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MAMMALIAN TOXICITY OF MUNITIONS COMPOUNDS
PHASE III: EFFECTS OF LIFE-TIME EXPOSURE
PART I: 2,4-DINITROTOLUENE

FINAL REPORT NO. 7

November 1979

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

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For

Contract Officer's Technical Representative:
Dr. Jack C. Dacre
Environmental Protection Research Division
U.S. Army medical Bioengineering Research
and Development Laboratory
For Detrick, Frederick, Maryland 21701

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Animal Experimentation: Animal experiments were conducted according to the "Guide for the Care and Use of Laboratory Animals" (1974) prepared by the Institute of Laboratory Animal Resources, National Research Council; the regulations and standards prepared by the Department of Agriculture; and Public Law 91-570, "Laboratory Animal Welfare Act," 1970.

Disclaimer: The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

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by

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Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, MD 21701

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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER Final Report No. 7	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Mammalian Toxicity of Munition Compounds Phase III • Effects of Lifetime Exposure • Part I • 2,4-Dinitrotoluene		5. TYPE OF REPORT & PERIOD COVERED Progress Report, July 1, 1975 to August 31, 1978
7. AUTHOR(s) H.V. Ellis III, J.H. Hagensen, J.R. Hodgson, J.L. Minor, C.B. Hong, E.R. Ellis, J.D. Girvin, D.O. Helton, B.L. Herndon, C.C. Lee		6. PERFORMING ORG. REPORT NUMBER MRI Project No. 3900-B
9. PERFORMING ORGANIZATION NAME AND ADDRESS Midwest Research Institute 425 Volker Boulevard Kansas City, MO 64110		8. CONTRACT OR GRANT NUMBER(s) DAMD-17-74-C-4873
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD 21701		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62720A 62758A 3A762720A83500.038 3A762758A83500.038
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) U.S. Army Medical Bioengineering Research and Development Laboratory Fort Detrick, Frederick, MD 21701		12. REPORT DATE September 1978
16. DISTRIBUTION STATEMENT (of this Report) Distribution Unlimited		13. NUMBER OF PAGES 296
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report) Harry V. /Ellis, III, Jack H. /Hagensen, John R. /Hodgson, Jan L. /Minor Chuen-Bin /Hong		15. SECURITY CLASS. (of this report) Unclassified
18. SUPPLEMENTARY NOTES 9 [Rept. no. 7 (Final), 1 Jul 75 - 31 Aug 78]		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) 2,4-Dinitrotoluene Reproductive Toxicity Risk Assessment Chronic Toxicity Mutagenesis Water Quality Criterion Carcinogenesis Metabolism 1-Methyl-2,4-dinitrobenzene (CAS Reg. No. 121-14-2)		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The effects of oral doses of 2,4-dinitrotoluene (2,4-DNT) after oral administration for up to 24 months were studied in dogs, rats and mice. Ancillary studies included cytogenetics analysis in dogs and rats and three generation reproduction, dominant lethal mutation and metabolism studies in rats. In dogs, 0.2 mg/kg/day by capsule had no apparent effects, 1.5 mg/kg/day was toxic to some and 10 mg/kg/day was toxic to all and lethal to some. In rats, 0.57 or 0.71 mg/kg/day in feed (males or females) had no apparent effects,		

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20. Abstract (Concluded)

3.9 or 5.1 mg/kg/day was toxic to some and 34 or 45 mg/kg/day was toxic to all and shortened lifespan. In mice, 13.5 mg/kg/day in feed was slightly toxic to some, 95 mg/kg/day toxic to all and 900 mg/kg/day halved lifespan.

Target organs included the blood (methemoglobinemia with Heinz bodies and other sequelae), central nervous system (incoordination and paralysis), liver (hepatocellular carcinoma in rats), kidney (cystic tumors in mice) and gonads (decreased spermatogenesis in males of all three species; decreased corpora lutea in female mice). Pigment deposits (from metabolites and/or methemoglobin) were found in livers, kidneys and other organs, especially in mice. Rats had an increased incidence of the background subcutaneous and mammary tumors. No specific effects were seen in the ancillary studies (cytogenetics, dominant lethal mutation, reproduction, metabolism).

From these data the concentration of 2,4-DNT in ambient water which would produce in man a risk of 1 in 100,000 of developing a tumor after lifetime exposure was estimated as 1.152 µg/liter.

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FOREWORD

The U.S. Army Medical Bioengineering Research and Development Laboratory (USAMBRDL), Fort Detrick, Frederick, MD, has been conducting a research program since 1973 for the purpose of developing the scientific data base from which water quality criteria for compounds unique to the munitions industry could be determined. A water quality criterion (as defined by the amended Clean Water Act, 1977) is a qualitative or quantitative estimate of the concentration of a pollutant in ambient waters that, when not exceeded, will ensure a water quality sufficient to protect a specified water use. The criterion is a scientific entity based solely on data and scientific judgment. It does not reflect considerations of economic or technological feasibility. Currently, a water quality criterion consists of two separate numerical limits, one for the protection of human health and the other for the protection of aquatic organisms. These numbers, when translated by the appropriate regulatory agency, can be the basis of enforceable discharge or effluent limitations in a point source discharge permit issued under the Clean Water Act.

Since a water quality criterion is to protect designated water uses, a diverse, multidisciplined research program was developed by USAMBRDL that includes "effects" studies on laboratory and domestic animals, wildlife species, aquatic organisms, plants, and economically important crops. In addition, extensive chemical and biological fate and persistence tests are conducted to provide information on the behavior of a pollutant in the aqueous environment. These kinds of data are especially useful for making site-specific translation of criteria into enforceable discharge limits.

This report represents a portion of the mammalian toxicology data base being developed by USAMBRDL on materials related to the use and disposal of 2,4-dinitrotoluene.

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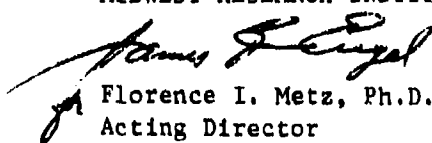
PREFACE

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 Medical Research and Development Command, Department of the Army. Dr. Jack C. Dacre, Environmental Protection Research Division, USAMBRDL, was the Contract Officer's Technical Representative for the project.

This work was conducted in the Biological Sciences Division, under the direction of Dr. William B. House, between July 1, 1975 and March 31, 1978, and Dr. Harold M. Hubbard, between April 1 and August 31, 1978. The experimental work was directed by Dr. Cheng-Chun Lee, Principal Advisor, with the assistance of Dr. Harry V. Ellis, III, Senior Pharmacologist. Mr. Jack H. Hagensen, Supervisor, supervised the animal experimentation with technical assistance of Karen J. Smith, E. Renee Walton, Darrel L. Lavish, Pam J. Saunders, Linda J. Ryhal and J. Christopher Unger. Dr. John R. Hodgson, Head, Biochemical and Developmental Pharmacology, supervised the studies on metabolism, cytogenesis and mutagenesis, with technical assistance of Daniel L. VanGoethem, Mary A. Kowalski, Maxine Hainje and Rita D. Freeman. Mr. Jan L. Minor, Assistant Toxicologist, supervised reproduction studies and the computer program and analysis of experimental data, with technical assistance of Timothy M. Unger. Dr. Danny O. Helton, Associate Chemist, performed the 2,4-DNT assay in feed. Dr. C. B. Hong, Senior Veterinary Pathologist, supervised the necropsy and the histology preparation and with Dr. Helmuth Sprinz, Consulting Pathologist, performed the microscopic examination, with technical assistance of Ellen R. Ellis, Kerry L. Crabb, Janet Kliethermes, Ernesto A. Castillo and Judith Shifrin. Miss Judith D. Girvin (ASCP certified M.T.), Laboratory Supervisor, supervised the hematology and clinical laboratory tests, with technical assistance of Ilonna S. Elwood, Duane R. Smith and Bhanu S. Gosalia. Dr. Betty L. Herndon, Associate Pharmacologist, prepared the water quality criteria.

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November 30, 1979

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EXECUTIVE SUMMARY

The effects of 2,4-dinitrotoluene (2,4-DNT) after oral administration for up to 2 years were investigated in dogs, rats and mice. Ancillary studies included a three-generation reproduction study in rats, cytogenetics as / in dogs and rats, dominant lethal mutation studies in rats, and metabolism studies in rats fed 2,4-DNT for various lengths of time.

In dogs, administration of 0.7 mg/kg/day for 2 years had no apparent adverse effect. Doses of 1.5 mg/kg/day were toxic to some, but not all dogs, while doses of 10 mg/kg/day were toxic to all dogs and lethal to some. The main target organs were the erythrocytes and the nervous system. The primary erythrocyte effect, methemoglobin, was seldom seen but sequelae were often obvious. These included Heinz bodies and reticulocytosis. In the second year of the study, these effects were minimal. The more serious was a neuromuscular effect seen as incoordination and paralysis. In all high-dose dogs (after weeks 8 to 20) and in one middle-dose dog (after week 66) there were intermittent episodes of symptoms involving the hind legs and finer control of lips and tongue. Three dogs, in weeks 8, 18 and 19, respectively, developed complete paralysis of limbs, trunk and neck, could neither eat nor drink, and were euthanized. Two of these dogs had degenerative lesions of the cerebellum. The lack of lesions in other dogs suggests the presence of a biochemical lesion not obvious to the microscope.

In rats, the 2,4-DNT intake of males and females fed the low dose was 0.57 and 0.71 mg/kg/day, respectively. This produced no effects. The intake from the middle dose, 3.9 and 5.1 mg/kg/day, respectively, caused some mild effects (decreased weight gain, liver toxicity, anemia, mammary tumors) to some susceptible individuals. The high dose, with intake of 34 and 45 mg/kg/day, respectively, was very toxic, causing severely decreased weight gain, shortened life span and a variety of pathological effects. Blood effects were like those in dogs, but anemia also occurred. The livers had the progressive development from hyperplastic areas through neoplastic nodules to hepatocellular carcinoma previously seen with other chemicals. Testes had decreased spermatogenesis, even aspermatogenesis. There were increases in some of the usual background tumors, fibromas in males and mammary fibroadenomas in females.

In mice, 2,4-DNT intake of those fed the low dose was 13.5 mg/kg/day. Some of these mice had a toxic nephropathy, excessive pigmentation, liver dysplasia, and (in the males) renal tumors not seen in the control mice. The middle dose, with 2,4-DNT intake of 95 mg/kg/day was very toxic, with a more extensive and intense incidence of lesions seen in the low dose mice (including cystic renal tumors in over half of the males) and also decreased spermatogenesis and atrophy in the testes. The high dose, 900 mg/kg/day,

caused a great decrease in weight gain with a corresponding decrease in feed consumption and shortened life span to only half that of other dose groups. These mice had anemia with many Heinz bodies. The lesions were like those of the middle dose group, but were seen in most of the mice. However, tumors were rare, probably because of the decreased life span. In addition, many females had nonfunctioning ovaries, and very few high dose mice of either sex had the pinworms commonly seen in the other groups.

The three generation reproduction study in rats found no specific reproductive effects of 2,4-DNT. There were only two generations in the high-dose group because of the combined effects of the overall toxicity of 2,4-DNT (decreased body weight, general debilitation and the antispermato-genesis effect noted above).

Cytogenetics assays of kidney and bone marrow cells from dogs and rats given 2,4-DNT in the chronic study found no toxicologically important effects.

Four dominant lethal mutation studies were done with male rats fed various doses of 2,4-DNT. Proper dose selection was hampered by the decreased spermatogenesis caused by 2,4-DNT. Finally, we concluded that there is no dominant lethal mutation effect of 2,4-DNT.

Metabolism studies on rats fed 2,4-DNT for 3, 9 or 20 months found results similar to those from rats not given 2,4-DNT chronically. The oral test dose was well absorbed, widely distributed with some concentration in the liver and kidney and extensively metabolized. Primary metabolic products from reduction of the nitro groups and/or oxidation of the methyl group. Most products were conjugated with glucuronate or sulfate before excretion, primarily in the urine.

Because 2,4-DNT has carcinogenic effects, an ambient water concentration of zero is necessary for maximum protection of human health. However, using EPA developed methodology, exposure to 1.152 $\mu\text{g/liter}$ of 2,4-DNT for a lifetime produces an estimated risk of 10^{-5} (1 in 100,000) that a tumor will develop in man. A tenfold decrease in dose would produce a tenfold decrease in the estimated risk. Because of the similarities between the isomeric DNT's, this limit for 2,4-DNT is appropriate for a normal mixture of DNT's.

I. INTRODUCTION

Under Contract No. DAMD-17-74-C-4073, entitled "Munition Compounds Mammalian Toxicity Study," we have performed a variety of studies, divided into three phases. Phase I, Effects of Acute Exposure, includes acute oral toxicity, primary skin and eye irritation, dermal sensitization, and disposition and metabolism studies. Results were reported in Progress Report No. 1.^{1/} Results on additional compounds plus in vitro mutagenic (Ames test) studies will be reported in Report No. 6.^{2/} Phase II, Effects of Multiple Exposure, includes subacute and subchronic toxicity, reversibility, immunologic response, chemical-biological interaction, mutagenicity, and disposition and metabolism studies. Results were presented in a series of reports on the compounds tested, trinitroglycerin (TNG),^{3/} 2,4-dinitrotoluene (2,4-DNT),^{4/} 2,6-dinitrotoluene,^{5/} and nitrocellulose (NC).^{6/} Phase III, Effects of Life-Time Exposure, includes chronic toxicity, reversibility, reproductive, cytogenetic, and metabolism studies on three of those compounds, 2,4-DNT, TNG and NC. This report contains the results of studies on 2,4-DNT.

II. MATERIALS AND METHODS

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II. MATERIALS AND METHODS

A. Animals

1. Sources

Young, healthy beagles were bought from Hazleton Research Animals (Cumberland, Virginia). Young, healthy CD® rats and CD-1® mice were bought from Charles River Breeding Laboratory (Wilmington, Massachusetts). All animals were maturing. They were conditioned in our animal quarters for at least 2 weeks.

2. Housing and Animal Husbandry

Dogs were kept in dog pens with outside runs. Up to 12 dogs shared 60 sq ft of heated inside space and 120 sq ft of outside space. Water was available continuously. Dogs were fed ad libitum or as described below under feed measurement. Runs were cleaned daily.

Rats and mice were kept in plastic cages with hardwood chip bedding, metal lids and filter tops. Bedding was steam sterilized before use and changed at least weekly. Cages, tops and water bottles were steam-sterilized before use and changed weekly. Feed and water were available at all times. Usually two male rats, three female rats or four mice were housed in each cage and differentiated by ear-punches. Some groups (especially male mice) were subdivided to prevent fighting. The rodent quarters are fully air conditioned, with 10 air changes per hour, maintained at $75 \pm 5^{\circ}\text{F}$ and $50 \pm 10\%$ relative humidity. The room air is passed through filters to remove 99.9% of all particles larger than 0.3μ . Lighting is controlled by a timer providing 12 hr on and 12 hr off.

All animals were observed daily for toxic signs and behavioral changes and were provided medical treatment as necessary for nontest injuries under the supervision of our veterinary pathologists. The typical case was injuries due to fighting, which may be treated by isolation, cleaning the wounds, and antibiotic therapy, systemic and local.

B. Basic Protocol

1. Dose Levels and Treatment

We usually had a control group and three treatment groups, spaced at equal logarithmic intervals (a factor of 7). With dogs the doses of 2,4-DNT in the respective treatment groups were 0.2 mg/kg/day (low), 1.5 mg/kg/day (middle) and 10.0 mg/kg/day (high). With rats, dosage levels of

0.0015% (15 ppm), 0.01% (100 ppm) and 0.07% (700 ppm) in the diet were used, respectively. Earlier studies^{1,4} showed that 2,4-DNT is less toxic to mice than to rats, because of decreased absorption from the gastrointestinal tract in the mice. Therefore, we chose the mouse dosage levels one step higher than the rat: 0.01% (100 ppm), 0.07% (700 ppm) and 0.5% (5,000 ppm), respectively.

Dogs were dosed daily by capsule. The capsule fills, consisting of 2,4-DNT diluted in lactose to give concentrations of 0, 0.4%, 3.0% and 20% 2,4-DNT, were prepared by mixing 2,4-DNT and lactose in a ball mill. After each weekly weighing, capsules were prepared for each dog for the following week. Rats diets were prepared weekly. Concentrates of 2,4-DNT in feed were prepared in a ball mill. These were then mixed with feed in a rotating box on a modified cement mixer to provide the proper dilution. For control groups or animals during recovery studies, lactose capsules or feed without 2,4-DNT, as appropriate, were given.

2. Number of Animals and Identification

Each group consisted of equal numbers of males and females. The beginning number of dogs was six of each sex per group, of rats 38 and of mice 58. Additional rats were included for the three-generation (20 females in each dosage group) and metabolism (12 males and 12 females, each, in the control, low dosage and high dosage group) studies. A few extra rodents were added to replace early losses.

Each animal is assigned a five digit number. The first two digits indicate the dosage groups for 2,4-DNT, i.e., 70, 71, 72 and 73 for the control, low, middle or high dose group, respectively. The last three digits are the animal number within each species.

3. Schedule

a. Dogs: All the dogs were bled from their jugular veins for hematology and clinical chemistry tests before dosing and at the end of 3, 6, 9, 12, 18 and 24 months during dosing. They were weighed weekly. Feed consumption was measured 1 week each month beginning in month 6. After 12 months dosing, one male and one female dog from each dosage group were killed for necropsy. The treatment of a second pair from each group was discontinued for 4 weeks. These dogs were used on a recovery study and killed for necropsy at the end of 13 months. After 24 months dosing, two males and two females from each dosage group were killed for necropsy. The remaining pair was used on a recovery study for 4 weeks and was terminated at the end of 25 months.

b. Rats: Four males and four females from each dosage group were bled for hematology by cutting off their tail tips before dosing and at the end of 3, 6, 9, 12, 18 and 24 months during dosing. As much as possible, the same rats were used at each bleeding. If a bled rat died or his tail became too short, another rat was substituted. Rats were weighed weekly for the first 6 months; after weight gain levelled off, they were weighed biweekly. Feed consumption was measured during the first 4 weeks and then during the last week of each month. After 12 months dosing, four males and four females from each dosage group were bled from their aortas for clinical chemistry and killed for necropsy. A second group of four male and four female rats from each dosage group was started on a recovery study without treatment for 4 weeks. These rats were terminated at 13 months. After 24 months dosing, a similar recovery study was started and the remaining surviving rats were killed for necropsy, with eight from each group bled for clinical chemistry. The recovery rats were terminated at the 25th month.

c. Mice: Mice were weighed weekly for the first 5 months; after their weight gain levelled off, they were weighed biweekly. Feed consumption was measured during the first 4 weeks and then for 1 week each month thereafter. After 12 months dosing, four males and four females from each dosage group were bled from their aortas for hematology and killed for necropsy. A second group of four male and four female mice from each dosage group was started on a recovery study. After 24 months dosing, a similar recovery study was started and the other surviving mice killed for necropsy, with eight mice from each dosage group bled for hematology. The recovery mice were terminated at the 13th or 25th month, respectively.

