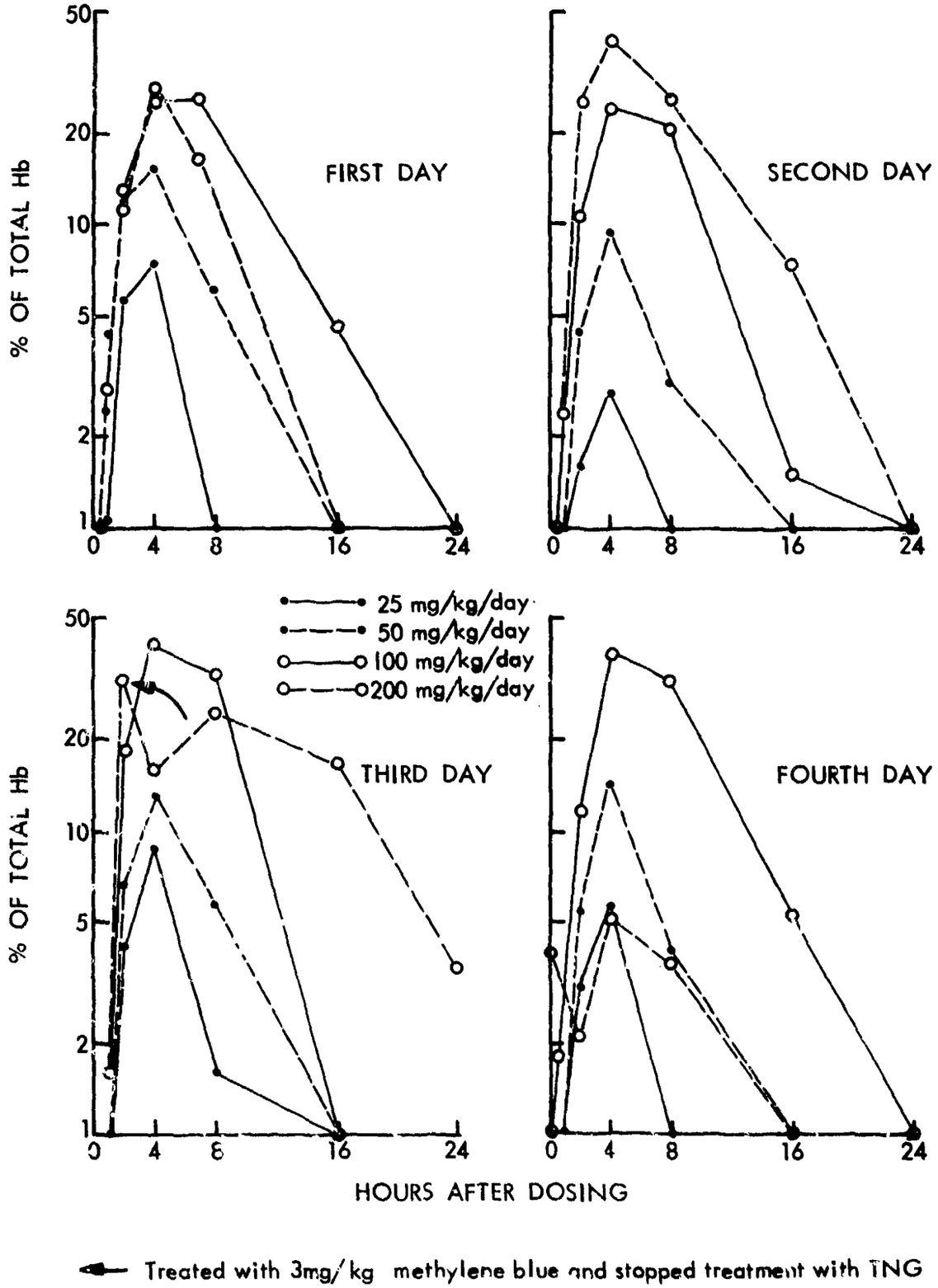

Figure 1 - Methemoglobin Concentration in Dogs Treated With Various Doses of TNG.

II. RATS

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II. RATS

A. Subacute and Subchronic Toxicities and Reversibility

1. Introduction

As for the dogs, these studies were performed to define the nature and extent of effects of TNG on the biological system at the biochemical and cellular levels and to elucidate the dose-response relationship in the rats fed TNG for 4 weeks and 13 weeks. The reversibility of any adverse effects was also studied after the feeding of TNG was discontinued for 4 weeks.

2. Material and Methods

a. Number of Rats, Sex and Treatment

A total of 64 male and 64 female young healthy CD® rats (Charles River Breeding Lab., Wilmington, Mass.) were used for this study. They were divided into four groups, each consisting of 16 males and 16 females. The average weights of all groups were kept close. Three groups of rats were fed 0.001, 0.01 or 0.1% TNG in the feed. At the end of 5 weeks, adverse effects were not observed in any rats. Starting the 6th week, TNG concentrations in the feed for all groups were increased 5-fold to 0.005, 0.05 or 0.5%, respectively. The 4th group, serving as the controls, was given the powdered standard rodent chow (Wayne Laboratory Meal) without TNG.

b. Animal Husbandry

Our animal quarters have a ventilation system with 10 air changes per hour. The room air is passed through filters to remove 99.9% of all particles larger than 0.3 μ. The temperature is maintained at 75 ± 5°F and the relative humidity at 50 ± 10%. The light cycle in all animal rooms is kept at 12-hour on and 12-hour off with a timer.

Upon arrival from the breeder, the rats were isolated and conditioned in our rodent quarter for at least 2 weeks before starting on the experiment. They were housed two per plastic cage with filter tops. Hardwood chips were used after steam-sterilization as bedding material and changed weekly. All cages, cover tops and water bottles were steam-sterilized before using and once every month. Feed and water were available at all times.

c. Feed Preparation

TNG was desensitized in lactose as 10% mixture (SDM No. 17, Atlas Chemicals Division, ICI America Inc., Wilmington, Del.) and the lactose mixture was added to powdered standard rodent chow at 1% by weight to yield

a diet containing the desired 0.1% TNG. The diet was placed in a wooden box (16 x 16 x 20 inches) until half full. The box was placed in a modified cement mixer and rotated about its long axis for 1 hour at a speed of 20 rpm. Subsequently, this diet was mixed with the standard chow at 10% by weight to yield 0.01% and again 0.001% of TNG, respectively. Starting the 6th week, the lactose mixture was added to the standard rodent chow at 5% by weight to yield a diet containing 0.5% of TNG. Accordingly, this diet was mixed with the standard rodent chow at 10% by weight to yield 0.05% and again 0.005% of TNG, respectively.

d. Stability of TNG in Feed Mixture

The stability of TNG in feed was determined and the assay procedure previously reported in Appendix I on The Identification and Assay of Nitrotoluenes and Nitroglycerines[1/] was used. Two feed levels of TNG were prepared. Duplicate samples were analyzed for TNG immediately and after various intervals under various conditions.

TNG concentration in feeder under normal feeding condition was found to decrease with time. The question of loss of TNG by evaporation or chemical reaction with rat feed components was examined by spiking a 0.5% TNG feed sample with TNG-1,3-^{14}C. The TNG-1,3-^{14}C in ethanol was added to 100 mg of lactose in 10 ml of ethanol. This mixture was evaporated to dryness under a stream of N_2 and added to 125 gm of 0.5% TNG in rat feed. After it was well mixed, triplicate samples of 100 mg of the feed mixture were taken and counted immediately in the scintillation solution using a Packard Tricarb 3375 liquid scintillation spectrophotometer described previously.[1/] The counting solution contained toluene in which TNG was well dissolved. For safety regulations, the TNG feed mixture containing TNG-1,3-^{14}C was stored in an operational fume hood. Duplicate samples were taken and counted for radioactivity at the end of 1, 4, 6 and 10 days.

e. Experimental Procedure

The experimental procedure for rats was the same as for dog, described in Section I.A.2.b., with the following exceptions:

(1) Feed consumption of all rats was measured throughout the experiment.

(2) Urine and fecal samples were not collected for examination.

(3) Blood samples were collected by cutting the tip of the tail at 0, 4, 8, 13 and/or 17 weeks for hematology tests. In addition, terminal blood was collected from the abdominal aorta under ether anesthesia for clinical chemistry tests.

(4) BSP retention test was not performed.

f. Experimental Design

The experimental design for rats was the same as for dogs, described in Section I.A.2.c., with the following exceptions:

(1) At the end of 4 and 13 weeks, four male and four females rats from each group were euthanized for necropsy.

(2) The treatment for four male and four female rats from each group were discontinued at the end of 4 weeks. They were kept for observation. Since adverse effects were not observed in any rats and TNG did not cause any lesions in the rats that were euthanized at the end of 4 weeks, the rats for the reversibility study were not necropsied for examination at 8 weeks as scheduled.

(3) Similarly, the treatment for four male and four female rats from each group were discontinued at the end of 13 weeks. These rats were euthanized for necropsy at the end of 17 weeks to study the reversibility of any adverse effects.

3. Results

a. Stability of TNG in Feed Mixture

The results on the stability of TNG in two feed mixtures are summarized in Table 26. The extraction efficiency of TNG from rat feed mixtures containing 0.01% or 1% TNG was 95 ± 4% and 99 ± 1%, respectively. TNG was stable in both the feed mixtures when frozen for 8 days. An average of 95% of the TNG remained at the end of 4 days when the feed mixtures were placed in the rat feeders under normal feeding conditions or in a capped metal can stored in room temperature. When the feed mixtures were stored in the capped metal can for 4 days plus in the rat feeder under normal feeding conditions for 4 additional days, an average of 82% TNG remained. The TNG remained in the metal can after 8 days in room temperature averaged 89%. When the 0.5% TNG feed mixture spiked with TNG-1,3-^{14}C was stored in an operating fume hood, the radioactivity remained in the mixture at the end of 1, 4, 6 and 10 days averaged 81, 67, 63 and 52%, respectively. This indicated that the loss of TNG in rat feed mixtures was due to evaporation. The larger loss of TNG as indicated by the loss of radioactivity was due to larger air flow in the fume hood.

For the feeding studies in rats and mice, TNG feed mixture was prepared weekly. The feed was stored in room temperature. The animal feeders were refilled after the 4th day and emptied completely after the 7th day before the freshly prepared TNG-feed mixture was used. The TNG intake of all animals was corrected for evaporation loss under the normal feeding condition. Amounts of TNG remaining in the feed mixture daily for 7 days were obtained from the decay graph. The average was found to be 93% which was used

to correct the evaporation loss of TNG in all feeding studies for rats and mice.

b. General Observation and Weight Gain

The control rats and rats fed various levels of TNG in the feed were healthy throughout the experimental periods of 4 or 13 weeks. The body weights of the male and female rats before, during and after treatment are summarized in Tables 27 and 28, respectively. The weight changes of these rats are better illustrated in Figure 2.

The weight gains of the male rats fed the low and middle levels of TNG were comparable to that of the controls. However, the weight gain of the rats receiving the high level of TNG was significantly depressed after 4 weeks. When the feeding of TNG was discontinued after the 4th or 13th weeks, the weight gain of these rats increased and was greater than the weight gain of the control rats. By the end of the 8th or 17th week, the body weights of these male rats were comparable to, or were only slightly smaller, than that of the controls.

As seen in the male rats, the high level of TNG in the feed also depressed the weight gain of the female rats after 4 weeks. However, this depressed effect on the weight gain in these females was only slight. After TNG feeding was discontinued, the weight gain of these rats also increased.

c. Feed Consumption and TNG Intake

Feed consumption of the rats fed various levels of TNG are summarized in Table 29. The male rats fed the low or middle level of TNG consumed comparable amounts of feed as the controls; whereas the males fed the high level of TNG consumed less feed between 5 and 13 weeks. In the female rats, the high level of TNG also decreased the feed consumption.

The TNG intake of the rats are summarized in Table 30. The TNG intake was corrected for evaporation loss as discussed in Section II.A.3.a. The TNG intake of the male rats fed 0.001, 0.01 or 0.1% TNG in feed during the first 5 weeks averaged 0.8, 6.0 or 59.0 mg/kg/day, respectivley. When the TNG concentrations in the feed were increased 5-fold starting the 6th week, the TNG intake of these rats increased to 2.5, 24.5 or 229.5 mg/kg/day, respectively. The TNG intake of the female rats fed the low, middle or high levels of TNG was comparable to that of the male rats. The intake averaged 0.9, 6.4 or 59.3 mg/kg/day, respectively, during the first 5 weeks; and averaged 3.1, 26.5 or 233.8 mg/kg/day, respectively, during the subsequent 8 weeks.

d. Blood Analyses

The hematology results of the control male rats and male rats fed various levels of TNG are summarized in Tables 31 through 34. The peripheral blood elements of these male rats were not apparently altered by TNG feeding for 13 weeks. However, when compared with the baseline levels within the groups or when compared with the control rats at the respective time intervals, there were a number of changes. The increases in erythrocyte count, hematocrit, hemoglobin concentration and/or the decrease in reticuloycte count in both the control and treated rats were related to the increase in age of these rats. The other changes were slight and inconsistent. The clinical blood chemistry values of the control male rats and male rats fed the high level of TNG are summarized in Table 35. Two rats (Nos. 181 and 183) fed TNG for 13 weeks had elevated SGOT of 220 or 415 IU/L, respectively. After allowed to recover for 4 weeks, the SGOT level of all four rats was not significantly different from that of the control males.

The hematology results of the control female rats and the female rats fed various levels of TNG are summarized in Tables 36 through 39. As seen in the male rats, the peripheral blood elements of the female rats were not apparently altered by TNG feeding for 13 weeks. When compared with the baseline levels or when compared with the control rats at the respective time intervals, a number of parameters were significantly altered. These changes were not related to TNG treatment. The clinical blood chemistry values of these female rats are summarized in Table 40. Two rats (Nos. 280 and 281) fed TNG for 13 weeks had elevated SGOT of 204 or 291 IU/L, respectively. As for the males, the SGOT level of all four female rats was not significantly different from that of the control females after they were allowed to recover for 4 weeks.

e. Organ Weights

The organ weights of the rats fed various levels of TNG for 4 and 13 weeks and for 13 weeks plus 4 weeks of recovery are summarized in Tables 41, 42 and 43, respectively. After 4 weeks, the liver weight of the female rats fed the middle level of TNG and the kidney weight of the female rats fed the low or high levels of TNG were larger than those of the control rats (Table 41). Based on the body weight, the liver weight of these rats fed the high level of TNG and the kidney weight of these rats fed the low level of TNG were larger than those of the control rats.

After 13 weeks, the adrenal weight of the male rats fed the high level of TNG, the thyroids weight of the male rats fed all levels of TNG, and the liver weight of the female rats fed the high level of TNG were smaller than those of the respective control rats (Table 42). Based on the body weight, the liver weight of male rats fed the low level of TNG, the thyroid weight of the male rats fed all levels of TNG, and the liver weight of the

female rats fed the low level of TNG were smaller than those of the respective control rats. On the other hand, the liver weight of both the male and the female rats fed the high level of TNG, the testis weight of the male rats fed the high level of TNG and the kidney weight of the female rats fed the high level of TNG were larger than those of the respective control rats.

After treatment for 13 weeks and recovery for 4 weeks, the heart weight of the male rats fed the low or middle level of TNG were larger than that of the control rats, and the liver and kidney weights of the male rats fed high level of TNG were smaller than those of the control rats (Table 43). Based on the body weight, the heart weights of the male rats fed the low or middle level of TNG, the liver weight of the male rats fed the middle level of TNG, and the heart weight of the female rats fed the middle level of TNG were larger than those of the respective control rats.

f. Gross and Microscopic Examination of Tissues

The rats fed various levels of TNG were in good nutritional condition at various times of necropsy. Microscopic examination of tissues revealed a number of lesions in both the control rats and rats fed TNG.

After TNG feeding for 4 weeks, spontaneous lesions occurred in the control rats and rats fed the high level of TNG in both sexes (Tables 44 and 45). These lesions included lymphoid hyperplasia and/or pneumonia in the lung, portal inflammation or necrosis of the hepatic cells in the liver, or extramedullary hematopoiesis in the spleen. The bone marrows and the M/E ratios of these rats were normal.

After feeding for 13 weeks, a number of spontaneous lesions also occurred in the heart, lung, liver and spleen of control rats and rats fed high level of TNG (Tables 46 and 47). One treated male rat also had mild acute inflammation in the pancreas. The bone marrows and the M/E ratios of these rats were normal.

Tissue lesions of male and female rats fed TNG for 13 weeks and allowed to recover for 4 weeks are summarized in Tables 48 and 49, respectively. As seen in the control rats and rats fed TNG for 4 or 13 weeks, a number of occasional lesions were also seen in the heart, lung, liver, spleen and/or kidney of both the control rats and the rats fed the high level of TNG for 13 weeks and allowed to recover for 4 weeks. These lesions were naturally-occurring and were not related to TNG. The bone marrows and the M/E ratios of these rats were normal.

4. Discussion and Conclusion

The TNG intake of the male rats fed the low, middle or high level of TNG in feed averaged 0.8, 6.0 or 59.0 mg/kg/day, respectively, during the first 5 weeks; and 2.6, 24.5 or 229.5 mg/kg/day, respectively, during the additional 8 weeks. The TNG intake of the female rats averaged 0.9, 6.4 or 59.3 mg/kg/day during the first 5 weeks and 3.1, 26.5 or 233.8 mg/kg/day during the additional 8 weeks.

Both the male and the female rats fed the low or the middle level of TNG did not show any adverse signs, any changes in peripheral blood elements or clinical laboratory tests, or lesions in any tissues related to TNG. The high level of TNG in feed decreased the feed consumption and retarded the weight gain of rats. These effects were more profound in the males than in the females and were reversible after discontinuation of TNG feeding for 4 weeks. High level TNG for 13 weeks also caused elevation of SGOT in some rats. However, these rats did not have any lesions in any organs. After allowed to recover for 4 weeks, the SGOT level of all rats was not significantly different from that of the control rats.

The absolute and/or relative weights of heart, liver, kidney, thyroid and/or testis of the male and/or female rats fed TNG for 4 or 13 weeks were significantly different from those of the respective control rats. However, these changes were not related to TNG feeding and were not considered to be clinically significant. First, the changes were slight and inconsistent. Second, these organs did not have any consistent lesions related to TNG feeding.

B. Additional Subchronic Toxicity

1. Introduction

As discussed in Section II.A.4., feeding of 0.5% TNG for 13 weeks depressed feed consumption and weight gain in rats. It is imperative to establish a dose-response for the adverse effects of TNG. In addition, the 0.5% TNG diet contained 4.5% lactose. The question arose whether lactose in the diet had any adverse effects. The objectives of these experiments were 2-fold. First, the TNG concentration in the diet was increased to 2.5% and the effects of feeding this diet for 13 weeks were studied. Second, the effects of feeding 25% lactose for 13 weeks were compared.

2. Material and Methods

In the first experiment, a total of 7 male and 7 female young healthy CD® rats (Charles River Breeding Lab.) were used. Four males and four females, serving as controls, were fed a powdered standard rodent chow (Wayne Laboratory Meal); three males and three females were fed 2.5% TNG in the feed. The preparation of the diet containing 2.5% TNG was similar to that for rats described in Section II.A.2.c. The 10% TNG lactose mixture was mixed with powdered standard chow at 25% by weight to yield the diet containing the desired 2.5% TNG every week. Feed consumption of all rats were measured throughout the experiments and body weights were recorded weekly. At the end of 13 weeks of feeding, arterial blood (abdominal aorta) of each rat was collected under ether anesthesia for hematology, clinical blood chemistry and measurement of serum electrolytes. Heart, liver, spleen and kidneys were weighed; various tissues were removed for histopathological examination.

In the second experiment, a total of 10 males and 10 females were used. The experimental design and procedure were similar to those described for the first experiment. Five males and five females, serving as controls, were fed the powdered standard chow; five males and five females were fed 25% of lactose in the feed. Lactose (USP) was mixed with the powdered standard chow at 25% by weight. In addition to the parameters studied in the first experiment, calcium content in the femur bone and iron content in the liver were determined at termination.

The femur was weighed, digested in concentrated nitric acid and analyzed for calcium with a Varian Model 6 atomic absorption spectrophotometer using the resonance line 2399 Å. A nitrous oxide-acetylene flame was used to eliminate any interference from phosphate. The liver was weighed, digested in concentrated nitric acid with the addition of 70% perchloric acid and analyzed for iron with the same spectrophotometer. An air-acetylene flame was used.

3. Results

a. Rats Fed 2.5% TNG

General observations, body weight, feed consumption and TNG intake: The body weights, feed consumption and TNG intake of the control rats and rats fed 2.5% TNG are summarized in Table 50. Both the male and the female rats fed TNG lost weight rapidly during the first 4 weeks and continued to lose weight through the 8th week. During this period, these rats were slightly less active, appeared in poor nutritional condition and had rough hair coat without any other adverse signs. Thereafter, the condition of these rats improved and they started to gain weight. By the 13th week, they

regained the lost weight. On the other hand, the male and female controls appeared healthy and persistently gained weight throughout the experiment. The weight changes of the control and treated rats are better illustrated in Figure 3.

Both the male and female rats fed 2.5% TNG consumed about one-half or less of the feed as the control group during the first 8 weeks. Thereafter, their feed consumption increased. This decrease and change in feed consumption of these rats correlated with their weight loss or the regained weight. The TNG intake of the male rats averaged 1,406 mg/kg/day and of the female rats averaged 1,416 mg/kg/day. As discussed in Section II.A.3.a., the TNG intake was corrected by a factor of 93% for the evaporation loss in the feeder during the feeding week.

Blood analysis: The results of hematology and clinical blood chemistry tests are summarized in Table 51. Both the male and the female rats fed TNG had increases in erythrocyte count, hematocrit and hemoglobin concentration. In addition, the fasting blood glucose of these rats decreased and the alkaline phosphatase increased as compared with those of the respective controls. In the males, the leukocyte count also decreased. However, the decrease in leukocyte counts were not considered clinically significant. Methemoglobin was not detected in the blood of any male or female rats.

Serum electrolytes of the control rats and rats fed TNG are summarized in Table 52. Concentrations of Na, K, Ca, Mg and Cl of rats fed 2.5% TNG for 13 weeks were not significantly altered.

Organ weights: The absolute and relative weights of various organs are summarized in Table 53. The absolute weights of brain, heart, liver and kidneys of the male rats fed 2.5% TNG for 13 weeks were smaller than those of the control males. The relative weights of brain, kidneys and spleen based on the body weight were larger than those of the controls, and the relative heart weight based on the brain weight was smaller than those of the controls.

The absolute weights of spleen and kidneys of the female rats fed 2.5% TNG were larger than those of control females. The relative weights of both organs, based on the body weight or the brain weight, were also larger than those of the controls.

Gross and microscopic examination of tissues: The control rats and rats fed 2.5% TNG were in good nutritional condition at time of necropsy. Tissue lesions in male and female rats are summarized in Tables 54 and 55, respectively. The males fed 2.5% TNG had moderate to severe testicular atrophy, mild to moderate testicular degeneration, and severe aspermatogenesis in the testes. In addition, there were hemosiderosis

in the spleen and liver. A number of spontaneous lesions also occasionally occurred in the male controls and/or the TNG treated males. These lesions included myocarditis, lymphoid hyperplasia and pneumonia in the lung, subacute inflammation in the liver, glomerulonephritis or pyelonephritis in the kidney, and/or vacuolar degeneration in the adrenal gland. The bone marrows and the M/E ratios of these males were normal.

The female rats fed 2.5% TNG had mild to severe hemosiderosis in the spleen and mild hemosiderosis or bile duct proliferation in the liver. As seen in the males, a number of spontaneous lesions occasionally occurred in the female controls and TNG treated females. These lesions included lymphoid hyperplasia, pneumonia and/or emphysema in the lung, subacute inflammation in the liver, interstitial nephritis in the kidney and/or keratosis, edema and inflammation of the stomach. The bone marrows and the M/E ratios of these females were normal.

b. Rats Fed 25% Lactose

General observation, body weight and feed consumption: The control rats and rats fed 25% lactose for 13 weeks appeared healthy throughout the experiment. The body weights and feed consumption of the rats are summarized in Table 56. Both the male and the female rats fed lactose persistently gained weight. Their weight gains, as better illustrated in Figure 3, were comparable to the male and female controls, respectively. Feed consumption of the rats fed lactose was also comparable to that of the controls.

Blood analysis: The results of hematology and clinical blood chemistry tests are summarized in Table 57. The peripheral blood elements and various clinical chemistry values of both the male and female rats fed 25% lactose for 13 weeks were not apparently altered as compared with those of the respective control rats. Methemoglobin was not found in any rats. The mean corpusular hemoglobin concentration of the female rats fed lactose was slightly but significantly less than that of the female controls. This change was not considered clinically significant.

Serum electrolytes of these rats are summarized in Table 58. Serum levels of Na, K, Ca, Mg and Cl of rats fed 25% lactose for 13 weeks were not significantly altered.

Calcium content in the bone and iron content in the liver of these rats are summarized in Table 59. These levels in the rats fed lactose were not significantly different from those of the control rats.

Organ weights: The absolute and relative weights of various organs are summarized in Table 60. The absolute and relative weights of caecum, based on body weight or brain weight, of rats fed 25% lactose for 13 weeks were significantly larger than those of the control rats. The other organ weights of rats fed lactose were not apparently altered.

Gross and microscopic examination of tissues: At termination, both the control rats and the rats fed 25% lactose were in good nutritional condition with normal deposits of body fat. Tissue lesions in male and female rats are summarized in Tables 61 and 62, respectively. A number of lesions occasionally occurred in these control rats and rats fed lactose. These lesions included focal myocarditis of the heart, lymphoid hyperplasia or pneumonia of the lung, inflammation or focal necrosis of the liver, and/or interstitial nephritis or mononuclear cell infiltration of the kidney. In addition, one female control had hemosiderosis in the spleen and mesenteric lymph node, and one female rat fed lactose had a fibroadenoma of the mammary gland. These lesions were mild to moderate; they were spontaneous and not related to lactose.

4. Discussion and Conclusions

The male and female rats fed 2.5% TNG for 13 weeks consumed an average of 1,406 or 1,416 mg/kg/day of TNG, respectively. Both the male and the female rats fed TNG consumed less feed and lost weight quickly. They continued to lose weight, were slightly less active, and had rough hair coat through the 8th week. Thereafter, these rats started to gain weight and their conditions improved.

These rats fed TNG had some changes in the blood and organ weights and lesions in the testis, liver and spleen. Erythrocyte count, hematocrit, hemoglobin concentration, and serum level of alkaline phosphatase increased and fasting blood glucose decreased in both the male and female rats. The erythrocyte count of both the male and the female controls were relatively low before treatment; however, the values were within normal limits for rats observed in our laboratory as shown in Table L in Appendix I. In the males, TNG feeding increased the relative weights of spleen, kidney and brain based on the body weight and decreased the relative heart weight based on the brain weight. In the females, TNG feeding increased the relative weights of spleen and kidney based on the body weight and increased the relative kidney weight based on the brain weight. TNG caused testicular atrophy and degeneration, and aspermatogenesis in the males. Both the males and the females fed TNG had hemosiderosis in the spleen and/or the liver. Hemosiderosis in these organs is often seen in rodents. However, it was not seen in these control rats or mice used for these studies.