C. Test Compound

1. Analysis of 2,4-DNT Bulk Samples

2,4-DNT was obtained commercially from K and K Laboratories (Plainview, NY). Appendix III contains reports on assays of three bulk lots of 2,4-DNT. In each case the assay indicated ~98% 2,4-DNT and ~2% 2,6-DNT. For intake calculations, the material was considered to be pure 2,4-DNT.

2. Analysis of 2,4-DNT Feed Samples

Discussed below are the extraction procedures used to remove 2,4-DNT from rat feed, the instrumental conditions, the extraction efficiency of this process, and the stability of 2,4-DNT on rat feed.

a. Extraction Procedure

A two gram sample was transferred to a 30 ml bottle fitted with a polyethylene seal cap. Twenty ml of acetone was added and the sample shaken for 20 min using a Burrell® wrist action shaker. A 5 ml aliquot was transferred to a 15 ml centrifuge tube and centrifuged for 10 min. A 1 ml aliquot was transferred to a volumetric flask and evaporated to dryness using an air jet. The sample was diluted with heptane to give a final dilution of about 5 ng 2,4-DNT/ml heptane.

b. Gas Chromatograph Conditions

Instrument: Bendix 2500 equipped with ^{63}Ni electron capture detector.

Column: Glass, 1.83 m x 2 mm i.d., packed with 1.5% DC LSX-3-0295 and 1.5% GE XE-60 on Gas Chrom Q.

Flow rate: 40 cc N_2 /min

Temperatures: Column - 150°
Injector - 150°
Detector - 200°

Results: 2,4-DNT eluted at 6.4 min and 2,6-DNT eluted at 3.6 min.

c. Extraction Efficiency

Duplicate rat feed samples were spiked with 2,4-DNT to make the following concentrations: 5, 1, 0.5, 0.1 and 0.01%. The entire feed sample was then extracted and assayed for 2,4-DNT. The results were:

<u>2,4-DNT Concentration</u>	<u>Average Percent 2,4-DNT Recovered</u>
5	100 $\pm$ 2
1	100 $\pm$ 1
0.5	103 $\pm$ 1
0.1	98 $\pm$ 1
0.01	64 $\pm$ 4

d. Stability of 2,4-DNT on Rat Feed

Sample of 1% 2,4-DNT/rat feed were assayed after storage for 0, 4 and 8 days in two rat cage feeders filled and stored in the normal manner. Samples taken at 0 and 4 days were frozen and assayed with the 8 day sample. The results were:

2,4-DNT LEVEL ABOUT 1%

<u>Storage Time in Days</u>	<u>% 2,4-DNT^{a/}</u>	<u>% Remaining</u>
0	0.94 $\pm$ 0.05	100 $\pm$ 5
4	0.93 $\pm$ 0.05	99 $\pm$ 5
8	0.90 $\pm$ 0.05	96 $\pm$ 5

a/ The deviation is an estimate of the error based on other work. This value is greater than or equal to the actual deviation.

D. Procedures

1. Observation

All animals were observed daily for toxic signs and changes in behavior and general health.

2. Body Weights

Body weights were taken as mentioned above. Dogs were weighed to 0.1 kg, rodents to 1 g.

3. Measurement of Feed Consumption

The feed consumption of the dogs was measured by placing them in a metabolism cage, giving them a measured amount of feed, waiting 0.5 hr, then returning them to their pen and estimating the remaining amount of feed by volume. This value was converted to weight by a factor determined by averaging the weight of 20 replicates of volume measurements. Feed consumption of the rodents was determined by weighing the feed and container placed in the cage and that remaining 1 week later.

4. Unscheduled Deaths

If an animal appeared moribund, he was killed and necropsied as described below. If an animal was found dead, he was necropsied as thoroughly as possible, but no blood samples or organ weights were taken. If an animal received a serious injury or lesion, causing pain and suffering (such as an ulcerated tumor), he was killed and necropsied as if moribund.

E. Hematology and Clinical Chemistry

1. Hematology

The hematology battery included erythrocyte, reticulocyte, leucocyte and platelet counts, hematocrit, hemoglobin, erythrocyte indices, methemoglobin, Heinz bodies and (for dogs) clotting time. Details of methodology are summarized in Appendix I.

2. Clinical Chemistry

The clinical chemistry battery included fasting blood glucose, serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, alkaline phosphatase, and blood urea nitrogen. Details of methodology appear in Appendix I.

3. Immunoglobulin E

Immunoglobulin E (IgE), the allergic or hypersensitive antibody, was associated with anaphylactic reactions in humans.^{7/} Serum concentrations of IgE were determined in all clinical chemistry samples, using the immunodiffusion technique of Mancini.^{8/}

4. Special Tests

If indicated by symptoms or other test results, special tests such as serum electrolytes were performed.

5. Statistics

Data were analyzed using Dunnett's multiple comparison procedure following an analysis of variance, as described in Appendix I.

F. Necropsies

1. Killing and Gross Examination

Rodents were killed with ether and dogs with an overdose of sodium pentobarbital. The necropsy was performed as soon after death as possible. Gross abnormalities in all tissues are observed and recorded.

2. Organ Weights

The brain, heart, liver, kidneys, spleen, gonads, and (dogs only) adrenals, thyroids and pituitary were trimmed free from surrounding tissues and weighed. The absolute weights and organ weight to body weight and/or brain weight ratios were analyzed statistically. Abnormal growths were measured and, if practical, trimmed and weighed.

3. Histopathology

Tissues routinely taken for histopathologic examination are listed in Table 1. In addition, all tissues with gross abnormalities were taken. Processing is detailed in Appendix I.

G. Recovery Studies

Recovery studies were performed after each scheduled necropsy (12 and 24 months). The compound treatment of one male and one female dog or four rodents of each sex was discontinued. They were given the control treatment (lactose capsules or feed without compound, as appropriate) for 28 days. During this period, their weight and feed consumption were determined weekly. At termination, blood samples were taken from jugular vein of dogs or aorta of rodents for hematology and (except mice) clinical chemistry. The animals were then killed and necropsied. Detailed procedures are as given above.

H. Three Generation Reproduction Study

1. Study Design

The study design is illustrated in Figure 1. The initial groups of rats used as the parental generation (F_0) were started at the same time

as the chronic toxicity study. Rats of each group, parents and offspring of each generation, received the same control or 2,4-DNT-containing diets as in the chronic study. For the F_0 generation, 10 males and 20 females from each dosage group were mated after receiving the test diets for 6 months. Each male was housed with two females from the same dosage group for 14 days. Offspring from the matings (F_{1a} , first litters) were discarded at weaning. The F_0 rats were again mated. Twenty to 24 offspring of each sex from this mating (F_{1b} , second litters) were randomly selected (with approximately equal numbers of pups from the various litters) from each dosage group at weaning. The F_0 females and surplus pups were discarded; the F_0 males were retained in the chronic study. Each F_{1b} male was mated with a female within the same dosage group for 14 days at 3 months of age. The F_{2a} generation was discarded at weaning and the F_{1b} rats were terminated at weaning of the F_{2b} pups. The F_{2b} rats were then selected and mated at 3 months of age according to the same procedure used for F_{1b} . The study was terminated upon weaning of the F_{3b} rats.

2. Evaluation

At birth, all offspring were examined for gross physical abnormalities and the number of live and dead pups of each litter were recorded. Survival and body weight were recorded at 0, 4 and 21 days.

Reproductive performance for each parental generation was quantified by: the mating ratio (the number of copulations to the number of male-female pairing), and fertility ratios for each sex (the number of males or females with offspring to the number of that sex mated). Reproductive performance for each litter was quantified by: the litter size, the liveborn index (the percentage of the total number of pups liveborn), the weight of liveborn pups at birth, the viability index (the percentage of liveborn pups surviving to 4 days), the lactation index (the percentage of the young alive at day 4 surviving to weaning), the weight at weaning, and the sex ratio (the number of males to the total number of offspring). Details of procedures are in Appendix II.

The general health of the parental generation was quantified by the weight at first mating.

I. Mutagenesis Studies

To assess the mutagenic potential of 2,4-DNT, we performed cytogenetic analysis of tissue cultures from dogs and rats from the chronic toxicity study, and dominant lethal mutation study in rats.

1. Cytogenetic Studies

a. Preparation of Cell Cultures

At the end of 1 year, blood samples were aseptically drawn from both control and treated dogs and rats. Blood was obtained from the tail vein of the rats and from the dogs' jugular veins. The lymphocytes were cultured by the method of Moorhead et al.^{9/} Bone marrow cells replaced peripheral blood lymphocytes as a source of mitotic chromosomes in the 2-year study. The use of bone marrow cells rather than peripheral blood lymphocytes has several advantages. Chromosomes will be obtained not only from lymphoid cells but also from cells of myeloid, erythroid, and reticuloendotheloid origin. Another advantage of bone marrow cells is that the culture time is reduced from 72 hr needed in lymphocyte cultures to 24 hr and no mitogenic agent is required to obtain metaphase chromosomes. Femur bone marrow was removed at necropsy and processed by the method of Eggen;^{10/} bone marrow cultures were maintained in nutrient mixture F-12 (HAM). Kidney tissue samples were removed at necropsy, cultured by the trypsinization method of Fernandes,^{11/} and maintained in Eagle's medium as modified by Dulbecco and Vogt.^{12/}

b. Chromosome Analysis

Actively dividing kidney cultures, bone marrow cells, and phytohemagglutinin-stimulated lymphocytes were arrested in metaphase by short-term colchicine treatment. The cells were removed from the culture flasks, swollen in hypotonic solution, and processed for spreading on glass slides by the method of Moorhead and Newell.^{13/} Slides were stained with Giemsa and scanned under low power optics. The slides showing minimum scattering of cells were selected for analysis under oil immersion optics. Cell ploidy was estimated by examination of 200 cells. Chromosomes were counted and morphological aberrations were examined from photographic negatives of up to 50 metaphase cells.

2. Dominant Lethal Mutation Studies

a. General Protocol

Groups of male Charles River CD® rats were fed 2,4-DNT in feed at various dosage levels or plain feed (as control) for 10 or 13 weeks, as specified below. Each male was then mated to two virgin females of the same strain. At mid-term of pregnancy, the females were killed and the following data collected: number of fertile males per number of males treated, number of pregnant females per number of mated females, number of corpora lutea per pregnant female, and the number of total implants, dead implants, and live implants per pregnant female. Methodology details are in Appendix II. Conclusive evidence for dominant lethality requires post-implantation losses. Increased preimplantation losses may be due to genetic damage.

b. Dosing Regimens and Special Studies

For the first dominant lethal mutation study, groups of four or five male rats were fed 0, 0.02 or 0.2% 2,4-DNT in feed for 10 weeks. For the second study, we used seven to 10 males from each of the dose groups of the chronic toxicity study (0, 0.0015, 0.01 and 0.07% 2,4-DNT in feed) after they had been fed the diets for 13 weeks. For the third study, groups of 10 rats were fed 0, 0.15 or 0.2% 2,4-DNT and a group of 15 rats (to allow for possible lethal effects^{4/}) was fed 0.5% 2,4-DNT in feed for 13 weeks. For the fourth and last study, groups of 24 male rats were fed 0, 0.07, 0.10 or 0.15% 2,4-DNT for 13 weeks. To get maximum information during the last study, we also measured body weight and feed consumption weekly during the last study. After mating, 10 of the males in each group were killed and necropsied for examination of any morphological changes in their genital organs. The remaining rats were fed plain feed without 2,4-DNT for a 13 week recovery period. They were then similarly necropsied. Methodology details are as described above for the chronic study.

J. Metabolism Studies

1. Experimental Procedure

Rats were fasted overnight for about 16 hr and given a single oral dose of approximately 1/10 of the LD₅₀ of 2,4-DNT. Males received a dose of 57 mg/kg and females a dose of 65 mg/kg. The compound, spiked with 25 μ Ci/kg of DNT-(ring-UL-¹⁴C, specific activity of 3.55 mCi/mM), was suspended in peanut oil and given via an intragastric tube in a volume of 10 ml/kg of body weight. Immediately after dosing, each rat was placed in a stainless steel metabolic cage for the separate collection of urine and feces. They were given feed and water ad libitum. At the end of 24 hr, the rats were anesthetized with ether and blood was collected from the abdominal aorta. Various tissues were removed, weighed and processed for analysis of radioactivity.

2. Sample Preparation and Analysis

Volumes of urine and urine rinse were measured. Feces and GI tract (plus contents) were weighed and homogenized separately in 10 volumes of 80% methanol in a Waring blender. Whole blood (200-400 μ l), fecal and GI homogenates (250-500 μ l) and tissue samples (30-120 mg) were digested in 0.2 ml of 70% perchloric acid and 0.4 ml of hydrogen peroxide with heating at 75 to 80°C for ~~2~~ 4 hr. Ten ml of a toluene-PP0-dimet⁴yl-POPOP cocktail containing 10% Beckman Biosolv BBS-3 were added to the digests or urine aliquot (100-200 μ l). Samples were counted in duplicate in a Packard Tricarb (Model 3375) liquid scintillation counter. The counts were corrected for background and the counting efficiency was determined from a calibration curve obtained from a ¹⁴C standard quench set (Amersham/Searle Corporation) using the external standard method.

3. Thin-Layer Chromatography (TLC) for Identification of Metabolites

Pre-coated silica gel plates (E. M. Laboratories, Inc., Elmsford, N. Y.) having 0.25 mm thickness were used. Samples of raw urine or urine extracts were spotted $\cong$ 2.0 cm from the bottom of the plate and developed for a minimum of 10 cm. Solvent systems used were: (a) benzene:ethylacetate (4:1, v/v); (b) ethylacetate:n-heptane (9:1, v/v); and (c) n-butanol:acetic acid:water (10:1:1, v/v/v). A sample of pure 2,4-DNT and reference standards available (diaminotoluene, amino and nitrobenzyl alcohols, nitrobenzoic acid) were spotted on each plate for reference. Nitrotoluenes were detected using 5% diphenylamine spray reagent followed by UV-irradiation. Plates were air-dried and scraped into zones which were added to scintillation cocktail and counted directly. Some urine samples were hydrolyzed by heating, for 1 hr at 100°C, with equal volumes of 5% HCl. The resulting solutions were adjusted to pH 9.0 with 2.5% NaOH and extracted with a mixture of chloroform:methanol (2:1). The two solvent layers were separated by centrifugation and concentrated into small volumes before spotting on TLC plates.

TABLE 1

ORGANS ROUTINELY TAKEN AT NECROPSY

Thyroid and parathyroids	Caecum
Pituitary	Colon
Adrenals	Urinary bladder
Lungs	Ureter ^{a/}
Liver and gallbladder	Diaphragm ^{a/}
Spleen	Skeletal muscle
Heart	Esophagus
Salivary glands	Tonsils ^{a/}
Pancreas	Mesenteric lymph node
Thymus	Tongue ^{a/}
Prescapular lymph node ^{a/}	Skin
Gonads	Mammary gland
Uterus or prostate and accessory organs	Brain
Stomach	Spinal cord ^{a/}
Duodenum	Sciatic nerve ^{a/}
Jejunum	Eyes
Ileum	Trachea
	Rib and bone marrow

a/ Not normally removed from rodents.

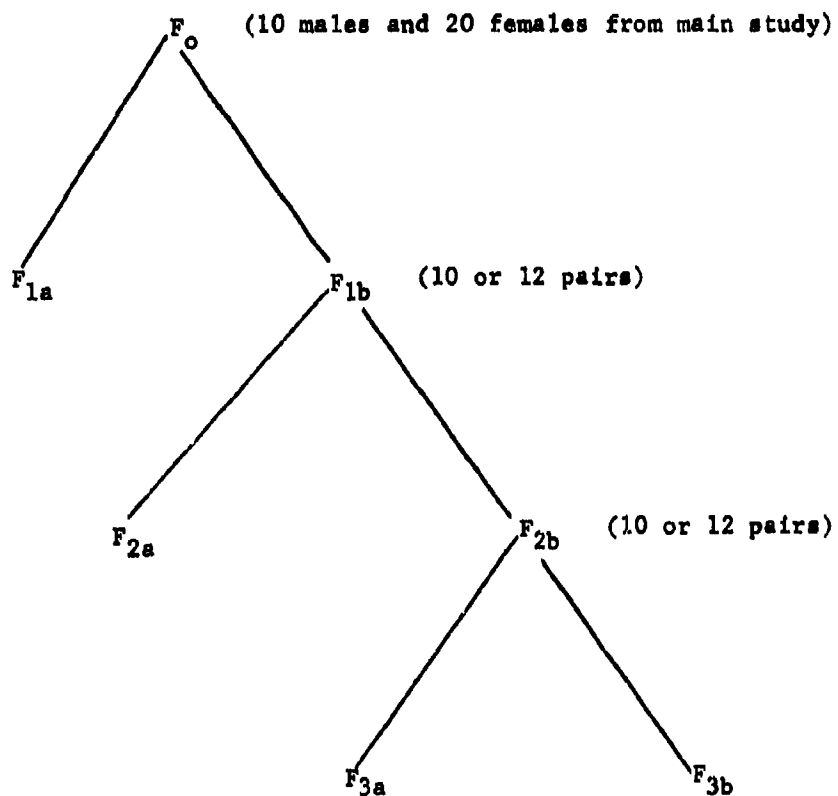


Figure 1 - Design of Three Generation Reproduction Study

III. DOG STUDIES

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III. DOG STUDIES

A. Observations and Toxic Signs

1. High Dose Dogs

The first toxic sign was observed in week 5, when the tongues of some of the high dose (10 mg/kg/day) dogs seemed bluish. Eight dogs (four each, control and high dose) were scheduled and bled for lymphocyte cultures, additional blood was taken and tested for methemoglobin and Heinz bodies. Methemoglobin was not found in any treated or control dogs. However, the high dose dogs tested had Heinz bodies, ranging from 0.4% to 8.8% of erythrocytes. No control dogs had Heinz bodies. Further scheduled tests are described below with other hematology results. The next morning, one high dose male dog (No. 73-187) was found dead in the run. The S-hook holding his number tag on his neckchain had snagged in the wire mesh fence of the run, and he had strangled. We consider this unrelated to treatment.

The first severe 2,4-DNT-related symptoms were seen in high dose male No. 73-191 during week 8. On Monday, he weighed 10.0 kg, down from 12.0 kg the previous week; he behaved normally. On Wednesday, he had intermittent, irregular tremors which could be called mild, ill-defined convulsions. Between the convulsions, his back was arched and his gait uncoordinated, especially the hind legs. His gums and coat appeared normal. The next morning (day 53) he was found lying on his right side, with his back arched and legs extended; his nose almost touched his hind paws. His abdominal and leg muscles were rigid. If placed on the left side, he flopped about like a fish until he had resumed his original position. His head, neck and eye reflexes and other functions appeared normal except for a regular cardiac arrhythmia (one slow beat after every 4 or 5 normal beats); similar patterns have been occasionally observed in the control dogs. By the afternoon of the next day, he had not eaten for over 24 hr. The rigid paralysis now included his neck; he was incapable of eating and drinking. He was killed for necropsy; the muscles relaxed at death. Blood samples taken 24 hr before necropsy had considerable amounts of Heinz bodies and some methemoglobin (Table 2). At necropsy, the blood was more dilute with no apparent methemoglobin. There was leukocytosis.