The male and female rats fed 25% lactose for 13 weeks did not show any adverse signs, any changes in the peripheral blood elements or clinical blood chemistry values, any effects on calcium content of the bone or iron content of the liver, or any lesions related to lactose. However, lactose feeding increased the weights of caecum of both the male and the female rats.

C. Cytogenetic and Mutagenic Effects of TNG

1 Introduction

The cytogenetic effect of TNG on somatic cell chromosomes was studied. The lymphocyte and kidney cultures from rats fed TNG were obtained and examined for any damages. In addition, the capability of TNG to induce single gene mutations was studied in Chinese hamster ovary cells in vitro. The Chinese hamster ovary system is capable of detecting mutations induced in nine different specific loci simutaneously.

2. Material and Methods

a. Cytogenetic Effects on Chromosomes

The procedure on the cytogenetic study described for dogs in Section I.C.2. was used. Peripheral lymphocyte and kidney cultures were obtained from rats fed the high level TNG for 4 and 13 weeks. The male rats were fed an average of 59.0 mg/kg/day TNG during the first 5 weeks and 229.5 mg/kg/day during the additional 8 weeks; the female rats were fed an average of 59.3 and 233.8 mg/kg/day for the respective periods. Accordingly, the lymphocytes or kidney cultures were activated, arrested, processed, stained and examined for both numerical and morphological aberrations of the chromosomes.

b. Mutagenic Effects on CHO-K1 Cells In Vitro

Wild type Chinese Hamster Ovary (CHO-K1) cells[11] capable of growth in both a minimal and an enriched medium were exposed to selected concentrations of TNG to test its ability to induce single gene mutations in mammalian somatic cells. The concentrations used were selected from a single cell survival curve obtained according to the method of Puck and Kao.[12] Potential mutants were isolated by the BUdR-visible light technique and confirmed by plating the cells in both media. A mutant was defined as having the capability of growth only in the enriched medium and not in the minimal medium. Mutagenesis was measured relative to the known mutagen ethyl methanesulfonate.[13]

3. Results

The results on numerical and morphological aberrations of chromosomes are shown in Tables 63 and 64, respectively. Rats fed TNG for 4 and 13 weeks did not show any changes in the chromosome frequency distribution or number of tetraploids, or any changes in the chromatid breaks or translocations, in the peripheral lymphocytes or kidney cultures.

The results of the in vitro single gene mutation study is shown in Table 65. No mutants were found in the cultures treated with TNG at concentrations which killed 65 and 99% of the cell population, respectively. Ethyl methanesulfonate, on the other hand, induced mutants at the frequency of 28.0×10^{-6} which is similar to that reported by Kao and Puck.[12/]

4. Discussion and Conclusion

An average of 59.0 to 59.3 mg/kg/day of TNG in feed for 5 weeks and 229.5 to 233.8 mg/kg/day for an additional 8 weeks to rats did not cause any numerical or morphological aberrations of chromosomes in the peripheral lymphocytes or kidney cultures.

Treatment of Chinese hamster ovary cells with TNG at concentrations which killed 65% and 99% of the population did not appear to induce any mutations. However, since this test system does not incorporate a metabolic activating system, no information on the mutagenicity of metabolites of TNG was obtained.

D. Immunologic Response to TNG

1. Introduction

Immunoglobin E (IgE), the allergic or hypersensitive antibody, was associated with anaphylactic reactions in human.[9/] Serum concentration of IgE of rats treated with TNG was determined.

2. Material and Method

As described for the dogs in Section I.D.2., the immunodiffusion technique of Mancini[10/] was used to determine the serum IgE of rats fed 2.5% TNG for 13 weeks. These rats were used for the subchronic toxicity study described in Section II.B.

3. Results and Conclusion

Serum concentrations of IgE of control rats and rats fed 2.5% TNG for 13 weeks are summarized in Table 66. TNG did not apparently alter the serum concentration of IgE. The TNG intake of the male rats averaged 1,406 mg/kg/day and in the female rat averaged 1,416 mg/kg/day.

TABLE 26

STABILITY OF TNG IN FEED MIXTURE FOR RATS

Sample	% NG	% Remaining
Feed Mixture No. 1		
1. Time zero-fresh	0.41[a/]	100
2. Time zero-frozen plus 8 days	0.41	100
3. 4 days in feeder	0.39	95
4. 4 days in capped can	0.41	100
5. Sample No. 4 after 4 days in feeder	0.36	88
6. 8 days in capped can	0.38	93
Feed Mixture No. 2		
1. Time zero-fresh	0.042	100
2. Time zero-frozen plus 8 days	0.042	100
3. 4 days in feeder	0.040	95
4. 4 days in capped can	0.038	90
5. Sample No. 4 after 4 days in feeder	0.031	74
6. 8 days in capped can	0.036	86

a/ Average of duplicate samples.

TABLE 27

BODY WEIGHTS OF MALE RATS FED TNG

% TNG in Feed[a/]	Body Weights (gm)				
	Initial	4 Weeks	8 Weeks	13 Weeks	17 Weeks
0	278±9 [b/]	441±10			
0.001-0.005	285±7	458±23			
0.01-0.05	278±9	423±21			
0.1-0.5	279±6	417±1			
0	276±10	434±9 [c/]	514±16		
0.001-0.005	289±6	458±16 [c/]	553±21		
0.01-0.05	267±7	410±6 [c/]	495±10		
0.1-0.5	290±6	422±11 [c/]	544±15		
0	270±7	446±20	532±27	555±22	
0.001-0.005	275±5	428±11	500±15	560±16	
0.01-0.05	276±9	429±10	509±18	552±28	
0.1-0.5	267±12	394±14	434±18	440±18	
0	288±9	462±10	542±18	603±20 [c/]	642±21
0.001-0.005	274±9	441±17	522±21	571±27 [c/]	617±32
0.01-0.05	281±11	427±14	501±20	564±18 [c/]	621±32
0.1-0.5	276±3	406±6	443±5	476±3 [c/]	576±7

a/ TNG concentrations in the feed were increased 5-fold starting the 6th week.

b/ Mean ± S.E. of four rats.

c/ TNG in feed discontinued thereafter.

TABLE 28

BODY WEIGHTS OF FEMALE RATS FED TNG

% TNG in Feed[a]	Body Weights (gm)				
	Initial	4 Weeks	8 Weeks	13 Weeks	17 Weeks
0	188±4[b]	244±7			
0.001-0.005	185±6	242±7			
0.01-0.05	199±5	267±4			
0.1-0.5	191±6	226±1			
0	185±3	243±7[c]	272±7		
0.001-0.005	186±6	248±4[c]	279±4		
0.01-0.05	198±4	258±10[c]	285±12		
0.1-0.5	204±9	251±11[c]	316±5		
0	195±7	268±9	266±18	299±11	
0.001-0.005	198±3	268±7	298±8	316±7	
0.01-0.05	193±6	252±6	275±4	291±5	
0.1-0.5	196±6	238±7	257±10	251±9	
0	190±4	257±9	291±9	308±9[c]	328±16
0.001-0.005	186±4	251±7	281±6	305±8[c]	342±13
0.01-0.05	187±4	253±10	273±9	294±12[c]	329±13
0.1-0.5	191±7	249±9	261±12	272±12[c]	315±12

a/ TNG concentrations in the feed were increased 5-fold starting the 6th week.

b/ Mean ± S.E. of four rats.

c/ TNG in feed discontinued thereafter.

TABLE 29

AVERAGE FEED CONSUMPTION (gm/day/rat) OF RATS FED TNG

% TNG In Feed[a]	Males		
	1-4[b]	5-8	9-13
0	28.4	26.9	26.5
0.001-0.005	28.0	26.5	27.5
0.01-0.05	26.4	26.9	26.3
0.1-0.5	27.1	22.6	22.6

% TNG In Feed[a]	Females		
	1-4	5-8	9-13
0	19.4	19.4	17.4
0.001-0.005	18.0	17.4	17.6
0.01-0.05	18.3	16.0	15.7
0.1-0.5	17.5	13.7	14.3

a/ TNG concentrations in the feed were increased 5-fold starting the 6th week.

b/ Weeks.

TABLE 30

AVERAGE TNG INTAKE (mg/kg/day) OF RATS DURING TREATMENT

% TNG In Feed[a/]	Males			
	1[b/]	4	8	13
0.001-0.005	0.9	0.7	2.7	2.5
0.01-0.05	6.5	5.4	22.6	20.6
0.1-0.5	64.1	54.0	207.9	196.8

% TNG In Feed[a/]	Females			
	1	4	8	13
0.001-0.005	0.9	0.8	3.2	2.9
0.01-0.05	6.7	6.0	23.1	23.6
0.1-0.5	56.1	62.5	228.9	183.5

a/ TNG concentrations in the feed were increased 5-fold starting the 6th week.

b/ Weeks.

TABLE 31

HEMATOLOGY DATA OF CONTROL MALE RATS FOR TNG

(B,N) BASELINE
(C,N) CONTROL
N = NUMBER OF RATS

	WKS 0 (B, 4)	WKS 4 (C, 4)	WKS 8 (C, 4)	WKS 13 (C, 4)	WKS 17 (C, 4)
ERYTHROCYTES ($\times 10^6$ /MM^3)	5.59 ± .29	6.67 ± .18[a]	7.66 ± .15[a]	7.80 ± .29[a]	7.92 ± .18[a]
RETICULOCYTES, %	1.83 ± .47	1.32 ± .07	1.16 ± .19	.74 ± .07[a]	.83 ± .19
HEMATOCRIT, VOL, %	50.5 ± 1.9	50.3 ± 1.4	51.8 ± .3	54.3 ± .9	51.5 ± 1.6
HEMOGLOBIN, GM, %	15.0 ± .4	15.5 ± .2	16.6 ± .2[a]	17.3 ± .2[a]	16.9 ± .2[a]
MCV, CUBIC MICRONS	90.6 ± 2.6	75.5 ± 3.4[a]	67.6 ± 1.2[a]	69.7 ± 2.1[a]	65.1 ± 2.0[a]
MCHB, MICRO MICROGMS,	26.9 ± .9	23.3 ± .3[a]	21.7 ± .4[a]	22.3 ± .7[a]	21.3 ± .4[a]
MCHBC, GM %	29.7 ± .7	31.0 ± 1.0	32.0 ± .2	32.0 ± .5	32.8 ± .7[a]
PLATELETS ($\times 10^5$ /MM^3)	8.0 ± .5	6.8 ± .3	5.7 ± .2[a]	6.9 ± .7	6.1 ± .5
LEUKOCYTES ($\times 10^3$ /MM^3)	17.3 ± .9	16.9 ± .7	26.5 ± 3.1[a]	21.1 ± 1.8	23.4 ± 2.1
NEUTROPHILS, %	15.5 ± 1.8	7.0 ± .4[a]	6.3 ± 1.2[a]	8.5 ± 2.8	12.8 ± 2.6
LYMPHOCYTES, %	79.8 ± 1.9	89.8 ± 1.1[a]	92.0 ± 1.8[a]	88.5 ± 4.2	82.3 ± 2.4
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.8 ± .3	.5 ± .3	1.8 ± 1.2	.8 ± .3	1.0 ± .4
BASOPHILS, %	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	4.0 ± .7	2.0 ± .8	0.0 ± 0.0[a]	2.3 ± 1.4	2.0 ± .6
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure [4]).

TABLE 32

HEMATOLOGY DATA OF MALE RATS BEFORE, DURING AND AFTER TNG FEEDING

DOSE 0.001-0.005% in Feed [a/]

(B,N) BASELINE
(T,N) TREATMENT
N = NUMBER OF RATS

	WKS 0 (B, 4)	WKS 4 (T, 4)	WKS 8 (T, 4)	WKS 13 (T, 4)	WKS 17 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	5.13 ± .42	6.42 ± .16[b/]	7.28 ± .12[b/]	7.80 ± .29[b/]	6.94 ± .44[b/]
RETICULOCYTES, %	1.57 ± .44	.95 ± .18	1.09 ± .13	.90 ± .19	.45 ± .16
HEMATOCRIT, VOL. %	46.8 ± .6	50.8 ± .6[b/]	50.5 ± .6[b/]	51.0 ± .4 [b,c/]	47.5 ± 1.7
HEMOGLOBIN, GM. %	14.7 ± .2	16.0 ± .2	15.9 ± .3	16.8 ± .1[b/]	15.2 ± .7[c/]
MCV, CUBIC MICRONS	93.1 ± 8.1	73.4 ± 1.8[b/]	69.4 ± .9[b/]	65.6 ± 2.2[b/]	69.0 ± 2.7[b/]
MCHB, MICRO MICROGMS.	29.3 ± 2.6	23.2 ± .5[b/]	21.8 ± .0[b/]	21.7 ± .8[b/]	22.1 ± .7[b/]
MCHBC, GM %	31.5 ± .6	31.7 ± .8	31.5 ± .4	33.0 ± .3	32.0 ± .3
PLATELETS ($X10^5$ /MM^3)	6.4 ± 1.0	5.4 ± .4	5.3 ± .3	6.9 ± .5	6.8 ± .9
LEUKOCYTES ($X10^3$ /MM^3)	14.2 ± 1.7	16.6 ± .9	26.9 ± 3.4[b/]	20.9 ± 1.5	25.6 ± .8[b/]
NEUTROPHILS, %	12.0 ± .8	12.0 ± 1.4[c/]	11.0 ± 2.5	12.5 ± 3.4	25.5 ± 4.3
LYMPHOCYTES, %	84.3 ± .6	87.0 ± 1.4	86.5 ± 2.9	86.5 ± 3.3	67.5 ± 9.1
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 1.0
EOSINOPHILS, %	0.0 ± 0.0	.5 ± .3	1.3 ± .4	.3 ± .3	2.3 ± .4[b/]
BASOPHILS, %	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	3.8 ± .8	.5 ± .5[b/]	1.3 ± .3[c/]	.8 ± .5	3.8 ± 1.5
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ TNG concentration was increased 5-fold starting the 6th week; treatment was discontinued after 13 weeks.
b/ Significantly different from the baseline level (Dunnett's multiple comparison procedure [4/]).
c/ Significantly different from the control rats at the respective time interval (Dunnett's multiple comparison procedure [4/]).

TABLE 33

HEMATOLOGY DATA OF MALE RATS BEFORE, DURING AND AFTER TNG FEEDING

LOSE 0.01-0.05% in Feed [a]

(B,N) BASELINE
(T,N) TREATMENT
N = NUMBER OF RATS

	WKS 0 (T, 4)	WKS 4 (T, 4)	WKS 6 (T, 4)	WKS 13 (T, 4)	WKS 17 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.78 ± .22[c]	7.06 ± .26	7.35 ± .23	7.71 ± .20[b]	7.84 ± .12[b]
RETICULOCYTES, %	.99 ± .12	1.23 ± .07	1.28 ± .12	.98 ± .12	1.32 ± .27
HEMATOCRIT, VOL. %	52.3 ± 1.4	49.3 ± 1.0	50.0 ± .7	51.0 ± .4[c]	52.5 ± .3
HEMOGLOBIN, GM. %	16.7 ± .2[c]	15.8 ± .1[b]	16.4 ± .0	16.7 ± .3	16.9 ± .2
MCV, CUBIC MICRONS	77.3 ± 3.4	69.9 ± 1.6	68.2 ± 2.6[b]	66.2 ± 1.7[b]	67.0 ± 1.3[b]
MCH, MICRO MICROGMS.	24.8 ± .8	22.4 ± .7[b]	22.4 ± .7[b]	21.6 ± .2[b]	21.6 ± .2[b]
MCHC, GM %	32.1 ± .7	32.1 ± .6	32.9 ± .4	32.7 ± .6	32.2 ± .4
PLATELETS ($X10^5$ /MM^3)	5.1 ± .2[c]	5.5 ± .6	6.0 ± .4	6.7 ± .1	5.4 ± .5
LEUKOCYTES ($X10^3$ /MM^3)	20.5 ± .3	18.5 ± 1.1	17.3 ± 1.4	22.0 ± 1.8	24.3 ± 3.8
NEUTROPHILS, %	10.5 ± 1.5	8.0 ± 1.2	8.0 ± .4	9.8 ± 1.9	10.5 ± .5
LYMPHOCYTES, %	87.5 ± 1.3[c]	89.3 ± 1.0	90.0 ± .7	87.8 ± 2.6	87.2 ± .8
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.3 ± .8	1.8 ± .5	2.0 ± .8	1.3 ± .5	1.5 ± .5
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.4 ± .3[c]	1.0 ± .4	0.0 ± 0.0	.5 ± .5	.5 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.8 ± .8	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

[a] TNG concentration was increased 5-fold starting the 6th week; treatment was discontinued after 13 weeks.
[b] Significantly different from the baseline level (Dunnett's multiple comparison procedure [4]).
[c] Significantly different from the control rats at the respective time interval (Dunnett's multiple comparison procedure [4]).

TABLE 34

HEMATOLOGY DATA OF MALE RATS BEFORE, DURING AND AFTER TNG FEEDING

DOSE 0.10-0.50% in Feed [a/]

(B.N) BASELINE
(T.N) TREATMENT
N = NUMBER OF RATS

	WKS 0 (B, 4)	WKS 4 (T, 4)	WKS 8 (T, 4)	WKS 13 (T, 4)	WKS 17 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.54 ± .11	7.08 ± .09[b/]	7.32 ± .10[b/]	7.48 ± .15[b/]	7.37 ± .15[b/]
RETICULOCYTES, %	1.03 ± .11	1.31 ± .08	1.01 ± .15	1.09 ± .14	.86 ± .12
HEMATOCRIT, VOL, %	48.3 ± .9	47.5 ± 1.2	46.0 ± 1.1[c/]	48.3 ± .6[c/]	49.5 ± 1.3
HEMOGLOBIN, GM, %	15.7 ± .2	14.8 ± .4	14.7 ± .1[c/]	15.6 ± .2[c/]	15.7 ± .4
MCV, CUBIC MICRONS	73.8 ± .9	67.0 ± 1.1[b,c/]	62.8 ± 1.7[b/]	64.5 ± 1.0[b/]	67.5 ± 1.4[b/]
MCHH, MICRO MICROGMS,	24.0 ± .5	20.9 ± .4[b,c/]	20.1 ± .4[b/]	20.8 ± .2[b/]	21.3 ± .5[b/]
MCHBC, GM %	32.5 ± .5[c/]	31.2 ± .3	32.0 ± .5	32.3 ± .2	31.6 ± .2
PLATELETS ($X10^5$ /MM^3)	5.5 ± .3[c/]	5.3 ± .2	6.5 ± .7	7.3 ± .5[b/]	7.3 ± .2[b/]
LEUKOCYTES ($X10^3$ /MM^3)	15.1 ± .7	16.9 ± 1.6	12.6 ± 1.1[c/]	17.4 ± .9	20.0 ± 1.2[b/]
NEUTROPHILS, %	8.8 ± 1.6[c/]	8.0 ± 1.4	12.5 ± 2.7	16.0 ± 1.1	16.3 ± 4.2
LYMPHOCYTES, %	89.0 ± 2.0[c/]	89.3 ± 1.4	86.3 ± 3.1	81.8 ± .9	82.3 ± 4.5
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	2.0 ± .4	2.3 ± .6[c/]	1.0 ± .7	1.8 ± .8	1.3 ± .5
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.3 ± .3[c/]	.5 ± .3	.3 ± .3	.3 ± .3	.3 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ TNG concentration was increased 5-fold starting the 6th week; treatment was discontinued after 13 weeks.
b/ Significantly different from the baseline level (Dunnett's multiple comparison procedure 4/).
c/ Significantly different from the control rats at the respective time interval (Dunnett's multiple comparison procedure 4/).

TABLE 35

THE CLINICAL BLOOD CHEMISTRY DATA OF CONTROL MALE RATS AND MALE RATS FED HIGH LEVEL TNG

% TNG in Feed[a/]	Fasting Glucose mg %)	SGOT (IU/L)	SGPT (IU/L)	Alkaline Phosphatase (IU/L)	BUN (mg %)
		Fed for 13 Weeks			
Control	144 ± 6[b/]	126 ± 10	36 ± 6	53 ± 2	11 ± 1
0.1 - 0.5	92 ± 21	262 ± 79	48 ± 9	75 ± 17	18 ± 3
		Fed for 13 Weeks and Allowed to Recover for 4 Weeks			
Control	172 ± 27	96 ± 6	28 ± 1	44 ± 3	18 ± 2
0.1 - 0.5	138 ± 8	131 ± 37	41 ± 11	44 ± 1	44 ± 1

a/ TNG concentration was increased 5-fold starting the 6th week.
b/ Mean ± S.E.

TABLE 36

HEMATOLOGY DATA OF CONTROL FEMALE RATS FOR TNG

(B,N) BASELINE
(C,N) CONTROL
N = NUMBER OF RATS

	WKS 0 (B, 4)	WKS 4 (C, 4)	WKS 8 (C, 4)	WKS 13 (C, 4)	WKS 17 (C, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	5.11 ± .61	6.07 ± .33	5.69 ± .26	7.10 ± .28[a/]	7.09 ± .28[a/]
RETICULOCYTES, %	1.20 ± .37	.97 ± .22	1.67 ± .16	.53 ± .06	1.19 ± .19
HEMATOCRIT, VOL, %	47.0 ± .7	46.3 ± 1.2	48.3 ± .5	48.3 ± 1.0	50.0 ± .4
HEMOGLOBIN, GM, %	15.2 ± .3	15.3 ± .4	15.8 ± .1	15.5 ± .6	16.3 ± .2
MCV, CUBIC MICRONS	96.6 ± 13.4	77.1 ± 5.6	85.8 ± 4.7	68.1 ± 1.5[a/]	70.8 ± 2.6
MCHB, MICRO MICROGMS.	31.1 ± 4.0	25.6 ± 1.8	28.0 ± 1.6	21.9 ± .6[a/]	23.0 ± .6
MCHBC, GM %	32.3 ± .4	33.2 ± .2	32.7 ± .3	32.2 ± 1.0	32.6 ± .3
PLATELETS ($X10^5$ /MM^3)	6.4 ± .2	6.3 ± .6	7.1 ± .4	5.8 ± .4	6.6 ± .7
LEUKOCYTES ($X10^3$ /MM^3)	18.7 ± 2.0	16.9 ± 1.7	21.0 ± 2.3	17.8 ± 1.4	18.1 ± 2.0
NEUTROPHILS, %	8.8 ± 2.3	17.3 ± 2.1	17.3 ± 2.9	10.8 ± 2.9	9.5 ± 2.9
LYMPHOCYTES, %	87.3 ± 2.0	80.8 ± 2.0	81.8 ± 2.8	88.5 ± 2.7	88.5 ± 3.3
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.8 ± .5	1.0 ± .7	.0 ± .5	0.0 ± 0.0	1.0 ± .4
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	3.3 ± 1.3	1.0 ± .5	.3 ± .3[a/]	.8 ± .5	.3 ± .3[a/]
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure [4/]).

TABLE 37

HEMATOLOGY DATA OF FEMALE RATS BEFORE, DURING AND AFTER TNG FEEDING

DOSE 0.001-0.005% in Feed [a/]

(B.N) BASELINE
(T.N) TREATMENT
N = NUMBER OF RATS

	WKS 0 (B, 4)	WKS 4 (T, 4)	WKS 8 (T, 4)	WKS 13 (T, 4)	WKS 17 (T, 4)
ERYTHROCYTES ($\times 10^6$ /MM^3)	5.32 ± .25	6.04 ± .37	5.82 ± .27	6.93 ± .16[b/]	7.17 ± .43[b/]
RETICULOCYTES, %	1.71 ± .41	1.01 ± .16	1.37 ± .21	1.05 ± .14[c/]	1.32 ± .22
HEMATOCRIT, VOL. %	46.8 ± 1.2	46.8 ± .8	48.8 ± .9	45.8 ± .5	49.5 ± .6
HEMOGLOBIN, GM. %	14.6 ± .1	15.8 ± .3[b/]	15.9 ± .4[b/]	15.4 ± .2	16.4 ± .3[b/]
MCV, CUBIC MICRONS	88.1 ± 2.1	78.2 ± 5.1	84.2 ± 2.8	66.1 ± 1.6[b/]	69.7 ± 3.5[b/]
MCHB, MICRU MICROGMS.	27.7 ± 1.2	26.4 ± 1.7	27.4 ± .7	22.3 ± .3[b/]	23.1 ± 1.0[b/]
MCHBC, GM %	31.4 ± .7	33.8 ± .4[b/]	32.6 ± .3	33.8 ± .4[b/]	33.1 ± .2[b/]
PLATELETS ($\times 10^5$ /MM^3)	7.1 ± .5	5.3 ± .1	6.9 ± .4	6.1 ± .5	4.4 ± 1.0[b/]
LEUKOCYTES ($\times 10^3$ /MM^3)	19.3 ± 2.1	15.4 ± 1.0	22.4 ± 2.4	15.1 ± 1.0	16.6 ± .9
NEUTROPHILS, %	11.0 ± 3.2	13.5 ± 2.9	16.5 ± 5.7	10.8 ± 3.2	5.0 ± .9
LYMPHOCYTES, %	83.5 ± 2.7	84.3 ± 3.7	81.0 ± 6.6	88.8 ± 3.0	92.3 ± 1.0
BANDS, %	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.5 ± .3	1.5 ± .9	1.3 ± .6	.3 ± .3	2.3 ± .3
BASUPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	4.8 ± .5	.8 ± .5[b/]	1.3 ± .5[b/]	.3 ± .3[b/]	.3 ± .3[b/]
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ TNG concentration was increased 5-fold starting the 6th week; treatment was discontinued after 13 weeks.
b/ Significantly different from the baseline level (Dunnett's multiple comparison procedure [4/]).
c/ Significantly different from the control rats at the respective time interval (Dunnett's multiple comparison procedure [4/]).