High dose female dog No. 73-192 showed symptoms during week 10. Her movements, especially the hind legs, were quite uncoordinated. This was worst 1 to 2 hr after dosing, but disappeared by the next morning. After a few days, the severity of symptoms decreased and none were apparent by week 13.

High dose male No. 73-197 showed severe toxicity during week 18. Symptoms were like those previously observed with occasional foamy, bilious vomitus. By the end of the week the paralysis had extended to his neck;

he was in rigid dorsiflexion making occasional thrashing movements. His blood sample had some Heinz bodies, considerable amount of methemoglobin and elevated SGPT (Table 2). The only remarkable finding on necropsy was a completely empty gastrointestinal tract, reflected in a weight drop from 10.6 kg to 9.3 kg during the 5 day period of symptoms.

High dose male No. 73-195 also had his first symptoms during week 18. His symptoms developed more slowly. He had profuse salivation and more frequent vomiting of white, foamy material. Unlike the other affected dogs, he was limp, rather than rigid. In 9 days, his weight dropped from 10.6 kg to 7.7 kg. He was killed for necropsy on the 13th day of symptoms during week 20. His blood samples had small amounts of Heinz bodies and methemoglobin, but were otherwise unremarkable (Table 2).

Because these deaths left us with only two high dose males, both were continued to the end of 24 months, where one was necropsied and the other placed on the recovery study. No high dose male was used for the 12 month necropsy and recovery study.

After this series of deaths, we continued to see intermittent symptoms in the surviving high dose dogs, but none were severe. As with No. 73-192 mentioned above, symptoms would disappear within a few days or, at most, weeks and reappear several weeks or even months later. The relatively severe, prolonged episodes were accompanied by weight losses, as when female No. 73-190 dropped from 8.4 kg in week 24 to 6.9 kg in week 26. Dogs showing symptoms were routinely isolated in individual cages, because their runmates would harass a weaker dog. During week 30, when female No. 73-192 was isolated, she could stand and walk, but with difficulty. She was losing 0.1 kg per day. She tried to eat the usual Purina Dog Chow (in chunks about 1 in. size) but could not pick them up with her lips and tongue, due to incoordination. We prepared Champion Dog Feed (in chunks about corn size) wetted to a soupy consistency. Within minutes, she had finished the entire bowl. More soft diet was supplied as necessary. She gained 1.0 kg in 1 day and recovered rapidly. Henceforth, the soft diet was routinely used on all dogs isolated for incoordination.

2. Middle Dose Dogs

During week 66, the first toxic signs in the middle dose group (1.5 mg/kg/day) were seen in male No. 72-181. As had happened with the high dose dogs, he lost most muscular control of his hindquarters and had occasional convulsive tremors. Within a week he appeared normal. It is noteworthy that he had received as much 2,4-DNT in 66 weeks as the high dose dogs (No. 73-191 and No. 73-192) had received in 10 weeks when they first showed symptoms. During the remainder of the study, No. 72-181 had occasional episodes of symptoms, but no other middle dose dog did.

In week 98, middle dose female No. 72-178 was found dead. No premonitory signs had been noted. Necropsy revealed no apparent cause of death. Nutritional status was fair. Since death was so near the end of the study, histopathology is discussed below with the 24-month terminations.

3. Recovery Dogs

On day 8 of the recovery study after 24 months dosing, high dose male No. 73-193 had the characteristic 2,4-DNT ataxia for several hours after dosing of control capsule. Chemical assay of his remaining capsules found no detectable 2,4-DNT. Extraction of these capsules with chloroform resulted in a clear solution and TLC gave no characteristic spot. On the other hand, the authentic high dose capsules gave a yellow extract and a large spot on the TLC. The sensitivity limit of the assay was less than 1% of the amount in a high dose capsule. It is not obvious if this transient ataxia was due to a delayed effect of the 2,4-DNT.

B. Body Weight and Feed Consumption

The average body weights of dogs given various doses of 2,4-DNT are shown in Figure 2. There were month-to-month variations. Notable low points took place in month 6 and 7 and 18 and 19, which correspond approximately to January and February. Month 18 was one of the most severe cold months recorded in this area. During one period of over 3 weeks, the temperature never rose to freezing and the wind chill often dropped to the vicinity of -40° . The dogs go outside despite these conditions.

Despite these changes in the body weights, the low dose (0.2 mg/kg/day) dogs tended to be the heaviest, followed by the controls, and the high dose (10.0 mg/kg/day) dogs. The middle dose (1.5 mg/kg/day) dogs were the lightest.

Average daily feed consumption is shown in Figure 3. These measurements were begun in week 23 at the request of the Advisory Committee. Variation from month to month was extremely high. There was some tendency for the low dose dogs, male and female, to eat the most, but this was not as conspicuous as with body weights.

C. Laboratory Data

Baseline values of hematology and clinical chemistry for male and female dogs are shown in Tables 3 and 4, respectively. The following tables show the values for these dogs after being treated with various doses of

2,4-DNT for 3 months (Tables 5 and 6), 6 months (Tables 7 and 8), 9 months (Tables 9 and 10), 12 months (Tables 11 and 12), 18 months (Tables 13 and 14) and 24 months (Tables 15 and 16).

1. Baseline Values

Before the start of the study, the only statistically significant difference between groups was slightly elevated fasting blood glucose in the high dose (10 mg/kg/day) males. However, all values were within expected limits (see Appendix I). One control male (No. 70-151) had extremely high SGPT (427 IU/ml) at this time, but not later. The average was calculated without this aberrant value.

2. Treatment-Related Changes

a. Erythrocytes: The primary blood effects of 2,4-DNT were on the erythrocytes. Perhaps the most evident examples are female dogs given 10 mg/kg/day of 2,4-DNT for 3 months (Table 6). There was anemia with the erythrocyte count depressed by 20%. The body compensated by increasing production of erythrocytes, thus increasing the proportion of immature erythrocytes (reticulocytes). These immature cells are larger, so the mean cell volume (MCV) and mean cell hemoglobin (MCHB) were increased and the total hemoglobin was decreased, although the amount (12-1/2%) was less than the decrease in the erythrocyte count (20%). Immature cells do contain some non-hemoglobin elements, reticulum (the network), which gives it its name, so its mean cell hemoglobin concentration (MCHBC) was decreased.

The cause of this anemia was reflected in the small, inconsistent methemoglobinemia and the more considerable, more consistent presence of Heinz bodies. Methemoglobin is difficult to measure in small quantities because the method involves the difference of two absorption measurements. Values up to 5% may be artifacts, since the smallest possible reading differences correspond to 2 to 2.5% methemoglobin. Since Heinz bodies are determined by counting cells picking up a special stain, low values are much more reliable. In addition, methemoglobin is transient, with even large levels being eliminated within 24 hr as we showed with trinitroglycerin.^{3/} In the later case, the blood samples taken almost 24 hr after the last dosing of trinitroglycerin may not show any methemoglobin even if large amounts were present a few hours after dosing.

As dosing continued, this qualitative pattern (anemia with reticulocytes and Heinz bodies) continued but the quantitative aspects changed. This was most obvious in the results after 18 and 24 months dosing (Tables 13 through 16). Despite continued dosing, the dogs now had only a slight anemia or none at all, near normal reticulocyte levels, no Heinz bodies and minimal methemoglobin. These minimal amounts of methemoglobin were found in most of the high dosage group samples, in about half of the middle dosage group samples and very few others. It is significant toxicologically, although not statistically.

b. Platelets: The other dose-related phenomena seen was an increase in platelet level. Except for the last sample (24 months of dosing) and the baseline, the high dose dogs had the highest platelet count in about half the time. The increase was small and had little clinical significance.

3. Other Changes

A variety of other changes were noted, but seemed unrelated to the 2,4-DNT. An increase in erythrocytes and a decrease in reticulocytes between different sampling periods were the results of the maturing of the beagles.

There was a statistical increase in the clotting time of the low dose dogs especially after 3 and 12 months of dosing. This effect was not consistent and not seen at higher doses. Furthermore, the clotting time is often quite variable due to temperature and other unknown variables. Thus, these changes are not considered toxicologically significant.

There were other random variations. As seen in Table 8, the eosinophil count was statistically high in the high dose females, but there is no consistency. The leukocyte count of the middle dose females was statistically low, but the decrease is clinically insignificant.

After 12 or more months dosing, the SGPT levels of the high and middle dose dogs were often elevated. This might be an effect of 2,4-DNT, but it was occasionally seen in only some of the dogs.

4. Recovery From Effects

Laboratory data from dogs allowed to recover for 1 month after 12 or 24 months dosing are shown in Tables 17 through 20. There was definite recovery from the anemia, with no Heinz bodies, no methemoglobin and control-like erythrocyte and reticulocyte counts in the high dose dogs.

D. Pathology

Data are presented on all dogs except No. 73-187, who was found partially autolyzed after accidental strangulation.

1. Treatment for 12 Months

A few gross changes, not related to 2,4-DNT treatment, were seen at necropsy. These included a congested lower gastrointestinal tract (colon and cecum) in the control male (No. 70-161), a hypertrophic nictitating gland of the right eye and areas of consolidation and firmness in the

apical lobe of the lungs of the low-dose female (No. 71-174), a dark red circular mass (5-7 mm diameter) on the mesentery of the middle-dose male (No. 72-185), a contracted spleen in the middle-dose female (No. 72-186), and some streaking of the renal corticomedullary junction and hepatic mottling in the high dose female (No. 73-198).

Treatment of 2,4-DNT for 12 months did not cause any obvious effects on the organ weights (Table 21). There were some variations in absolute and relative organ weights among individual dogs.

Tissue lesions in these dogs were few (Table 22) and generally corresponded to the grossly obvious lesions. Male dog No. 70-161 had ascariasis in the intestine and a parasite migration scar in his liver, female dog No. 71-174 had lymphoid hyperplasia and some foreign body granulomas consisting of mononucleated macrophages and fibrous tissue surrounding what appears to be hairshafts in the lung, and female dog No. 72-185 had an accessory spleen. Other occasional changes occurred in the liver, pancreas, stomach, intestine, kidney, prostate, pituitary, adrenal gland and lymph node. The lesions were mild and not related to the treatment. The only effects which, in view of later observation, related to the 2,4-DNT treatment were the bile duct hyperplasia and the minimal pigment deposits in the liver of the one high-dose dog, No. 73-198. These lesions were consistently present in high dose dogs after treatment of 2,4-DNT for 24 months. The bone marrow of the dogs terminated at 1 year was normal and the myeloid/erythroid (M/E) ratios were within normal ranges.

Recovery dogs: The results from the dogs given 2,4-DNT for a year and allowed to recover for a month are given in Tables 23 (Organ Weights) and 24 (Lesions). The only gross lesion was the parasites in the control male dog No. 70-159, which proved to be a few ascarids and cestodes. There was some variation among individual dogs in the absolute and relative organ weights. Bile duct hyperplasia was seen in one low dose female (No. 71-172) and pigment deposits in one middle dose female (No. 72-184) and one high dose female (No. 73-196). A number of other lesions in various tissues were not related to treatment. These lesions also occurred in control dogs or were not persistent in treated dogs. The bone marrow and M/E ratios of these dogs were normal.

2. Treatment For 24 Months

At necropsy, the obvious lesions were nodules and/or white patches in the lungs of about half of the dogs, a wart which proved to be a papilloma on the tip of the right ear of a middle dose female (No. 72-180), and "cherry eyes" in a control female (No. 70-152) and a low dose male (No. 71-165).

The absolute and the relative organ weights of the dogs terminated at the end of 2 years were unremarkable (Table 25). There was some variation among individual dogs.

Histopathologic examination revealed several lesions which were related to the 2,4-DNT treatment (Table 26). There were a mild bile duct hyperplasia and clusters of brown pigment-laden Kupffer cells in the livers of all three high dose dogs and in one low dose dog. Cystic hyperplasia of the epithelium occurred in the gallbladder of all high dose dogs, one middle dose dog and one control dog. Brown epithelial pigmentation was seen in the gallbladder of two high dose dogs and three middle dose dogs and in the kidney of two high dose dogs. Excessive pigment was also seen in the spleens of two high dose dogs. A variety of lesions, not related to the 2,4-DNT treatment, were seen in other tissues of the dogs terminated at the end of 2 years. The white patches in the lungs were focal subpleural fibrosis. The lung nodules comprised several lesions. Some were foreign body granulomas due to inhaled hair shafts. Many were focal interstitial fibrosis, with or without eosinophilic infiltration, suggesting old parasite-related lesions. These lesions were mild, also seen in the control dogs, and/or not persistently occurred in the treated dogs. The bone marrow and the M/E ratio of these dogs were normal.

Recovery dogs: Dogs given 2,4-DNT for 2 years and allowed to recover for a month were grossly similar to those terminated at end of 2 years. Several dogs had lung nodules and a scattering of other changes including an ectopic spleen in a control female (No. 70-156) and uterine cysts in both low dose females (Nos. 71-168 and 71-170) with a cystic ovary in the latter.

Histopathologic examination (Table 28) revealed the same treatment-related lesions including bile duct hyperplasia, cystic hyperplasia of the gallbladder epithelium, pigmentation in the liver, gallbladder and kidney and excessive pigment in the spleen. However, the gallbladder pigmentation and splenic hemosiderosis were seen in several control and low dose dogs. There is no indication of recovery from the relatively mild lesions found after 2 years' treatment of 2,4-DNT. As seen in other groups of dogs, a number of lesions, not related to treatment, occurred in other tissues of these dogs.

3. Unscheduled Deaths

As discussed above, three male high dose dogs became totally paralyzed; they could not lift up their heads to lap up water. These dogs were killed for necropsy. Gross examination was unremarkable, except for the gastrointestinal tracts, which contained only their own secretions. Histopathological examination revealed a number of tissues related to the paralysis (Table 29). There were a generalized vacuolation with some resemblance to encephalomalacia, hypertrophy and mitosis of the endothelium and gemastocytosis (enlarged astrocytes) in the cerebellum, and some perivascular hemorrhage in the cerebellum and the brain stem. These changes

were not seen in dog No. 73-191 who died first, and were only minimal in dog No. 73-195, who died within a week of first symptoms. The lesions were most severe in dog No. 73-197 who survived almost 2 weeks with increasing paralysis. They were not seen in any dogs without paralysis as described above. Pigmentation also occurred in the liver and/or spleen of these three dogs and bile duct hyperplasia occurred in one dog (No. 73-197). The occasional lesions in other tissues of these dogs were not related to 2,4-DNT treatment.

E. Cytogenetics

The results of the chromosome analysis of the bone marrow and kidney cultures treated with 2,4-DNT for 24 months are shown in Table 30. The kidney cultures from the treated dogs had slightly increased tetraploids. The increase was not statistically significant. In addition, the bone marrow cultures were normal. Administration of 10 mg/kg/day of 2,4-DNT did not cause any morphological aberrations of the chromosomes.

F. Discussion and Conclusions

Repeated oral administration of 2,4-DNT was toxic to dogs. The high dose (10 mg/kg/day) was toxic to all dogs, and lethal to some. The middle dose (1.5 mg/kg/day) was toxic to some dogs, but not all. The low dose (0.7 mg/kg/day) had no apparent adverse effects. Chronic administration of 10 mg/kg/day of 2,4-DNT for 24 months had no apparent mutagenic effect on the chromosomes.

There were three target organs after chronic administration of 2,4-DNT: the erythrocytes, the biliary tract and the nervous system.

1. Effects on Erythrocytes

Repeated oral administration of 10 mg/kg/day of 2,4-DNT caused the destruction of erythrocytes resulting in anemia. The effects of toxic agents on the red cell have been well understood for some time. The process is sometimes called "anilinism," and is seen with aromatic nitro- and amino-compounds, inorganic nitrites and nitrates (reduced to nitrite by gut bacteria), and other oxidizing agents. The relative importance of the various effects varies with the compound, but the qualitative picture is constant.^{14/}

The initial biochemical lesion was the oxidation of the ferrous ion in hemoglobin to produce methemoglobin. The likeliest oxidizing chemical species after the administration of 2,4-DNT was a hydroxylamine, an intermediate in the reduction of nitros to amines. Within limits, the body could

reduce this methemoglobin to hemoglobin. However, some was destroyed to produce Heinz bodies, small granules of degenerate hemoglobin within the red cell. Because of the increased formation, there was an increase in red cell destruction. Therefore, pigment deposits (derived from hemoglobin and/or 2,4-DNT metabolic products) were found in various tissues, including Kupffer cells and epithelium of the gallbladder and kidney. The bone marrow increased the production of erythrocytes; there was an increased proportion of immature erythrocytes (reticulocytes), which, in turn, caused increases in mean cell volume and mean cell hemoglobin, and decreases in total hemoglobin and mean cell hemoglobin concentration. If the hemolysis is extreme, the marrow cannot increase erythrocyte production enough, and anemia is seen. If the hemolysis is not severe, a "compensated anemia," normal erythrocyte count with increased reticulocytes resulted.

As the treatment of 2,4-DNT is continued, the dogs might have developed "tolerance." There were only a slight anemia or none at all, near normal reticulocyte levels, no Heinz bodies and minimal methemoglobin.

2. Effect on Biliary Tract

Hyperplasia of both the biliary tract and the gallbladder epithelium was noted in most of high dose dogs (10 mg/kg/day) and a few middle dose dogs (1.5 mg/kg/day). There was no indication of recovery when the treatment of 2,4-DNT was discontinued for 1 month. This lesion in the biliary tract and gallbladder was a very mild effect. Its significance in the dog after prolonged administration of 2,4-DNT is unknown.

3. Neurotoxicity

In dogs repeated oral administration of 2,4-DNT caused characteristic neurotoxicity. The effects appeared to be a cumulative effect with a wide range of individual variation. The affected middle dose dog was first affected after a total dose of about 700 mg/kg, the first four affected high dose dogs had received similar total doses of 510 mg/kg, 680 mg/kg, and 1,240 mg/kg at onset of symptoms. No other middle dose dogs had symptoms by the end of the dosing period (1,092 mg/kg total dose), but all high dose dogs had such symptoms by the end of the third quarter (2,730 mg/kg total dose). Therefore, it seems that a total dose of 500 to 3,000 mg/kg would produce these symptoms in most beagles.

The primary symptom seen was a loss of muscle control, producing ataxia and/or incoordination. The hind limbs were affected more than the forward parts of the body. The muscles usually became rigid in extension. Thus, a characteristic posture consisted of the dog sitting down with his hind limbs protruding at odd angles. If the dog walked or ran, a strange hopping gait was seen because the hind legs were stiffly held, moving less frequently than the front legs, and completely out of synchrony. In dogs,

the most delicate muscular control and coordination, analogous to the human hand, is that of the lips and tongue picking up feed. The 2,4-DNT induced nervous system effects interfered with this process, making feed unavailable to the dogs.

A remarkable aspect of this neurotoxicity was its waxing and waning, despite continuing dosing. There was no obvious explanation, but it occurred quite regularly. If parenteral nutrition ("hyperalimentation") was used, it was possible that even the severely paralyzed dogs might have recovered.