TABLE 38

HEMATOLOGY DATA OF FEMALE RATS BEFORE, DURING AND AFTER TNG FEEDING

DOSE 0.01-0.05% in Feed [a]

(B.H) BASELINE
(T.M) TREATMENT
N = NUMBER OF RATS

	WKS 0 (B, 4)	WKS 4 (T, 4)	WKS 8 (T, 4)	WKS 13 (T, 4)	WKS 17 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	5.44 ± .23	5.90 ± .71	5.82 ± .19	7.39 ± .12[b]	6.54 ± .44
RETICULOCYTES, %	2.20 ± .30	1.02 ± .04[b]	1.13 ± .22[b]	1.24 ± .12[b,c]	.47 ± .20[b]
HEMATOCRIT, VOL. %	49.5 ± 1.2	48.0 ± .7	51.3 ± .3	48.0 ± .7	45.3 ± 2.3
HEMOGLOBIN, GM. %	15.8 ± .3	16.4 ± .2[c]	16.1 ± .2	16.0 ± .1	15.7 ± .4
MCV, CUBIC MICRONS	91.7 ± 5.7	85.7 ± 12.1	88.3 ± 2.8	65.0 ± 1.0	70.9 ± 2.1
MCH, MICRO MICROGMS.	29.2 ± 1.4	29.4 ± 4.2	27.8 ± .9	21.6 ± .2	23.0 ± .4
MCHBC, GM %	31.9 ± .8	34.3 ± .2[b,c]	31.5 ± .3	33.3 ± .5	32.5 ± .5
PLATELETS ($X10^5$ /MM^3)	6.7 ± .1	5.4 ± .4	6.2 ± .7	4.6 ± .5[b]	6.6 ± .4
LEUKOCYTES ($X10^3$ /MM^3)	19.3 ± 1.3	16.6 ± .8	19.5 ± 2.1	15.7 ± 1.4	21.2 ± 2.2
NEUTROPHILS, %	14.3 ± 2.1	16.5 ± 2.4	12.0 ± 2.3	12.0 ± 2.3	8.3 ± .5
LYMPHOCYTES, %	82.3 ± 2.0	81.3 ± 2.5	86.0 ± 2.1	87.0 ± 3.1	88.8 ± 1.1
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.0 ± 0.0	1.0 ± .6	1.5 ± .6	.5 ± .5	3.0 ± .4
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	2.5 ± .9	1.3 ± .6	.5 ± .3[b]	0.0 ± 0.0[b]	0.0 ± 0.0[b]
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ TNG concentration was increased 5-fold starting the 6th week; treatment was discontinued after 13 weeks.
b/ Significantly different from the baseline level (Dunnett's multiple comparison procedure [47]).
c/ Significantly different from the control rats at the respective time interval (Dunnett's multiple comparison procedure [47]).

TABLE 39

HEMATOLOGY DATA OF FEMALE RATS BEFORE, DURING AND AFTER TNG FEEDING

DOSE 0.10-0.50% in Feed [a/]

(B.N) BASELINE
(T.N) TREATMENT
N = NUMBER OF RATS

	WKS 0 (B, 4)	WKS 4 (T, 4)	WKS 8 (T, 4)	WKS 13 (T, 4)	WKS 17 (T, 4)
ERYTHROCYTES ($\times 10^6$ /MM^3)	5.73 ± .28	6.24 ± .24	6.22 ± .24	7.59 ± .10[b/]	7.50 ± .09[b/]
RETICULOCYTES, %	2.04 ± .21	.80 ± .12[b/]	1.63 ± .05	1.25 ± .17[b,c/]	1.10 ± .28[b/]
HEMATOCRIT, VOL. %	49.3 ± 1.4	46.8 ± .9	48.3 ± 1.7	47.5 ± 1.4	51.5 ± 1.5
HEMOGLOBIN, GM. %	15.6 ± .3	15.5 ± .2	15.4 ± .4	16.1 ± .4	17.0 ± .5
MCV, CUBIC MICRONS	86.2 ± 2.0	75.2 ± 2.6[b/]	77.7 ± .9[b/]	62.6 ± 1.9[b/]	68.6 ± 1.4[b/]
MCH, MICRO MICROGMS.	27.4 ± 1.0	24.9 ± .9	24.9 ± .5	21.2 ± .3[b/]	22.7 ± .4[b/]
MCHC, GM %	31.4 ± .5	33.2 ± .3	32.0 ± .7	34.0 ± 1.0	33.1 ± .4
PLATELETS ($\times 10^5$ /MM^3)	5.6 ± .4	4.8 ± .5	6.3 ± .3	5.3 ± .5	5.5 ± .6
LEUKOCYTES ($\times 10^3$ /MM^3)	20.0 ± 2.0	14.6 ± 1.3	17.3 ± 1.3	14.8 ± 1.8	15.9 ± 2.4
NEUTROPHILS, %	8.8 ± 2.3	10.8 ± 1.9	11.5 ± 2.7	23.8 ± 4.6[b,c/]	13.0 ± 3.3
LYMPHOCYTES, %	86.5 ± 1.3	87.3 ± 2.4	87.3 ± 3.0	74.3 ± 5.0[c/]	83.3 ± 3.4
BANDS, %	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.5 ± 1.0	1.8 ± .9	1.3 ± .5	2.0 ± .4[c/]	2.8 ± .8
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	3.0 ± 1.2	.3 ± .3[b/]	0.0 ± 0.0[b/]	0.0 ± 0.0[b/]	1.0 ± .7
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ TNG concentration was increased 5-fold starting the 6th week; treatment was discontinued after 13 weeks.
b/ Significantly different from the baseline level (Dunnett's multiple comparison procedure [47]).
c/ Significantly different from the control rats at the respective time interval (Dunnett's multiple comparison procedure [47]).

TABLE 40

THE CLINICAL BLOOD CHEMISTRY DATA OF CONTROL FEMALE RATS AND FEMALE RATS FED HIGH LEVEL TNG

% TNG in Feed[a]	Fasting Glucose (mg %)	SGOT (IU/L)	SGPT (IU/L)	Alkaline Phosphatase (IU/L)	BUN (mg %)
		Fed for 13 Weeks			
Control	107 ± 3[b]	155 ± 31	51 ± 20	29 ± 1	10 ± 3
0.1 - 0.5	87 ± 4	248 ± 44	36 ± 2	29 ± 2	14 ± 2
		Fed for 13 Weeks and Allowed to Recover for 4 Weeks			
Control	163 ± 16	111 ± 19	29 ± 1	38 ± 2	16 ± 2
0.1 - 0.5	131 ± 4	104 ± 13	30 ± 1	32 ± 2	18 ± 1

a/ TNG concentration was increased 5-fold starting the 6th week.

b/ Mean ± S.E.

TABLE 41

ABSOLUTE AND RELATIVE ORGAN WEIGHTS
OF RATS FED TNG FOR 4 WEEKS

Sex	% TNG In Feed	Terminal	Absolute Organ Weight (gm) Liver	Kidney	Spleen
Male	Control	441 ± 10[a/]	18.2 ± 1.6	1.74 ± 0.05	0.77 ± 0.04
	0.001	458 ± 23	19.4 ± 1.4	1.88 ± 0.11	0.80 ± 0.10
	0.01	423 ± 22	19.0 ± 1.8	1.72 ± 0.11	0.78 ± 0.09
	0.1	408 ± 9	19.0 ± 1.7	1.53 ± 0.13	0.95 ± 0.11
Female	Control	244 ± 7	8.6 ± 0.3	0.78 ± 0.05	0.54 ± 0.04
	0.001	242 ± 7	8.8 ± 0.3	0.98 ± 0.05[b/]	0.51 ± 0.03
	0.01	267 ± 4	9.9 ± 0.1[b/]	1.05 ± 0.05[b/]	0.59 ± 0.04
	0.1	226 ± 1	9.3 ± 0.1	0.80 ± 0.03	0.51 ± 0.03

Sex	% TNG In Feed	Relative Organ Weights (gm/11gm Body Weight) Liver	Kidney	Spleen
Male	Control	4.1 ± 0.3	0.40 ± 0.01	0.17 ± 0.01
	0.001	4.2 ± 0.2	0.41 ± 0.01	0.18 ± 0.02
	0.01	4.5 ± 0.3	0.41 ± 0.03	0.18 ± 0.02
	0.1	4.6 ± 0.3	0.37 ± 0.02	0.23 ± 0.02
Female	Control	3.5 ± 0.2	0.32 ± 0.03	0.22 ± 0.01
	0.001	3.6 ± 0.3	0.41 ± 0.02[b/]	0.21 ± 0.02
	0.01	3.7 ± 0.6	0.39 ± 0.01	0.22 ± 0.02
	0.1	4.1 ± 0.06[b/]	0.35 ± 0.01	0.23 ± 0.01

a/ Mean ± S.E.

b/ Significantly different from the control rats (Dunnett's multiple comparison procedure[4/])

TABLE 42

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF RATS FED TNG FOR 13 WEEKS

Sex	% TNG in Feed[a/]	Terminal Body Weight	Absolute Organ Weight (gm)						
			Heart	Liver	Kidneys	Spleen	Gonads	Adrenals	Thyroids
Male	Control	555±22[b/]	1.53±0.10	16.5±0.5	3.86±0.14	0.84±0.02	3.10±0.30	67±2	35±2
	0.001-0.005	560±16	1.47±0.08	13.7±0.8	3.75±0.22	0.81±0.04	3.20±0.07	58±3	21±3[c/]
	0.01-0.05	519±34	2.15±0.38	13.7±1.8	4.89±1.09	0.88±0.03	2.54±0.65	49±4	20±2[c/]
	0.1-0.5	444±18[c/]	1.27±0.12	15.2±1.0	3.70±0.54	0.82±0.05	3.35±0.09	39±9[c/]	21±1[c/]
Female	Control	299±11	0.98±0.07	8.3±0.2	2.02±0.09	0.59±0.05	0.14±0.02	80±6	26±4
	0.001-0.005	316±7	0.91±0.02	7.3±0.2	1.94±0.02	0.52±0.02	0.14±0.02	76±1	26±2
	0.01-0.05	291±5	0.89±0.04	7.6±0.3	1.88±0.07	0.57±0.04	0.12±0.02	68±6	21±2
	0.1-0.5	251±9[c/]	0.83±0.01[c/]	9.1±0.6	1.97±0.06	0.53±0.02	0.11±0.01	61±5	18±1

Sex	% TNG in Feed	Relative Organ Weights (gm/100 gm body weight)						
		Heart	Liver	Kidneys	Spleen	Gonads	Adrenals	Thyroids
Male	Control	0.27±0.01	3.0±0.0	0.70±0.02	0.15±0.01	0.56±0.05	12±1	6.3±0.6
	0.001-0.005	0.26±0.01	2.4±0.1[c/]	0.67±0.02	0.14±0.00	0.57±0.02	10±0	3.8±0.5[c/]
	0.01-0.05	0.43±0.11	2.6±0.2	0.98±0.29	0.17±0.02	0.48±0.10	10±1	3.8±0.2[c/]
	0.1-0.5	0.30±0.03	3.4±0.1[c/]	0.82±0.08	0.18±0.01	0.76±0.02[c/]	9±2	4.6±0.3[c/]
Female	Control	0.34±0.02	2.8±0.1	0.67±0.02	0.20±0.02	0.05±0.00	27±3	8.5±1.2
	0.001-0.005	0.29±0.01	2.3±0.1[c/]	0.61±0.02	0.16±0.01	0.04±0.01	24±0	8.2±0.7
	0.01-0.05	0.31±0.02	2.6±0.1	0.65±0.03	0.20±0.01	0.04±0.01	23±2	7.2±0.7
	0.1-0.5	0.33±0.01	3.6±0.2[c/]	0.79±0.04[c/]	0.21±0.01	0.04±0.01	24±2	7.3±0.8

a/ TNG concentrations were increased 5-fold starting the 6th week.

b/ Mean ± S.E.

c/ Significantly different from the control rats (Dunnett's multiple comparison procedure[4/]).

TABLE 43

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF RATS FED TNG FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Sex	% TNG In Feed[a]	Terminal Body Weight	Absolute Organ Weight (gm) Heart	Liver	Spleen	Kidneys
Male	Control	642±21[b]	0.96±0.06	18.30±0.98	0.72±0.08	4.43±0.30
	0.001-0.005	616±25	1.36±0.06[c]	19.47±0.87	1.01±0.11	3.95±0.32
	0.01-0.05	615±31	1.29±0.05[c]	20.46±0.90	1.07±0.03	4.06±0.04
	0.1-0.5	576±6	0.85±0.11	14.29±0.63[c]	0.81±0.15	2.91±0.26[c]
Female	Control	328±16	0.90±0.04	8.30±0.98	0.90±0.05	1.84±0.11
	0.001-0.005	341±13	1.00±0.02	10.23±0.95	0.86±0.09	2.04±0.41
	0.01-0.05	319±18	1.02±0.05	8.75±2.13	0.86±0.08	1.86±0.20
	0.1-0.5	315±12	0.96±0.07	8.33±0.34	0.89±0.06	1.66±0.18

Sex	% TNG In Feed	Relative Organ Weights (gm/100gm Body Weight) Heart	Liver	Spleen	Kidneys
Male	Control	0.15±0.01	2.85±0.13	0.11±0.01	0.70±0.07
	0.001-0.005	0.22±0.00[c]	3.16±0.07	0.16±0.02	0.65±0.07
	0.01-0.05	0.21±0.01[c]	3.34±0.15[c]	0.18±0.01	0.66±0.03
	0.1-0.5	0.15±0.02	2.65±0.06	0.14±0.03	0.75±0.05
Female	Control	0.27±0.00	2.77±0.12	0.27±0.01	0.57±0.05
	0.001-0.005	0.30±0.01	2.99±0.17	0.25±0.02	0.59±0.10
	0.01-0.05	0.32±0.01[c]	3.21±0.27	0.27±0.02	0.58±0.06
	0.1-0.5	0.30±0.01	2.65±0.06	0.28±0.04	0.53±0.05

a/ TNG concentrations were increased 5-fold starting the 6th week.

b/ Mean + S.E.

c/ Significantly different from the control rats (Dunnett's multiple comparison procedure[4]).

TABLE 44

SUMMARY OF TISSUE LESIONS IN MALE RATS
FED TNG FOR 4 WEEKS

Lesions[a/]	Rat No:	Controls				0.1% TNG in Feed	
		113	114	115	116	187	188
Lung							
Lymphoid hyperplasia		+			+		+
Pneumonia			+	+++		++	
Liver							
Portal inflammation							+
Hepatic cell necrosis		+					
Spleen							
Extramedullary hematopoiesis						±	
Bone Marrow							
M/E Ratio		1.6	1.6	1.4	1.3	1.3	1.1

Tissues not listed were normal.

a/ Severity of lesions: + = mild; ++ = moderate; +++ = severe; ++++ = very severe; ± = questionable.

TABLE 45

SUMMARY OF TISSUE LESIONS IN FEMALE RATS
FED TNG FOR 4 WEEKS

Lesions[a/]	Rat No:	Controls				0.1% TNG in Feed	
		213	214	215	216	287	288
Lung							
Lymphoid hyperplasia			+		+		+
Pneumonia		++					
Bone Marrow							
M/E Ratio		b/	1.3	b/	1.6	b/	1.3

Tissues not listed were normal.

a/ Severity of lesions: + = mild; ++ = moderate; +++ = severe; ++++ = very severe; ± = questionable.

b/ Marrow smear was not made.

TABLE 46

SUMMARY OF TISSUE LESIONS IN MALE RATS
FED TNG FOR 13 WEEKS

Lesions[a]	Rat No:	Controls 105	106	107	108	0.1-0.5% TNG in Feed[b] 180	181	182	183
Lung									
Lymphoid hyperplasia		+			+	+	+	+-	
Pneumonia						+			
Heart									
Myocarditis			+-	+					+
Liver									
Hepatic cell necrosis			+	+		+	+	++	
Spleen									
Hemosiderosis						+		±	+
Pancreas									
Acute inflammation								±	
Bone Marrow									
M/E Ratio		1.4	1.3	1.6	1.4	1.5	1.4	1.5	1.4

Tissues not listed were normal.

a/ Severity of lesions: + = mild; ++ = moderate; +++ = severe; +-+++ = very severe; ± = questionable.

b/ TNG concentration was increased 5-fold starting the 6th week.

TABLE 47

SUMMARY OF TISSUE LESIONS IN FEMALE RATS
FED TNG FOR 13 WEEKS

Lesions[a] / Rat No:	Controls				0.1-0.5% TNG in Feed[b]			
	205	206	207	208	280	281	282	283
Heart								
Myocarditis	+	+						
Lung								
Lymphoid hyperplasia					+	+	+	
Pneumonia			++					
Liver								
Hepatic cell necrosis		+		+	++	+	+	
Hemosiderosis						+		
Spleen								
Hemosiderosis			++		+++	+	+	+
Extramedullary hemotopoiesis		+						
Bone Marrow								
M/E Ratio	1.2	1.6	1.6	1.4	1.5	1.4	1.5	1.6

Tissues not listed were normal.

a/ Severity of lesions: + = mild; ++ = moderate; +++ = severe; ++++ = very severe; ± = questionable.

b/ TNG concentration was increased 5-fold starting the 6th week.

TABLE 48

SUMMARY OF TISSUE LESIONS IN MALE RATS
FED TNG FOR 13 WEEKS AND ALLOWED
TO RECOVER FOR 4 WEEKS

Lesions[a]	Rat No:	Controls 101	102	103	104	0.1-0.5% TNG in Feed[b] 176	177	178	179
Heart									
Myocarditis			++	++		++	+		+
Lung									
Lymphoid hyperplasia						++	+		
Pneumonia								+	
Liver									
Hepatic cell necrosis			++				++		+
Spleen									
Hemosiderosis						+			+
Kidney									
Glomerulonephritis									+
Bone Marrow									
M/E Ratio		1.4	1.6	1.6	1.7	1.5	1.6	1.4	1.6

Tissues not listed were normal.

a/ Severity of lesions: + = mild; ++ = moderate; +++ = severe; ++++ = very severe; ± = questionable.

b/ TNG concentration was increased 5-fold starting the 6th week.

TABLE 49

SUMMARY OF TISSUE LESIONS IN FEMALE RATS FED TNG
FOR 13 WEEKS AND ALLOWED TO RECOVER
FOR 4 WEEKS

Lesions[a] / Rat No:	Controls 201	202	203	204	0.1-0.5% TNG in Feed[b] 276	277	278	279
Heart								
Myocarditis			±					
Lung								
Lymphoid hyperplasia			+	+		+		
Pneumonia	+							
Liver								
Hepatic cell necrosis						+		
Spleen								
Hemosiderosis	+			+	++		++	
Bone Marrow								
M/E Ratio	1.4	1.5	1.3	1.6	1.4	1.4	1.5	1.5

Tissues not listed were normal.

a/ Severity of lesions: + = mild; ++ = moderate; +++ = severe; ++++ = very severe; ± = questionable.

b/ TNG concentration was increased 5-fold starting the 6th week.

TABLE 50

BODY WEIGHTS (GM) OF RATS FED 2.5% TNG

Sex	Treatment[a/]	Treatment Weeks 0	4	8	13
Male	Control	316±9[b/]	454±10	525±7	584±5
	TNG	306±7	233±3[c/]	212±8[d/]	322±3[d/]
Female	Control	185±5	226±6	246±8	252±10
	TNG	202±7	148±5[d/]	134±5[d/]	202±9[d/]

FEED CONSUMPTION (GM/RAT/DAY) OF RATS FED 2.5% TNG

		Treatment Weeks 0-1	2-4	5-8	9-13
Male	Control	28.8[c/]	28.7	31.0	30.5
	TNG	12.4	12.8	12.2	22.0
Female	Control	14.7	14.8	15.2	14.7
	TNG	7.5	7.4	7.1	15.4

TNG INTAKE (MG/KG/DAY) OF RATS FED 2.5% TNG

		Treatment Weeks 0-1	2-4	5-8	9-13	Average
Male	TNG	1176[c/]	1277	1339	1588	1406
Female	TNG	1076	1163	1252	1773	1416

a/ Four rats for control groups and three rats for TNG groups.
b/ Mean ± S.E.
c/ Mean
d/ Significantly different from the controls (Student "t" test).

TABLE 51

LABORATORY DATA OF CONTROL RATS AND RATS FED TNG FOR 13 WEEKS

(C,N) Control (T,N) Treated N = Number of Rats

DOSE: % in Feed	Male 0.0 (C,4)	Male 2.5 (T,3)	Female 0.0 (C,4)	Female 2.5 (T,3)
ERYTHROCYTES ($X10^6$ /MM^3)	4.14 ± .25	5.44 ± .23[a/]	3.88 ± .51	6.12 ± .19[a/]
RETICULOCYTES, %	1.47 ± .09	2.47 ± .13[a/]	1.69 ± .11	2.75 ± .45[a/]
HEMATOCRIT, VOL. %	46.8 ± .5	57.3 ± 4.1[a/]	43.5 ± .9	61.0 ± 1.7[a/]
HEMOGLOBIN, GM. %	15.6 ± .1	17.8 ± .8[a/]	14.2 ± .4	18.8 ± .4[a/]
MCV, CUBIC MICRONS	114.0 ± 7.3	97.9 ± 3.2	117.0 ± 12.4	99.7 ± 2.4
MCHM, MICRO MICROGMS.	38.0 ± 2.5	30.4 ± .3	38.0 ± 3.6	30.7 ± .5
MCHBC, GM %	33.3 ± .2	31.2 ± .6[a/]	32.6 ± .4	30.8 ± .4[a/]
PLATELETS ($X10^5$ /MM^3)	3.7 ± .4	4.3 ± .6	5.7 ± .5	4.8 ± .3
LEUKOCYTES ($X10^3$ /MM^3)	12.1 ± .9	7.5 ± .7[a/]	7.4 ± .6	8.6 ± .3
NEUTROPHILS, %	11.0 ± 1.4	12.7 ± 3.7	14.3 ± 2.6	14.0 ± .6
LYMPHOCYTES, %	86.3 ± 2.6	85.7 ± 5.4	88.0 ± 2.6	85.0 ± 1.0
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.3 ± .7	.3 ± .3	.5 ± .5	1.0 ± .6
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0
MONOCYTES, %	1.8 ± .8	1.3 ± 1.3	1.0 ± .4	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
GLUCOSE (FASTING), MG %	121.3 ± 4.0	82.3 ± 2.9[a/]	113.5 ± 7.4	83.3 ± 4.7[a/]
SGOT, IU/L	69.3 ± 7.4	87.7 ± 18.4	75.8 ± 7.0	57.3 ± 3.9
SGPT, IU/L	34.0 ± 1.7	31.0 ± 1.7	24.3 ± 2.4	23.3 ± 2.3
ALK. PHOS., IU/L	49.3 ± 2.8	76.3 ± 4.7[a/]	25.3 ± 3.4	44.0 ± 4.5[a/]
BUN, MG %	17.3 ± 1.7	20.0 ± .6	18.3 ± 1.3	23.0 ± 1.5

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the respective control rats (Dunnett's multiple comparison procedure [4]).

TABLE 52

SERUM ELECTROLYTES OF RATS FED 2.5% TNG FOR 13 WEEKS

Sex	Treatment[a/]	Serum Electrolytes (meq/L)				
		Na	K	Ca	Mg	Cl
Male	Control	142±1[b/]	4.9±0.1	4.8±0.0	1.7±0.0	102±1
	TNG	149±5	5.5±0.5	5.0±0.2	2.2±0.3	100±3
Female	Control	146±1	5.6±0.1	5.2±0.1	2.0±0.1	106±2
	TNG	147±1	5.9±0.3	5.1±0.1	2.3±0.1	101±2

a/ Four rats for control groups and three rats for TNG groups.
b/ Mean ± S.E. (number of rats).

TABLE 53

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF RATS FED 2.5% TNG FOR 13 WEEKS

Sex	Treatment[a]	Terminal	Absolute Weight (gm) Brain	Heart	Liver	Kidneys	Spleen	Adrenal
Male	Control	584±5[b]	2.21±0.04	1.65±0.08	15.58±0.97	3.70±0.13	0.88±0.03	0.077±0.013
	TNG	322±3[c]	1.99±0.03[c]	1.05±0.10[c]	11.47±1.12[c]	3.07±0.18[c]	0.85±0.09	0.054±0.006
Female	Control	252±10	1.81±0.04	0.71±0.05	6.07±0.34	1.58±0.02	0.47±0.03	0.065±0.008
	TNG	202±9[c]	1.78±0.05	0.71±0.03	8.30±1.31	2.04±0.10[c]	0.77±0.13[c]	0.051±0.002

Sex	Treatment	Relative Weights (gm/100 gm body weight) Brain	Heart	Liver	Kidney	Spleen	Adrenal
Male	Control	0.38±0.00	0.28±0.01	2.67±0.01	0.64±0.02	0.15±0.01	0.013±0.002
	TNG	0.62±0.02[c]	0.03±0.03	3.57±0.37	0.96±0.06[c]	0.26±0.03[c]	0.017±0.002
Female	Control	0.72±0.04	0.28±0.02	2.43±0.20	0.63±0.02	0.19±0.02	0.026±0.003
	TNG	0.89±0.07	0.35±0.01	4.07±0.50	1.01±0.01[c]	0.38±0.05[c]	0.025±0.002

Sex	Treatment	Relative Weights (gm/gm brain weight) Heart	Liver	Kidney	Spleen	Adrenal
Male	Control	0.75±0.03	7.07±0.50	1.68±0.07	0.40±0.02	0.035±0.006
	TNG	0.53±0.05[c]	5.77±0.56	1.55±0.08	0.43±0.05	0.027±0.003
Female	Control	0.39±0.02	3.36±0.18	0.87±0.03	0.26±0.01	0.036±0.004
	TNG	0.40±0.02	4.71±0.83	1.15±0.09[c]	0.44±0.08[c]	0.029±0.002

a/ Four rats for control groups and three rats for TNG groups.

b/ Mean ± S.E.

c/ Significantly different from that of the control rats (Dunnett's multiple

TABLE 54

SUMMARY OF TISSUE LESIONS IN MALE RATS FED
2.5% TNG FOR 13 WEEKS

Lesions[a/]	Control				2.5% TNG in Feed		
Rat No:	117	118	119	120	195	196	197
Heart							
Myocarditis	++		+				
Lung							
Lymphoid hyperplasia		+			++		+
Pneumonia	++		++	++			
Liver							
Subacute inflammation	+	+			+		+
Hemosiderosis						+	
Kidney							
Glomerulonephritis				+			
Pyelonephritis		++					
Adrenal							
Vacuolar degeneration		++					
Spleen							
Hemosiderosis					+	+	
Testis							
Atrophy					++	+++	++
Testicular degeneration					+	++	+
Aspermatogenesis					+++	+++	+++
Bone Marrow							
M/E Ratio	1.7	1.6	1.8	1.6	1.7	1.6	1.4

Tissues not listed were normal.

a/ Severity of lesions: + = mild; ++ = moderate; +++ = severe; ++++ = very severe; ± = questionable.

TABLE 55

SUMMARY OF TISSUE LESIONS IN FEMALE RATS FED
2.5% TNG FOR 13 WEEKS

Lesions[a/]	Rat No:	Control				2.5% TNG in Feed		
		217	218	219	220	295	296	297
Lung								
Lymphoid hyperplasia		+		++			+	
Pneumonia			++		++	++		++
Emphysema		+						
Liver								
Subacute inflammation		+	++			+		+
Hemosiderosis							+	
Bile duct proliferation								±
Kidney								
Interstitial nephritis					++			
Spleen								
Hemosiderosis						+	+++	+++
Stomach								
Keratosis							+	
Edema and inflammation							+	
Bone Marrow								
M/E Ratio		1.5	1.4	1.6	1.7	1.6	b/	b/

Tissues not listed were normal.

a/ Severity of lesions: + = mild; ++ = moderate; +++ = severe; ++++ = very severe; ± = questionable.

b/ Marrow smear was not made.