The three high dose dogs (10 mg/kg/day) with paralysis, killed for necropsy during the 8th, 18th and 19th weeks of treatment, had generalized vacuolization, hypertrophy and mitosis of the endothelium, and gemastocytosis in the cerebellum. There was also perivascular hemorrhage in the cerebellum and the brain stem. These lesions were probably responsible for the incoordination, ataxia and paralysis produced in all the high dose dogs. There was a lack of any other apparent lesion, biochemical or histopathological. Secondly, the paralysis ceased when the barbiturate overdose used for euthanasia began. Anatomically, the cerebellum provides coordination for muscular movement, and the physiological effects are an impairment of this coordination. Finally, there were similarities, in both histopathology and pathophysiology, to the syndrome known as "encephalomalacia" in horses and chickens. The primary argument against this relationship is the lack of the lesions in most dogs. If development of a visible lesion requires a week or more of severe toxic signs, the results are explicable. Presumably, lesser degrees of toxicity involve lesions at the biochemical scale which are not apparent to light microscopy.

4. Prediction for Human Toxicity

If one were looking for 2,4-DNT toxicity in man, the most useful tests would seem to be a coordination test (hand-eye) to detect the neurotoxicity, and blood analyses for Heinz bodies, erythrocytes, hemoglobin and reticulocytes, to detect and evaluate the severity of the anemia.

TABLE 2

LABORATORY DATA OF DOGS GIVEN 10 MG/KG/DAY WITH SEVERE TOXIC SIGNS

Dog No.:	73-191	73-191	73-197	73-195	73-195
Study Day:	53	54 ^a /	131 ^a /	136	138 ^a /
Erythrocytes, x 10 ⁶ /mm ³	6.18	4.74	6.39	6.82	7.02
Heinz bodies, %	6.67	8.00	2.30	0.11	0.33
Reticulocytes, %	1.10	0.89	0.26	0.11	0.04
Hematocrit, vol. %	48	36	50	50	53
Hemoglobin, gm %	16.4	12.4	16.6	18.4	18.2
Methemoglobin, %	7.9	0.0	21.1	4.9	7.1
MCV, cubic microns	77.7	75.9	78.2	73.3	75.5
MCHB, micromicrograms	26.5	26.2	26.0	27.0	25.9
MCHBC, gm %	34.2	34.4	33.2	36.8	34.3
Platelets, x 10 ⁵ /mm ³	3.05	1.20	2.80	1.80	1.88
Leukocytes, x 10 ³ /mm ³	9.8	19.9	7.4	14.2	14.4
Neutrophils, %	80	66	84	76	77
Lymphocytes, %	19	27	11	18	19
Bands, %	0	0	0	0	0
Monocytes, %	0	1	5	5	3
Eosinophils, %	1	6	0	1	1
Basophils, %	0	0	0	0	0
Atypical, %	0	0	0	0	0
Nucleated RBC, %	0	0	0	0	0
Glucose, mg %	119	108	165	118	98
SGOT, IU/l	21	21	62	15	21
SGPT, IU/l	52	40	136	34	31
Alkaline phosphatase, IU/l	38	33	22	13	12
BUN, mg %	14	15	24	12	12
Bilirubin total, mg %	0.0	0.0	0.2	0.5	0.3
Bilirubin, direct, mg %	0.0	0.0	0.0	0.3	0.0
IgE, IU/l	2350	2725	1800	1450	1675
Ca, meq/l	-- ^b /	4.9	5.3	5.1	5.0
Mg, meq/l	--	1.6	2.1	2.0	1.9
K, meq/l	--	4.8	4.1	4.4	5.2
Na, meq/l	--	149	153	147	144
Cl, meq/l	--	114	108	104	110
BSP retention, %	--	--	--	11	--
CPK, IU/l	--	--	--	12	24

^a/ Killed for necropsy this day.

^b/ Not measured.

TABLE 3

LABORATORY DATA OF MALE DOGS BEFORE ADMINISTRATION OF 2,4-DNT
(C,N) CONTROL (T,N) TREATED N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C, 6)	0.2 (T, 6)	1.5 (T, 6)	10 (T, 6)
ERYTHROCYTES ($\times 10^6$ /MM ³)	5.70 $\pm$.11	5.91 $\pm$.08	5.69 $\pm$.10	5.74 $\pm$.10
HEINZ BODIES, %	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
RETICULOCYTES, %	.87 $\pm$.08	.96 $\pm$.05	.87 $\pm$.07	.83 $\pm$.09
HEMATOCRIT, VOL. %	42.6 $\pm$.7	43.2 $\pm$.4	42.9 $\pm$.7	43.2 $\pm$.8
HEMOGLOBIN, GM. %	14.5 $\pm$.3	14.9 $\pm$.2	14.6 $\pm$.3	14.7 $\pm$.3
METHEMOGLOBIN, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	1.3 $\pm$.5
MCV, CUBIC MICRONS	74.7 $\pm$ 1.0	73.1 $\pm$.5	75.5 $\pm$ 1.1	75.3 $\pm$ 1.1
MCHM, MICRO MICROGMS.	25.4 $\pm$.3	25.3 $\pm$.1	25.7 $\pm$.5	25.6 $\pm$.3
MCHBC, GM. %	34.0 $\pm$.1	34.6 $\pm$.1	34.0 $\pm$.3	34.1 $\pm$.3
PLATELETS ($\times 10^3$ /MM ³)	2.1 $\pm$.2	2.2 $\pm$.1	2.5 $\pm$.3	2.4 $\pm$.2
LEUKOCYTES ($\times 10^3$ /MM ³)	17.1 $\pm$.7	14.8 $\pm$.7	16.6 $\pm$ 1.5	14.3 $\pm$.7
NEUTROPHILS, %	61.0 $\pm$ 3.4	59.1 $\pm$ 2.9	56.0 $\pm$ 2.4	60.9 $\pm$ 3.0
LYMPHOCYTES, %	34.4 $\pm$ 4.0	36.8 $\pm$ 2.9	29.9 $\pm$ 2.9	36.9 $\pm$ 2.9
EOSINOPHILS, %	0.0 $\pm$ 0.0	.1 $\pm$.1	.2 $\pm$.2	0.0 $\pm$ 0.0
MONOCYTES, %	3.6 $\pm$.4	3.8 $\pm$ 1.0	3.4 $\pm$ 1.2	1.8 $\pm$.5
BASOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
ATYPICAL, %	1.0 $\pm$.2	.5 $\pm$.2	.7 $\pm$.4	.4 $\pm$.2
NUCLEATED RBC, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
CLOTTING TIME, MIN.	6.4 $\pm$.3	6.4 $\pm$.5	6.4 $\pm$.3	6.6 $\pm$.2
GLUCOSE (FASTING), MG. %	92.4 $\pm$.6	95.9 $\pm$ 2.5	94.0 $\pm$ 2.4	99.7 $\pm$ 1.7 ^a
SGOT, IU/L	44.1 $\pm$ 13.6	29.3 $\pm$ 1.5	43.0 $\pm$ 11.3	27.7 $\pm$ 1.2
SGPT, IU/L	35 $\pm$ 2 (3)	35 $\pm$ 2	39 $\pm$ 5	75 $\pm$ 2
ALK. PHOS., IU/L	73 $\pm$ 17	62 $\pm$ 5	60 $\pm$ 4	55 $\pm$ 4
BUN, MG. %	12.9 $\pm$.5	12.5 $\pm$.7	13.0 $\pm$ 1.2	13.4 $\pm$ 1.3
IMMUNOGLOBULIN E, IU/ML	885 $\pm$ 126 (5)			738 $\pm$ 93

^a/ SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

ENTRIES ARE MEAN $\pm$ STANDARD ERROR.

TABLE 4

LABORATORY DATA OF FEMALE DOGS BEFORE ADMINISTRATION OF 2,4-DNT
(C.N) CONTROL (T.N) TREATED N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C, 6)	0.2 (T, 6)	1.5 (T, 6)	10 (T, 6)
ERYTHROCYTES ($\times 10^6$ /MM ³)	5.89 $\pm$.10	5.86 $\pm$.15	5.89 $\pm$.08	5.98 $\pm$.11
WEINZ BODIES, %	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
RETICULOCYTES, %	1.38 $\pm$.16	1.12 $\pm$.11	1.14 $\pm$.12	1.01 $\pm$.07
HEMATOCRIT, VOL. %	42.5 $\pm$.7	42.9 $\pm$ 1.2	43.5 $\pm$.6	42.7 $\pm$.8
HEMOGLOBIN, GM. %	14.7 $\pm$.2	14.8 $\pm$.5	15.0 $\pm$.2	14.7 $\pm$.3
METHEMOGLOBIN, %	.7 $\pm$.7	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MCV, CUBIC MICRONS	72.1 $\pm$.5	73.3 $\pm$.8	73.9 $\pm$.8	71.4 $\pm$.6
MCH, MICRO MICROGMS.	25.0 $\pm$.2	25.4 $\pm$.3	25.4 $\pm$.2	24.6 $\pm$.2
MCHC, GM %	34.6 $\pm$.2	34.6 $\pm$.2	34.4 $\pm$.1	34.5 $\pm$.2
PLATELETS ($\times 10^3$ /MM ³)	3.5 $\pm$.2	3.0 $\pm$.4	3.4 $\pm$.2	2.7 $\pm$.1
LEUKOCYTES ($\times 10^3$ /MM ³)	14.8 $\pm$.7	16.0 $\pm$ 1.2	15.0 $\pm$ 1.6	14.7 $\pm$.9
NEUTROPHILS, %	63.8 $\pm$ 1.9	61.6 $\pm$ 2.0	61.6 $\pm$ 1.7	64.4 $\pm$ 1.9
LYMPHOCYTES, %	32.0 $\pm$ 2.0	34.3 $\pm$ 2.6	34.6 $\pm$ 1.8	31.3 $\pm$ 2.1
BANDS, %	0.0 $\pm$ 0.0	.2 $\pm$.1	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
EOSINOPHILS, %	2.7 $\pm$.9	2.6 $\pm$.8	1.6 $\pm$.4	2.2 $\pm$.3
BASOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MONOCYTES, %	1.6 $\pm$.4	1.3 $\pm$.2	1.5 $\pm$.2	1.8 $\pm$.3
ATYPICAL, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
NUCLEATED RBC, %	.1 $\pm$.1	0.0 $\pm$ 0.0	.1 $\pm$.1	0.0 $\pm$ 0.0
CLOTTING TIME, MIN.	5.7 $\pm$.1	6.0 $\pm$.2	5.0 $\pm$.2	5.9 $\pm$.2
GLUCOSE (FASTING), MG %	94.8 $\pm$ 4.1	94.6 $\pm$ 4.4	101.4 $\pm$ 3.1	96.9 $\pm$ 3.3
SGOT, IU/L	31.7 $\pm$ 1.2	29.5 $\pm$ 1.9	26.5 $\pm$ 1.4	29.8 $\pm$ 2.5
SGPT, IU/L	39.0 $\pm$ 4.7	36.5 $\pm$ 2.6	34.5 $\pm$ 1.6	37.2 $\pm$ 3.0
ALK. PHOS., IU/L	46 $\pm$ 3	53 $\pm$ 7	52 $\pm$ 3	49 $\pm$ 5
BUN, MG %	17.2 $\pm$.5	18.4 $\pm$ 1.2	16.8 $\pm$ 1.1	19.0 $\pm$ 2
IMMUNOGLOBULIN E, IU/ML	925 $\pm$ 46 (5)			805 $\pm$ 79 (5)

* SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

ENTRIES ARE MEAN $\pm$ STANDARD ERROR.

TABLE 5

LABORATORY DATA OF MALE DOGS AFTER ADMINISTRATION OF 2,4-DNT FOR 3 MONTHS
(C.N) CONTROL (T.N) TREATED N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C, 6)	0.2 (T, 6)	1.5 (T, 6)	10 (T, 4)
ERYTHROCYTES ($\times 10^6$ /MM ³)	6.25 $\pm$.13	6.15 $\pm$.12	6.13 $\pm$.11	5.40 $\pm$.04
MEINZ BODIES, %	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	.47 $\pm$.67
RETICULOCYTES, %	.59 $\pm$.06	.73 $\pm$.06	.65 $\pm$.12	1.22 $\pm$.28 ^{a/}
HEMATOCRIT, VOL. %	44.3 $\pm$.8	43.9 $\pm$.8	43.7 $\pm$ 1.1	44.5 $\pm$ 1.0
HEMOGLOBIN, GM. %	16.0 $\pm$.3	15.3 $\pm$.3	15.1 $\pm$.3	14.7 $\pm$.3 ^{a/}
METHEMOGLOBIN, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	.6 $\pm$.6
MCV, CUBIC MICRONS	74.2 $\pm$.4	71.3 $\pm$.4	71.3 $\pm$ 1.4	76.7 $\pm$ 1.2
MCH, MICRO MICROGRMS.	25.6 $\pm$.2	24.9 $\pm$.2	24.7 $\pm$.1 ^{a/}	25.4 $\pm$.4
MCHC, GM %	34.6 $\pm$.2	35.0 $\pm$.2	34.7 $\pm$.7	33.1 $\pm$.0
PLATELETS ($\times 10^9$ /MM ³)	2.0 $\pm$.2	2.0 $\pm$.2	2.2 $\pm$.2	2.6 $\pm$.3
LEUKOCYTES ($\times 10^3$ /MM ³)	14.4 $\pm$.9	12.1 $\pm$.8	12.5 $\pm$.4	15.4 $\pm$.7
NEUTROPHILS, %	64.3 $\pm$ 1.6	55.6 $\pm$ 3.1	54.2 $\pm$ 2.6	63.0 $\pm$ 5.6
LYMPHOCYTES, %	31.0 $\pm$.8	36.1 $\pm$ 3.0	39.3 $\pm$ 2.0	32.8 $\pm$ 4.4
BANDS, %	.2 $\pm$.2	.8 $\pm$.3	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
EOSINOPHILS, %	3.3 $\pm$ 1.3	4.7 $\pm$ 1.2	5.7 $\pm$.4	4.0 $\pm$ 1.7
BASOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MONOCYTES, %	1.2 $\pm$.4	3.0 $\pm$.8	.8 $\pm$.7	.3 $\pm$.3
ATYPICAL, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
NUCLEATED HBC, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	.3 $\pm$.3
CLOTTING TIME, MIN.	7.0 $\pm$.4	13.0 $\pm$.6 ^{a/}	6.8 $\pm$.3	5.9 $\pm$.7
GLUCOSE (FASTING), MG %	47.5 $\pm$ 5.2	101.0 $\pm$ 2.8	48.5 $\pm$ 3.2	92.8 $\pm$ 2.8
SGOT, IU/L	27.2 $\pm$ 2.4	24.6 $\pm$ 2.1	33.5 $\pm$ 1.8	50.3 $\pm$ 14.7 ^{a/}
SGPT, IU/L	39.2 $\pm$ 4.9	35.3 $\pm$ 7.1	59.5 $\pm$ 5.8	61.0 $\pm$ 11.9
ALK. PHOS., IU/L	30 $\pm$ 3	49 $\pm$ 5 ^{a/}	32 $\pm$ 4	29 $\pm$ 5
BUN, MG %	18.5 $\pm$.4	13.9 $\pm$.6 ^{a/}	16.8 $\pm$.7	18.8 $\pm$ 2.7
IMMUNOGLOBULIN E, IU/ML	2090 $\pm$ 54 (5)			2550 $\pm$ 50 ^{a/}

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

ENTRIES ARE MEAN $\pm$ STANDARD ERROR.

TABLE 6

LABORATORY DATA OF FEMALE DOGS AFTER ADMINISTRATION OF 2,4-DNT FOR 3 MONTHS

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

DOSE: MG/KG/DAY	0 (C. 6)	0.2 (T. 5)	1.5 (T. 6)	10 (T. 4)
ERYTHROCYTES (X10 ⁶ /MM ³)	5.81 ± .20	6.30 ± .11	6.00 ± .20	6.61 ± .36 ^{a/}
HEINZ BODIES. %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.94 ± 1.63
RETICULOCYTES. %	.62 ± .13	.68 ± .06	.58 ± .12	1.49 ± .42 ^{a/}
HEMATOCRIT. VOL. %	41.8 ± 1.3	45.6 ± .7	45.0 ± 1.2	38.8 ± 2.1
HEMOGLOBIN. GM. %	14.4 ± .5	15.9 ± .1	15.4 ± .4	12.6 ± .7 ^{a/}
METHEMOGLOBIN. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 1.0
MCV. CUBIC MICRONS	72.1 ± .8	72.5 ± 1.2	75.1 ± 1.0	65.4 ± 4.1 ^{a/}
MCHN. MICRO MICROGMS.	24.9 ± .2	25.3 ± .4	25.6 ± .3	27.6 ± 1.3 ^{a/}
MCHRC. GM %	34.5 ± .4	34.9 ± .3	34.2 ± .3	32.4 ± .2 ^{a/}
PLATELETS (X10 ³ /MM ³)	2.7 ± .2	2.4 ± .3	2.8 ± .4	4.0 ± .4
LEUKOCYTES (X10 ³ /MM ³)	13.9 ± .9	12.2 ± 1.2	11.5 ± .4	14.9 ± 2.6
NEUTROPHILS. %	59.5 ± 3.9	61.2 ± 2.4	64.2 ± 3.4	61.5 ± 3.7
LYMPHOCYTES. %	37.7 ± 4.0	35.2 ± 2.4	30.5 ± 4.4	35.7 ± 3.6
EOSINOPHILS. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES. %	2.0 ± .8	1.4 ± .4	4.5 ± 1.5	1.8 ± .3
ATYPICAL. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC. %	.2 ± .2	.2 ± .2	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME. MIN.	7.1 ± .9	14.8 ± .4 ^{a/}	7.3 ± .5	6.5 ± .7
GLUCOSE (FASTING). MG %	93.2 ± 3.1	100.0 ± 2.8	83.5 ± 2.9	78.8 ± 2.2 ^{a/}
SODIUM. IU/L	26.7 ± 1.5	23.0 ± 1.4	24.8 ± 2.2	22.8 ± 4.1
POT. IU/L	32.3 ± 2.8	38.2 ± 4.2	35.5 ± 2.5	32.3 ± 3.1
ALK. PHOS. IU/L	31 ± 1	52 ± .4 ^{a/}	32 ± 6	35 ± 4
BUN. MG %	19.0 ± 1.2	15.6 ± .4	17.2 ± 1.8	17.2 ± 1.0
IMMUNOGLOBULIN E. IU/ML	2140 ± 94 (5)			2692 ± 133 ^{a/}

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

ENTRIES ARE MEAN ± STANDARD ERROR.