TABLE 56

BODY WEIGHTS (GM) OF RATS FED 25% LACTOSE

Sex	Treatment[a/]	Treatment Week 0	4	8	13
Male	Control	262±17[b/]	403±17	486±20	524±24
	Lactose	270±17	401±10	480±18	510±19
Female	Control	210±5	257±5	284±8	294±6
	Lactose	204±6	269±5	295±9	299±7

FEED CONSUMPTION (GM/RAT/DAY) OF RATS FED 25% LACTOSE

Sex	Treatment[a/]	Treatment Week 1	4	8	13
Male	Control	22.9[c/]	26.1	27.2	24.2
	Lactose	17.2	28.3	25.9	21.7
Female	Control	16.8	18.1	18.0	16.7
	Lactose	13.9	20.3	18.1	16.3

a/ Five rats per group.
b/ Mean ± S.E.
c/ Mean.

TABLE 57

LABORATORY DATA OF CONTROL RATS AND RATS FED LACTOSE FOR 13 WEEKS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

	MALES		FEMALES	
DOSE: % IN FEED	0.0 (C, 5)	25.0 (T, 5)	0.0 (C, 5)	25.0 (T, 5)
ERYTHROCYTES ($\times 10^6$ /MM^3)	4.64 ± .28	4.21 ± .09	3.40 ± .32	3.13 ± .25
RETICULOCYTES, %	1.36 ± .15	1.12 ± .17	1.45 ± .37	1.18 ± .19
HEMATOCRIT, VOL. %	47.0 ± .7	47.0 ± 1.3	42.4 ± .2	44.0 ± .8
HEMOGLOBIN, GM. %	16.1 ± .2	15.4 ± .3	14.6 ± .1	14.7 ± .2
MCV, CUBIC MICRONS	101.2 ± 4.3	111.4 ± 3.1	128.7 ± 11.1	141.8 ± 10.0
MCH, MICRO MICROGMS.	34.7 ± 1.8	37.6 ± .8	43.6 ± 5.2	47.6 ± 3.4
MCHC, GM %	34.3 ± .2	33.7 ± .3	34.3 ± .3	33.4 ± .2[a/]
PLATELETS ($\times 10^5$ /MM^3)	6.5 ± .4	6.0 ± .9	5.8 ± .7	5.6 ± .3
LEUKOCYTES ($\times 10^3$ /MM^3)	14.3 ± 1.5	11.2 ± 1.2	6.5 ± .6	7.4 ± .3
NEUTROPHILS, %	9.0 ± 2.8	10.2 ± 1.6	9.2 ± 2.0	15.0 ± 4.4
LYMPHOCYTES, %	87.8 ± 3.3	88.6 ± 1.3	89.4 ± 1.5	84.0 ± 4.5
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	2.0 ± .9	1.0 ± .5	1.0 ± .6	.6 ± .4
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.2 ± .5	.2 ± .2	.4 ± .4	.4 ± .2
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
GLUCOSE (FASTING), MG %	110.6 ± 2.4	113.4 ± 2.5	114.7 ± 3.7	106.8 ± 2.6
SGOT, IU/L	86.4 ± 5.9	81.4 ± 6.4	83.3 ± 4.9	87.6 ± 20.4
SGPT, IU/L	37.4 ± 10.0	32.0 ± 2.2	33.3 ± 2.5	46.4 ± 11.7
ALK. PHOS., IU/L	69 ± 4	60 ± 2	38 ± 6	43 ± 5
BUN, MG %	18.2 ± 1.6	16.2 ± .6	14.3 ± .4	12.2 ± .6

ENTRIES ARE MEAN ± STANDARD ERROR

[a/] Significantly different from the respective control rats (Dunnett's multiple comparison procedure [2/]).

TABLE 58

SERUM ELECTROLYTES OF RATS FED 25% LACTOSE FOR 13 WEEKS

Sex	Treatment[a/]	Serum Electrolytes (meg/L)				
		Na	K	Ca	Mg	Cl
Male	Control	149±2[b/]	6.2±0.1	5.3±0.1	2.5±0.1	97±1
	Lactose	153±1	5.6±0.1	5.6±0.1	2.4±0.2	101±1
Female	Control	151±1	5.2±0.3	5.3±0.1	2.2±0.1	103±1
	Lactose	149±1	5.2±0.4	5.4±0.1	2.3±0.1	96±1

a/ Five rats per group.
b/ Mean ± S.E.

TABLE 59

BONE CALCIUM AND LIVER IRON OF RATS FED 25% LACTOSE

Sex	Treatment[a/]	Bone Ca (%)	Liver Fe (µg/gm)
Male	Control	9.24 ± 1.14[b/]	151 ± 18
	Lactose	9.41 ± 0.91	158 ± 18
Female	Control	9.06 ± 0.64	390 ± 28
	Lactose	8.87 ± 0.89	446 ± 47

a/ Five rats per group.
b/ Mean ± S.E.

TABLE 60

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF RATS FED 2.5% LACTOSE FOR 13 WEEKS

Sex	Treatment[a]	Terminal Body Wt.	Absolute Organ Weight (gm)					
			Brain	Heart	Liver	Kidneys	Spleen	Cecum
Male	Control	524±24[b]	1.95±0.06	1.51±0.18	12.73±0.78	3.09±0.21	0.70±0.04	6.19±0.64
	Lactose	510±19	1.96±0.06	1.35±0.06	13.14±0.92	3.04±0.20	0.70±0.02	8.50±0.55[c]
Female	Control	294±6	1.94±0.05	0.88±0.05	7.81±0.23	1.74±0.06	0.57±0.03	5.14±0.46
	Lactose	299±7	1.93±0.04	0.92±0.05	7.40±0.42	1.82±0.09	0.61±0.03	7.67±0.56[c]

Sex	Treatment	Relative Organ Weights (gm/100 gm body weights)					
		Brain	Heart	Liver	Kidney	Spleen	Cecum
Male	Control	0.37±0.01	0.29±0.02	3.27±0.87	0.59±0.02	0.11±0.01	1.18±0.11
	Lactose	0.38±0.01	0.27±0.02	2.60±0.23	0.60±0.05	0.14±0.01	1.63±0.15[c]
Female	Control	0.66±0.01	0.30±0.01	2.66±0.09	0.59±0.02	0.19±0.01	1.75±0.16
	Lactose	0.65±0.01	0.31±0.02	2.48±0.14	0.61±0.03	0.21±0.01	2.44±0.22[c]

Sex	Treatment	Relative Organ Weights (gm/gm brain weight)				
		Heart	Liver	Kidney	Spleen	Cecum
Male	Control	0.77±0.08	6.51±0.27	1.58±0.07	0.36±0.02	3.15±0.27
	Lactose	0.69±0.05	6.76±0.59	1.56±0.13	0.36±0.02	4.10±0.44[c]
Female	Control	0.45±0.02	4.04±0.12	0.90±0.02	0.29±0.01	2.66±0.24
	Lactose	0.48±0.03	3.83±0.19	0.94±0.05	0.32±0.02	3.99±0.33[c]

a/ Five rats per group.
b/ Mean ± S.E.
c/ Significantly different from the controls (Dunnett's multiple comparison procedure).[4]

TABLE 61

SUMMARY OF TISSUE LESIONS IN MALE RATS RECEIVING
25% LACTOSE IN THE FEED FOR 13 WEEKS

Lesions[a/]	Controls					25% Lactose in Feed				
Rat No:	121	122	123	124	125	171	172	173	174	175
Heart										
Focal myocarditis	+						±			
Lung										
Lymphoid hyperplasia	+			+		+	+	+	+	+
Pneumonia		++	++							
Liver										
Subacute inflammation	+	+			++					
Focal necrosis						±				
Kidney										
Interstitial nephritis								±		
Bone Marrow										
M/E Ratio	1.7	1.8	1.8	1.5	1.6	1.6	1.8	1.7	1.5	1.4

Tissue not listed were normal.

a/ Severity of lesions: + = mild; ++ = moderate; +++ = severe; ++++ = very severe; ± = questionable.

TABLE 62

SUMMARY OF TISSUE LESIONS IN FEMALE RATS FED
25% LACTOSE FOR 13 WEEKS

Lesions[a]	Rat No:	Controls					25% Lactose in Feed				
		221	222	223	224	225	271	272	273	274	275
Heart											
Focal myocarditis							+				
Lung											
Lymphoid hyperplasia		+	+	++	+	+	+		+		
Pneumonia								+++			++
Liver											
Acute inflammation		±		±				++			+
Spleen											
Hemosiderosis			±								
Lymph Node											
Hemosiderosis			±								
Kidney											
Mononuclear cell infiltration						±		++			
Mammary Gland											
Fibroadenoma									b/		
Bone Marrow											
M/E Ratio		1.7	1.7	1.5	1.7	1.5	1.5	2.3	1.4	c/	1.4

Tissues not listed were normal.

a/ Severity of lesions: + = mild; ++ = moderate; +++ = servere; +++ = very severe; ± = questionable.

b/ Fibroadenoma.

c/ Marrow smear was not made.

TABLE 63

CHROMOSOMES DERIVED FROM RATS
FED TNG IN THE DIET

Treatment[a]	Number of Rats	Chromosome Frequency <40	41	42	43	>44	Tetraploids Per 100 Cells
Control							
Lymphocyte	5	1[b]	3	44	2	--	0.50±0.16[c]
Kidney	4	4	4	42	1	--	0.59±0.19
TNG for 4 weeks							
Lymphocyte	5	3	3	39	3	1	0.54±0.18
Kidney	3	3	1	44	1	--	0.65±0.33
TNG for 13 weeks							
Lymphocyte	4	1	3	44	2	--	0.38±0.23
Kidney	4	4	2	43	1	--	0.75±0.25

a/ TNG was fed at 0.1% for 5 weeks; the concentration was increased 5-fold at the start of the sixth week.

b/ Mean

c/ Mean ± S.E.

TABLE 64

MORPHOLOGICAL ABERRATIONS OF CHROMOSOMES DERIVED FROM RATS FED TNG IN THE DIET

Treatment[a/]	Number of Rats	Chromatid Breaks and Gaps Per 50 Cells	Translocations Per 50 Cells	Total Aberrations Per 50 Cells
Control				
Lymphocyte	5	1.0±0.3[b/]	0.2±0.2	1.2±0.4
Kidney	4	1.5±0.3	0.5±0.3	2.0±0.3
TNG for 4 weeks				
Lymphocyte	5	0.2±0.2	0.2±0.2	0.4±0.2
Kidney	3	1.0±0.6	1.0±1.0	2.0±1.3
TNC for 13 weeks				
Lymphocyte	4	1.0±0.4	0.3±0.3	1.3±0.6
Kidney	4	1.0±0.7	0.5±0.3	1.5±0.9

a/ TNG was fed at 0.1% for 5 weeks; the concentration was increased 5-fold at the start of the sixth week.

b/ Mean ± S.E.

TABLE 65

MUTATION FREQUENCY OF CHO-K1 CELLS TREATED WITH TNG

Treatment	Mean Lethal Concentration (D_o) (µg/ml)	Concentration Tested (µg/ml)	Survival (%)	Summed Mutation Frequency Per D_o Per Cell For All Loci Tested x 10^{-6}
Ethyl Methanesulfonate (standard mutagen)	67.5	124.0	15	28.0[a/]
TNG	47.0	50.0	35	0
		144.8	1	0

a/ Corrected for loss during mutant isolation.

TABLE 66

SERUM IgE OF RATS FED 2.5% TNG FOR 13 WEEKS

Sex	Treatment[a/]	IgE (IU/ml)
Males	Control	1250 ± 46[b/]
	TNG	1333 ± 44
Females	Control	1263 ± 24
	TNG	1350 ± 44

a/ Five rats for control groups and three rats for TNG group.

b/ Mean ± S.E.

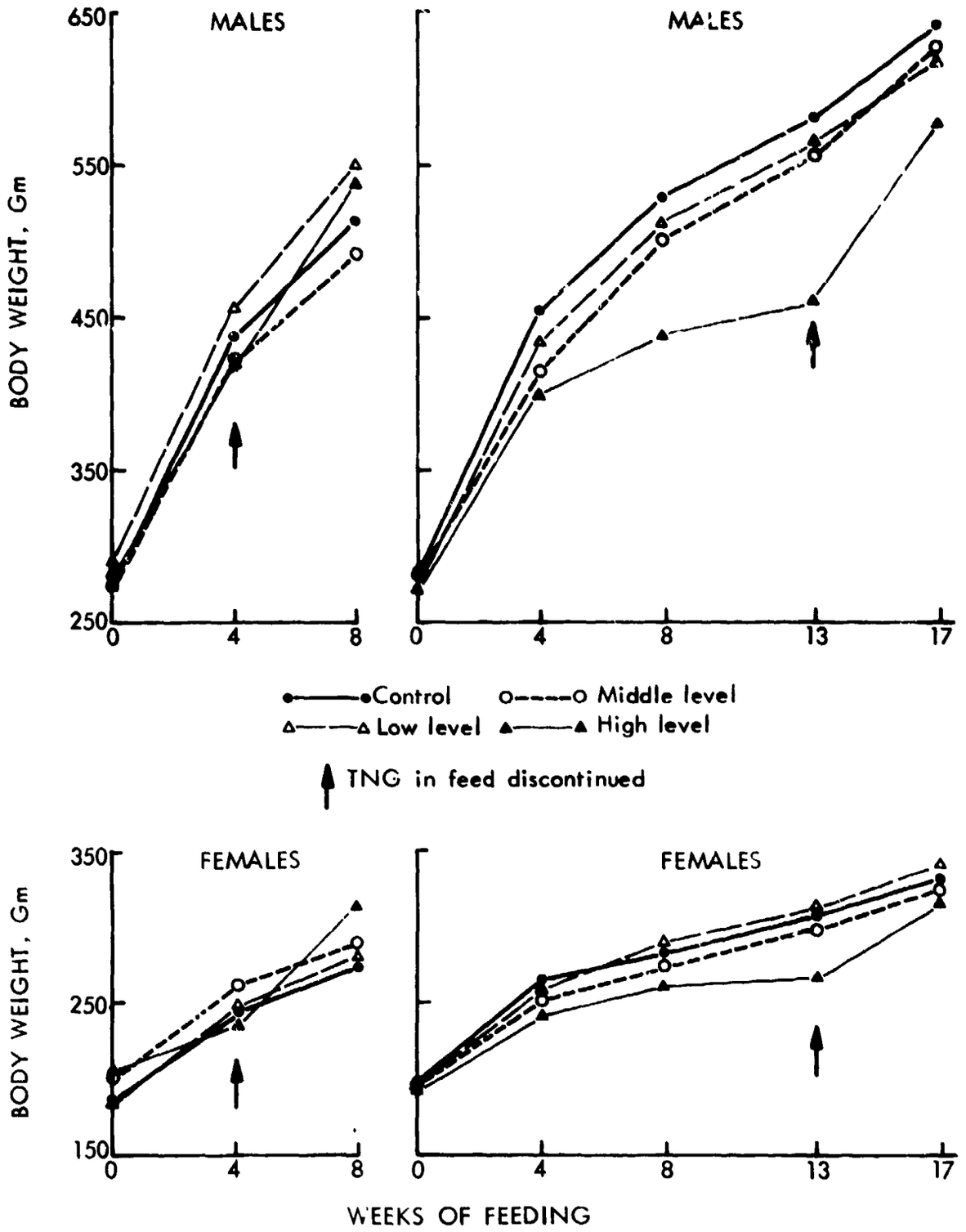

Figure 2 - Body Weights of Rats Fed Various Levels of TNG

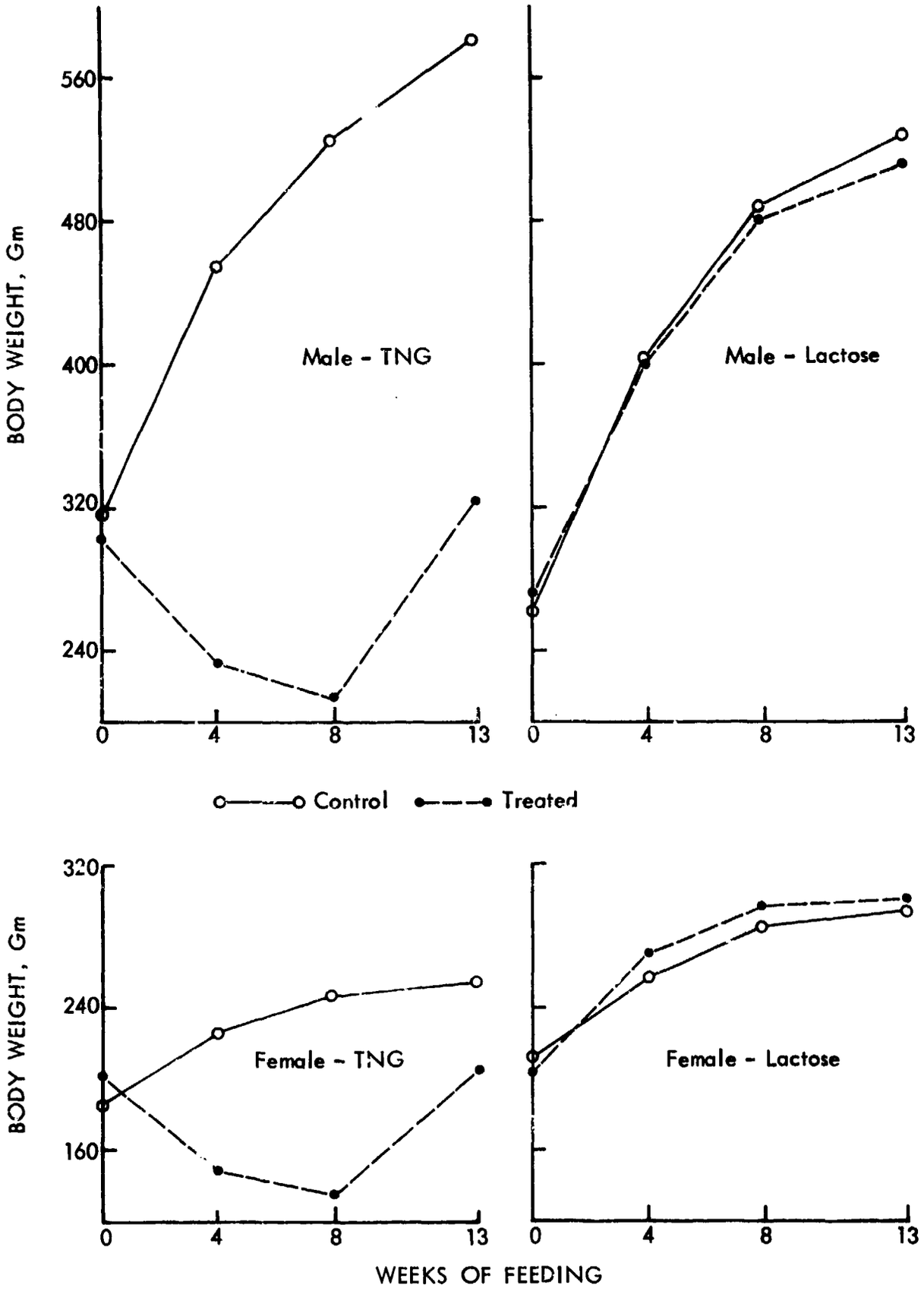

Figure 3 - Body Weights of Rats Fed 2.5% TNG or 25% Lactose

III. MICE

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III. MICE

A. Subacute and Subchronic Toxicities and Reversibility

1. Introduction

As for the dogs and mice, these studies were performed to define the nature and extent of effects of TNG on the biological system at the biochemical and cellular levels and to elucidate the dose-response relationship in the mice fed TNG for 4 and 13 weeks. The reversibility of any adverse effects was also studied in mice after the feeding of TNG was discontinued for 4 weeks.

2. Material and Methods

The basic design and procedure for these experiments in mice were similar to those described for rats in Section II.A.2. with the following exceptions:

a. A total of 64 male and 64 female young healthy albino swiss mice (National Laboratory Animals, O'Fallon, Missouri) were used for this study. They were divided into four groups, each consisting of 16 males and 16 females. The average weights of all groups were kept close. Three groups of mice were fed 0.001, 0.01, or 0.1% of TNG in powdered standard rodent chow (Wayne Laboratory Meal). At the end of 3 weeks, adverse effects were not observed in any mice. Starting the 4th week, TNG concentrations in feed for all groups were increased 5-fold to 0.005, 0.05 or 0.5%, respectively. The 4th group served as controls and was given the powered standard rodent chow.

b. Mice were kept in a separate room of our rodent quarters. They were housed four per plastic cage with filter tops.

c. Blood samples were collected by heart puncture under ether anesthesia at termination for hematology. Clinical blood chemistry tests in mice were not performed.

d. Since adverse effects were not observed in any mice and TNG did not cause any lesions in any mice that were terminated at the end of 4 weeks, the mice for the reversibility study were not necropsied for examination at 8 weeks as scheduled.

3. Results

a. General Observation and Weight Gain

The control mice and mice fed various levels of TNG were healthy throughout the experiment. The body weights of the male and female mice before, during and after treatment are summarized in Tables 67 and 68, respectively. The weight gains of both the male and the female mice fed the low, middle or high level of TNG were comparable to those of the controls.

b. Feed Consumption and TNG Intake

Feed consumption of the mice fed TNG are summarized in Table 69. Both the male and female mice fed various levels of TNG consumed comparable amounts of feed as the controls throughout the experiment.

TNG intake of these mice are summarized in Table 70. The TNG intake of the male mice fed 0.001, 0.01 or 0.1% TNG during the first 3 weeks averaged 1.3, 11.5 or 106.7 mg/kg/day, respectively. When the TNG concentrations in the feed were increased 5-fold starting the 4th week, the TNG intake of these mice increased and averaged 6.4, 60.2 or 607.6 mg/kg/day, respectively. The TNG intake of the female mice fed the low, middle or high level was comparable and averaged 1.3, 10.9 or 94.9 mg/kg/day during the first 3 weeks, and averaged 6.9, 58.7, or 561.2 mg/kg/day, respectively, during the subsequent 10 weeks.

c. Blood Analysis

The hematology results of the control mice and male mice fed various levels of TNG for 4 or 13 weeks, or for 13 weeks and allowed to recover for 4 weeks, are summarized in Tables 71, 72, or 73, respectively. The peripheral blood elements were not apparently altered by TNG. When compared with the control males, however, there were a few occasional differences at the various time intervals. These differences were slight and were inconsistent.

The hematology results of the control female mice and female mice fed various levels of TNG are summarized in Tables 74 through 76. As for the males, the peripheral blood elements of the females were not apparently altered by TNG. When compared with the control females, there were a few occasional differences at the various time intervals. The differences were slight and were not consistent.

d. Organ Weights

The organ weights of the mice fed various levels of TNG for 13 weeks are summarized in Table 77. The absolute and relative spleen weights, based on the body weight, of the female mice fed TNG were larger than those of the control females. However, due to large individual variation, the increase in spleen weights was not statistically significant. The absolute and relative weights of other organs of mice fed TNG were not apparently altered as compared with those of the control mice.

After feeding for 13 weeks and allowed to recover for 4 weeks, the absolute kidney weight of the female mice fed the middle level of TNG was significantly larger than that of the control mice (Table 78). Based on the body weight, this difference was not statistically significant. Since absolute kidney weight was not increased in the female mice fed the low or high level of TNG or in the male mice fed any levels of TNG and since no lesion was found in the kidneys of these mice, the increase of the absolute kidney weight was not considered clinically significant and was not related to TNG. The slight but not significant increases in the absolute and relative spleen weight, seen in the female mice fed TNG for 13 weeks, was not apparent in the mice after they were allowed to recover for 4 weeks.

e. Gross and Microscopic Examination of Tissues

At necropsy, the control mice and mice fed various levels of TNG were in good nutritional condition at various time periods. Microscopic examination of tissues revealed a number of tissues in both the control mice and mice fed TNG for 4 weeks. In the males, 3 of 4 control mice had microfoci of subacute inflammation in the liver (Table 79). One mouse also had a focal chronic perivasculitis of the kidney. Two of the 4 male mice fed the high level of TNG had focal chronic interstitial nephritis. In the females, 2 control mice had microfoci of subacute inflammation in the liver (Table 80). Another control had a chronic interstitial nephritis and tubular basophilia of the kidney. Three of the 4 female mice fed the high level of TNG had focal chronic murine pneumonia, microfoci of subacute inflammation in the liver, and/or chronic interstitial nephritis. These occasional lesions were spontaneous and are commonly seen in the mice.

After TNG feeding for 13 weeks, a number of spontaneous lesions also occurred in the control mice and mice fed TNG. In the males, there were microfoci of subacute inflammation in the liver and/or interstitial nephritis (Table 81). In the females, there were murine pneumonia with tracheitis, microfoci of subacute inflammation and/or pinworms in the large intestine (Table 82). In addition, extramedullary hematopoiesis occurred in the liver and spleen of the male and female mice fed the high and middle levels TNG and in the spleen of one male and one female mouse fed the low

level TNG. Bone marrows of the control mice and mice fed high levels of TNG were normal; the M/E ratios were within normal ranges.

After TNG feeding for 13 weeks and allowed to recover for 4 weeks, a number of spontaneous lesions were also seen in these mice (Tables 83 and 84). These lesions included lymphoid hyperplasia or focal pneumonia in the lung, foci of subacute inflammation in the liver, interstitial nephritis in the kidney, and/or fatty degeneration in the adrenal. These lesions occurred in the control mice and/or in the mice fed the TNG and allowed to recover for 4 weeks. As seen in the mice fed TNG for 13 weeks, extramedullary hematopoiesis occurred in the liver and spleen of these male and female mice. The bone marrows of the control mice and mice fed the high level of TNG, were normal; the M/E ratios were within normal ranges.

B. Discussion and Conclusions

Both the male and female mice fed the low, middle or high level of TNG did not show any adverse signs, any changes in hematology or clinical blood chemistry tests. The TNG intake of the male mice fed the low, middle and high levels in the feed averaged 1.3, 11.5, or 106.7 mg/kg/day, respectively, during the first 3 weeks; and 6.4, 60.2 or 607.2 mg/kg/day, respectively, during the subsequent 10 weeks. The TNG intake of the female mice averaged 1.3, 10.9 or 94.9 mg/kg/day, respectively, during the first 3 weeks; and averaged 6.9, 58.7 or 561.2 mg/kg/day, respectively, during the subsequent 10 weeks.

The absolute and relative spleen weights of female mice fed TNG for 13 weeks were larger than those of the control mice. However, the increases were not statistically significant. After feeding for 13 weeks and allowed to recover for 4 weeks, the slight increases in the absolute and relative spleen weights were not apparent. Extramedullary hematopoiesis was seen in the liver and spleen of mice fed TNG for 13 weeks and for 13 weeks plus recovery for 4 weeks. The significance of this extranmedullary hematopoiesis was not clear. First, extramedullary hematopoiesis is often seen in rodents. Second, the extramedullary hematopoiesis in the liver and spleen of these mice was mild. Third, there was no dose-relationship in the extramedullary hematopoiesis in these mice fed the high, middle or low levels of TNG.