TABLE 7

LABORATORY DATA OF MALE DOGS AFTER ADMINISTRATION OF 2,4-DNT FOR 6 MONTHS
(C.N) CONTROL (T.N) TREATED N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C. 6)	0.2 (T. 6)	1.5 (T. 6)	10 (T. 2)
ERYTHROCYTES ($\times 10^6$ /MM ³)	5.70 $\pm$.11	5.50 $\pm$.20	4.10 $\pm$.13	5.26 $\pm$.18
HEINZ BODIES, %	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	1.02 $\pm$.65 ^{a/}
RETICULOCYTES, %	.46 $\pm$.09	.46 $\pm$.04	.97 $\pm$.10	1.04 $\pm$.48 ^{a/}
HEMATOCRIT, VOL. %	46.3 $\pm$ 1.1	42.8 $\pm$ 1.0	47.5 $\pm$.7	44.0 $\pm$ 3.0
HEMOGLOBIN, GM. %	15.1 $\pm$.3	14.5 $\pm$.7	15.9 $\pm$.4	14.2 $\pm$.7
METHEMOGLOBIN, %	1.0 $\pm$.5	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	.8 $\pm$.8
MCV, CUBIC MICRONS	81.3 $\pm$.9	78.0 $\pm$ 1.2	78.0 $\pm$ 1.0	83.5 $\pm$ 2.9
MCHM, MICRO MICROGMS.	26.5 $\pm$.2	26.4 $\pm$.5	26.1 $\pm$.3	27.0 $\pm$.4
MCHMC, GM %	32.6 $\pm$.3	33.8 $\pm$.2	33.5 $\pm$.4	32.3 $\pm$.6
PLATELETS ($\times 10^5$ /MM ³)	1.6 $\pm$.1	1.8 $\pm$.1	1.9 $\pm$.2	3.1 $\pm$.3 ^{a/}
LEUKOCYTES ($\times 10^3$ /MM ³)	13.2 $\pm$.6	14.9 $\pm$.5 ^{a/}	12.1 $\pm$.8	13.9 $\pm$ 1.8
NEUTROPHILS, %	43.0 $\pm$ 2.5	53.8 $\pm$ 3.6	67.5 $\pm$ 1.4	55.0 $\pm$ 7.0
LYMPHOCYTES, %	29.8 $\pm$ 3.3	40.8 $\pm$ 3.7	24.3 $\pm$ 3.4	40.5 $\pm$ 7.5
MONOS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	.3 $\pm$.3	0.0 $\pm$ 0.0
EOSINOPHILS, %	6.7 $\pm$ 1.1	5.3 $\pm$.8	7.5 $\pm$ 2.1	4.0 $\pm$ 0.0
BASOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MONOCYTES, %	.5 $\pm$.3	0.0 $\pm$ 0.0	.3 $\pm$.2	.5 $\pm$.5
ATYPICAL, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
NUCLEATED PRC, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
CLOTTING TIME, MIN.	8.0 $\pm$.2	9.3 $\pm$.4 ^{a/}	7.7 $\pm$.2	7.0 $\pm$.5
GLUCOSE (FASTING), MG %	102.7 $\pm$ 1.3	91.8 $\pm$ 3.3 ^{a/}	99.2 $\pm$ 2.4	98.0 $\pm$ 3.0
SGOT, IU/L	24.7 $\pm$.7	26.2 $\pm$ 2.3	28.8 $\pm$ 1.8	32.5 $\pm$ 1.5
SGPT, IU/L	39.0 $\pm$ 2.0	37.5 $\pm$ 2.4	45.7 $\pm$ 20.6	41.5 $\pm$ 4.5
ALK. PHOS., IU/L	22 $\pm$ 5	26 $\pm$ 2	31 $\pm$ 4	36 $\pm$ 4
BUN, MG %	14.0 $\pm$.9	14.5 $\pm$.5	13.5 $\pm$.5	15.5 $\pm$.5
IMMUNOGLOBULIN E, IU/ML	2740 $\pm$ 139			1918 $\pm$ 187 ^{a/}

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

ENTRIES ARE MEAN $\pm$ STANDARD ERROR.

TABLE 8

LABORATORY DATA OF FEMALE DOGS AFTER ADMINISTRATION OF 2,4-DNT FOR 6 MONTHS
(C.N) CONTROL (T.N) TREATED N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C. 6)	0.2 (T. 6)	1.5 (T. 4)	10 (T. 6)
ERYTHROCYTES ($\times 10^6$ /MM ³)	5.90 $\pm$.08	5.45 $\pm$.11	5.67 $\pm$.24	5.21 $\pm$.13 ^{a/}
WEINZ RODIES. %	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	1.48 $\pm$.39 ^{a/}
RETICULOCYTES. %	.84 $\pm$.22	.57 $\pm$.04	.66 $\pm$.19	2.94 $\pm$.60 ^{a/}
HEMATOCRIT. VOL. %	47.5 $\pm$ 1.0	43.3 $\pm$.9	47.3 $\pm$ 1.7	44.8 $\pm$.9
HEMUGLOLIN. GM. %	15.5 $\pm$.3	14.6 $\pm$.3	15.4 $\pm$.4	14.6 $\pm$.3
METHENUGLOLIN. %	1.2 $\pm$.8	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	2.0 $\pm$.9
MCV. FURIC MICRONS	80.5 $\pm$ 1.3	79.6 $\pm$ 1.5	83.8 $\pm$ 2.6	86.1 $\pm$.9
MCHC. MICHU MICROGMS.	26.3 $\pm$.3	26.9 $\pm$.6	27.3 $\pm$.4	28.0 $\pm$.3
MCHC. GM %	32.8 $\pm$.3	33.8 $\pm$.3 ^{a/}	32.6 $\pm$.4	32.5 $\pm$.1
PLATELETS ($\times 10^3$ /MM ³)	2.1 $\pm$.2	2.2 $\pm$.3	2.5 $\pm$.4	3.0 $\pm$.2
LEUKOCYTES ($\times 10^3$ /MM ³)	14.3 $\pm$.9	11.9 $\pm$.7	10.6 $\pm$.5 ^{a/}	12.4 $\pm$ 1.1
NEUTROPHILS. %	65.0 $\pm$ 3.9	63.0 $\pm$ 4.3	62.0 $\pm$ 3.3	59.2 $\pm$ 2.8
LYMPHOCYTES. %	40.0 $\pm$ 7.9	33.5 $\pm$ 4.3	29.7 $\pm$ 3.5	31.5 $\pm$ 2.8
BANDS. %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	1.3 $\pm$.7	.5 $\pm$.2
EOSINOPHILS. %	2.3 $\pm$.6	3.0 $\pm$.4	6.0 $\pm$ 1.9	8.5 $\pm$ 1.7 ^{a/}
BASOPHILS. %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MONOCYTES. %	1.0 $\pm$.5	.5 $\pm$.5	1.0 $\pm$.5	.3 $\pm$.2
ATYPICAL. %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
NUCLEATED RBC. %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	.3 $\pm$.3
CLOTTING TIME. MIN.	8.2 $\pm$.4	7.2 $\pm$.5	9.5 $\pm$.4	8.2 $\pm$.6
GLUCOSE (FASTING). MG %	104.7 $\pm$ 3.8	93.2 $\pm$ 2.6 ^{a/}	100.7 $\pm$ 1.2	98.8 $\pm$ 1.9
SGOT. IU/L	28.2 $\pm$ 1.9	27.3 $\pm$ 1.6	23.7 $\pm$ 1.1	25.5 $\pm$ 2.5
SGPT. IU/L	35.0 $\pm$ 5.6	44.0 $\pm$ 2.5	37.5 $\pm$ 2.1	38.2 $\pm$ 4.5
ALK. PHOS. IU/L	37 $\pm$ 5	33 $\pm$ 5	29 $\pm$ 5	31 $\pm$ 5
BUN. MG %	12.7 $\pm$ 1.0	15.7 $\pm$ 1.7	14.2 $\pm$ 1.4	14.0 $\pm$.7
IMMUNOGLOBULIN E. IU/ML	2425 $\pm$ 128			1979 $\pm$ 115 ^{a/}

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

ENTRIES ARE MEAN $\pm$ STANDARD ERROR.

TABLE 9

LABORATORY DATA OF MALE DOGS AFTER ADMINISTRATION OF 2,4-DNT FOR 9 MONTHS

(C.N) CONTROL

(T.N) TREATED

N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C. 4)	0.2 (T. 6)	1.5 (T. 6)	10 (T. 2)
ERYTHROCYTES (X10 ⁶ /MM ³)	5.51 ± .12	4.23 ± .18	5.36 ± .13 ^a	5.58 ± .52 ^{a/}
WEINZ RBCIES. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.8 ± .4 ^{a/}
RETICULOCYTES. %	.69 ± .15	.95 ± .09	.57 ± .11	1.39 ± .47
HEMATOCRIT. VOL. %	46.7 ± 1.0	45.3 ± 1.1	45.0 ± .9	42.0 ± 3.0
HEMOGLOBIN. GM. %	16.5 ± .3	15.4 ± .4	15.7 ± .3	14.1 ± 1.4 ^{a/}
METHEMOGLOBIN. %	0.0 ± 0.0	0.0 ± 0.0	.9 ± .4	2.8 ± .3 ^{a/}
MCV. CUBIC MICRONS	71.6 ± .5	72.8 ± .5	70.8 ± .5	75.3 ± 1.6 ^{a/}
MCH. MICRO MICROGRAMS.	25.3 ± .2	24.7 ± .1	24.7 ± .3	25.2 ± .2
MCHC. GM. %	35.3 ± .2	33.9 ± .1	34.9 ± .3	33.5 ± .9 ^{a/}
PLATELETS (X10 ³ /MM ³)	2.3 ± .1	2.2 ± .2	2.6 ± .2	3.3 ± .0 ^{a/}
LEUKOCYTES (X10 ³ /MM ³)	12.6 ± .7	13.1 ± .5	10.0 ± .3 ^{a/}	10.5 ± .9
NEUTROPHILS. %	58.7 ± 2.8	61.3 ± 3.2	60.8 ± 1.6	63.5 ± 8.5
LYMPHOCYTES. %	34.3 ± 1.0	35.8 ± 2.7	33.8 ± 1.4	28.5 ± 7.5
BANDS. %	.2 ± .2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS. %	3.5 ± 1.6	2.5 ± .9	4.8 ± 1.1	7.0 ± 2.0
BASOPHILS. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES. %	1.3 ± .4	.3 ± .2	.5 ± .2	1.0 ± 1.0
ATYPICAL. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED WBC. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.5 ± .5 ^{a/}
CLOTTING TIME. MIN.	4.4 ± .3	9.3 ± .7	9.5 ± .5	9.0 ± .5
GLUCOSE (FASTING). MG. %	91.7 ± 2.3	95.8 ± 1.1	94.2 ± 1.0	90.0 ± 1.0
SGOT. IU/L	25.5 ± 2.5	28.8 ± 1.4	27.2 ± 1.6	43.5 ± 26.5 ^{a/}
SGPT. IU/L	40 ± 4	39 ± 2	54 ± 4	172 ± 46 ^{a/}
ALK. PHOS. IU/L	24 ± 2	24 ± 2	29 ± 4	39 ± 14
HUN. MG. %	14.2 ± 1.3	15.2 ± .9	14.0 ± .9	14.5 ± .5
IMMUNOGLOBULIN E. IU/ML	800 ± 52 (5)			1048 ± 187

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

ENTRIES ARE MEAN ± STANDARD ERROR.

TABLE 10

LABORATORY DATA OF FEMALE DOGS AFTER ADMINISTRATION OF 2,4-DNT FOR 9 MONTHS

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

DOSE: MG/KG/DAY	0 (C. 6)	0.2 (T. 6)	1.5 (T. 5)	10 (T. 6)
ERYTHROCYTES (X10 ⁶ /MM ³)	5.49 ± .24	5.40 ± .17	5.78 ± .21	5.05 ± .17 ^{a/}
MEINZ ROUTES. A	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	.04 ± .23 ^{a/}
RETICULOCYTES. A	.00 ± .00	.70 ± .17	.05 ± .10	1.33 ± .19 ^{a/}
HEMATOCRIT. VOL. A	46.0 ± 1.3	42.8 ± 1.7	42.5 ± 1.2	37.2 ± 1.6 ^{a/}
HEMOGLOBIN. GM. A	16.1 ± .5	14.7 ± .6	14.8 ± .5	13.3 ± .5 ^{a/}
METHEMOGLOBIN. A	.4 ± .4	0.0 ± 0.0	1.3 ± .6	2.8 ± .7 ^{a/}
MCV. CUBIC MICRONS	71.0 ± .8	72.4 ± 1.1	73.6 ± 1.1	73.6 ± 2.0
MCH. MICRO MICROMS.	24.9 ± .3	24.4 ± .4	25.7 ± .4	26.3 ± .2 ^{a/}
MCHC. GM. A	35.0 ± .2	34.3 ± .4	34.9 ± .3	35.9 ± 1.2
PLATELETS (X10 ³ /MM ³)	2.4 ± .1	2.4 ± .1	2.6 ± .3	3.3 ± .1
LEUKOCYTES (X10 ³ /MM ³)	13.0 ± 1.1	14.7 ± 1.3	9.8 ± .4	12.0 ± .7
NEUTROPHILS. A	58.2 ± 2.4	61.2 ± 3.4	62.2 ± 2.2	63.5 ± 2.6
LYMPHOCYTES. A	39.2 ± 2.0	35.3 ± 3.1	32.5 ± 2.4	32.0 ± 2.4
MONOS. A	.2 ± .2	0.0 ± 0.0	.2 ± .2	0.0 ± 0.0
EOSINOPHILS. A	1.4 ± .9	1.7 ± .4	1.3 ± .4	3.5 ± 1.0
PLASMODIUM. A	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES. A	.7 ± .3	1.8 ± .9	.5 ± .3	1.0 ± .3
ATYPICAL. A	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED -MC. A	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.2 ± .2
CLOTTING TIME. MIN.	10.4 ± .2	8.3 ± .4 ^{a/}	9.1 ± .4 ^{a/}	9.1 ± .4 ^{a/}
GLUCOSE (FASTING). MG. A	13.3 ± 4.2	48.2 ± 2.7	94.8 ± 2.9	93.8 ± 2.2
SALT. IU/L	24.2 ± 1.7	25.4 ± 1.9	39.3 ± 12.4	28.7 ± 3.1
SGPT. IU/L	31.8 ± 2.3	37.0 ± 2.2	50.3 ± 14.5	44.2 ± 9.1
ALA. PHOS. IU/L	30 ± 2	29 ± 4	27 ± 5	24 ± 2
BUN. MG. A	13.7 ± .4	13.7 ± .4	14.5 ± 1.0	12.2 ± .9
IMMUNOGLOBULIN E. IU/ML	750 ± 62 (5)			725 ± 62

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

ENTRIES ARE MEAN ± STANDARD ERROR.

TABLE 11

LABORATORY DATA OF MALE DOGS AFTER ADMINISTRATION OF 2,4-DNT FOR 12 MONTHS
(C.N) CONTROL (T.N) TREATED N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C. 6)	0.2 (T. 6)	1.5 (T. 6)	10 (T. 2)
ERYTHROCYTES ($\times 10^6$ /MM ³)	5.96 $\pm$.22	5.33 $\pm$.16	5.69 $\pm$.19	5.22 $\pm$.19
HEINZ BODIES, %	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	.92 $\pm$.37 ^{a/}
RETICULOCYTES, %	.40 $\pm$.09	.78 $\pm$.13	.66 $\pm$.11	1.23 $\pm$.23 ^{a/}
HEMATOCRIT, VOL. %	45.2 $\pm$.9	41.8 $\pm$ 1.2	44.7 $\pm$.8	44.0 $\pm$ 4.0
HEMOGLOBIN, GM. %	15.1 $\pm$.4	14.1 $\pm$.3	14.8 $\pm$.4	14.2 $\pm$ 1.2
METHEMOGLOBIN, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MCV, CURIC MICRONS	76.0 $\pm$ 1.9	78.5 $\pm$.5	78.8 $\pm$ 1.8	84.0 $\pm$ 4.5
MCHM, MICRO MICROGMS.	25.4 $\pm$.5	26.5 $\pm$.3	26.0 $\pm$.4	27.1 $\pm$ 1.3
MCHC, GM %	33.5 $\pm$.3	33.8 $\pm$.3	33.1 $\pm$.4	32.3 $\pm$.2
PLATELETS ($\times 10^3$ /MM ³)	1.7 $\pm$.2	1.7 $\pm$.1	1.4 $\pm$.2	3.3 $\pm$.6 ^{a/}
LEUKOCYTES ($\times 10^3$ /MM ³)	9.0 $\pm$.3	7.4 $\pm$ 1.5	9.1 $\pm$.4	9.3 $\pm$ 1.6
NEUTROPHILS, %	62.0 $\pm$ 2.3	58.3 $\pm$ 4.2	61.7 $\pm$ 3.0	72.5 $\pm$ 1.5
LYMPHOCYTES, %	32.0 $\pm$ 1.9	37.7 $\pm$ 3.5	33.2 $\pm$ 2.7	23.5 $\pm$ 1.5
BANDS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
EOSINOPHILS, %	5.0 $\pm$ 1.6	3.8 $\pm$.9	5.0 $\pm$.6	3.0 $\pm$ 3.0
BASOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MONOCYTES, %	1.0 $\pm$.4	.2 $\pm$.2	.2 $\pm$.2	1.0 $\pm$ 0.0
ATYPICAL, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
NUCLEATED RBC, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
CLOTTING TIME, MIN.	7.3 $\pm$.3	10.1 $\pm$.5 ^{a/}	7.6 $\pm$.2	9.5 $\pm$ 1.5
GLUCOSE (FASTING), MG %	90.2 $\pm$ 3.4	91.2 $\pm$ 2.2	91.7 $\pm$ 2.1	87.5 $\pm$ 4.5
SGOT, IU/L	28.2 $\pm$ 1.4	25.5 $\pm$ 2.2	29.7 $\pm$ 3.4	28.0 $\pm$ 7.0
SGPT, IU/L	55.5 $\pm$ 9.5	35.5 $\pm$ 1.3	66.5 $\pm$ 11.8	52.5 $\pm$ 6.5
ALK. PHOS., IU/L	25 $\pm$ 3	21 $\pm$ 3	37 $\pm$ 4	32 $\pm$ 2
BUN, MG %	13.2 $\pm$ 1.4	14.8 $\pm$ 1.4	13.0 $\pm$.9	13.0 $\pm$ 2.0
IMMUNOGLOBULIN F, IU/ML	1521 $\pm$ 31			1613 $\pm$ 288

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

ENTRIES ARE MEAN $\pm$ STANDARD ERROR.