C. Other Studies

Metabolic studies are described in Section IV. Mutagenic and immunologic studies were not performed in mice.

TABLE 67

BODY WEIGHTS OF MALE MICE FED TNG

% TNG in Feed[a]	Body Weights (gm)				
	Initial	4 Weeks	8 Weeks	13 Weeks	17 Weeks
0	29.3±1.4[b]	34.0±0.6			
0.001-0.005	26.8±1.5	30.0±1.6			
0.01-0.05	27.5±1.5	31.0±1.6			
0.1-0.5	28.0±1.3	30.3±0.8			
0	26.8±0.8	32.0±0.4[c]	33.8±1.0		
0.001-0.005	27.5±0.6	31.5±1.3[c]	33.3±1.7		
0.01-0.05	23.3±1.4	29.3±1.5[c]	28.7±0.9		
0.1-0.5	27.5±0.7	31.8±0.9[c]	33.5±1.2		
0	26.0±1.4	31.5±1.6	33.5±1.8	28.8±1.6	
0.001-0.005	27.3±1.3	32.0±1.0	33.3±1.7	29.0±1.7	
0.01-0.05	25.5±1.0	30.5±1.6	32.3±0.9	29.3±0.9	
0.1-0.5	25.0±1.2	28.5±0.7	31.0±0.7	28.3±0.8	
0	27.8±0.6	29.8±1.0	32.8±1.1	40.5±0.6[c]	36.8±0.3
0.001-0.005	29.0±1.5	32.0±2.0	35.0±2.1	36.3±1.3[c]	38.8±1.3
0.01-0.05	26.5±1.2	27.8±1.8	33.5±1.9	35.8±1.1[c]	38.0±1.4
0.1-0.5	28.0±0.8	30.3±0.5	31.8±0.5	36.3±0.6[c]	35.5±1.0

a/ TNG concentrations in the feed were increased 5-fold starting the 4th week.

b/ Mean ± S.E. of four mice.

c/ TNG in feed discontinued thereafter.

TABLE 68

BODY WEIGHTS OF FEMALE MICE FED TNG

% TNG in Feed[a]	Initial	4 Weeks	8 Weeks	13 Weeks	17 Weeks
0	25.8±1.1[b]	27.3±1.3			
0.001-0.005	23.0±0.4	25.5±0.9			
0.01-0.05	22.8±0.8	21.8±1.0			
0.1-0.5	23.3±1.3	25.3±1.1			
0	24.3±0.5	26.8±0.3[c]	27.0±0.4		
0.001-0.005	23.5±1.3	25.5±1.3[c]	28.5±1.7		
0.01-0.05	23.3±1.0	25.5±0.9[c]	30.5±0.9		
0.1-0.5	22.8±1.2	26.0±0.7[c]	28.0±1.8		
0	23.5±0.7	26.8±0.3	28.5±0.7	26.3±0.6	
0.001-0.005	22.0±0.6	23.3±1.6	27.3±0.8	25.3±1.0	
0.01-0.05	23.3±1.0	25.3±1.1	29.0±2.2	28.0±1.1	
0.1-0.5	25.3±0.8	26.5±0.3	29.5±0.3	27.0±2.0	
0	22.5±0.5	24.3±0.6	25.8±0.3	30.3±0.5[c]	27.8±0.9
0.001-0.005	21.5±0.5	24.3±1.1	26.5±1.0	28.5±0.7[c]	29.5±1.3
0.01-0.05	23.8±0.5	25.0±0.7	28.5±1.2	32.0±1.2[c]	31.3±1.4
0.1-0.5	24.5±0.9	26.0±1.1	28.5±0.3	32.3±0.9[c]	32.0±0.9

a/ TNG concentrations in the feed were increased 5-fold starting the 4th week.

b/ Mean ± S.E. of four mice.

c/ TNG in feed discontinued thereafter.

TABLE 69

AVERAGE FEED CONSUMPTION (gm/day/mouse) OF MICE FED TNG

% TNG In Feed[a/]	Males			
	1-4[b/]	5-8	9-13	17th
0	3.8	4.5	4.4	4.1
0.001-0.005	3.7	4.8	3.7	4.8
0.01-0.05	3.7	4.4	4.3	4.3
0.1-0.5	3.7	4.5	4.1	4.1

% TNG In Feed[a/]	Females			
	1-4	5-8	9-13	17th
0	3.5	3.5	3.5	3.2
0.001-0.005	3.6	4.1	3.3	2.9
0.01-0.05	3.8	4.6	3.9	4.0
0.1-0.5	3.4	4.6	3.6	3.6

a/ TNG concentrations in the feed were increased 5-fold starting the 4th week.

b/ Weeks.

TABLE 70

AVERAGE TNG INTAKE (mg/kg/day) of MICE
DURING TREATMENT

% TNG In Feed[a]	Males		
	0-3[b]	4-8	9-13
0.001-0.005	1.3	7.0	5.7
0.01-0.05	11.5	54.2	66.2
0.1-0.5	106.7	580.5	634.7

% TNG In Feed[a]			
	0-3	4-8	9-13
0.001-0.005	1.3	7.6	6.2
0.01-0.05	10.9	64.7	52.6
0.1-0.5	94.9	642.3	480.1

a/ TNG concentrations in the feed were increased 5-fold starting the 4th week.

b/ Weeks.

TABLE 71

HEMATOLOGY DATA OF MALE MICE FED TNG FOR 4 WEEKS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF MICE

DOSE: % TNG in Feed [a]	0.0 (C. 4)	0.1-0.5 (T. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	4.90 ± .41	4.13 ± .36
RETICULOCYTES, %	3.54 ± .24	2.56 ± .54
HEMATOCRIT, VOL. %	44.5 ± .3	41.0 ± 1.5
HEMOGLOBIN, GM. %	14.8 ± .2	12.8 ± .6 [b]
MCV, CUBIC MICRONS	92.8 ± 8.1	101.7 ± 10.6
MCHB, MICRO MICROGMS.	30.9 ± 2.7	.9 ± 3.6
MCHBC, GM %	33.3 ± .3	31.2 ± .4 [b]
PLATELETS ($X10^5$ /MM^3)	6.7 ± .8	6.5 ± .4
LEUKOCYTES ($X10^3$ /MM^3)	9.5 ± .8	9.4 ± 1.2
NEUTROPHILS, %	18.3 ± 3.4	21.5 ± 4.7
LYMPHOCYTES, %	79.8 ± 4.	78.0 ± 4.4
BANDS, %	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.5 ± .4	.3 ± .3
EOSINOPHILS, %	1.5 ± .3	.3 ± .3 [b]
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

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a/ TNG concentration was increased 5-fold starting the 4th weeks.
b/ Significantly different from the control mice (Dunnett's multiple comparison procedure [4]).

TABLE 72

HEMATOLOGY DATA OF MALE MICE FED TNG FOR 13 WEEKS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF MICE

DOSE: % TNG in Feed [a]	0.0 (C. 4)	0.001-0.005 (T. 3)	0.01-0.05 (T. 4)	0.1-0.5 (T. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.74 ± .47	6.43 ± .96	7.11 ± .07	7.19 ± .22
RETICULOCYTES, %	1.39 ± .13	5.48 ± 2.60	1.53 ± .25	2.30 ± .57
HEMATOCRIT, VOL. %	41.9 ± 2.4	41.7 ± 4.4	42.0 ± .4	45.5 ± .5
HEMOGLOBIN, GM. %	13.4 ± .5	12.8 ± 1.8	13.5 ± .2	14.8 ± .1
MCV, CUBIC MICRONS	59.8 ± 1.0	65.8 ± 3.6	59.0 ± .7	63.4 ± 2.2
MCHB, MICRO MICROGMS.	20.0 ± .7	19.9 ± .4	19.0 ± .1	20.6 ± .5
MCHBC, GM %	33.4 ± 1.2	30.4 ± 1.3	32.2 ± .6	32.5 ± .4
PLATELETS ($X10^5$ /MM^3)	4.2 ± 1.7	6.3 ± 2.1	6.4 ± 1.5	7.2 ± .9
LEUKOCYTES ($X10^3$ /MM^3)	7.8 ± .2	7.6 ± .2	7.8 ± .6	7.3 ± .4
NEUTROPHILS, %	8.5 ± .6	7.3 ± 1.5	18.3 ± 6.9	18.0 ± 2.7 [b]
LYMPHOCYTES, %	91.0 ± .9	92.7 ± 1.5	81.8 ± 6.9	81.8 ± 3.0 [b]
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.5 ± .5	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control.

b/ TNG concentrations were increased 5-fold starting the 4th week.

TABLE 73

HEMATOLOGY DATA OF MALE MICE FED TNG FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF MICE

DOSE: % TNG in Feed [a]	0.0 (C. 4)	0.001-0.005 (T. 4)	0.01-0.05 (T. 4)	0.1-0.5 (T. 4)
ERYTHROCYTES ($\times 10^6$ /MM^3)	7.38 ± .30	7.44 ± .38	7.46 ± .34	7.30 ± .24
RETICULOCYTES, %	1.53 ± .48	1.28 ± .25	2.87 ± 1.10	1.48 ± .18
HEMATOCRIT, VOL. %	43.0 ± 1.1	46.3 ± 2.1	43.5 ± 1.4	43.0 ± 1.1
HEMOGLOBIN, GM. %	14.3 ± .6	15.9 ± .8	14.9 ± .4	14.9 ± .1
MCV, CUBIC MICRONS	58.3 ± 1.1	58.3 ± .2	58.5 ± 1.1	59.0 ± 1.0
MCH, MICRO MICROGMS.	19.3 ± .2	20.0 ± .1	20.1 ± .4	20.5 ± .5
MCHC, GM %	33.2 ± .7	34.3 ± .3	34.4 ± .3	34.7 ± .7
PLATELETS ($\times 10^5$ /MM^3)	8.4 ± .5	6.6 ± 1.1	7.8 ± .7	6.2 ± .7
LEUKOCYTES ($\times 10^3$ /MM^3)	6.1 ± .1	6.7 ± 1.2	10.8 ± 1.4[b]	6.9 ± .6
NEUTROPHILS, %	34.0 ± 10.0	34.0 ± 8.2	39.5 ± 2.6	24.3 ± 7.4
LYMPHOCYTES, %	65.3 ± 10.0	64.8 ± 8.6	57.8 ± 2.7	75.0 ± 7.3
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	.8 ± .5	.5 ± .3	0.0 ± 0.0
EOSINOPHILS, %	.8 ± .3	.5 ± .3	2.3 ± 1.0	.8 ± .5
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ TNG concentrations were increased 5-fold starting the 4th week.
b/ Significantly different from the control mice (Dunnett's multiple comparison procedure [4]).

TABLE 74

HEMATOLOGY DATA OF FEMALE MICE FED TNG FOR 4 WEEKS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF MICE

DOSE: % TNG in Feed[a]	0.0 (C. 4)	0.1-0.5 (T. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	4.48 ± .13	5.39 ± .29[b]
RETICULOCYTES, %	.70 ± .04	1.34 ± .36
HEMATOCRIT, VOL. %	46.0 ± 1.2	46.8 ± 1.8
HEMOGLOBIN, GM. %	15.4 ± .4	15.9 ± .6
MCV, CUBIC MICRONS	102.8 ± 4.1	87.1 ± 3.1[b]
MCHB, MICRO MICROGMS.	34.4 ± 1.4	29.6 ± .9[b]
MCHBC, GM %	33.4 ± .3	34.0 ± .4
PLATELETS ($X10^5$ /MM^3)	5.8 ± .7	5.2 ± .5
LEUKOCYTES ($X10^3$ /MM^3)	8.9 ± 1.5	9.8 ± .9
NEUTROPHILS, %	13.3 ± 4.7	18.0 ± 4.9
LYMPHOCYTES, %	82.5 ± 2.5	81.8 ± 5.1
BANDS, %	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.3 ± .3	.3 ± .3
EOSINOPHILS, %	1.3 ± .5	0.0 ± 0.0[b]
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ TNG concentrations were increased 5-fold starting the 4th week.
b/ Significantly different from the control mice (Dunnett's multiple comparison procedure [4]).

TABLE 75

HEMATOLOGY DATA OF FEMALE MICE FED TNG FOR 13 WEEKS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF MICE

DOSE: % TNG in Feed[a]	0.0 (C. 4)	0.001-0.005 (T. 4)	0.01-0.05 (T. 4)	0.1-0.5 (T. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.98 ± .11	6.87 ± .31	7.60 ± .11	7.35 ± .13
RETICULOCYTES, %	2.00 ± .45	1.51 ± .24	1.20 ± .18	2.48 ± 1.37
HEMATOCRIT, VOL. %	42.0 ± .4	43.8 ± .9	46.0 ± 1.2	44.3 ± 1.5
HEMOGLOBIN, GM. %	14.0 ± .3	13.9 ± .2	15.1 ± .3[b]	14.4 ± .3
MCV, CUBIC MICRONS	60.2 ± .5	64.1 ± 3.1	60.5 ± 1.1	60.2 ± 1.7
MCHB, MICRO MICROGMS.	20.1 ± .1	20.3 ± .9	19.9 ± .2	19.6 ± .3
MCHBC, GM %	33.4 ± .3	31.7 ± .3[b]	32.9 ± .4	32.6 ± .6
PLATELETS ($X10^5$ /MM^3)	6.2 ± .8	3.3 ± .9[b]	5.6 ± .4	6.3 ± .2
LEUKOCYTES ($X10^3$ /MM^3)	5.8 ± .5	6.3 ± .4	7.2 ± .8	8.8 ± .6[b]
NEUTROPHILS, %	10.8 ± 3.9	15.3 ± 2.6	14.3 ± 1.4	19.0 ± 2.5
LYMPHOCYTES, %	89.0 ± 3.8	84.3 ± 2.5	85.3 ± 1.0	80.8 ± 2.4
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	.3 ± .3	.3 ± .3	0.0 ± 0.0
EOSINOPHILS, %	.3 ± .3	.3 ± .3	.3 ± .3	.3 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ TNG concentrations were increased 5-fold starting the 4th week.
b/ Significantly different from the control mice (Dunnett's multiple comparison procedure [4]).

TABLE 76

LABORATORY DATA OF FEMALE MICE FED TNG FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF MICE

DOSE: % TNG in Feed[a/]	0.0 (C. 4)	0.001-0.005 (T. 4)	0.01-0.05 (T. 5)	0.1-0.5 (T. 3)
ERYTHROCYTES ($X10^6$ /MM^3)	8.27 ± .20	7.84 ± .26	7.68 ± .16	7.48 ± .10
RETICULOCYTES, %	1.23 ± .36	1.03 ± .14	1.00 ± .09	1.30 ± .20
HEMATOCRIT, VOL. %	45.7 ± .7	45.3 ± .8	45.0 ± .6	42.7 ± .3[a/]
HEMOGLOBIN, GM. %	15.8 ± .4	15.7 ± .3	15.4 ± .3	14.9 ± .2
MCV, CUBIC MICRONS	55.3 ± .5	57.8 ± 1.1	58.6 ± .5[b/]	57.1 ± 1.2
MCHB, MICRO MICROGMS.	19.2 ± .1	20.1 ± .4	20.0 ± .2	19.9 ± .4
MCHBC, GM %	34.7 ± .5	34.8 ± .5	34.2 ± .5	34.9 ± .3
PLATELETS ($X10^5$ /MM^3)	7.4 ± .3	7.4 ± .4	7.0 ± .8	5.6 ± .3
LEUKOCYTES ($X10^3$ /MM^3)	7.4 ± .5	9.0 ± 1.2	10.9 ± 1.7	7.8 ± .6
NEUTROPHILS, %	13.8 ± 4.2	15.0 ± 1.8	17.4 ± 3.3	11.7 ± .9
LYMPHOCYTES, %	83.8 ± 5.4	82.5 ± 2.3	80.6 ± 3.4	88.0 ± 1.2
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.5 ± .3	0.0 ± 0.0	.2 ± .2	0.0 ± 0.0
EOSINOPHILS, %	2.0 ± 1.1	2.3 ± 1.1	1.8 ± .4	.3 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ TNG concentration was increased 5-fold starting the 4th week.
b/ Significantly different from the control mice (Dunnett's multiple comparison procedure [4/]).

TABLE 77

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MICE FED TNG FOR 13 WEEKS

Sex	% TNG In Feed[a/]	Terminal Body Weight	Absolute Weight (gm)			
			Heart	Liver	Kidneys	Spleen
Male	Control	28.8±1.7[b/]	0.16±0.01	1.25±0.03	0.55±0.02	0.14±0.01
	0.001-0.005	29.5±1.3	0.17±0.02	1.21±0.08	0.55±0.06	0.15±0.04
	0.01-0.05	29.3±0.9	0.15±0.01	1.28±0.07	0.58±0.03	0.12±0.01
	0.1-0.5	28.3±0.8	0.15±0.01	1.39±0.10	0.56±0.04	0.10±0.01
Female	Control	26.3±0.6	0.13±0.01	1.19±0.09	0.33±0.01	0.07±0.01
	0.01-0.05	28.0±1.1	0.15±0.01	1.36±0.09	0.42±0.03	0.14±0.01
	0.1-0.5	30.0±2.0	0.17±0.01	1.54±0.22	0.42±0.03	0.18±0.06

Sex	% TNG In Feed	Relative Organ Weights (gm/100 gm body weight)			
		Heart	Liver	Kidneys	Spleen
Male	Control	0.55±0.02	4.37±0.20	1.95±0.16	0.49±0.04
	0.001-0.005	0.58±0.04	4.16±0.13	1.90±0.12	0.53±0.14
	0.01-0.05	0.52±0.02	4.38±0.18	1.98±0.03	0.40±0.04
	0.1-0.5	0.54±0.04	4.92±0.34	1.98±0.18	0.36±0.02
Female	Control	0.51±0.05	4.67±0.45	1.31±0.05	0.27±0.03
	0.01-0.05	0.52±0.03	4.86±0.35	1.50±0.13	0.50±0.04
	0.1-0.5	0.55±0.03	5.21±0.83	1.43±0.16	0.62±0.22

a/ TNG concentrations were increased 5-fold starting the 4th week.
b/ Mean ± S.E.

TABLE 78

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MICE FED TNG FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Sex	% TNG In Feed[a]	Terminal Body Weight	Absolute Weight (gm) Heart	Liver	Kidneys	Spleen
Male	Control	31.1±0.3[b]	0.17±0.01	1.37±0.13	0.64±0.01	0.15±0.07
	0.001-0.005	35.0±1.9	0.21±0.03	1.57±0.13	0.63±0.01	0.11±0.01
	0.01-0.05	33.0±1.6	0.22±0.03	1.45±0.05	0.73±0.06	0.13±0.02
	0.1-0.5	31.0±0.7	0.19±0.01	1.38±0.05	0.56±0.02	0.12±0.01
Female	Control	24.3±0.6	0.14±0.01	1.05±0.06	0.36±0.01	0.08±0.01
	0.001-0.005	24.8±1.3	0.14±0.01	1.24±0.06	0.40±0.02	0.14±0.02
	0.01-0.05	26.5±1.6	0.16±0.01	1.24±0.08	0.45±0.04[c]	0.12±0.02
	0.1-0.5	29.0±1.1[c]	0.14±0.01	1.26±0.04	0.38±0.01	0.12±0.02

Sex	% TNG In Feed[a]	Relative Organ Weights (gm/100 gm body weight) Heart	Liver	Kidneys	Spleen
Male	Control	0.56±0.03	4.40±0.43	2.07±0.06	0.47±0.22
	0.001-0.005	0.60±0.07	4.50±0.36	1.81±0.10	0.32±0.02
	0.01-0.05	0.65±0.07	4.42±0.19	2.21±0.15	0.39±0.05
	0.1-0.5	0.62±0.03	4.46±0.13	1.79±0.02	0.40±0.02
Female	Control	0.58±0.05	4.32±0.20	1.48±0.06	0.31±0.03
	0.001-0.005	0.59±0.03	5.05±0.28	1.61±0.06	0.56±0.09
	0.01-0.05	0.60±0.04	4.69±0.27	1.70±0.06	0.47±0.07
	0.1-0.5	0.51±0.06	4.40±0.31	1.34±0.08	0.41±0.10

a/ TNG concentrations were increased 5-fold starting the 4th week.

b/ Mean ± S.E.

c/ Significantly different from the control mice (Dunnett's multiple comparison procedure[4]).

TABLE 79

SUMMARY OF TISSUE LESIONS IN MALE MICE
FED TNG FOR 4 WEEKS

Lesions[a/]	Mice No:	Controls				0.1-0.5% TNG in Feed[b/]			
		301	302	303	304	376	377	378	379
Liver									
Microfoci of subacute inflammation			+	+++	+				
Kidney									
Focal, chronic perivasculitis				+					
Focal, chronic interstitial nephritis						+		+	

Tissues not listed were normal.

a/ Severity of lesions: + = mild; ++ = moderate; +++ = severe; ++++ = very severe; ± = questionable.

b/ TNG concentration was increased 5-fold starting with the 4th week.

TABLE 80

SUMMARY OF TISSUE LESIONS IN FEMALE MICE
FED TNG FOR 4 WEEKS

Lesions[a] / Mice No:	Controls				0.1-0.5% TNG in Feed[b]			
	401	402	403	404	476	477	478	479
Lung								
Focal, Chronic murine pneumonia							±	+
Liver								
Microfoci of subacute inflammation		+		+++	++			++
Kidney								
Chronic interstitial nephritis			+				+	
Tubular basophilia			±					

Tissues not listed were normal.

a/ Severity of lesions: + = mild; ++ = moderate; +++ = severe; ++++ = very severe; ± = questionable.

b/ TNG concentration was increased 5-fold starting with the 4th week.

TABLE 81

SUMMARY OF THE TISSUE LESIONS IN MALE MICE RED TNG FOR 13 WEEKS

Lesions[a]	Mice No:	TNG % in Feed[b]															
		Controls				0.001-0.005				0.01-0.05				0.1-0.5			
		309	310	311	312	334	335	336	337	359	360	361	362	384	385	386	387
Liver																	
Microfoci of subacute inflammation		+	+	+		+						+	+	+			+
Extramedullary hematopoiesis		+								+	+	+	+	+	+	+	+
Spleen																	
Extramedullary hematopoiesis							±			+	+	+	+	+	+	+	+
Kidneys																	
Interstitial nephritis		++															
Bone Marrow																	
M/E ratio		1.6	1.5	1.7	1.8	c/	c/	c/	c/	c/	c/	c/	c/	1.6	1.7	1.5	1.8

Tissues not listed were normal.

a/ Severity of lesions: + = mild; ++ = moderate; +++ = severe; ++++ = very severe; ± = questionable.

b/ TNG concentrations were increased 5-fold starting the 4th week.

BLE 82

SUMMARY OF TISSUE LESIONS IN FEMALE MICE FED TNG FOR 13 WEEKS

Lesions[a/] / Mice No:	Controls		TNG % in Feed[b/] 0.001-0.005				0.01-0.05				0.1-0.5			
	409	410	434	435	436	437	459	460	461	462	484	485	486	487
Lung														
Murine pneumonia			+						+		+			
Trachea														
Tracheitis						+				+	+	+		
Liver														
Microfoci of subacute inflammation							+	+	++	+		+	+	++
Extramedullary hematopoiesis						+	+	+	+	+	+	+	+	+++
Large Intestine														
Pinworms	+							+				+		
Spleen														
Extramedullary hematopoiesis						+	+	+	+	+	++	+	++	++
Bone Marrow														
M/E ratic	1.6	1.9	c/	c/	c/	c/	c/	c/	c/	c/	1.4	1.7	1.7	1.4

Tissues not listed were normal.

a/ Severity of Lesions: + = mild; ++ = moderate; +++ = severe; ++++ = very severe; ± = questionable.

b/ TNG concentrations were increased 5-fold starting the 4th week.

c/ Bone marrow smear was not prepared.

TABLE 83

SUMMARY OF THE TISSUE LESIONS IN MALE MICE FED TNG FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Lesions[a] / Mice No:	TNG % in Feed[b]															
	Controls				0.001-0.005				0.01-0.05				0.1-0.5			
	313	314	315	316	338	339	340	341	363	364	365	366	388	389	390	391
Lung																
Lymphoid hyperplasia															+	
Focal pneumonita													+++			
Liver																
Foci of subacute inflammation			+	++	+				+		+++		+			
Extramedullary hematopoiesis										+	+			+	+	
Kidneys																
Interstitial nephritis															+	+
Spleen																
Extramedullary hematopoiesis						+		+		+	+	+		+	+	+
Bone Marrow																
M/E ratio	1.6	1.9	1.9	1.7	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	1.6	1.7	1.5	1.8

Tissues not listed were normal.

a/ Severity of lesions: + = mild; ++ = moderate; +++ = severe; ++++ = very severe; ± = questionable.

b/ TNG concentrations were increased 5-fold starting the 4th week.

c/ Bone marrow smear was not prepared.

TABLE 84

SUMMARY OF TISSUE LESIONS IN FEMALE MICE FED TNG FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Lesions[a]	TNG % in Feed[b]															
	Controls				0.001-0.005				0.01-0.05				0.1-0.5			
Mice No:	413	414	415	416	438	439	440	441	463	464	465	466	488	489	490	491
Liver																
Microfoci of subacute inflammation		+	+		+	+	+	+	+	++	+			+	+	+
Extramedullary hematopoiesis						±	+	±	+	±	+			±	+	±
Adrenals																
Fatty degeneration	+															
Spleen																
Extramedullary hematopoiesis						±				±	+				+	±
Bone marrow																
M/E ratio	1.5	1.7	1.4	1.9	c/	c/	c/	c/	c/	c/	c/	c/	c/	1.6	1.7	1.6	1.5

Tissues not listed were normal.

a/ Severity of lesions: + = mild; ++ = moderate; +++ = severe; ++++ = very severe; ± = questionable.

b/ TNG concentrations were increased 5-fold starting the 4th week.

c/ Marrow smear was not made.

IV. DISPOSITION AND METABOLISM

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IV. DISPOSITION AND METABOLISM

A. Disposition and Metabolism of TNG in Various Species

1. Introduction

Absorption, distribution, biotransformation, and excretion of TNG were studied in rats[1]. TNG and its metabolites were identified by the available conventional analytical methods and radioassay. In these experiments, similarities and differences in the disposition and pathways of biotransformation were investigated in mice, rabbits, dogs and monkeys.

2. Material and Methods

The procedure and methods described for rats[1] were generally used to study the detailed disposition and metabolism of TNG in female albino Swiss mice (National Laboratory Animals, O'Fallan, MO) weighing 20 to 25 gm, male and female New Zealand rabbits (Small Stock Ind., Pea Ridge, AR) weighing 2.30 to 2.37 kg, male and female beagle dogs (Hazelton Research Animals, Cumberland, VA) weighing 9.0 to 9.2 kg, and male and female rhesus monkeys (Primate Imports, Port Washington, NY) weighing 3.8 to 3.9 kg.

Each animal was fasted overnight. A single oral dose of TNG approximately 10% of the acute LD_{50}, spiked with 10 μCi of TNG-1,3-^{14}C (specific activity of 53.25 mCi/mM) was given via an intragastric metal or rubber tube. The TNG with TNG-1,3-^{14}C was suspended in peanut oil and given at a volume of 0.2 ml/10 gm for mice, 1 ml/100 gm for rats, or 1 ml/kg for rabbits, dogs and monkeys. After dosing, the mouse or rat was placed immediately in a "Roth-Delmar" metabolism cage.[14] The rabbit, dog or monkey was placed in a stainless steel animal cage (24 in. x 24 in. x 20 in.) inside of a closed air-tight Plexiglass box. All animals were given food and water ad libitum. The chambers were vented continuously with CO_2-free air and the expired CO_2 was collected and absorbed in 5% NaOH for rodents and in LiOH for the larger animals. Feces and urine were collected separately in the apparatus. At termination, each animal was anesthetized with ether or pentobarbital sodium. Aortic blood was collected and various tissues including feces were removed, weighed, and digested. Aliquots of tissue digests, filtrates, blood, plasma and urine were decolored with H_2O_2 and counted in the scintillation solution using a Packard Tricarb 3375 liquid scintillation spectrophotometer. $^{14}CO_2$ samples from the air trap were spotted on filter paper, dried and counted. Various

techniques including TLC, GLC, chemical detection tests, B-glucuronidase treatment and tissue extracts described previously[17] were used to identify TNG and its metabolites.

3. Results

a. Distribution and Excretion

The results on the disposition and metabolism of TNG in rats reported previously[17] are included for discussion in the present report. The distribution and excretion of radioactivity after a single oral dose of TNG in various species are summarized in Table 85. In mice, an average of 30.7% of the administered dose was recovered in the gastrointestinal tract plus contents and feces in 24 hours, whereas in rats, rabbits, dogs and monkeys less than 12% was recovered.

The total recovery of radioactivity in tissues and excreta averaged from 78.5 to 83.3% in mice, rats and monkeys, 91.4% in rabbits and 99.2% in dogs. This suggests that the absorption of TNG in mice averaged 50 to 70% of the administered dose in 24 hours, whereas the absorption in other species was somewhat larger, averaging 75 to 95% of the dose. In rats and mice, the majority of the absorbed radioactivity was excreted in the urine and expired air. In 24 hours, mice excreted 19.2 and 18.8% of the dose in the urine and expired air, respectively, whereas rats excreted 25.5 or 39.8% of the dose in the urine and expired air, respectively. In monkeys, dogs and rabbits, most of the absorbed radioactivity was excreted in the urine, averaging 45.0 to 72.0% of the dose in 24 hours. Only 2.5 to 7.8% of the administered radioactivity were recovered in the expired air during this period in these species. At the end of 24 hours, the liver of all species contained significant amounts of radioactivity, ranging from 4.3 to 6.8% of the dose. Dogs and monkeys retained an average of 9.5 or 13.0% of the administered radioactivity in the skeletal muscle, whereas only 2.1 to 2.8% of the dose were recovered in the skeletal muscle of rabbits, rats or mice. Kidneys, spleen, lung and brain of all species contained only small amounts of radioactivity.

The tissue/plasma radioactivity ratios after a single oral dose of TNG-1,3-^{14}C in various species are summarized in Table 86. Radioactivity was highly concentrated in the liver of all species. The tissue/plasma concentration ratio of radioactivity in the liver of rabbit or mouse was 21.0 or 30.1, respectively. The concentration ratios in the liver of rats, dogs and monkeys ranged from 7.4 to 7.8. The concentration ratio of radioactivity was also relatively high in the kidney, probably reflecting the excretion of radioactivity in the urine. The radioactivity was also concentrated in the spleen and lung in which the concentration

ratios were greater than one. The concentration ratios in the brain of mice, rats and rabbits were greater than one and of dogs and monkeys were less than one; the concentration ratio in the skeletal muscle of rabbits was greater than one and of the other species were less than one.

b. Metabolites in Urine

Metabolites of TNG in the 24-hour urine of various species are summarized in Table 8?. The parent compound, TNG, was detected only in small amounts, averaging less than 0.1% of the dose in the 24-hour urine of mice, rats and rabbits, and 0.3 to 0.8% in dogs and monkeys. The free dinitroglycerines (DNGs) were also excreted in small amounts in the urine, averaging less that 0.1% in mice, 0.3% in rabbits, 1.1% in rats, 2.5% in monkeys, and 3.6% in dogs.

Most of the urinary metabolites in mice were unidentified polar compounds, averaging 12.0% of the administered dose. One component moved in the ethyl acetate and petroleum ether solvent system and accounted for 11.2% of the dose. Other urinary metabolites included 2.0% of the dose as free mononitroglycerines (MNGs) 2.4% as DNG-glucuronides, 0.2% as MNG-glucuronides and 2.1% as glycerol.

Most of the urinary metabolites in rats were free MNGs (10.6% of the administreed dose) and 1,2-DNG-glucuronides (10.0%). Other urinary metabolites included 3.5% of the dose as 1,3-DNG-glucuronide and 1.5% of the dose as MNG-glucuronide. Glycerol accounted for 6.9% of the dose and the unidentified polar components accounted for 6.2% of the dose.

The unidentified polar compounds in the urine of rabbits accounted for most of the urinary radioactivity, averaging 44.8% of the administered dose. The major component moved in the ethyl acetate and pertroleum ether solvent system. Rabbits also excreted large amounts of 1,3-DNG-glucuronide and glycerol, averaging 11.2% and 8.8% of the dose, respectively. Free MNGs, 1,2-DNG-glucuronide and MNG-glucuronide accounted for 2.4%, 2.8% and 1.6% of the dose, respectively.

Dogs and monkeys excreted more MNGs and more MNG-glucuronides than other species. Free MNGs in the urine of dogs and monkeys accounted for 18.2% and 15.8% of the dose, respectively; and MNG-glucuronides accounted for 5.2% and 7.5%, respectively. Dogs also excreted 8.9% of the dose as DNG-glucuronides; and monkeys excreted only 0.9% as the DNG-glucuronides. Glycerol accounted for 8.8% and 4.8% of the dose in dogs and monkeys, respectively; whereas the unidentified polar compounds accounted for 22.0% and 12.6% in dogs and monkeys, respectively.

4. Discussion and Conclusions

The metabolic pathways were similar in rats, mice, rabbits, dogs and monkeys. About 50 to 70% of an oral dose of TNG-1,3-^{14}C was absorbed in 24 hours in mice, whereas 75 to 95% was absorbed in rats, rabbits, dogs and monkeys. In rats and mice, the majority of the absorbed radioactivity was excreted in the urine and expired air. In rabbits, dogs and monkeys, most of the absorbed radioactivity was excreted in the urine with only small amounts in the expired air. The radioactivity was highly concentrated in the liver of all species. Other tissues, including kidney, spleen, lung and/or skeletal muscle, also contained significant amounts of radioactivity.

TNG and free DNGs were excreted only in small amounts in the urine of mice, rats and rabbits, and in slight amounts greater in the urine of dogs and monkeys. Large amounts of unidentified polar compounds and glycerol were found in all species. Mice excreted only small amounts of free MNG, MNG-glucuronides and DNG-glucuronides, indicating relatively complete biotransformation of TNG. Most of the urinary metabolites in rats and rabbits were free MNG and DNG-glucuronides. Dogs and monkeys excreted mostly free MNGs and MNG-glucuronides.

The lesser amount of absorption and complete metabolism of TNG in mice reflects the relative toxicity of TNG in this species. As reported previously, the acute oral toxicity of TNG was less in mice than in rats. In the present studies, the subacute and subchronic toxicities of TNG were more in dogs and rats than in mice as described in Sections I, II, and III.

B. Biliary Excretion of TNG, DNGs and MNGs in Rats

1. Introduction

As discussed in the preceding Section IV.A., TNG was highly concentrated in the liver of various species. Liver serves both as a site for metabolic biotransformation of foreign compounds and as an organ for excretion. In these experiments, biliary excretion of TNG, its dinitroisomers (DNGs) and mononitro-isomers (MNGs) in rats were compared.

2. Material and Methods

Female CD® rats (Charles River Breeding Laboratories, Wilmington, MA), weighing 280 to 320 gm, were fasted overnight before use. Under ether anesthesia, the common bile duct was cannulated with PE 10 plastic tubing through a midline abdominal incision. After the incision

had been closed, a dose of TNG, DNGs or MNGs approximating 10% of the acute LD_{50}, spiked with about 10 μCi of the respective ^{14}C-labeled compounds (specific activity of 36.67 to 53.25 mCi/mM), was dissolved in peanut oil and administered orally by intragastric intubation. The rats were then confined individually in restrictive animal holders (Stoelting Company, Chicago, IL). Purina Rodent Chow and water were freely accessible to the animals.

Bile was collected for the predetermined intervals and the amount of bile was measured by weighing. Small volume (200 μl) blood samples were obtained periodically from the rats by cutting off the tips of their tails and heparinized. At the end of 24 hours, the rats were removed from the holders and anesthetized with ether. Blood was collected from the abdominal aorta with heparinized syringe. Entire length of gastrointestinal tract including their contents was removed and combined with the feces which were collected without urinary contamination.

Radioactivities in the bile, blood, plasma and the GI tract were measured using a Packard Tricarb 3375 liquid scintillation spectrophotometer as described in Section IV.A.2. Bile was counted directly in the scintillation solution. Blood, plasma and the GI tract were digested with NaOH, decolored with H_2O_2, solubilized in the scintillation solution and counted.

3. Results

a. Biliary Excretion

The biliary excretion of radioactivity in female rats after oral administration of TNG-1,3-^{14}C is summarized in Table 88. The radioactivity appeared in the bile within 15 minutes after dosing. The rate of biliary excretion increased with time and reached a peak in 3 hours. Thereafter, the rate of excretion decreased. The total biliary excretion averaged 13.50% of the dose in 24 hours. The blood concentration of radioactivity continued to increase through the 5th to 6th hour and decreased thereafter.

The biliary excretions of radioactivity after oral administration of 1-MNG, 2-MNG, 1,2-DNG and 1,3-DNG (NG-1,3-^{14}C) are summarized in Table 89. The biliary excretion of radioactivity was high for 2-MNG and 1,2-DNG, averaging 18.90% and 11.20% of the administered dose in 24 hours, respectively. The biliary excretion was low for 1-MNG and 1,3-DNG, averaging 3.81% and 1.63% of the dose, respectively. The recovery of radioactivity in the gastrointestinal tract plus contents and feces was 4.64% of the dose for 1-MNG and less than 1% for 2-MNG, 1,2-DNG or 1,3-DNG.

b. Metabolites of TNG in Bile

Metabolites of TNG in the 24-hour bile of rats are summarized in Table 90. As described in the urine in Section IV.A.2.b., TNG was detected only in small amounts in the 24-hour bile of rats, less than 0.1% of the administered dose. The free DNGs were also excreted in small amounts in the bile, averaging 0.3% of the dose or less. In addition to 1,2-DNG-glucuronide (3.9% of the dose), 1,3-DNG-glucuronide (2.5%), glycerol (2.8%) and unidentified polar compounds (2.4%), other major metabolites in the bile were MNG-glucuronides (2.0%). Free MNGs accounted for 1.0% of the dose.

Separate experiments showed that the concentration of 1,3-DNG in the first-hour bile was higher than that in the 5th-hour bile, whereas the concentration of MNG was higher in the 5th-hour bile than in the first-hour bile. This indicates the progressive denitration of the DNG to MNG.

4. Discussion and Conclusion

After oral administration of TNG-1,3-^{14}C to rats, the radioactivity appeared in the bile in 15 minutes. The rate of biliary excretion increased with time and reached a peak in 3 hours. The blood concentration of radioactivity continued to increase through the 5th to 6th hour. The biliary excretion of radioactivity was high for 2-MNG, TNG and 1,2-MNG and low for 1-MNG and 1,3-DNG. The amount of radioactivity remained in the gastrointestinal tract plus contents and in the feces was small. This suggests relatively complete absorption after oral administration of these compounds.

C. Metabolism of TNG In Vitro

1. Introduction

This phase of the study was to describe the in vitro metabolism of TNG by homogenates of livers from various species. These data in conjunction with the in vivo observations may be utilized to predict how humans metabolize TNG. In addition, metabolism of TNG by placentas and tissues of several species during development was investigated.

2. Material and Methods

Animals were sacrificed by decapitation (rats), cervical dislocation (mice), air embolism (rabbits), or an overdose of magnesium sulfate (dogs and monkeys) and the livers removed, weighed, and homogenized in three volumes of 1.15% KCl. Human liver was obtained from autopsy

samples and similarly processed. The *in vitro* system, as modified from Needleman and Krantz,[15] contained 1 mM TNG-1,3-^{14}C or 1-MNG-1,3-^{14}C, 8 mM reduced gluthathione, 8 mM potassium cyanide, phosphate buffer and liver homogenate at a final pH of 7.4. The incubations were conducted at 37°C for 10 minutes, except where specified, and terminated with mercuric chloride and absolute ethanol. Metabolites were resolved by silica gel thin-layer chromatography in a solvent system of benzene and ethyl acetate (4:1). In this system, TNG, 1,3-DNG, and 1,2-DNG are resolved on the plate while MNGs, glycerol and glucuronides remain at the origin. The Lowry[16] protein assay was used to measure protein assay. The ability of livers to metabolize TNG was expressed as nanomoles metabolites per milligram protein. Since there were no sex differences in TNG metabolism, the values for both males and females were combined.

3. Results

a. Metabolism by Rat Liver

The *in vitro* system metabolized TNG primarily to 1,3-DNG and 1,2-DNG after a 15-minutes' incubation. The results are shown in Table 91. If the reactions were continued for up to 2 hours, then the amount of DNGs decreased and the amount of polar components at the origin increased as a function of time. The material at the origin probably represented MNGs since 94% of 1-MNG incubated in the same system was present as the parent compound after a 90-minute incubation. These observations suggest that the liver *in vitro* was not able to completely denitrate TNG as the *in vivo* system.

These results regarding the ability of livers to metabolize TNG are in general agreement with the reports of others[15,17,18], Since liver homogenates metabolized glycerol-^{14}C, but not TNG-^{14}C, to $^{14}CO_2$, they concluded that TNG was not denitrated to glycerol in the liver. In addition, the conversion of TNG-^{14}C to $^{14}CO_2$ which occurred in the intact animal was blocked when rats were eviscerated. Since the removal of liver, stomach, intestines, pancreas, spleen and kidneys interferred with the metabolism of TNG-^{14}C, it was possible that these organs acted in conjunction with the liver to metabolize TNG. This proposal was tested in the *in vitro* system by incubating homogenates of the liver and various organs with TNG-1,3-^{14}C and 1-MNG-1,3^{14}C. As shown in Tables 92 and 93, the addition of none of the tissues which were removed during evisceration modified the ability of the liver to metabolize either substrate. The results of this experiment still leave in question the role of the liver and various other organs in the complete denitration of TNG to glycerol and ultimately to CO_2.

b. Metabolism by Liver of Various Species

Comparative studies on TNG metabolism were conducted by incubating homogenates of livers from various species with TNG-1,3-^{14}C or 1-MNG-1,3-^{14}C for 10 minutes. Livers from rats, dogs, rabbits, and monkeys were not able to metabolize ^{14}C-1-MNG to glycerol. As shown in Table 94, the primary metabolites of TNG were 1,3-DNG and 1,2-DNG. Polar components at the origin represented 5% or less of the total radioactivity and were excluded from the comparison. No sex difference in TNG metabolism was observed for any of the animal species tested and the results for males and females were combined. Livers from rats and mice produced more 1,3-DNG than 1,2-DNG. In contrast, more 1,2-DNG was produced by livers from rabbits, dogs, monkeys and humans. Livers from mice and humans had a low ability to metabolize TNG. In addition, the human livers had a lower ability to form 1,3-DNG than any of the other species. The formation of 1,2-DNG by livers from rats and humans was equivalent.

c. Metabolism During Development

Since the metabolism of many compounds changes during development, studies were conducted to determine the ability of various developing tissues to metabolize TNG. Placenta from mice, rats, and humans had a poor ability, relative to the liver, to produce 1,3-DNG and 1,2-DNG. The results are shown in Table 95. Low levels of TNG metabolism were found in mouse embryos on day 12 of gestation and in mouse livers and carcasses on day 18 of gestation. The results are shown in Table 96. TNG metabolism in rat livers increased between 1 and 7 days after birth and did not change between 7 and 21 days after birth. Livers from rats at 3 weeks of age produced less DNGs than the adult liver and the ratio of 1,3-DNG to 1,2-DNG was 1.2. These results suggest that at 3 weeks of age rats do not produce the adult pattern of TNG metabolites.

4. Discussion and Conclusions

TNG was primarily metabilized to DNGs *in vitro* by livers of various species. Livers from rats and mice produced more 1,3-DNG than 1,2-DNG; whereas, livers from rabbits, dogs, monkeys and humans produced more 1,2-DNG than 1,3-DNG. Livers from mice and humans had a low ability to metabolize TNG.

The rat liver quickly metabolized TNG *in vitro* to DNGs in 15 minutes. Then the amount of DNGs decreased when the incubation was continued for up to 2 hours. MNG was not appreciably metabolized by liver *in vitro*. These results suggested that the liver *in vitro* was not able to completely denitrate TNG as *in vivo* system. The addition of other tissue homogenates, including stomach and intestines, or pancreas, spleen and kidneys, did not modify the ability of the liver to metabolize TNG or MNGs *in vitro*.

Placentas from mice, rats or humans and mouse embryo, liver or carcass during late gestation had a poor ability, relative to liver, to metabolize TNG *in vitro*. TNG metabolism in rat livers increased with time after birth up to 7 days. The metabolism did not change between 7 and 21 days. The ability of rat liver at 21 days after birth to metabolize TNG was lower than that of the adult liver.

D. Effect of TNG on Drug Metabolizing Enzyme

1. Introduction

The effect of TNG on the ability of rats to metabolize test compounds was studied. The test compounds selected for this study were zoxazolamine and nitroanisole. The *in vivo* metabolism of zoxazolamine was followed by the duration of the loss of the righting reflex following treatment. The *in vitro* metabolism of nitroanisole in liver was measured in terms of the formation of *p*-nitrophenol.

2. Material and Methods

Male rats were fed diets that contained 0.5% TNG for 2 weeks. Rats in the positive control group received 50 mg/kg of phenobarbital sodium twice daily for 3 days. At the end of treatment, the zoxazolamine paralysis time and nitroanisole O-demethylase activity were determined.

Zoxazolamine was administered i.p. to three rats at a dose of 45 mg/kg in a vehicle of 0.2 N HCl. The duration of paralysis was measured in terms of the loss of the righting reflex. The values are reported as the mean ± S.E. and the test of significance was the two-sample rank[19] test. The level of significance was selected at $P < 0.05$.

The metabolism of nitroanisole by livers was measured in an *in vitro* system. Rats were sacrificed by decapitation and the livers were removed, weighed, and homogenized in 4 volumes of 1.15% potassium chloride. The homogenate was centrifuged at 9,000 x g for 30 minutes. The incubation medium contained 15 µmoles magnessium chloride, 15 µmoles glucose-6-phosphate, 3 µmoles *p*-nitroanisole, 0.5 ml of the 9,000 x g liver supernatant, and 0.5 ml of 0.5 M sodium phosphate buffer pH 7.8. Reactions were conducted for 20 minutes in a shaking water bath at 37°C. The reaction was terminated by the addition of 0.5 ml of 40% formalin and the color was developed with 0.5 ml of 0.8 N sodium hydroxide. The product formed was measured spectrophotometrically at 420 nm. The

relationship of μmoles p-nitrophenol formed = Absorbance Units/10.22 was used to quantitate product formed. The Lowry protein assay[16] was used to measure protein content. The activity was expressed as nanomoles p-nitrophenol/mg protein. The values are reported as the mean ± S.E. The test and level of significance were the same as described above.

3. Results

The results on the zoxazolamine paralysis in rats are summarized in Table 97. Pretreatment of phenobarbital sodium significantly simulated the metabolism of zoxazolamine and reduced the duration of paralysis as compared to control rats. On the other hand, feeding of 0.5% TNG for 2 weeks did not affect the duration of zoxazolamine paralysis. This result indicates that the liver enzyme(s) to metabolize zoxazolamine was not affected by TNG.

The results of nitroanisole O-demethylase activity in rat livers are summarized in Table 98. The nitroanisole O-demethylase activity in livers of rats fed 0.5% TNG for 2 weeks was not significantly different from that of the control rats.

4. Conclusion

Feeding of 0.5% TNG for 2 weeks did not affect the liver enzyme(s) to metabolize zoxazolamine *in vivo*, nor affected the O-demethylase activity in the liver *in vitro*.

TABLE 85

DISTRIBUTION AND EXCRETION OF RADIOACTIVITY IN VARIOUS SPECIES OF ANIMALS 24 HR AFTER ORAL ADMINISTRATION OF A SINGLE DOSE OF TNG-1,3-^{14}C

	% of Administered Dose				
	Mice	Rats	Rabbits	Dogs	Monkeys
GI plus Contents	2.2 ± 0.1[c]	3.0 ± 0.2[c]	3.7[d]	4.8[d]	8.4 ± 3.5[e]
Feces	28.5 ± 4.2	6.3 ± 1.0	0.1	1.8	3.2 ± 1.6
Whole Blood[a]	0.3 ± 0.0	0.7 ± 0.0	0.6	1.6	2.1 ± 0.1
Expired Air	19.2 ± 1.2	25.5 ± 1.5	7.8	2.5	3.6 ± 0.6
Urine	18.8 ± 7.5	39.8 ± 2.6	72.0	71.6	45.0 ± 0.9
Liver	6.3 ± 0.6	4.3 ± 0.9	4.8	6.4	6.8 ± 2.2
Kidneys	0.2 ± 0.0	0.3 ± 0.0	0.2	0.4	0.3 ± 0.0
Spleen	< 0.1	--	<0.1	0.1	<0.1
Lungs	0.1 ± 0.0	0.1 ± 0.0	0.1	0.3	0.2 ± 0.0
Brain	0.1 ± 0.0	0.1 ± 0.0	<0.1	0.2	0.7 ± 0.1
Muscle[b]	2.5 ± 0.7	2.8 ± 0.2	2.1	9.5	13.0 ± 1.0
Recovery	78.5 ± 9.7	82.9 ± 6.4	91.4	99.2	83.3 ± 3.8

a/ Based on 7% of the body weight.
b/ Based on 40% of the body weight.
c/ Mean ± S.E. of four animals.
d/ Mean of two animals.
e/ Mean ± S.E. of three animals.

TABLE 86

TISSUE/PLASMA RADIOACTIVITY RATIOS[a] IN VARIOUS SPECIES OF ANIMALS 24 HR AFTER ORAL ADMINISTRATION OF A SINGLE DOSE OF TNG-1,3-^{14}C

Tissue	Mice	Rats	Rabbits	Dogs	Monkeys
Liver	30.1 ± 5.6[b]	7.8 ± 1.7[b]	21.0[c]	7.5[c]	7.4 ± 2.8[d]
Kidney	4.1 ± 1.1	2.8 ± 0.3	4.3	2.7	2.2 ± 0.2
Spleen	2.6 ± 0.7	--	3.3	1.8	1.3 ± 0.1
Lungs	2.8 ± 0.7	1.7 ± 0.1	2.8	1.4	1.3 ± 0.1
Brain	1.2 ± 0.2	1.0 ± 0.1	1.5	0.7	0.9 ± 0.3
Muscle	1.8 ± 0.6	0.6 ± 0.1	0.6	0.8	0.8 ± 0.1

a/ Radioactivity in 1 gm of wet tissue per radioactivity in 1 ml of plasma.
b/ Mean ± S.E. of four animals.
c/ Mean of two animals.
d/ Mean ± S.E. of three animals.

TABLE 87

METABOLITES OF TNG IN URINE OF VARIOUS SPECIES COLLECTED FOR 24 HR AFTER ORAL ADMINISTRATION OF A SINGLE DOSE OF TNG-1,3-^{14}C

Metabolite	% of Administered Dose				
	Mice	Rats	Rabbits	Dogs	Monkeys
TNG	<0.1[a]	<0.1[a]	0.1[b]	0.8[b]	0.3[a]
1,3-DNG	<0.1	0.4	0.2	2.8	1.3
1,2-DNG	<0.1	0.7	0.1	0.7	1.2
MNGs	2.0	10.6	2.4	18.2	15.8
1,3-DNG-glucuronide	1.5	3.5	11.2	5.5	0.4
1,2-DNG-glucuronide	0.9	10.0	2.8	3.4	0.5
MNG-glucuronides	0.2	1.5	1.6	5.2	7.5
Glycerol	2.1	6.9	8.8	8.8	4.8
Polar Compounds					
R_f 0.0 in Solvent C	0.8	4.9	5.4	18.6	12.4
R_f 0.6 in Solvent C	11.2	1.3	39.4	3.4	0.2

a/ Mean of three animals.
b/ Mean of two animals.

TABLE 88

BILIARY EXCRETION OF RADIOACTIVITY IN RATS AFTER ORAL ADMINISTRATION OF A SINGLE DOSE OF TNG-1,3-^{14}C

Time After Dosing (hours)	Bile Volume (ml)	Excretion in Bile (% of Dose)	Excretion in Bile (Cumulative %)	Excretion Rate (% of dose/min)	Blood Concentration (dpm/ml)
1/4	0.26 ± 0.06[a/]	0.04 ± 0.02	0.04 ± 0.02	$3.0 \pm 0.9 \times 10^{-3}$	$1.6 \pm 0.4 \times 10^{3}$
1/2	0.26 ± 0.06	0.11 ± 0.03	0.15 ± 0.04	$7.3 \pm 2.0 \times 10^{-3}$	$2.6 \pm 0.6 \times 10^{3}$
1	0.60 ± 0.07	0.41 ± 0.12	0.56 ± 0.16	$14.6 \pm 3.9 \times 10^{-3}$	$4.4 \pm 0.6 \times 10^{3}$
1-1/2	0.60 ± 0.07	0.53 ± 0.16	1.09 ± 0.29	$17.7 \pm 5.2 \times 10^{-3}$	$5.9 \pm 1.0 \times 10^{3}$
2	0.57 ± 0.06	0.83 ± 0.14	1.92 ± 0.42	$27.7 \pm 7.6 \times 10^{-3}$	$9.0 \pm 1.6 \times 10^{3}$
3	1.11 ± 0.11	1.89 ± 0.63	3.81 ± 1.03	$31.3 \pm 10.3 \times 10^{-3}$	$15.2 \pm 2.8 \times 10^{3}$
4	1.04 ± 0.18	1.72 ± 0.29	5.53 ± 1.27	$28.6 \pm 4.6 \times 10^{-3}$	$23.4 \pm 3.1 \times 10^{3}$
5	0.91 ± 0.14	1.16 ± 0.17	6.69 ± 1.34	$19.3 \pm 2.9 \times 10^{-3}$	$25.9 \pm 1.1 \times 10^{3}$
6	0.83 ± 0.13	1.15 ± 0.27	7.84 ± 1.13	$19.1 \pm 6.4 \times 10^{-3}$	$26.0 \pm 2.2 \times 10^{3}$
23	9.02 ± 0.90	5.61 ± 1.75	13.45 ± 1.41	$5.3 \pm 1.4 \times 10^{-3}$	--
24	0.44 ± 0.07	0.05 ± 0.01	13.50 ± 1.42	$0.8 \pm 0.2 \times 10^{-3}$	$13.0 \pm 0.6 \times 10^{3}$

a/ Mean ± S.E. of three rats.