TABLE 12

LABORATORY DATA OF FEMALE DOGS AFTER ADMINISTRATION OF 3,4-DNT FOR 12 MONTHS

(C.N) CONTROL

(T.N) TREATED

N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C. 6)	0.2 (T. 6)	1.5 (T. 6)	10 (T. 6)
ERYTHROCYTES ($\times 10^6$ /MM ³)	5.87 $\pm$.20	5.54 $\pm$.14	4.64 $\pm$.25 ^{a/}	4.45 $\pm$.25 ^{a/}
HEINZ BODIES, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	.5 $\pm$.2 ^{a/}
RETICULOCYTES, %	.39 $\pm$.04	.84 $\pm$.07 ^{a/}	.45 $\pm$.04	1.54 $\pm$.14 ^{a/}
HEMATOCRIT, VOL. %	45.8 $\pm$ 1.1	44.7 $\pm$ 1.3	41.8 $\pm$ 1.2 ^{a/}	40.8 $\pm$.7 ^{a/}
HEMOGLOBIN, GM. %	15.1 $\pm$.4	15.1 $\pm$.4	13.7 $\pm$.4 ^{a/}	12.9 $\pm$.2 ^{a/}
METHEMOGLOBIN, %	0.0 $\pm$ 0.0	.6 $\pm$.6	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MCV, CUBIC MICRONS	78.4 $\pm$ 1.9	80.7 $\pm$ 1.7	89.8 $\pm$ 2.1	93.5 $\pm$ 4.9 ^{a/}
MCHM, MICRO MICROGMS.	24.9 $\pm$.7	27.2 $\pm$.5	29.3 $\pm$.7	29.5 $\pm$ 1.8
MCHMC, GM. %	33.1 $\pm$.2	33.7 $\pm$.3	32.6 $\pm$.2	31.5 $\pm$.2 ^{a/}
PLATELETS ($\times 10^3$ /MM ³)	2.0 $\pm$.1	2.3 $\pm$.2	2.2 $\pm$.3	3.0 $\pm$.1 ^{a/}
LEUKOCYTES ($\times 10^3$ /MM ³)	10.4 $\pm$ 1.3	10.0 $\pm$.9	9.4 $\pm$ 1.0	10.8 $\pm$.4
NEUTROPHILS, %	66.8 $\pm$ 3.2	60.3 $\pm$ 4.4	63.2 $\pm$ 2.7	66.3 $\pm$ 2.8
LYMPHOCYTES, %	29.5 $\pm$ 3.5	37.5 $\pm$ 4.0	31.2 $\pm$ 4.5	30.3 $\pm$ 2.9
HANDS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
EOSINOPHILS, %	3.2 $\pm$.4	2.0 $\pm$ 1.1	5.5 $\pm$ 2.3	2.8 $\pm$.6
BASOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.1	0.0 $\pm$ 0.0
MONOCYTES, %	.5 $\pm$.3	.2 $\pm$.2	.2 $\pm$.2	.5 $\pm$.3
ATYPICAL, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
NUCLEATED RBC, %	.2 $\pm$.2	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
CLOTTING TIME, MIN.	7.4 $\pm$.4	10.1 $\pm$.1 ^{a/}	7.0 $\pm$.2	7.3 $\pm$.4
GLUCOSE (FASTING), MG %	92.8 $\pm$ 2.4	79.1 $\pm$ 4.1	87.4 $\pm$ 6.2	89.7 $\pm$ 3.2
SGOT, IU/L	29.7 $\pm$ 2.4	24.4 $\pm$ 2.9	27.3 $\pm$.7	31.3 $\pm$ 1.8
SGPT, IU/L	36.5 $\pm$ 1.4	43.5 $\pm$ 4.5	36.5 $\pm$ 3.2	51.7 $\pm$ 4.3 ^{a/}
ALK. PHOS., IU/L	27 $\pm$ 2	24 $\pm$ 3	23 $\pm$ 3	21 $\pm$ 2
BUN, MG %	11.7 $\pm$.7	13.3 $\pm$ 1.1	11.8 $\pm$ 1.2	11.5 $\pm$.6
IMMUNOGLOBULIN E, IU/ML	1447 $\pm$ 47			1933 $\pm$ 60

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).ENTRIES ARE MEAN $\pm$ STANDARD ERROR.

TABLE 13

LABORATORY DATA OF MALE DOGS AFTER ADMINISTRATION OF 2,4-DNT FOR 18 MONTHS
(C.N) CONTROL (T.N) TREATED N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C, 4)	0.2 (T, 4)	1.5 (T, 4)	10 (T, 2)
ERYTHROCYTES (X10 ⁶ /MM ³)	6.80 ± .28	6.59 ± .08	6.59 ± .27	6.37 ± .18
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.47 ± .03	.31 ± .07	.57 ± .07	.71 ± .31
HEMATOCRIT, VOL. %	49.0 ± 2.9	47.3 ± 1.1	45.3 ± 1.6	46.0 ± 2.0
HEMOGLOBIN, GM. %	17.1 ± .8	16.1 ± .4	16.1 ± .4	15.9 ± .8
METHENOGLOBIN, %	.9 ± .3	2.9 ± .6 ^{1/}	.7 ± .4	2.1 ± .9
MCV, CUBIC MICRONS	71.9 ± 1.6	71.7 ± 1.2	68.8 ± 2.0	72.2 ± 1.1
MCHB, MICRO MICROGMS.	25.2 ± .4	24.4 ± .4	24.5 ± .3	24.4 ± .5
MCHBC, GM %	35.0 ± .5	34.1 ± .6	35.7 ± 1.0	34.5 ± .1
PLATELETS (X10 ⁵ /MM ³)	1.8 ± .1	2.0 ± .2	2.3 ± .2	3.5 ± .1 ^{1/}
LEUKOCYTES (X10 ³ /MM ³)	10.0 ± .6	8.2 ± 1.2	8.3 ± .4	8.6 ± .2
NEUTROPHILS, %	73.3 ± 4.6	61.5 ± 4.6	69.5 ± 3.8	68.0 ± 1.0
LYMPHOCYTES, %	22.8 ± 5.3	33.8 ± 2.3	23.5 ± 3.5	25.5 ± .5
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	3.5 ± 1.0	4.5 ± 2.4	6.3 ± 2.3	6.0 ± 0.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.5 ± .5	.3 ± .3	.8 ± .5	.5 ± .5
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.	6.4 ± .2	8.9 ± 1.5	7.1 ± .6	8.8 ± .8
GLUCOSE (FASTING), MG %	91.3 ± 2.3	98.8 ± 1.7 ^{1/}	92.5 ± 2.4 ^{1/}	93.0 ± 0.0 ^{1/}
SGOT, IU/L	36.3 ± 3.3	25.3 ± 2.5 ^{2/}	40.0 ± 1.7	44.5 ± 1.5
SGPT, IU/L	44.5 ± 1.9	32.5 ± 2.9	61.8 ± 12.3	85.0 ± 25.0
ALK. PHOS., IU/L	24 ± 5	30 ± 6	31 ± 4	34 ± 10
BUN, MG %	13.8 ± 1.7	14.0 ± 2.5	13.8 ± .5	14.0 ± 1.0
IMMUNOGLOBULIN E, IU/ML	850 ± 0			650 ± 200

^{1/} SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

ENTRIES ARE MEAN ± STANDARD ERROR.

TABLE 14

LABORATORY DATA OF FEMALE DOGS AFTER ADMINISTRATION OF 2,4-DNT FOR 18 MONTHS

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF DOGS	
DOSE: MG/KG/DAY	0 (C, 4)	0.2 (T, 4)	1.5 (T, 4)	10 (T, 4)
ERYTHROCYTES (X10 ⁶ /MM ³)	6.86 ± .14	5.90 ± .26 ^{a/}	5.06 ± .21	6.31 ± .23
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.49 ± .17	.57 ± .15	.35 ± .07	.74 ± .18
HEMATOCRIT, VOL. %	49.0 ± .9	43.3 ± 2.4	42.3 ± 1.3	45.5 ± 1.8
HEMOGLOBIN, GM. %	17.4 ± .3	14.8 ± .8 ^{a/}	15.1 ± .4	15.4 ± .6
METHEMOGLOBIN, %	1.0 ± .7	5.4 ± .9 ^{a/}	.7 ± .4	2.1 ± 1.3
MCV, CUBIC MICRONS	71.4 ± .8	73.2 ± 1.2	69.8 ± 1.4	72.1 ± .4
MCHB, MICRO MICROGMS.	25.4 ± .3	25.0 ± .5	24.9 ± .4	24.4 ± .0
MCHWC, GM %	35.6 ± .1	34.2 ± .3 ^{a/}	35.8 ± .6	33.8 ± .3 ^{a/}
PLATELETS (X10 ⁵ /MM ³)	2.2 ± .2	2.7 ± .5	3.3 ± .4	3.5 ± .2
LEUKOCYTES (X10 ³ /MM ³)	11.1 ± 1.6	9.9 ± 1.0	7.3 ± .6	9.0 ± 2.1
NEUTROPHILS, %	67.0 ± 2.3	63.8 ± 5.4	62.3 ± 3.0	66.8 ± 4.3
LYMPHOCYTES, %	28.6 ± 2.1	31.5 ± 5.2	26.8 ± 3.6	30.8 ± 4.7
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EUSINOPHILS, %	2.8 ± .9	3.0 ± 1.1	10.0 ± 4.9	2.3 ± .6
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.5 ± .6	1.8 ± .5	1.0 ± .4	.3 ± .3
ATYPICAL, %	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	.3 ± .3
CLOTTING TIME, MIN.	6.5 ± .5	7.4 ± 1.5	7.8 ± .3	9.4 ± .3
GLUCOSE (FASTING), MG %	91.0 ± 2.7	98.0 ± 4.1	91.8 ± 1.4	93.3 ± 3.1
SGOT, IU/L	33.3 ± 1.4	28.0 ± 2.9	35.5 ± .9	62.5 ± 27.6
SGPT, IU/L	38.5 ± 1.5	46.0 ± 5.3	43.8 ± 3.9	75.5 ± 20.3
ALK. PHOS., IU/L	24 ± 2	24 ± 5	35 ± 9	21 ± 2
BUN, MG %	12.5 ± 1.3	15.3 ± 4.3	12.8 ± 2.6	11.5 ± .6
IMMUNOGLOBULIN E, IU/ML	676 ± 174			917 ± 67 (3)

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

ENTRIES ARE MEAN ± STANDARD ERROR.

TABLE 15

LABORATORY DATA OF MALE DOGS AFTER ADMINISTRATION OF 2,4-DNT FOR 24 MONTHS
(C.N) CONTROL (T.N) TREATED N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C, 4)	0.2 (T, 4)	1.5 (T, 4)	10 (T, 2)
ERYTHROCYTES ($\times 10^6$ /MM ³)	5.86 $\pm$.15	6.11 $\pm$.27	5.87 $\pm$.22	6.65 $\pm$.51
HEINZ BODIES, %	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
RETICULOCYTES, %	.73 $\pm$.14	.75 $\pm$.19	.62 $\pm$.16	.49 $\pm$.05
HEMATOCRIT, VOL. %	46.3 $\pm$ 1.3	45.0 $\pm$ 1.3	44.0 $\pm$ 1.5	48.5 $\pm$ 1.5
HEMOGLOBIN, GM. %	15.5 $\pm$.6	14.9 $\pm$.6	14.7 $\pm$.6	16.3 $\pm$.8
METHEMOGLOBIN, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	1.2 $\pm$.7	1.4 $\pm$ 1.4
MCV, CUBIC MICRONS	78.9 $\pm$.7	73.8 $\pm$ 1.3 ^{a/}	75.0 $\pm$.3	73.2 $\pm$ 3.4 ^{a/}
MCHC, MICRO MICROGMS.	26.5 $\pm$.4	24.4 $\pm$.2 ^{a/}	25.1 $\pm$.2 ^{a/}	24.5 $\pm$.6 ^{a/}
MCHBC, GM %	33.5 $\pm$.3	33.1 $\pm$.5	33.5 $\pm$.4	33.5 $\pm$.7
PLATELETS ($\times 10^5$ /MM ³)	2.1 $\pm$.2	2.0 $\pm$.1	2.3 $\pm$.2	3.0 $\pm$.4 ^{a/}
LEUKOCYTES ($\times 10^3$ /MM ³)	11.7 $\pm$ 1.1	8.9 $\pm$.6 ^{a/}	8.4 $\pm$.4 ^{a/}	8.5 $\pm$.1
NEUTROPHILS, %	60.5 $\pm$ 3.9	63.0 $\pm$ 4.0	58.5 $\pm$ 3.3	54.0 $\pm$ 2.0
LYMPHOCYTES, %	34.8 $\pm$ 2.7	33.5 $\pm$ 3.9	37.5 $\pm$ 3.2	40.5 $\pm$ 1.5
BANDS, %	.5 $\pm$.5	.3 $\pm$.3	1.3 $\pm$.6	1.0 $\pm$ 0.0
EOSINOPHILS, %	4.0 $\pm$ 1.5	2.0 $\pm$.7	2.8 $\pm$.5	4.5 $\pm$.5
BASOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MONOCYTES, %	.3 $\pm$.3	1.3 $\pm$.5	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
ATYPICAL, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
NUCLEATED RBC, %	.3 $\pm$.3	.3 $\pm$.3	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
CLOTTING TIME, MIN.	6.1 $\pm$.2	7.4 $\pm$.4	6.6 $\pm$.4	7.0 $\pm$.5
GLUCOSE (FASTING), MG %	97.8 $\pm$ 6.8	91.0 $\pm$ 1.8	84.5 $\pm$ 4.5	81.0 $\pm$ 3.0
SGOT, IU/L	28.5 $\pm$ 4.0	25.3 $\pm$ 4.4	27.8 $\pm$ 1.4	29.5 $\pm$ 1.5
SGPT, IU/L	51.8 $\pm$ 13.8	34.8 $\pm$ 2.8	95.8 $\pm$ 20.3	67.5 $\pm$ 15.5
ALK. PHOS., IU/L	20 $\pm$ 3	20 $\pm$ 4	28 $\pm$ 6	27 $\pm$ 5
BSP, %	5.0 $\pm$ 1.0 (2)	4.5 $\pm$.5 (2)	5.0 $\pm$.6 (3)	7.0 (1)
BUN, MG %	17.0 $\pm$ 2.1	17.0 $\pm$ 3.6	15.3 $\pm$.6	15.5 $\pm$.5
IMMUNOGLOBIN E, IU/ML	1863 $\pm$ 160			975 $\pm$ 221 ^{a/}

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

ENTRIES ARE MEAN $\pm$ STANDARD ERROR.

TABLE 16

LABORATORY DATA OF FEMALE DOGS AFTER ADMINISTRATION OF 2,4-DNT FOR 24 MONTHS

(C,N) CONTROL

(T,N) TREATED

N = NUMBER OF DOGS

DOSE: MG/KG/DAY	0 (C, 4)	0.2 (T, 4)	1.5 (T, 3)	10 (T, 4)
ERYTHROCYTES (X10 ⁶ /MM ³)	6.32 ± .06	5.78 ± .21	5.70 ± .15	6.53 ± .41
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.36 ± .12	.25 ± .02	.00 ± .03	.55 ± .10
HEMATOCRIT, VOL. %	48.3 ± 1.0	41.5 ± 1.6 ^{a/}	44.3 ± .7	47.3 ± 2.7
HEMOGLOBIN, GM. %	16.4 ± .3	14.2 ± .6	14.9 ± .2	16.1 ± 1.1
METHEMOGLOBIN, %	1.0 ± 1.0	0.0 ± 0.0	1.4 ± .8	2.0 ± .4
MCV, CUBIC MICRONS	76.4 ± 1.1	71.9 ± 1.8	77.9 ± 1.6	72.5 ± .9
MCHC, MICRO MICROGMS.	26.0 ± .4	24.6 ± .9	26.2 ± .6	24.7 ± .2
MCHC, GM. %	34.0 ± .4	34.1 ± .5	33.6 ± .2	34.1 ± .6
PLATELETS (X10 ³ /MM ³)	2.5 ± .2	2.9 ± .4	3.2 ± .5	2.4 ± .3
LEUKOCYTES (X10 ³ /MM ³)	11.5 ± 1.7	12.4 ± 1.6	7.4 ± .4	8.1 ± .3
NEUTROPHILS, %	57.5 ± 5.3	56.3 ± 3.3	56.3 ± 4.3	49.0 ± 1.7
LYMPHOCYTES, %	39.0 ± 4.9	42.3 ± 3.9	40.7 ± 2.9	46.5 ± 1.4
BANDS, %	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	3.0 ± 1.1	1.5 ± .9	3.0 ± 1.5	4.5 ± 1.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.3 ± .3	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	.3 ± .3	0.0 ± 0.0
CLOTTING TIME, MIN.	5.6 ± .1	7.9 ± .2 ^{a/}	6.2 ± .4	5.8 ± .1
GLUCOSE (FASTING), MG %	92.3 ± 4.1	94.5 ± 4.4	86.0 ± 4.4	91.3 ± 3.7
SGOT, IU/L	23.3 ± .8	19.9 ± .9 ^{a/}	28.0 ± 0.0 ^{a/}	25.0 ± 1.0
SGPT, IU/L	32.5 ± 3.6	29.8 ± 4.5 ^{a/}	25.7 ± 2.3	30.3 ± 1.4
ALK. PHOS., IU/L	24 ± 2	25 ± 3	36 ± 12	26 ± 3
BSP, %	5.5 ± .5 (2)	6.5 ± .5 (2)	4.0 (1)	6.0 ± 1.0 (2)
BUN, MG %	14.0 ± 1.2	15.5 ± 1.3	13.7 ± 1.8	12.5 ± 1.0
IMMUNOGLOBULIN E, IU/ML	1463 ± 296			1766 ± 224 (3)

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

ENTRIES ARE MEAN ± STANDARD ERROR.

TABLE 17

LABORATORY DATA OF MALE DOGS AFTER ADMINISTRATION OF 2,4-DNT FOR 12 MONTHS AND ALLOWING TO RECOVER FOR 1 MONTH

	0.0 (C. 1)	0.2 (T. 1)	1.5 (T. 1)
DOSE: MG/KG/DAY			
ERYTHROCYTES (X10 ⁶ /MM ³)	6.21	5.37	6.80
HEINZ BODIES, %	0.00	0.00	0.00
RETICULOCYTES, %	0.46	0.28	0.47
HEMATOCRIT, VOL. %	45.6	42.0	47.0
HEMOGLOBIN, GM. %	14.9	14.4	16.0
METHEMOGLOBIN, %	0.0	0.0	0.0
MCV, CUBIC MICRONS	72.4	78.2	69.1
MCH, MICRO MICROGRAMS	24.0	26.4	23.5
MCHC, GM. %	33.1	34.3	34.0
PLATELETS (X10 ⁵ /MM ³)	3.0	3.1	2.3
LEUKOCYTES (X10 ³ /MM ³)	7.4	11.2	8.5
NEUTROPHILS, %	62.0	45.0	60.0
LYMPHOCYTES, %	34.0	32.0	37.0
BANDS, %	0.0	0.0	0.0
EOSINOPHILS, %	3.0	0.0	3.0
BASOPHILS, %	0.0	0.0	0.0
MONOCYTES, %	1.0	0.0	0.0
ATYPICAL, %	0.0	0.0	0.0
NUCLEATED WBC, %	0.0	0.0	0.0
CLOTTING TIME, MIN.	4.0	10.0	10.0
GLUCOSE (FASTING), MG. %	102.0	97.0	90.0
SGOT, IU/L	21.0	24.0	18.0
SGPT, IU/L	31.0	37.0	46.0
ALK. PHOS., IU/L	21	24	25
BUN, MG. %	4.0	11.0	10.0

TABLE 18

LABORATORY DATA OF FEMALE DOGS AFTER ADMINISTRATION OF 2,4-DNT FOR 12 MONTHS AND ALLOWING TO RECOVER FOR 1 MONTH

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF DOGS	
DOSE: MG/KG/DAY	0 (C. 1)	0 (T. 1)	2 (T. 1)	10 (T. 1)
ERYTHROCYTES (X10 ⁶ /MM ³)	5.44	6.17	6.55	6.14
WEINZ BODIES. %	0.00	0.00	0.00	0.00
RETICULOCYTES. %	.71	.07	1.14	.75
HEMATOCRIT. VOL. %	42.0	46.0	46.0	42.0
HEMOGLOBIN. GM. %	14.1	15.1	15.5	14.5
METHEMOGLOBIN. %	0.0	0.0	0.0	0.0
MCV. CUBIC MICRONS	71.4	74.4	70.2	68.0
MCH. MICRO MICROGM.	24.1	24.4	23.7	23.5
MCHC. GM %	33.4	32.8	33.7	34.5
PLATELETS (X10 ³ /MM ³)	3.3	3.8	2.6	3.4
LEUKOCYTES (X10 ³ /MM ³)	4.5	11.4	10.5	8.6
NEUTROPHILS. %	41.6	64.0	71.0	47.0
LYMPHOCYTES. %	13.0	24.0	28.0	35.0
MONO. %	0.0	0.0	0.0	0.0
EOSINOPHILS. %	4.0	2.0	0.0	7.0
BASOPHILS. %	0.0	0.0	0.0	0.0
MONOCYTES. %	0.0	1.0	1.0	1.0
ATYPICAL. %	0.0	0.0	0.0	0.0
NUCLEATED WBC. %	0.0	0.0	0.0	0.0
CLOTTING TIME. MIN.	11.5	10.0	10.0	11.0
GLUCOSE (FASTING). MG %	103.0	46.0	112.0	95.0
SGOT. IU/L	24.0	31.0	24.0	15.0
SGPT. IU/L	34.0	52.0	34.0	31.0
ALK. PHOS. IU/L	25	30	25	27
BUN. MG %	10.0	10.0	8.0	10.0

TABLE 19

LABORATORY DATA OF MALE DOGS AFTER ADMINISTRATION OF 2,4-DNT FOR 24 MONTHS AND ALLOWING TO RECOVER FOR 1 MONTH

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF DOGS	
DOSE: MG/KG/DAY	0 (C. 2)	0.2 (T. 2)	1.5 (T. 2)	10 (T. 1)
ERYTHROCYTES (X10 ⁶ /MM ³)	6.48 ± .19	6.43 ± .68	5.90 ± .01	6.31
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
RETICULOCYTES, %	.33 ± .04	.71 ± .65	.14 ± .03	.26
HEMATOCRIT, VOL. %	43.5 ± .5	47.0 ± 4.0 ^{2/}	37.5 ± 2.5	42.0
HEMOGLOBIN, GM. %	15.6 ± .1	17.0 ± 1.7	13.8 ± .3	15.3
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	.9 ± .8	0.0
MCV, CUBIC MICRONS	67.1 ± 1.1	68.9 ± 1.0	63.6 ± 4.3	66.6
MCHC, MICRO MICROGMS.	24.1 ± .5	25.0 ± .1	23.3 ± .6	24.2
MCHC, GM %	35.9 ± .2	36.2 ± .6	36.8 ± 1.5	36.4
PLATELETS (X10 ⁵ /MM ³)	2.7 ± .2	2.0 ± .1	1.7 ± .1	2.1
LEUKOCYTES (X10 ³ /MM ³)	11.0 ± 1.1	7.0 ± 1.1	12.5 ± 3.2	9.0
NEUTROPHILS, %	42.5 ± 4.5	45.0 ± 8.0	78.0 ± 8.0	79.0
LYMPHOCYTES, %	15.5 ± 2.5	29.0 ± 6.0	18.0 ± 5.0	20.0
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
EOSINOPHILS, %	2.0 ± 2.0	5.5 ± 2.4	3.5 ± 2.5	0.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
MONOCYTES, %	0.0 ± 0.0	.5 ± .5	.5 ± .5	1.0
ATYPICAL, %	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
CLOTTING TIME, MIN.	6.5 ± .5	5.5 ± .5 ^{2/}	10.3 ± 1.4	10.5
GLUCOSE (FASTING), MG %	105.0 ± 1.0	99.0 ± 2.0	95.5 ± 1.5	106.0
SBOT, IU/L	13.5 ± 1.5	24.5 ± 3.5 ^{2/}	24.5 ± 6.5 ^{2/}	21.0
SOPT, IU/L	38.5 ± 4.5	29.0 ± 5.0	46.5 ± 40.5	40.0
ALK. PHOS., IU/L	14 ± 0	19 ± 9	26 ± 6	28
BSP, %	4.5 ± .5	7.5 ± .5	5.0 ± 1.0	7.0
BUN, MG %	8.5 ± 1.5	12.5 ± 1.5	10.5 ± .5	12.0
IMMUNOGLOBULIN E, IU/ML	975 ± 238			800

^{2/} SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

ENTRIES ARE MEAN ± STANDARD ERROR.

TABLE 20

LABORATORY DATA OF FEMALE DOGS AFTER ADMINISTRATION OF 2,4-DNT FOR 24 MONTHS AND ALLOWING TO RECOVER FOR 1 MONTH

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF DOGS	
DOSE: MG/KG/DAY	0 (C. 2)	0.2 (T. 2)	1.5 (T. 1)	10 (T. 2)
ERYTHROCYTES (X10 ⁶ /MM ³)	6.92 ± .83	6.10 ± .51	6.53	6.19 ± .20
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0
RETICULOCYTES, %	.64 ± .13	1.03 ± .45	.84	.33 ± 0.00
HEMATOCRIT, VOL. %	47.5 ± 6.5	45.0 ± 4.0	47.0	42.5 ± 2.5
HEMOGLOBIN, GM. %	17.1 ± 2.1	16.0 ± 1.2	16.9	15.1 ± 1.0
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	68.4 ± 1.2	73.7 ± .4	72.0	68.5 ± 1.9
MCHM, MICRO MICROGMS.	24.8 ± .0	26.2 ± .2	25.9	24.3 ± .9
MCHMC, GM %	36.2 ± .6	35.6 ± .5	36.0	35.4 ± .4
PLATELETS (X10 ⁵ /MM ³)	2.1 ± .0	2.3 ± .2	3.0	2.0 ± .4
LEUKOCYTES (X10 ³ /MM ³)	11.1 ± 1.4	9.7 ± 3.1	9.8	10.3 ± 1.6
NEUTROPHILS, %	56.0 ± 4.0	73.0 ± 1.0	74.0	69.0 ± 11.0
LYMPHOCYTES, %	41.0 ± 3.0	26.0 ± 1.0	23.0	29.0 ± 12.0
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0
EOSINOPHILS, %	3.0 ± 1.0	1.0 ± 0.0	2.0	2.0 ± 1.0
MASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	0.0 ± 0.0	1.0	0.0 ± 0.0
ATYPICAL, %	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
CLOTTING TIME, MIN.	8.5 ± .5	7.0 ± 0.0	6.4	8.5 ± 1.0
GLUCOSE (FASTING), MG %	95.5 ± .5	94.5 ± 4.5	103.0	101.0 ± 7.0
SGOT, IU/L	18.0 ± 0.0	23.0 ± 5.0	18.0	35.0 ± 11.0
SGPT, IU/L	27.5 ± 3.5	24.5 ± 6.5	37.0	47.5 ± 7.5
ALK. PHOS., IU/L	27 ± 4	22 ± 5	44	23 ± 10
BSP, %	4.0 ± 1.0	5.5 ± .5	4.0	5.5 ± .5
BUN, MG %	11.0 ± 0.0	8.5 ± .5	10.0	9.5 ± .5
IMMUNOGLOBULIN E, IU/ML	800 ± 0			850 ± 175

±/ SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

ENTRIES ARE MEAN ± STANDARD ERROR.

TABLE 21

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF DOGS GIVEN 2,4-DNT FOR 12 MONTHS

Dose (mg/kg/day)	Dog No.	Terminal Body Weight (kg)	Absolute Organ Weight (gm)									
			Brain	Heart	Liver	Kidney	Spleen	Adrenal	Pituitary	Thyroid	Testis	Ovary
0	161	13.6	94.6	105.7	339.9	80.4	91.9	1.74	0.08	0.93	16.5	
0	162	9.0	71.0	67.0	258.5	49.8	48.2	1.48	0.06	0.70		0.60
0.2	173	15.5	79.0	121.7	310.2	60.8	85.0	1.70	0.05	0.99	22.0	
0.2	174	9.7	84.1	82.5	212.2	45.0	64.8	1.44	0.04	0.94		1.15
1.5	185	11.8	83.7	93.4	360.2	52.1	79.9	1.44	0.09	0.74	19.3	
1.5	186	8.9	73.2	63.8	235.7	39.3	21.0	1.56	0.05	0.69		0.91
10.0	198	10.1	76.0	84.1	337.0	51.9	49.3	1.49	0.06	0.55		1.12

Dose (mg/kg/day)	Dog No.	Relative Organ Weight (gm/kg Body Weight)									
		Brain	Heart	Liver	Kidney	Spleen	Adrenal	Pituitary	Thyroid	Testis	Ovary
0	161	6.96	7.77	25.0	5.91	6.76	0.128	0.006	0.068	1.21	
0	162	7.89	7.44	28.7	5.53	5.36	0.164	0.007	0.078		0.067
0.2	173	5.10	7.85	20.0	3.92	5.48	0.110	0.003	0.064	1.42	
0.2	174	8.67	8.51	21.9	4.64	6.68	0.149	0.004	0.097		0.119
1.5	185	7.09	7.92	30.5	4.42	6.77	0.122	0.008	0.063	1.64	
1.5	186	8.22	7.17	26.5	4.42	2.36	0.175	0.006	0.078		0.102
10.0	198	7.53	8.33	33.4	5.14	4.88	0.148	0.006	0.055		0.111

Dose (mg/kg/day)	Dog No.	Relative Organ Weight (gm/gm Brain Weight)								
		Heart	Liver	Kidney	Spleen	Adrenal	Pituitary	Thyroid	Testis	Ovary
0	161	1.117	3.59	0.850	0.97	0.0184	0.0008	0.0098	0.174	
0	162	0.944	3.64	0.701	0.68	0.0208	0.0008	0.0099		0.0085
0.2	173	1.541	3.93	0.770	1.08	0.0215	0.0006	0.0125	0.279	
0.2	174	0.981	2.52	0.535	0.77	0.0171	0.0005	0.0112		0.0137
1.5	185	1.116	4.30	0.623	0.95	0.0172	0.0011	0.0088	0.231	
1.5	186	0.872	3.22	0.537	0.29	0.0213	0.0007	0.0094		0.0124
10.0	198	1.107	4.43	0.683	0.65	0.0196	0.0008	0.0072		0.0147

TABLE 22

SUMMARY OF LESIONS AND M/E RATIOS IN DOGS GIVEN 2,4-DNT FOR 12 MONTHS

Dosage (mg/kg/day):	0		0.2		1.5		10.0
Dog No.:	161	162	173	174	185	186	198
Sex:	M	F	M	F	M	F	F
<u>Treatment-Related Lesions</u> ^{a/}							
Liver							
Bile duct hyperplasia							1
<u>Pigment deposits</u>							1
<u>Other Lesions</u>							
Lung							
Parabronchiolar lymphoid hyperplasia				2			
<u>Granuloma</u>				1			
Liver							
Parasite migration scar	2						
<u>Fatty change</u>					1		1
Pancreas							
<u>Mononuclear cell foci</u>				1			
Stomach							
<u>Gastritis</u>				1			
Intestine							
Ascariasis	1						
<u>Lymphoid hyperplasia</u>				1			
Kidney							
<u>Fatty change</u>						2	
Prostate							
<u>Focal interstitial prostatitis</u>			1				
Pituitary							
Colloid cyst					1		
<u>Mononuclear cell foci</u>					1		
Adrenal Gland							
<u>Focal fatty change</u>				2			
Lymph Node							
<u>Eosinophilic granuloma</u>	1						
Spleen							
<u>Accessory spleen</u>					1		
Eye							
<u>Uveitis</u>					1		
Bone Marrow Smear							
<u>M/E ratio</u>	2.2	1.7	1.1	2.3	1.1	1.2	1.3

Tissues not listed were normal.

^{a/} Severity of lesions: 1 = mild; 2 = moderate; 3 = severe; 4 = very severe;

+ = questionable; X = present.

TABLE 23

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF DOGS GIVEN 2,4-DNT FOR 12 MONTHS AND ALLOWED TO RECOVER FOR 1 MONTH

Dose (mg/kg/day)	Dog No.	Terminal Body Weight (kg)	Absolute Organ Weight (gm)									
			Brain	Heart	Liver	Kidney	Spleen	Adrenal	Pituitary	Thyroid	Testis	Ovary
0	159	13.2	81.4	93.9	328.0	65.9	121.7	1.39	0.08	1.14	19.0	
0	160	8.4	81.6	78.4	244.0	46.0	38.9	1.31	--	0.82		1.30
0.2	171	13.4	79.4	115.4	274.2	70.4	115.4	1.12	0.08	1.08	23.6	
0.2	172	9.0	73.6	93.6	252.6	59.0	81.6	1.10	0.06	1.59		1.10
1.5	183	11.5	82.3	93.0	320.0	77.2	88.9	1.51	0.07	0.85	21.5	
1.5	184	8.4	74.5	61.0	297.8	38.6	56.4	1.30	0.05	0.60		1.02
10.0	196	10.0	79.0	82.4	304.3	57.7	73.5	1.67	0.08	0.84		3.24

Dose (mg/kg/day)	Dog No.	Relative Organ Weight (gm/kg Body Weight)									
		<u>Brain</u>	<u>Heart</u>	<u>Liver</u>	<u>Kidney</u>	<u>Spleen</u>	<u>Adrenal</u>	<u>Pituitary</u>	<u>Thyroid</u>	<u>Testis</u>	<u>Ovary</u>
0	159	6.17	7.11	24.8	4.99	9.22	0.105	0.006	0.086	1.44	
0	160	9.71	9.33	29.0	5.48	4.63	0.156	--	0.098		0.155
0.2	171	5.93	8.61	20.5	5.25	8.61	0.084	0.006	0.081	1.76	
0.2	172	8.18	10.40	28.1	6.56	9.07	0.122	0.007	0.177		0.122
1.5	183	7.16	8.09	27.8	6.71	7.73	0.131	0.006	0.074	1.87	
1.5	184	8.87	7.26	35.5	4.60	6.71	0.155	0.006	0.071		0.121
10.0	196	7.90	8.24	30.4	5.77	7.35	0.167	0.008	0.084		0.324

Dose (mg/kg/day)	Dog No.	Relative Organ Weight (gm/gm Brain Weight)								
		Heart	Liver	Kidney	Spleen	Adrenal	Pituitary	Thyroid	Testis	Ovary
0	159	1.154	4.03	0.810	1.50	0.0171	0.0010	0.0140	0.233	
0	160	0.961	2.99	0.564	0.48	0.0161	--	0.0100		0.0159
0.2	171	1.453	3.45	0.887	1.45	0.0141	0.0010	0.0136	0.297	
0.2	172	1.272	3.43	0.802	1.11	0.0149	0.0008	0.0216		0.0149
1.5	183	1.130	3.89	0.938	1.08	0.0183	0.0009	0.0103	0.261	
1.5	184	0.819	4.00	0.518	0.76	0.0174	0.0007	0.0081		0.0137
10.0	196	1.043	3.85	0.730	0.93	0.0211	0.0010	0.0106		0.0410

TABLE 24

SUMMARY OF LESIONS AND M/E RATIOS IN RECOVERY DOGS GIVEN 2,4-DNT FOR
12 MONTHS AND ALLOWED TO RECOVER FOR 1 MONTH

Dosage (mg/kg/day):	0		0.2		1.5		10.0
Dog No.:	159	160	171	172	183	184	196
Sex:	M	F	M	F	M	F	F
<u>Treatment-Related Lesions^{a/}</u>							
Liver							
Bile duct hyperplasia			1				
<u>Pigment deposits</u>					1		1
Kidney							
<u>Pigment deposits</u>					2		1
<u>Other Lesions</u>							
Lung							
Parabronchiolar lymphoid							
<u>hyperplasia</u>			1				
Liver							
Fatty change					1		2
<u>Portal inflammation</u>			1		1		
Salivary Gland							
<u>Mononuclear cell foci</u>					1	1	
Intestine							
Lymphoid hyperplasia	1						
Ascariasis	1						
<u>Cestodiasis</u>	1						
Kidney							
Fatty change		1				1	1
Prostate							
Focal interstitial prostatitis	2				1		
<u>Hyperplasia</u>			2				
Thyroid							
Chronic lymphocytic thyroiditis	4		4				
<u>Adenoma</u>			2				
Adrenal Gland							
Fatty change		3					
Spleen							
<u>Hemosiderotic plaque</u>			1				
Epididymis							
<u>Epithelial vacuolization</u>					1		
Bone Marrow Smear							
<u>M/E ratio</u>	1.9	1.7	1.3	1.5	b/	b/	1.6

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = severe; 4 = very severe;
+ = questionable; x = present.

b/ Smear not readable.