TABLE 89

TOTAL RADIOACTIVITY EXCRETED IN THE BILE AND REMAINING IN THE GASTROINTESTINAL TRACT IN RATS 24 HOURS AFTER ORAL ADMINISTRATION OF A SINGLE DOSE OF DNGS- OR MNGS-1,3^{14}C

Compound	Excretion in Bile (% of Dose)			Radioactivity in GI Tract[b/] (% of Dose)
	0-4 hr	4-24 hr	Total	
1-MNG	0.56 ± 0.64[a/]	3.25 ± 0.86	3.81 ± 0.89	4.64 ± 0.94
2-MNG	2.50 ± 0.50	16.40 ± 1.94	18.90 ± 1.73	0.10 ± 0.03
1,2-DNG	4.30 ± 0.19	6.90 ± 0.97	11.20 ± 0.57	0.37 ± 0.01
1,3-DNG	0.66 ± 0.07	0.97 ± 0.17	1.63 ± 0.14	0.01 ± 0.00

a/ Mean ± S.E. of three rats.
b/ Includes gastrointestinal contents and feces.

TABLE 90

METABOLITES OF TNG IN RAT BILE COLLECTED FOR 24 HOURS AFTER ORAL ADMINISTRATION OF A SINGLE DOSE OF TNG-1,3-^{14}C

Metabolites	Radioactivity in Bile	
	% in Bile	% of Dose
TNG	< 0.1	< 0.1
1,3-DNG	1.7 ± 0.3	0.3 ± 0.1
1,2-DNG	0.4 ± 0.1	0.1 ± 0.0
MNGs	6.4 ± 1.6	1.0 ± 0.2
1,3-DNG glucuronide	16.3 ± 2.6	2.5 ± 0.4
1,2-DNG glucuronide	25.9 ± 3.8	3.9 ± 0.6
MNG glucuronides	13.2 ± 4.7	2.0 ± 0.7
Glycerol	18.9 ± 4.2	2.8 ± 0.6
Polar compounds	17.2 ± 4.3	2.6 ± 0.6

a/ Mean ± S.E. of three rats.

TABLE 91

METABOLISM OF TNG-1,3-^{14}C IN RAT LIVER AS A FUNCTION OF TIME

Time (minutes)	nMoles/mg Protein		
	1,3-DNG	1,2-DNG	Origin
15	36.8[a/] ± 2.6	24.9 ± 1.9	2.8 ± 0.4
30	29.8 ± 1.8	20.2 ± 1.4	6.4 ± 0.8
60	19.4 ± 1.2	8.6 ± 0.8	13.3 ± 1.6
120	7.1 ± 0.5	5.7 ± 0.7	21.7 ± 2.4

a/ Mean ± S.E. for three livers from male rats.

TABLE 92

METABOLISM OF TNG-1,3-^{14}C IN LIVERS OF FEMALE RATS IN THE PRESENCE AND ABSENCE OF VARIOUS TISSUE PREPARATIONS

Tissue	nMoles/mg Protein 1,3 DNG	1,2 DNG
Liver[a/]	44.3[d/] ± 2.2	29.6 ± 1.8
Liver + Preparation 1[b/]	31.3 ± 0.7	23.5 ± 0.1
Liver + Preparation 2[c/]	49.5 ± 5.3	33.6 ± 3.4

a/ One volume liver homogenate.

b/ 0.5 volume liver homogenate and 0.5 volume homogenate of stomach and intestines.

c/ 0.5 volume liver homogenate and 0.5 volume homogenate of pancreas, spleen, and kidneys.

d/ Mean ± S.E. for three observations.

TABLE 93

METABOLISM OF 1-MNG-1,3-^{14}C IN LIVERS FROM FEMALE RATS IN THE PRESENCE AND ABSENCE OF VARIOUS TISSUE PREPARATIONS

Tissue	nMoles/mg Protein	
	1-MNG	Origin
Liver[a/]	93[d/] ±5	1.6 ± 0.3
Liver + Preparation 1[b/]	93 ± 5	1.3 ± 0.2
Liver + Preparation 2[c/]	93 ± 5	1.3 ± 0.2

a/ One volume liver homogenate.

b/ 0.5 volume liver homogenate and 0.5 volume homogenate of stomach and intestines.

c/ 0.5 volume liver homogenate and 0.5 volume homogenate of pancreas, spleen, and kidneys.

d/ Mean ± S.E. for three observations.

TABLE 94

METABOLISM OF TNG-1,3-^{14}C IN LIVERS FROM DIFFERENT SPECIES

Species	Number of Determinations	nMoles/mg Protein 1,3-DNG	1,2-DNG	1,3-DNG/1,2-DNG
Rat	7	40.6 ± 1.9[b]	28.0 ± 1.3	1.4
Mouse	6	16.7 ± 0.9	7.0 ± 0.3	2.4
Rabbit	6	18.3 ± 0.8	51.3 ± 2.2	0.36
Dog	7	21.1 ± 0.7	46.8 ± 2.0	0.45
Monkey	5	16.3 ± 2.1	52.7 ± 5.2	0.31
Human[a]	4	12.5 ± 2.9	32.5 ± 4.9	0.38

a/ Autopsy samples from a 65-year old Negro male died of a dissecting aneurysm of the aorta, a 21-year old white male died of a gunshot wound, a 67-year old white female died with CNS seizures, and a 60-year old female died with a lung carcinoma.

b/ Mean ± S.E. or mean of the indicated number of determinations. Values for males and females were combined.

TABLE 95

METABOLISM OF TNG-1,3-^{14}C IN PLACENTAS FROM VARIOUS SPECIES

Species	nMoles/mg Protein 1,3-DNG	1,2-DNG
Mouse		
Day 12	1.5 ± 0.8[a/]	3.9 ± 0.9
Day 18	3.1 ± 0.1	5.2 ± 0.4
Rat		
Day 19	2.5 ± 0.6	3.8 ± 0.2
Human		
Term	0.7 ± 0.4	4.7 ± 1.3

a/ Mean ± S.E. for three determinations.

TABLE S6

METABOLISM OF TNG-1,3-^{14}C DURING DEVELOPMENT IN MICE AND RATS

Species	Tissue	Days After Birth	nMoles/mg Protein 1,3-DNG	1,2-DNG
Mouse				
	Embryo	-7	0	3.8 ± 0.9
	Liver	-1	7.4 ± 0.6[a/]	5.9 ± 0.2
	Carcass	-1	3.4 ± 1.2	4.9 ± 0.4
Rat				
	Liver	-2	12.2 ± 0.8	9.4 ± 1.0
	Liver	1	14.6 ± 0.4	9.1 ± 0.1
	Liver	7	27.0 ± 1.0	23.1 ± 0.6
	Liver	14	26.9 ± 1.2	21.4 ± 2.0
	Liver	21	29.1 ± 1.0	24.8 ± 1.8

a/ Mean ± S.E. for three determinations.

TABLE 97

DURATION OF ZOXAZOLAMINE PARALYSIS IN MALE RATS PRETREATED WITH PHENOBARBITAL SODIUM OR FED A CONTROL DIET OR A DIET CONTAINING 0.5% TNG

Treatment	Duration of Paralysis (Minutes)
Experiment I	
Control	98 ± 11 (10)[c]
Phenobarbital[a]	34 ± [illegible] (7)[d]
Experiment II	
Control	134 ± 14 (8)
0.5% TNG[b]	149 ± 11 (8)

a/ 50 mg/kg, I.P., twice daily for 3 days.
b/ Incorporated into diet and fed for 2 weeks.
c/ Mean + S.E. (number of observations).
d/ Significantly different from control (two rank test[19]).

TABLE 98

NITROANISOLE O-DEMETHYLASE ACTIVITY IN LIVERS OF RATS FED A CONTROL DIET OR A DIET CONTAINING 0.5% TNG

Treatment	Activity[b]
Control	1.32 ± 0.17 (3)[c]
0.5% TNG[a]	1.14 ± 0.01 (3)

a/ Incorporated into diet and fed for 2 weeks.
b/ nMoles p-nitrophenol/mg protein in 9,000 x g supernatant.
c/ Mean ± S.E. (number of observations).

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APPENDIX I

MANUAL FOR

HEMATOLOGY, CLINICAL BLOOD CHEMISTRY, URINALYSIS, HISTOPATHOLOGY, STATISTICAL ANALYSIS, AND NORMAL VALUES

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May 1975

APPENDIX I

MANUAL FOR

HEMATOLOGY, CLINICAL LABORATORY TESTS, HISTOPATHOLOGY, STATISTICAL ANALYSIS, AND NORMAL VALUES

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HEMATOLOGY, CLINICAL LABORATORY TESTS, HISTOPATHOLOGY, STATISTICAL ANALYSIS, AND NORMAL VALUES

I. HEMATOLOGY AND CLINICAL LABORATORY TESTS

The usual blood sample from dogs is 8 ml, from monkeys 4 ml, and from rats 0.3 ml for hematology and about 8 ml for full analysis at termination.

A. Hematology

The following hematological analyses are performed on all blood samples from rats, dogs and monkeys.

1. Erythrocyte and leukocyte counts: A Coulter Electronic Particle Counter with 100 μ aperture is used.[1] Particle-free diluents (Isoton for RBC, Zap-Oglobin in Isoton for WBC, Coulter Electronics, Inc.) are counted to establish the background. Each blood sample is counted in duplicate. For each test day, two control blood samples (Diagnostic Technology, Inc.) are counted separately in duplicate.

2. Hematocrit: Hematocrit is determined in capillary tubes using a microcapillary centrifuge (International Equipment Company, Model MB). Two control blood samples (Diagnostic Technology, Inc.) are measured separately in duplicate.

3. Hemoglobin: Hemoglobin is measured as cyanomethemoglobin.[2] Each blood sample is measured in duplicate. Cyanomethemoglobin (Coulter Electronics, Inc.) is used as the standard. For each assay, two levels of the standard are used and two control blood samples (Diagnostic Technology, Inc.) are measured in duplicate.

4. Methemoglobin (Met-Hb): Met-Hb is measured by the method of Dubowski.[3] A positive control is made by adding potassium ferricyanide to control blood.

5. Heinz bodies: Heinz bodies are stained with methyl violet and the percent of Heinz bodies is calculated.

6. Mean corpuscular volume (MCV): MCV is calculated as follows:

$$\text{MCV } (\mu^3) = \frac{\text{Hematocrit x 10}}{\text{Erythrocytes in millions/mm}^3}$$

7. Mean corpuscular hemoglobin (MCHb): MCHb is calculated as follows:

$$\text{MCHb } (\mu\mu g) = \frac{\text{Hemoglobin (gm \%) x 10}}{\text{Erythrocytes in millions/mm}^3}$$

8. Mean corpuscular hemoglobin concentration (MCHbC): MCHbC is calculated as follows:

$$\text{MCHbC (gm \%)} = \frac{\text{Hemoglobin (gm \%) x 100}}{\text{Hematocrit}}$$

9. Differential leukocyte counts: Wright's stain is used to stain the leukocytes for examination.

10. Reticulocyte count: Reticulocytes are counted by the methylene blue method using the Miller disc.[4]

11. Platelet count: A Coulter Electronic Particle Counter with 70 μ aperture is used.[5] Particle-free Isoton is used as diluent and counted to establish the background. At weekly intervals, platelets are also visually counted in a hemocytometer with a phase microscope for comparison.[6]

12. Clotting time (dog and monkey): Clotting time is determined by the capillary tube procedure using two capillary tubes.[7] The time elapsed from the appearance of the blood from the animal and coagulation in either tube is measured.

B. Clinical Blood Tests

The following clinical blood chemistry tests are performed on all blood samples from dogs and monkeys and on blood samples from rats at termination.

1. Blood glucose: Fasting blood glucose is determined by Stein's hexokinase method.[8] Standard glucose solution (Dade) is used to establish a standard curve. For each assay, one level of the standard and two controls (Reference Serum, Worthington; and Validate, General Diagnostics) are measured.

2. Serum glutamic-oxaloacetic transaminase (SGOT): SGOT is measured by the method of Amador and Wacker.[9] Validate and Reference Serum are used as the enzyme reference for each assay.

3. Serum glutamic-pyruvic transaminase (SGPT): SGPT is measured by the method of Henry et al.[10] Validate and Reference Serum are used as the enzyme reference for each assay.

4. Alkaline phosphatase: Alkaline phosphatase is measured by the method of Bowers and McComb.[11] Validate and Reference Serum are used as the enzyme reference for each assay.

5. BUN: BUN is measured using the BUN Strate Kit (General Diagnostic) which is based on the urease method.[12] Three levels of Calibrate (General Diagnostics) are used to establish a standard curve. For each assay, two controls (Calibrate I and Validate) are used as the reference.

6. Creatinine: Creatinine is measured by a modified kinetic alkaline picrate procedure.[13] Creatinine Standard Solutions (Sigma Chemical Company) are used to establish a standard curve. For each assay, two levels of the standard and two controls (Calibrate I and Validate) are used as reference.

7. Lactate dehydrogenase (LDH): LDH is measured by the method of Wacker et al.[14] Precinorm E and Precipath E (Boehringer, Mannheim Corporation) are used as the enzyme controls for each assay.

8. α-Hydroxybutyrate dehydrogenase (α-HBDH): α HBDH is measured by the method of Rosalki and Wilkinson.[15] Precinorm E and Precipath E are used as the enzyme controls for each assay.

9. Creatine phosphokinase (CPK): CPK is measured by the improved procedure of Rosalki[16] based on the methods of Oliver.[17] Precinorm E and Precipath E are used as the enzyme controls for each assay.

C. Urinalysis

Urine samples are collected from animals before and during treatment as are the blood samples. The urine from rats is collected by slight manipulation of their body, and samples within each group are pooled. The monkeys and dogs are placed individually in metabolism cages, and urine is collected in the stainless steel pan. The urine from each dog and the pooled urine from rats are tested and examined for the following:

1. Protein: Urinary protein is determined with Labstix (Ames Company, Elkhart, Indiana).

2. Sugar: Urinary glucose and reducing substance are determined with Labstix (Ames Company).

3. Microscopic examination: Urine samples are centrifuged and the supernatant discarded. The residue is resuspended and examined microscopically for the presence of erythrocytes, leukocytes, epithelial cells, and crystals under high power field and for casts under low power field.

A positive urine control prepared with known amounts of protein and glucose in saline adjusted to pH 6.0 is run with each assay to check the reliability of the Labstix.

D. Occult Blood in Feces

Fecal samples are collected from animals before and during treatment as are the blood and urine samples. Occult blood in the feces is determined with Hematest Reagent Tablets (Ames Company, Elkhart, Indiana). A positive control (whole blood) and a negative control (distilled water) are included with each assay to check the reliability of the Hematest tablets.

E. Precision of Hematology and Clinical Blood Chemistry Tests

1. Reproducibility

For erythrocyte and leukocyte counts, hematocrit, hemoglobin, and the various clinical blood chemistry tests, the same control blood samples or control standards are used for day-to-day assays. The replication of results are excellent and are summarized in Table A.

The determination of differential leukocyte counts and reticulocyte counts are performed by experienced personnel. At weekly intervals, a blood sample is counted by two or more personnel to confirm the accuracy of the counting. Also at weekly intervals, the platelet counts obtained from a Coulter Electronic Particle Counter are compared with the direct visual counts in a hemocytometer using a phase microscope.

2. Reproducibility Within a Test Day

At monthly intervals, a blood sample is taken from a control dog and six or more determinations for erythrocyte, leukocyte, reticulocyte, and platelet counts, hemoglobin, and various clinical blood chemistry tests are performed to establish the reproducibility within an assay. The results are summarized in Table B.

3. Proficiency Test Service

We subscribe to the Proficiency Test Service of the Institute for Clinical Science, Hahnemann Medical College, Philadelphia, Pennsylvania (F. Wm. Sunderman, M.D., Director). On the first day of each month, this service sends two samples containing two different sera or solutions to all subscribers for measurements of one or more of the parameters usually analyzed in clinical laboratories. Participants report their results on a form furnished by the service. On the 15th day of the month, each participant receives a report from the service which includes: the results of a statistical analysis of the values reported by all the participating laboratories; a current review of pertinent methodology; a comprehensive bibliography; and validation of the results which the participating laboratory reported. This service enables each participating laboratory to obtain an unbiased and critical assessment of its proficiency in relation to that of 1,000 or so other clinical laboratories throughout the country. The service has been in continuous operation since 1949 and was given endorsement by the American Society of Clinical Pathologists in 1952 and by the Association of Clinical Scientists in 1957 and 1968. Our results have been found to be satisfactory and are summarized in Table C.

II. HISTOPATHOLOGY

A. Necropsy and Gross Examination

At termination or prior to imminent death, rats are killed with ether, and dogs and monkeys with an overdose of sodium pentobarbital. Animals that die on tests are kept refrigerated but not frozen until necropsy. The general physical condition and nutritional status of each animal at the time of death or termination are observed and recorded. Necropsy is performed as soon as possible after death. Gross changes of all tissues are carefully examined and recorded.

B. Organ Weights

The brain, liver, spleen, kidneys, adrenals, thyroids and gonads are trimmed free from surrounding tissues and weighed. The organ weight to body weight and/or brain weight ratios are then calculated.

C. Tissues for Microscopic Examination

Tissues to be examined include the eye, skin (breast), trachea, lung, tongue (except rat), salivary gland, liver, gallbladder (except rats), pancreas, esophagus, fundic and pyloric stomach, duodenum, jejunum, ileum, cecum, colon, kidneys, urinary bladder, gonads, and accessory organs, diaphragm and gracilis muscle, anterior pituitary, thyroids/parathyroids, adrenals, tonsil (except rat), thymus, spleen, prescapular (except rats) and mesenteric lymph nodes, rib bone with bone marrow, brain (sagittal section for rats; coronal sections of cerebral cortex, cerebellum, and brain stem for dog and monkey), spinal cord (lumbosacral plexus, dog and monkey), sciatic nerve and any other structures not mentioned which show abnormal gross changes.

D. Fixation and Staining of Tissues

All tissues are cut not to exceed 1 cm in thickness for fixation. For most tissues, neutral buffered 10% formalin is used. Sufficient volume of fixing solution is used and the tissues are changed to a fresh solution after 24 hours. The fixed tissues are processed in an Autotechnicon for dehydration, clearing, and infiltration and then embedded in paraffin. Routine H & E staining is used to stain the sectioned tissues for microscopic examination.

Supplementary tissue fixatives and staining techniques may be employed for more positive identification of special lesions such as calcification, pigments, fat deposition and other abnormal changes.

III. STATISTICAL ANALYSIS

Data are analyzed statistically using the Dunnett's multiple comparison procedure following an analysis of variance,[18] or our modification of this procedure for uneven numbers among groups. The chosen criterion significance is $p < 0.05$. The means of each group at various intervals during treatment are compared with pretreatment levels. For most experiments in beagles, three baseline (pretreatment) levels are obtained. The baseline levels for each animal are averaged and the mean is used in the analysis. In addition, the means of the various treated groups are compared with that of the control group at the respective time intervals.

IV. NORMAL VALUES

A. Hematology, Clinical Laboratory Tests and Bone Marrow

Since June 1971, we have used about 180 rhesus monkeys (Woodard Research Corporation, Herndon, Virginia, Primate Imports, Port Washington, New York, and PrimeLabs, Inc., Farmingdale, New Jersey) for various studies. The peripheral blood elements and clinical blood chemistry values of these monkeys before treatment and the myeloid/erythroid (M/E) ratio of the bone marrow of the monkeys used as normal controls varied among individual animals. The mean ± S.D. and the range of the various parameters for the males and females are summarized in Tables D and E, respectively.

Since September 1971, we have used about 525, 5 to 9 months old, beagles dogs (AKC registered, Hazelton Research Animals, Inc.). The peripheral blood elements, clinical blood chemistry values and the M/E ratio of the bone marrow varied considerably among individual dogs. The mean ± S.D. and the ranges of the various parameters for the males and females are summarized in Tables H and I, respectively.

During the same period, we have used about 500, 7 to 10 weeks old, male albino rats (CD® Strain, Charles River Breeding Laboratories). As for the dogs, the individual variations of the peripheral blood elements, clinical blood chemistry values and the M/E ratio of the bone marrow were large. The mean ± S.D. and the ranges of the various parameters for these male rats are summarized in Table L.

B. Absolute and Relative Organ Weights

Organ weights, both absolute and relative to body weight, of rhesus monkeys, beagle dogs, and albino rats are summarized in Tables F and G, J and K, and M, respectively. These were control animals used between June 1971 and December 1976.

C. Presence of Various Substances in the Urine

Various substances occasionally occurred in the urine of monkeys, dogs and rats. The results are summarized in Table N. Large percentage of urine samples from monkeys contained epithelial cells, i.e., 34.7% to 52.0%. Other substances occurred in 8.1% or less of the urine samples.

In dogs, protein, erythrocytes, leukocytes and epithelial cells were present in 19.1 to 21.6%, 16.5 to 19.8%, 22.6 to 24.6% or 24.7 to 25.7%, respectively, of the samples from dogs collected for analysis. Glucose,

crystals, and casts occurred in less than 2% of these samples. Some dogs had been bled and returned to the metabolism cages before the urine was removed for analysis. The high incidence of some of these substances in the urine of these dogs might be due to contamination with the fecal material and traces of blood dropped in the cage. Special care to avoid contamination has been undertaken.

In rats, large percentage of urine samples contained protein, i.e., 29.8 to 36.0%. A few samples contained erythrocytes, leukocytes, epithelial cells and crystals.

D. Occult Blood in the Feces

Less than 10% of the feces samples from monkeys or dogs was positive with the Hematest for occult blood. The results are summarized in Table O.

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TABLE A

REPRODUCIBILITY AMONG TEST DAYS ON THE SAME CONTROL SAMPLES OR STANDARDS[a]

	No. of Determinations	Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)			
Normal level	20	4.51 ± 0.07	4.36 - 4.67
Abnormal level	20	2.32 ± 0.04	2.25 - 2.40
Hematocrit (vol %)			
Normal level	20	44.3 ± 0.40	44 - 45
Abnormal level	20	22.8 ± 0.60	22 - 24
Hemoglobin (gm %)			
Normal level	20	14.2 ± 0.20	13.6 - 14.5
Abnormal level	20	7.4 ± 0.20	6.9 - 7.8
Leukocyte Counts (x 10^3/mm^3)			
Normal level	20	7.3 ± 0.50	6.8 - 8.7
Abnormal level	20	17.6 ± 0.80	16.3 - 18.7
Fasting Blood Glucose (mg %)	20	163.0 ± 7.5	151 - 178
SGOT (IU/ℓ)	23	61.7 ± 3.9	55 - 68
SGPT (IU/ℓ)	23	51.3 ± 2.6	46 - 55
Creatinine (·	18	2.2 ± 0.3	1.6 - 2.6
BUN (mg %)	19	9.8 ± 0.2	9.5 - 10.2
Bilirubin (mg %)	11	0.8 ± 0.1	0.8 - 1.0
Alkaline Phosphatase (IU/ℓ)	22	71.6 ± 5.4	62 - 80
CPK	11	153.0 ± 7.7	139 - 161
LDH	8	98.0 ± 2.4	95 - 101
HBDH	8	226.0 ± 7.2	214 - 238

a/ Performed in December 1976.

TABLE B

REPRODUCIBILITY WITHIN A TEST DAY
ON THE SAME SPECIMEN[a/]

	Mean ± S.D.[b/]	Range
Erythrocytes (x 10^6/mm^3)	5.90 ± 0.14	5.73 - 6.08
Reticulocytes (%)	0.63 ± 0.12	0.44 - 0.79
Hematocrit (vol %)	46.8 ± 0.6	46.0 - 47.5
Hemoglobin (gm %)	16.1 ± 0.2	15.8 - 16.1
Platelets (x 10^5/mm^3)	1.56 ± 0.07	1.49 - 1.66
Leukocytes (x 10^3/mm^3)	10.8 ± 0.4	10.2 - 11.3
Bands (%)	0 ± 0	0 - 0
Neutrophils (%)	64.3 ± 3.1	61 - 69
Lymphocytes (%)	29.0 ± 4.9	23 - 35
Eosinophils (%)	3.2 ± 0.8	2 - 4
Basophils (%)	0 ± 0	0 - 0
Monocytes (%)	3.4 ± 0.9	3 - 5
Atypical (%)	0 ± 0	0 - 0
Nucleated RBC (%)	0 ± 0	0 - 0
Methemoglobin (gm %)	0 ± 0	0 - 0
Fasting Glucose (mg %)	96.7 ± 3.0	32 - 101
SGOT (IU/ℓ)	23.2 ± 2.8	21 - 28
SGPT (IU/ℓ)	25.3 ± 2.1	24 - 28
Creatinine (mg %)	0.6 ± 0.1	0.5 - 0.6
BUN (mg %)	9.0 ± 0.0	9 - 9
Alkaline Phosphatase (IU/ℓ)	63.5 ± 1.1	62 - 65
CPK	44.0 ± 1.6	43 - 46
LDH	38.5 ± 1.6	37 - 40
HBDH	42.0 ± 1.6	40 - 43

a/ Performed in October 1976.
b/ Six determinations from an adult beagle blood sample.

TABLE C

PROFICIENCY TEST SERVICE (PTS) REPORTS (1975-1976)[a/]

Unknowns	MRI Results	PTS Results	Participating Laboratories (10-90 Percentiles) Median	Mean	Acceptable Performance[b/]
Hemoglobin	13.8 gm %	13.8	13.8	13.8	13.6 - 14.0
	18.1 gm %	17.9	17.9	17.8	17.6 - 18.2
Serum Protein	6.6 mg %	7.1	7.0	7.0	6.7 - 7.3
Fasting Glucose	272.0 mg %	264.5	266.0	263.0	240 - 290
	229.0 mg %	221.4	220.5	222.5	200 - 240
BUN	12.1 mg %	12.0	12.0	12.2	11.0 - 13.0
	38.4 mg %	40.1	40.3	39.2	36.0 - 44.0
Creatinine	1.0 mg %	1.0	1.0	1.0	0.8 - 1.3
	4.3 mg %	4.4	4.5	4.4	3.9 - 4.9
Bilirubin	3.9 mg %	4.16	4.15	4.14	3.5 - 4.6
	1.3 mg %	1.78	1.80	1.77	1.5 - 2.1
Cholesterol	175.0 mg %	161.4	161.0	162.0	145 - 175
	100.0 mg %	109.8	109.4	111.0	98 - 120
Ca	15.7 meq/ℓ	15.4	15.4	15.3	14.1 - 16.4
	9.5 meq/ℓ	9.8	9.8	9.8	9.2 - 10.3
Na	156.0 meq/ℓ	155.8	156.0	155.5	153 - 158
K	7.3 meq/ℓ	7.5	7.5	7.5	7.3 - 7.7
Cl	96.0 meq/ℓ	97.8	98.0	97.5	96 - 101
	78.0 meq/ℓ	79.4	79.0	80.0	77 - 83
Mg	1.0 meq/ℓ	1.1	1.1	1.2	0.9 - 1.4
	1.9 meq/ℓ	2.0	2.0	2.1	1.8 - 2.3

a/ To date, we have received unknowns for phosphorus, uric acid, and serum iron. We do not routinely perform these determinations.

b/ Based on values submitted by participants by 10th of month.

TABLE D

HEMATOLOGY, CLINICAL BLOOD CHEMISTRY VALUES, AND BONE MARROW (MYELOID/ERYTHROID) RATIOS OF MALE RHESUS MONKEYS[a/]

	Male Rhesus Monkeys			
	Number Studied	Body Weight (kg) Mean ± S.D.	Observed Results Mean ± S.D.	Range
Erythrocytes (x $10^6/mm^3$)	108	3.74 ± 0.50	5.51 ± 0.45	3.75 - 6.61
Reticulocytes (%)	108	3.74 ± 0.50	0.97 ± 0.82	0.07 - 2.41
Hematocrit (vol %)	108	3.74 ± 0.50	43.0 ± 2.6	37.0 - 50.0
Hemoglobin (gm %)	108	3.74 ± 0.50	13.4 ± 0.8	10.8 - 15.4
MCV (μ^3)	108	3.74 ± 0.50	77.8 ± 7.0	69.6 - 117.3
MCHb ($\mu\mu g$)	108	3.74 ± 0.50	24.4 ± 1.8	21.0 - 33.6
MCHbC (mg %)	108	3.74 ± 0.50	31.4 ± 1.3	27.2 - 34.1
Platelets (x $10^5/mm^3$)	99	3.74 ± 0.50	3.08 ± 0.45	0.80 - 4.10
Leukocytes (x $10^3/mm^3$)	108	3.74 ± 0.50	10.4 ± 4.9	3.8 - 30.1
Neutrophils I (%)	108	3.74 ± 0.50	0.18 ± 0.45	0 - 2
Neutrophils M (%)	108	3.74 ± 0.50	39.30 ± 17.72	10 - 83
Lymphocytes (%)	108	3.74 ± 0.50	56.83 ± 17.74	13 - 84
Eosinophils (%)	108	3.74 ± 0.50	1.91 ± 2.42	0 - 13
Monophils (%)	108	3.74 ± 0.50	1.37 ± 1.58	0 - 7
Basophils (%)	108	3.74 ± 0.50	0.04 ± 0.20	0 - 2
Atypical cells (%)	108	3.74 ± 0.50	0.00 ± 0.00	0 - 0
Nucleated RBC (%)		3.74 ± 0.50	0.00 ± 0.00	0 - 0
Fasting Glucose (mg %)	100	3.76 ± 0.51	96.9 ± 15.2	59 - 127
SGOT (IU/ℓ)	100	3.76 ± 0.51	33.7 ± 9.2	20 - 60
SGPT (IU/ℓ)	100	3.76 ± 0.51	31.3 ± 7.8	15 - 46
Alkaline Phosphatase (IU/ℓ)	100	3.76 ± 0.51	360.0 ± 116.0	143 - 501
BUN (mg %)	100	3.76 ± 0.51	19.5 ± 7.5	12 - 65
Proth. Time (sec)	62	3.91 ± 0.44	10.2 ± 0.7	9.3 - 11.9
Serum Creat. (mg %)	100	3.76 ± 0.51	1.1 ± 0.3	0.6 - 1.8
Bilirubin				
Total (mg %)	62	3.91 ± 0.44	0.1 ± 0.2	0.0 - 0.8
Direct (mg %)	62	3.91 ± 0.44	0.0 ± 0.0	0.0 - 0.0
BSP 15 min (% ret.)	62	3.91 ± 0.44	18.0 ± 7.4	2 - 34
Na (mEq/ℓ)	62	3.91 ± 0.44	154.0 ± 19.1	144 - 179
K (mEq/ℓ)	62	3.91 ± 0.44	4.8 ± 0.6	3.9 - 5.7
Cl (mEq/ℓ)	62	3.91 ± 0.44	109.0 ± 6.4	93 - 118
Ca (mEq/ℓ)	62	3.91 ± 0.44	5.2 ± 0.4	4.2 - 6.3
Mg (mEq/ℓ)	62	3.91 ± 0.44	1.6 ± 0.1	1.2 - 1.8
Bone Marrow				
Myeloid/erythroid ratio	15	3.65 ± 0.41	1.5 ± 0.3	1.5 - 2.1

a/ Data collected between June 1971 and December 1976.

TABLE E

HEMATOLOGY, CLINICAL BLOOD CHEMISTRY VALUES, AND BONE MARROW (MYELOID/ERYTHROID) RATIOS OF FEMALE RHESUS MONKEYS[a/]

	Female Rhesus Monkeys			
	Number Studied	Body Weight (kg) Mean ± S.D.	Observed Results Mean ± S.D.	Range
Erythrocytes (x $10^6/mm^3$)	81	3.51 ± 0.48	5.33 ± 0.40	4.25 - 6.03
Reticulocytes (%)	81	3.51 ± 0.48	1.07 ± 0.51	0.35 - 3.31
Hematocrit (vol %)	81	3.51 ± 0.48	41.5 ± 2.8	30.0 - 46.0
Hemoglobin (gm %)	81	3.51 ± 0.48	13.7 ± 1.0	7.9 - 14.1
MCV (μ^3)	81	3.51 ± 0.48	77.7 ± 5.3	66.5 - 95.2
MCHb ($\mu\mu g$)	81	3.51 ± 0.48	24.6 ± 1.7	17.6 - 29.7
MCHbC (mg %)	81	3.51 ± 0.48	31.6 ± 1.4	26.6 - 34.2
Platelets (x $10^5/mm^3$)	81	3.51 ± 0.48	3.11 ± 1.23	1.85 - 7.90
Leukocytes (x $10^3/mm^3$)	81	3.51 ± 0.48	9.5 ± 3.9	3.2 - 24.8
Neutrophils J (%)	81	3.51 ± 0.48	0.10 ± 0.43	0 - 3
Neutrophils M (%)	81	3.51 ± 0.48	36.41 ± 13.32	13 - 56
Lymphocytes (%)	81	3.51 ± 0.48	60.38 ± 13.26	41 - 79
Eosinophils (%)	81	3.51 ± 0.48	2.28 ± 3.10	0 - 18
Monophils (%)	81	3.51 ± 0.48	0.75 ± 0.98	0 - 4
Basophils (%)	81	3.51 ± 0.48	0.05 ± 0.22	0 - 1
Atypical cells (%)	81	3.51 ± 0.48	0.00 ± 0.00	0 - 0
Nucleated RBC (%)	74	3.56 ± 0.50	0.00 ± 0.00	0 - 0
Fasting Glucose (mg %)	81	3.51 ± 0.48	92.1 ± 15.3	57 - 116
SGOT (IU/ℓ)	81	3.51 ± 0.48	32.1 ± 7.6	20 - 70
SGPT (IU/ℓ)	81	3.51 ± 0.48	30.1 ± 7.6	12 - 39
Alkaline Phosphatase (IU/ℓ)	81	3.51 ± 0.48	349.9 ± 112.3	148 - 572
BUN (mg %)	81	3.51 ± 0.48	17.3 ± 4.2	13 - 29
Proth. Time (sec)	59	3.56 ± 0.43	10.5 ± 0.9	9.7 - 12.3
Serum Creat. (mg %)	81	3.51 ± 0.48	1.1 ± 0.3	0.6 - 1.7
Bilirubin				
Total (mg %)	81	3.51 ± 0.48	0.1 ± 0.1	0.0 - 0.8
Direct (mg %)	81	3.51 ± 0.48	0.0 ± 0.0	0.0 - 0.0
BSP 15 min (% ret.)	59	3.56 ± 0.43	10.4 ± 8.3	5 - 34
Na (mEq/ℓ)	59	3.56 ± 0.43	158.2 ± 6.5	147 - 174
K (mEq/ℓ)	59	3.56 ± 0.43	4.8 ± 0.7	3.9 - 6.2
Cl (mEq/ℓ)	59	3.56 ± 0.43	109.0 ± 6.1	95 - 113
Ca (mEq/ℓ)	59	3.56 ± 0.43	5.2 ± 0.5	4.3 - 6.3
Mg (mEq/ℓ)	59	3.56 ± 0.43	1.6 ± 0.2	1.3 - 2.0
Bone Marrow				
Myeloid/erythroid ratio	11	3.49 ± 0.62	1.4 ± 0.3	1.0 - 1.8

a/ Data collected between June 1971 and December 1976.

TABLE F

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MALE RHESUS MONKEYS[a/]

Organ Weight	Absolute Mean ± S.D.	Range
Liver (gm)	82 ± 17	64 - 122
Spleen (gm)	4.6 ± 1.8	2.0 - 9.3
Kidneys (gm)	15.1 ± 3.8	8.0 - 22.0
Adrenals (gm)	0.73 ± 0.15	0.45 - 0.86
Thyroids (gm)	0.57 ± 1.30	0.37 - 0.81
Testes (gm)	1.29 ± 0.57	0.53 - 3.30
	Relative (per kg body weight)	
	Mean ± S.D.	**Range**
Liver (gm)	23.4 ± 2.5	18.8 - 30.4
Spleen (gm)	1.25 ± 0.47	0.57 - 2.38
Kidneys (gm)	4.13 ± 0.92	2.20 - 6.43
Adrenals (mg)	201 ± 44	129 - 254
Thyroids (mg)	154 ± 42	86 - 250
Testes (gm)	0.34 ± 0.11	0.18 - 0.53

a/ Data collected between September 1971 and December 1976 from 17 monkeys weighing 3.71 ± 0.48 kg, used as control animals.

TABLE G

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF FEMALE RHESUS MONKEYS[a/]

Organ Weight	Absolute Mean ± S.D.	Range
Liver (gm)	83 ± 17	64 - 122
Spleen (gm)	3 ± 1.4	2.0 - 6.0
Kidneys (gm)	14.5 ± 2.8	11.0 - 20.0
Adrenals (gm)	0.68 ± 0.16	0.53 - 1.14
Thyroids (gm)	0.60 ± 0.20	0.37 - 1.11
Ovaries (gm)	0.28 ± 0.10	0.14 - 0.45
	Relative (per kg body weight)	
	Mean ± S.D.	**Range**
Liver (gm)	25.4 ± 5.8	19.2 - 37.4
Spleen (gm)	1.16 ± 0.49	0.60 - 1.89
Kidneys (gm)	4.40 ± 0.86	3.20 - 6.25
Adrenals (mg)	212 ± 80	138 - 438
Thyroids (mg)	173 ± 66	97 - 346
Ovaries (mg)	82 ± 28	43 - 140

a/ Data collected between September 1971 and December 1976 from 11 monkeys weighing 3.39 ± 0.58 kg, used as controls.

TABLE H

HEMATOLOGY, CLINICAL BLOOD CHEMISTRY VALUES, AND BONE MARROW (MYELOID/ERYTHROID) RATIOS OF MALE BEAGLE DOGS[a]

	Male Beagle Dogs				
	Number Studied	Age (months)	Body Weight (kg) Mean ± S.D.	Observed Results Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)	276	4 - 7	8.3 ± 1.7	5.55 ± 0.73	3.62 - 7.60
Reticulocytes (%)	284	4 - 7	8.3 ± 1.7	0.72 ± 0.46	0.04 - 4.35
Hematocrit (vol %)	276	4 - 7	8.3 ± 1.7	41.6 ± 3.5	31 - 50
Hemoglobin (gm %)	276	4 - 7	8.3 ± 1.7	13.5 ± 1.4	10.0 - 16.9
MCV (μ^3)	276	4 - 7	8.3 ± 1.7	75.6 ± 8.3	56.7 - 127.1
MCHb ($\mu\mu$g)	276	4 - 7	8.3 ± 1.7	24.6 ± 3.0	17.1 - 41.7
MCHbC (mg %)	276	4 - 7	8.3 ± 1.7	32.5 ± 1.5	28.1 - 40.3
Platelets (x 10^5/mm^3)	270	4 - 7	8.4 ± 1.7	2.91 ± 1.02	0.93 - 6.35
Leukocytes (x 10^3/mm^3)	284	4 - 7	8.3 ± 1.7	11.9 ± 3.5	4.6 - 24.6
Neutrophils I (%)	284	4 - 7	8.3 ± 1.7	0.55 ± 1.06	0 - 6
Neutrophils M (%)	284	4 - 7	8.3 ± 1.7	56.81 ± 9.47	22 - 80
Lymphocytes (%)	284	4 - 7	8.3 ± 1.7	37.94 ± 9.26	13 - 71
Eosinophils (%)	284	4 - 7	8.3 ± 1.7	2.76 ± 2.93	0 - 16
Monophils (%)	284	4 - 7	8.3 ± 1.7	1.78 ± 1.84	0 - 11
Basophils (%)	284	4 - 7	8.3 ± 1.7	0.01 ± 0.10	0 - 2
Atypical cells (%)	284	4 - 7	8.3 ± 1.7	0.11 ± 0.37	0 - 2
Nucleated RBC (%)	284	4 - 7	8.3 ± 1.7	0.02 ± 0.10	0 - 2
Fasting Glucose (mg %)	284	4 - 7	8.3 ± 1.7	100.9 ± 12.6	66 - 134
SGOT (IU/ℓ)	276	4 - 7	8.3 ± 1.7	23.2 ± 7.4	11 - 59
SGPT (IU/ℓ)	276	4 - 7	8.3 ± 1.7	25.7 ± 7.9	8 - 46
Alkaline Phosphatase (IU/ℓ)	276	4 - 7	8.3 ± 1.7	73.3 ± 18.5	21 - 133
BUN (mg %)	284	4 - 7	8.3 ± 1.7	12.1 ± 3.3	4 - 23
Bone Marrow					
Myeloid/erythroid ratio	34	5 - 9	9.4 ± 1.6	1.6 ± 0.4	1.1 - 3.0

a/ Data collected between September 1971 and December 1976.

TABLE I

HEMATOLOGY, CLINICAL BLOOD CHEMISTRY VALUES, AND BONE MARROW (MYELOID/ERYTHROID) RATIOS OF FEMALE BEAGLE DOGS[a/]

	Female Beagle Dogs				
	Number Studied	Age (months)	Body Weight (kg) Mean ± S.D.	Observed Results Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)	257	4 - 7	6.9 ± 1.3	5.59 ± 0.73	3.27 - 7.75
Reticulocytes (%)	265	4 - 7	6.9 ± 1.3	0.74 ± 0.52	0.04 - 5.05
Hematocrit (vol %)	257	4 - 7	6.9 ± 1.3	42.3 ± 3.5	32 - 51
Hemoglobin (gm %)	257	4 - 7	6.9 ± 1.3	13.7 ± 1.3	11.0 - 18.6
MCV (μ^3)	257	4 - 7	6.9 ± 1.3	76.7 ± 9.7	55.8 - 128.4
MCHb ($\mu\mu$g)	257	4 - 7	6.9 ± 1.3	24.8 ± 3.3	17.1 - 41.6
MCHbC (mg %)	257	4 - 7	6.9 ± 1.3	32.3 ± 1.6	28.7 - 40.4
Platelets (x 10^5/mm^3)	227	4 - 7	6.9 ± 1.3	3.08 ± 1.15	1.08 - 7.95
Leukocytes (x 10^3/mm^3)	265	4 - 7	6.9 ± 1.3	10.9 ± 3.4	3.8 - 20.9
Neutrophils I (%)	265	4 - 7	6.9 ± 1.3	0.54 ± 1.16	0 - 7
Neutrophils M (%)	265	4 - 7	6.9 ± 1.3	57.08 ± 10.10	31 - 85
Lymphocytes (%)	265	4 - 7	6.9 ± 1.3	37.15 ± 10.46	10 - 61
Eosinophils (%)	265	4 - 7	6.9 ± 1.3	2.37 ± 2.25	0 - 13
Monophils (%)	265	4 - 7	6.9 ± 1.3	1.94 ± 2.01	0 - 9
Basophils (%)	265	4 - 7	6.9 ± 1.3	0.01 ± 0.09	0 - 1
Atypical cells (%)	265	4 - 7	6.9 ± 1.3	0.11 ± 0.43	0 - 4
Nucleated RBC (%)	265	4 - 7	6.9 ± 1.3	0.03 ± 0.17	0 - 2
Fasting Glucose (mg %)	248	4 - 7	6.9 ± 1.3	99.6 ± 14.4	55 - 130
SGOT (IU/ℓ)	257	4 - 7	6.9 ± 1.3	23.5 ± 7.2	6 - 52
SGPT (IU/ℓ)	257	4 - 7	6.9 ± 1.3	25.3 ± 7.0	8 - 49
Alkaline Phosphatase (IU/ℓ)	257	4 - 7	6.9 ± 1.3	73.5 ± 19.2	30 - 146
BUN (mg %)	265	4 - 7	6.9 ± 1.3	12.4 ± 3.3	4 - 26
Bone Marrow					
Myeloid/erythroid ratio	34	5 - 9	7.8 ± 1.4	1.4 ± 0.3	1.1 - 2.4

a/ Data collected between September 1971 and December 1976.

TABLE J

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MALE BEAGLE DOGS[a/]

Organ Weight	Absolute Mean ± S.D.	Range
Liver (gm)	264 ± 51	166 - 384
Spleen (gm)	58 ± 25	22 - 167
Kidneys (gm)	53 ± 10	32 - 71
Adrenals (gm)	1.12 ± 0.26	0.74 - 1.75
Thyroids (gm)	1.03 ± 0.32	0.55 - 2.50
Testes (gm)	6.60 ± 4.56	1.32 - 18.00
	Relative (per kg body weight) Mean ± S.D.	Range
Liver (gm)	27.9 ± 4.2	19.6 - 42.3
Spleen (gm)	6.0 ± 2.0	2.8 - 12.5
Kidneys (gm)	5.6 ± 0.8	4.0 - 7.7
Adrenals (mg)	117 ± 25	70 - 165
Thyroids (mg)	108 ± 34	56 - 211
Testes (gm)	0.67 ± 0.39	0.13 - 1.67

a/ Data collected between September 1971 and December 1976 from 51 dogs, weighing 9.3 ± 1.8 kg, used as control animals.

TABLE K

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF FEMALE BEAGLE DOGS[a/]

Organ Weight	Absolute Mean ± S.D.	Range
Liver (gm)	218 ± 51	106 - 322
Spleen (gm)	48 ± 21	16 - 103
Kidneys (gm)	43 ± 9	24 - 71
Adrenals (gm)	1.04 ± 0.26	0.49 - 1.65
Thyroids (gm)	0.88 ± 0.25	0.55 - 1.91
Ovaries (gm)	0.74 ± 0.24	0.38 - 1.27
	Relative (per kg body weight) Mean ± S.D.	Range
Liver (gm)	28.2 ± 5.0	20.7 - 38.8
Spleen (gm)	6.0 ± 2.3	3.1 - 10.9
Kidneys (gm)	5.5 ± 0.9	3.7 - 7.9
Adrenals (mg)	135 ± 35	67 - 215
Thyroids (mg)	112 ± 31	75 - 219
Ovaries (mg)	96 ± 33	54 - 222

a/ Data collected between September 1971 and December 1976 from 49 dogs, weighing 7.7 ± 1.5 kg, used as control animals.

TABLE L

HEMATOLOGY, CLINICAL BLOOD CHEMISTRY VALUES, AND BONE MARROW (MYELOID/ERYTHROID) RATIOS OF MALE ALBINO RATS[a/]

	Male Rats			Observed Results	
	Number Studied	Age (weeks)	Body Weight (gm) Mean ± S.D.	Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)	527	5 - 7	16_ . 22	5.84 ± 0.54	3.24 - 7.60
Reticulocytes (%)	461	5 - 7		3.04 ± 1.80	0.30 - 6.83
Hematocrit (vol %)	525	5 - 7	168 ± 22	45.1 ± 3.2	40 - 58
Hemoglobin (gm %)	525	5 - 7	168 ± 22	13.7 ± 0.9	11.8 - 17.1
MCV (μ^3)	525	5 - 7	168 ± 22	78.1 ± 16.3	62.3 - 104.6
MCHb ($\mu\mu$g)	525	5 - 7	168 ± 22	23.7 ± 2.6	19.2 - 41.0
MCHbC (mg %)	525	5 - 7	168 ± 22	30.5 ± 1.8	21.1 - 36.9
Platelets (x 10^5/mm^3)	473	5 - 7	164 ± 24	4.93 ± 1.23	2.30 - 7.95
Leukocytes (x 10^3/mm^3)	448	5 - 7	164 ± 24	15.4 ± 4.0	6.3 - 20.8
Neutrophils I (%)	448	5 - 7	164 ± 24	0.07 ± 0.31	0 - 3
Neutrophils M (%)	448	5 - 7	164 ± 24	14.1 ± 6.2	4 - 29
Lymphocytes (%)	448	5 - 7	164 ± 24	83.63 ± 6.75	52 - 96
Eosinophils (%)	448	5 - 7	164 ± 24	0.64 ± 0.91	0 - 6
Monophils (%)	448	5 - 7	164 ± 24	1.23 ± 1.73	0 - 13
Basophils (%)	448	5 - 7	164 ± 24	0.01 ± 0.15	0 - 2
Atypical cells (%)	448	5 - 7	164 ± 24	0.01 ± 0.12	0 - 2
Nucleated RBC (%)	448	5 - 7	164 ± 24	0.10 ± 0.42	0 - 4
Fasting Glucose (mg %)	125	10 - 12	348 ± 72	130.9 ± 17.2	94 - 165
SGOT (IU/ℓ)	125	10 - 12	348 ± 72	108.2 ± 34.5	63 - 223
SGPT (IU/ℓ)	125	10 - 12	348 ± 72	34.2 ± 16.5	17 - 120
Alkaline Phosphatase (IU/ℓ)	125	10 - 12	348 ± 72	94.9 ± 30.0	32 - 153
BUN (mg %)	125	10 - 12	348 ± 72	16.4 ± 4.7	8 - 41
Bone Marrow					
Myeloid/erythroid ratio	109	10 - 12	349 ± 63	1.7 ± 0.5	1.0 - 2.6

a/ Data collected between September 1971 and December 1976.

TABLE M

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MALE ALBINO RATS[a/]

Organ Weight	Absolute Mean ± S.D.	Absolute Range
Liver (gm)	10.89 ± 2.87	7.18 - 15.09
Spleen (gm)	0.65 ± 0.11	0.34 - 0.89
Kidneys (gm)	2.64 ± 0.37	1.84 - 3.58
Adrenals (mg)	63.6 ± 9.5	21.9 - 73.5
Thyroids (mg)	26.3 ± 5.8	14.3 - 37.7
Testes (gm)	2.98 ± 0.51	1.76 - 3.81
	Relative (per 100 gm body weight)	
	Mean ± S.D.	**Range**
Liver (gm)	2.96 ± 0.42	2.09 - 4.01
Spleen (gm)	0.19 ± 0.08	0.10 - 0.30
Kidneys (gm)	0.76 ± 0.10	0.22 - 0.88
Adrenals (mg)	18.6 ± 5.8	5.8 - 22.4
Thyroids (mg)	7.6 ± 2.7	4.2 - 12.7
Testes (gm)	0.87 ± 0.15	0.23 - 1.09

a/ Data collected between September 1971 and December 1976 from 139 rats, weighing 352 ± 59 gm, used as control animals.

TABLE N

PRESENCE OF VARIOUS SUBSTANCES IN THE URINE OF MALE AND FEMALE MONKEYS, DOGS AND MALE RATS

Species:	Monkeys		Dogs		Rats[a]	
No. of Animals:	141[b]	18	615[b]	112	84[b]	18
No. of Collections:	141	98[c]	615	565[c]	84	56[d]
Glucose: < 250 mg %	0[e]	2.0 (2)	0.2 (1)	0.7 (4)	0	0
> 250 mg %	0	0	0.5 (3)	0.2 (1)	0	0
Protein: < 100 mg %	3.5 (5)	6.1 (6)	19.3 (119)	17.3 (98)	29.8 (25)	36.0 (18)
> 100 mg %	0	2.0 (2)	2.3 (14)	1.8 (10)	0	0
RBC:[f] Moderate	1.4 (2)	3.1 (3)	16.4 (101)	13.3 (75)	3.6 (3)	8.0 (4)
Excessive	0	0	3.4 (21)	3.2 (18)	0	0
WBC:[f] Moderate	1.4 (2)	2.0 (2)	18.7 (115)	20.9 (118)	0	4.0 (2)
Excessive	0	0	3.9 (24)	3.7 (21)	0	0
Epithelium:[g] Moderate	31.2 (44)	44.9 (44)	21.0 (129)	21.9 (124)	0	8.0 (4)
Excessive	3.5 (5)	7.1 (7)	4.7 (29)	2.8 (16)	0	0
Crystal:[h] Moderate	0.7 (1)	2.0 (2)	0.2 (1)	0.7 (4)	0	2.0 (1)
Excessive	0	0	0.2 (1)	0.7 (4)	0	2.0 (1)
Casts: Positive	0.7 (1)	5.1 (5)	0	0.9 (5)	0	0

a/ Pooled sample of 4-20 rats.
b/ Baseline data collected from all animals employed between September 1971 and December 1976.
c/ Data collected at weekly intervals for 4-7 collections from controls employed between September 1971 and December 1976.
d/ Data collected at 2-week intervals for 2-4 collections from control rats employed between September 1971 and December 1976.
e/ Percent of total (number of samples).
f/ Normal, 10 or less cells; moderate, 10-100 cells; excessive, > 100 cells/field (x 440).
g/ Normal, 5 or less cells; moderate, 5-25 cells; excessive, > 25 cells/field (x 100).
h/ Normal, none; moderate, 1-5 crystals; excessive, > 5 crystals/field (x 100).

TABLE O

PRESENCE OF OCCULT BLOOD IN THE FECES OF MALE AND FEMALE MONKEYS AND DOGS

Species:		Monkeys		Dogs	
No. of Animals:		44[a]	8	118[a]	30
No. of Collections:		44	48[b]	118	156[b]
Occult Blood:	Negative	90.9 (40)[c]	95.8 (46)	94.1 (111)	91.7 (143)
	Positive	9.1 (4)	4.2 (2)	5.9 (7)	8.3 (13)

a/ Baseline data collected from all animals employed between July 1974 and December 1976.

b/ Data collected at weekly intervals for 4-7 collections from controls employed between July 1974 and December 1976.

c/ Percent of total (number of samples).