TABLE 25

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF DOGS GIVEN 2,4-DNT FOR 24 MONTHS

Dose (mg/kg/day)	Dog No.	Terminal Body Weight (kg)	Absolute Organ Weight (g)									
			Brain	Heart	Liver	Kidney	Spleen	Adrenal	Pituitary	Thyroid	Testis	Ovary
0	151	12.1	87.4	90.9	354.8	64.1	85.0	1.63	0.09	0.73	14.2	
0	152	8.7	77.0	61.0	273.2	38.2	26.2	1.26	0.08	0.52		1.63
0	153	12.2	86.4	100.0	308.4	54.1	100.0	1.19	0.08	0.93	17.5	
0	154	10.1	86.3	91.5	318.8	55.4	125.8	2.23	0.07	0.61		1.01
0.2	163	14.8	81.5	108.3	385.8	60.8	140.3	1.25	0.07	0.89	16.7	
0.2	164	10.6	85.9	64.0	389.3	55.8	51.5	1.77	0.06	1.05		1.43
0.2	165	13.7	74.3	112.3	339.1	61.2	83.4	1.33	0.07	0.63	19.2	
0.2	166	11.6	87.0	73.3	369.6	47.0	19.9	1.43	0.08	0.48		1.89
1.5	175	8.8	71.7	68.9	232.2	49.5	58.0	1.04	0.04	0.50	15.7	
1.5	176	7.7	81.0	60.3	263.9	43.7	52.6	1.22	0.07	0.52		1.54
1.5	177	11.4	81.0	85.1	284.8	53.5	80.5	1.31	0.06	0.92	16.5	
1.5	180	7.6	78.9	63.1	242.7	47.0	39.9	1.00	0.06	0.57		1.50
10.0	188	8.0	73.0	80.9	303.8	45.2	70.5	1.48	0.08	0.68		1.31
10.0	189	11.2	74.0	94.8	312.5	51.4	75.5	1.49	0.06	0.91	16.9	
10.0	190	10.2	74.0	87.4	309.2	55.5	72.2	1.49	0.08	0.58		1.14

Dose (mg/kg/day)	Dog No.	Relative Organ Weight (g/kg body weight)									
		Brain	Heart	Liver	Kidney	Spleen	Adrenal	Pituitary	Thyroid	Testis	Ovary
0	151	7.22	7.31	29.3	5.30	7.02	0.135	0.007	0.060	1.17	
0	152	8.85	7.01	31.4	4.39	3.01	0.145	0.009	0.060		0.187
0	153	7.08	8.20	25.3	4.43	8.20	0.098	0.007	0.076	1.43	
0	154	8.56	9.06	31.6	5.49	12.46	0.223	0.007	0.060		0.100
0.2	163	5.51	7.32	26.1	4.11	9.48	0.085	0.005	0.060	1.13	
0.2	164	8.10	6.04	36.7	5.26	4.86	0.167	0.006	0.099		0.135
0.2	165	5.42	8.20	24.8	4.47	6.09	0.097	0.005	0.046	1.40	
0.2	166	7.50	6.32	31.9	4.05	1.72	0.123	0.007	0.041		0.163
1.5	175	8.15	7.83	26.4	5.63	6.59	0.118	0.005	0.057	1.78	
1.5	176	10.52	7.83	34.3	5.68	6.83	0.158	0.009	0.068		0.200
1.5	177	7.11	7.46	25.0	4.69	7.06	0.115	0.005	0.081	1.45	
1.5	180	10.38	8.30	31.9	6.18	5.25	0.132	0.008	0.075		0.197
10.0	188	9.13	10.11	38.0	5.66	8.81	0.185	0.010	0.085		0.164
10.0	189	6.61	8.46	27.9	4.58	6.74	0.133	0.005	0.081	1.51	
10.0	190	7.25	8.57	30.3	5.44	7.08	0.146	0.008	0.057		0.112

Dose (mg/kg/day)	Dog No.	Relative Organ Weight (g/g brain weight)								
		Heart	Liver	Kidney	Spleen	Adrenal	Pituitary	Thyroid	Testis	Ovary
0	151	1.040	4.06	0.733	0.97	0.0186	0.0010	0.0084	0.163	
0	152	0.792	3.55	0.496	0.34	0.0164	0.0010	0.0068		0.0212
0	153	1.157	3.57	0.626	1.16	0.0138	0.0009	0.0108	0.203	
0	154	1.058	3.69	0.641	1.45	0.0260	0.0008	0.0071		0.0117
0.2	163	1.329	4.73	0.746	1.72	0.0153	0.0009	0.0109	0.205	
0.2	164	0.745	4.53	0.650	0.60	0.0206	0.0007	0.0122		0.0166
0.2	165	1.511	4.56	0.824	1.12	0.0179	0.0009	0.0085	0.258	
0.2	166	0.843	4.25	0.540	0.23	0.0164	0.0009	0.0055		0.0217
1.5	175	0.961	3.24	0.690	0.81	0.0145	0.0006	0.0070	0.219	
1.5	176	0.744	3.26	0.540	0.65	0.0151	0.0009	0.0064		0.0190
1.5	177	1.051	3.52	0.661	0.99	0.0162	0.0007	0.0114	0.204	
1.5	180	0.800	3.08	0.596	0.51	0.0127	0.0008	0.0072		0.0190
10.0	189	1.108	4.16	0.620	0.97	0.0203	0.0011	0.0093		0.0179
10.0	189	1.281	4.22	0.694	1.02	0.0201	0.0008	0.0123	0.228	
10.0	190	1.181	4.18	0.750	0.98	0.0201	0.0011	0.0078		0.0154

TABLE 26

SUMMARY OF LESIONS AND M/E RATIOS OF DOGS GIVEN 2,4-DNT FOR 24 MONTHS

	0						0.2						1.5						10.0			1.5 178 F ^b
	151	153	152	154	163	165	164	166	175	177	176	180	189	188	190	178	190	178	M	F	F	
Dosage (mg/kg/day):	M	M	F	F	M	M	F	F	M	M	FF	F	M	F	F	F	F	F	M	F	F	
Dog No.:																						
Sex:																						
<u>Treatment-Related Lesions^a</u>																						
Liver																						
- Bile duct hyperplasia								1											1	1	1	
- Pigment deposit								1											1	2	1	
- Gallbladder																						
- Cystic hyperplasia of epithelium			1																2	1	1	
- Epithelial pigmentation									1		1	1							1	1	2	
- Spleen																						
- Excessive pigment																			1	1		
- Kidney																						
- Epithelial pigmentation																				1	1	
<u>Other Lesions</u>																						
Adrenal																						
- Vasculation									1													
- Pituitary																						
- Cyst formation									1			1										1
- Thyroid																						
- Hypothyroidism																						3
- Chronic lymphocytic thyroiditis																						
- Lung																						
- Focal fibrosis (pleura)			1			1		1		1		1							1		1	
- Granulomatous pneumonia								1														
- Peribronchiolar or perivascular mononuclear cell aggregation			1	1	1	1																
- Interstitial pneumonia			1																			
- Muscular hypertrophy of bronchioles			1	1	1	1	1	1		1									1		2	1
- Bronchopneumonia																						
- Liver																						
- Vacuolization of hepatocyte (centrilobular)		1	1	1	1	1	1	1		1	1	1							1	1	1	
- Portal inflammation																						
- Gallbladder																						
- Lymphoid hyperplasia																						
- Spleen																						
- Hematopoiesis																						
- Pancreas																						
- Foci of mononuclear cells																						

TABLE 26 (Continued)

Lesion	Dosage (mg/kg/day):									
	0					0.2				
	151	153	152	154	163	165	164	166	175	177
Dog No:	M	M	F	F	M	M	F	F	M	M
Sex:	M	M	F	F	M	M	F	F	M	M
Thymus										
Hemorrhage										
Testis										
Aspermatogenesis	2									
Prostate										
Foci of mononuclear cells		1								
Stomach										
Lymphoid hyperplasia	1									
Intestine										
Enteritis										
Ascariasis							1			
Kidney										
Microcalculi		1		1	1		1	1	1	1
Tonsil										
Focal tonsillitis			1		1					
Rib										
Hemosiderosis										
Ear										
Papilloma										X
Eye										
"Cherry eye" (hyperplasia of glans nictitans)			1			1				
Keratitis			1							
Calcification of pigment epithelium			1							
Lymph Node										
Hemosiderocytosis							1			
Lymphoid hyperplasia										
Lymphadenitis (mesenteric lymph node)										
Bone Marrow Smear										
H/E Ratio	1.0	1.0	2.0	1.0	1.1	1.4	0.9	1.8	1.3	1.0
									0.9	0.9
									1.1	0.9
										1.4

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = severe; 4 = very severe; † = questionable; x = present.

b/ This dog died in week 98.

TABLE 27

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF DOGS GIVEN 2,4-DNT FOR 24 MONTHS AND
ALLOWED TO RECOVER FOR 1 MONTH

Dose (mg/kg/day)	Dog No.	Terminal Body Weight (kg)	Absolute Organ Weight (g)									
			Brain	Heart	Liver	Kidney	Spleen	Adrenal	Pituitary	Thyroid	Testis	Ovary
0	155	11.3	70.7	89.6	246.9	55.3	60.0	0.92	0.08	0.53	15.7	
0	156	9.4	78.2	71.5	270.9	43.4	46.6	1.31	0.07	0.79		0.88
0	157	12.2	84.6	107.0	308.5	59.5	65.0	1.05	0.10	0.49	18.0	
0	158	9.3	75.0	53.0	340.0	49.4	83.7	1.65	0.07	0.94		1.26
0.2	167	10.8	76.7	102.8	333.7	61.0	51.3	1.73	0.06	1.04	22.7	
0.2	168	10.4	73.9	85.7	259.2	46.4	58.6	1.35	0.08	0.61		2.09
0.2	169	15.0	71.4	122.1	389.2	65.8	57.7	1.81	0.08	0.87	21.8	
0.2	170	10.2	77.6	83.9	274.7	51.8	80.6	1.90	0.07	1.29		1.44
1.5	179	11.6	79.0	100.0	328.9	57.3	18.5	1.45	0.08	0.74	19.0	
1.5	181	10.7	74.4	80.8	289.0	54.7	66.2	1.25	0.08	1.02	12.3	
1.5	182	8.4	72.2	65.3	271.7	38.2	17.9	1.28	0.06	0.64		1.38
10.0	192	8.1	69.3	73.2	241.4	37.0	68.3	1.21	0.06	0.64		0.85
10.0	193	12.4	75.3	103.8	329.8	57.4	90.0	1.39	0.07	0.85	16.6	
10.0	194	7.7	81.5	73.8	213.2	41.5	39.7	1.22	0.05	0.61		0.98

Dose (mg/kg/day)	Dog No.	Relative Organ Weight (g/kg body weight)									
		Brain	Heart	Liver	Kidney	Spleen	Adrenal	Pituitary	Thyroid	Testis	Ovary
0	155	6.26	7.93	21.8	4.89	5.31	0.081	0.007	0.047	1.39	
0	156	8.32	7.61	28.8	4.62	4.96	0.139	0.007	0.084		0.094
0	157	6.93	8.77	25.3	4.88	5.33	0.086	0.008	0.040	1.48	
0	158	8.06	5.70	36.6	5.31	9.00	0.177	0.008	0.101		0.243
0.2	167	7.10	9.52	30.9	5.64	4.75	0.160	0.006	0.096	2.10	
0.2	168	7.10	8.24	24.9	4.46	5.63	0.130	0.007	0.059		0.201
0.2	169	4.76	8.14	25.9	4.39	3.05	0.121	0.005	0.061	1.45	
0.2	170	7.61	8.22	26.9	5.08	7.90	0.186	0.007	0.127		0.141
1.5	179	6.81	8.62	28.4	4.94	1.59	0.125	0.007	0.064	1.64	
1.5	181	6.95	7.55	27.0	5.11	6.18	0.117	0.008	0.095	1.15	
1.5	182	8.60	7.77	32.3	4.55	2.13	0.152	0.007	0.076		0.164
10.0	192	8.56	9.04	29.8	4.57	8.43	0.149	0.007	0.079		0.105
10.0	193	6.07	8.37	26.6	4.63	7.26	0.112	0.006	0.069	1.34	
10.0	194	10.38	9.58	27.7	5.39	5.16	0.127	0.007	0.079		0.127

Dose (mg/kg/day)	Dog No.	Relative Organ Weight (g/g brain weight)								
		Heart	Liver	Kidney	Spleen	Adrenal	Pituitary	Thyroid	Testis	Ovary
0	155	1.267	3.49	0.732	0.849	0.0130	0.0011	0.0073	0.222	
0	156	0.914	3.46	0.555	0.596	0.0168	0.0009	0.0101		0.0113
0	157	1.263	3.65	0.703	0.768	0.0124	0.0012	0.0058	0.213	
0	158	0.707	4.53	0.659	1.116	0.0220	0.0009	0.0123		0.0301
0.2	167	1.340	4.35	0.795	0.668	0.0225	0.0008	0.0136	0.296	
0.2	168	1.160	3.51	0.627	0.793	0.0183	0.0010	0.0083		0.0283
0.2	169	1.710	5.45	0.922	0.808	0.0254	0.0011	0.0127	0.305	
0.2	170	1.081	3.54	0.668	1.039	0.0245	0.0009	0.0166		0.0186
1.5	179	1.266	4.16	0.725	0.234	0.0184	0.0010	0.0094	0.241	
1.5	181	1.086	3.80	0.735	0.890	0.0168	0.0011	0.0137	0.165	
1.5	182	0.904	3.76	0.529	0.248	0.0177	0.0008	0.0089		0.0191
10.0	192	1.056	3.48	0.534	0.986	0.0175	0.0009	0.0092		0.0123
10.0	193	1.379	4.38	0.764	1.195	0.0185	0.0009	0.0113	0.221	
10.0	194	0.906	2.62	0.509	0.487	0.0150	0.0006	0.0073		0.0120

TABLE 28

SUMMARY OF LESIONS AND M/E RATIOS IN DOGS GIVEN 2,4-DNT FOR 24 MONTHS
AND ALLOWED TO RECOVER FOR 1 MONTH

Dosage (mg/kg/day):														
Dog No.:														
Sex:														
Treatment-Related Lesions ^{a/}														
Liver														
Bile duct hyperplasia														
Pigment deposition														
Gallbladder														
Cystic hyperplasia of epithelium														
Epithelial pigmentation														
Spleen														
Excessive pigment														
Kidney														
Epithelial pigmentation														
Other Lesions														
Adrenal Gland														
Vacuolation														
Thyroid														
Chronic lymphocytic thyroiditis														
Lung														
Focal fibrosis (pleura)														
Granulomatous pneumonia														
Peribronchiolar or perivascular mononuclear cells aggregation														
Interstitial pneumonia														
Muscular hypertrophy of bronchioles														
Bronchopneumonia														
Liver														
Vacuolization of hepatocytes (centrilobular)														
Portal inflammation														
Gallbladder														
Lymphoid hyperplasia														
Spleen														
Hematopoiesis (extramedullary)														
Ectopic nodule														
Testis														
Testicular degeneration														
Epididymis														
Foci of mononuclear cells														
Uterus														
Cystic gland														

TABLE 29

SUMMARY OF LESIONS IN DOGS GIVEN 10 MG/KG/DAY OF 2,4-DNT
AND DYING AT UNSCHEDULED TIMES

Dog number:	191	195	197
Week of death:	8	19	20

Treatment-Related Lesions^{a/}

Liver

Pigmentation	1		
<u>Bile duct hyperplasia</u>			1

Spleen

<u>Pigmentation</u>	1	1	1
---------------------	---	---	---

Cerebellum

Vacuolation		±	2
Hypertrophy and mitosis of endothelium			2
Gemastocytosis		1	2
<u>Perivascular hemorrhage</u>		1	1

Brain Stem

<u>Perivascular hemorrhage</u>		1	1
--------------------------------	--	---	---

Other Lesions

Liver

Vacuolation of hepatocytes		1	1
<u>Microgranuloma</u>		1	

Intestine

<u>Lymphoid hyperplasia of Peyer's patches</u>		2	
--	--	---	--

Tonsil

<u>Focal tonsillitis</u>		1	1
--------------------------	--	---	---

Lymph Node

<u>Erythrophagocytosis</u>			1
----------------------------	--	--	---

Urinary Bladder

Cytoplasmic vacuolation of <u>epithelium</u>			1
---	--	--	---

Adrenal Gland

Cytoplasmic vacuolation in zona <u>glomerulosa</u>		1	
---	--	---	--

Skeletal Muscle

<u>Degeneration</u>		±	
---------------------	--	---	--

Organs not listed were normal.

^{a/} Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe;
 ± = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 30

CHROMOSOMES AND THEIR MORPHOLOGICAL ABERRATIONS FROM DOGS FED 2,4-DNT FOR 24 MONTHS

Dose (mg/kg/day)	Tissue Culture	Number of Dogs	Tetraploids per 100 Cells	Chromatid Breaks and Gaps per 50 Cells	Translocations per 50 Cells	Total Aberrations Per 50 Cells
0	Bone marrow	3	0.17 ± 0.17^a	0	0	0
	Kidney	4	0.62 ± 0.24	0	0	0
10	Bone marrow	3	0.17 ± 0.17	0.3 ± 0.3	0	0.3 ± 0.3
	Kidney	3	1.50 ± 0.58	0	0	0

^a/ Mean $\pm$ standard error.

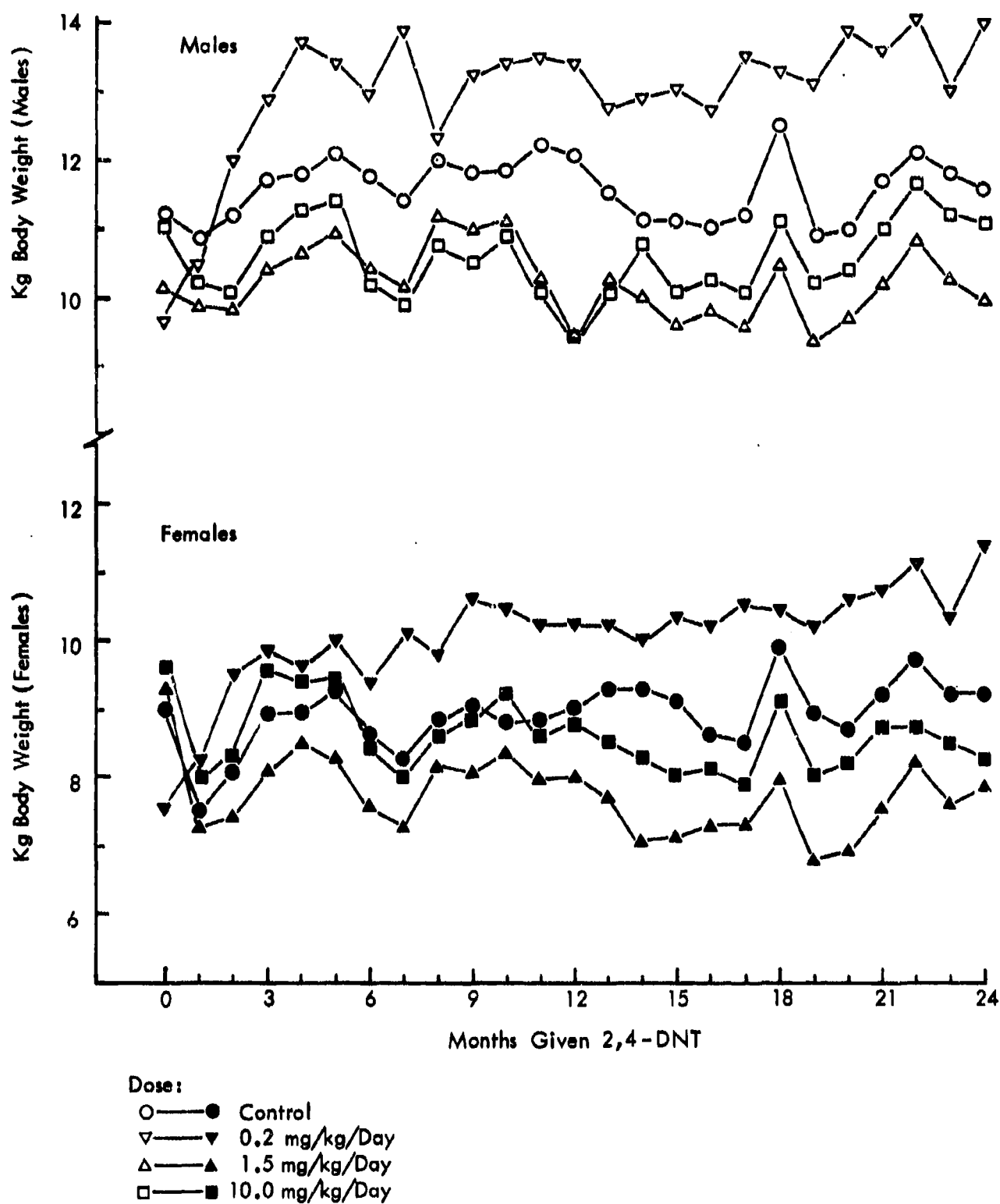


Figure 2 - Average Body Weights of Dops Given 2,4-DNT

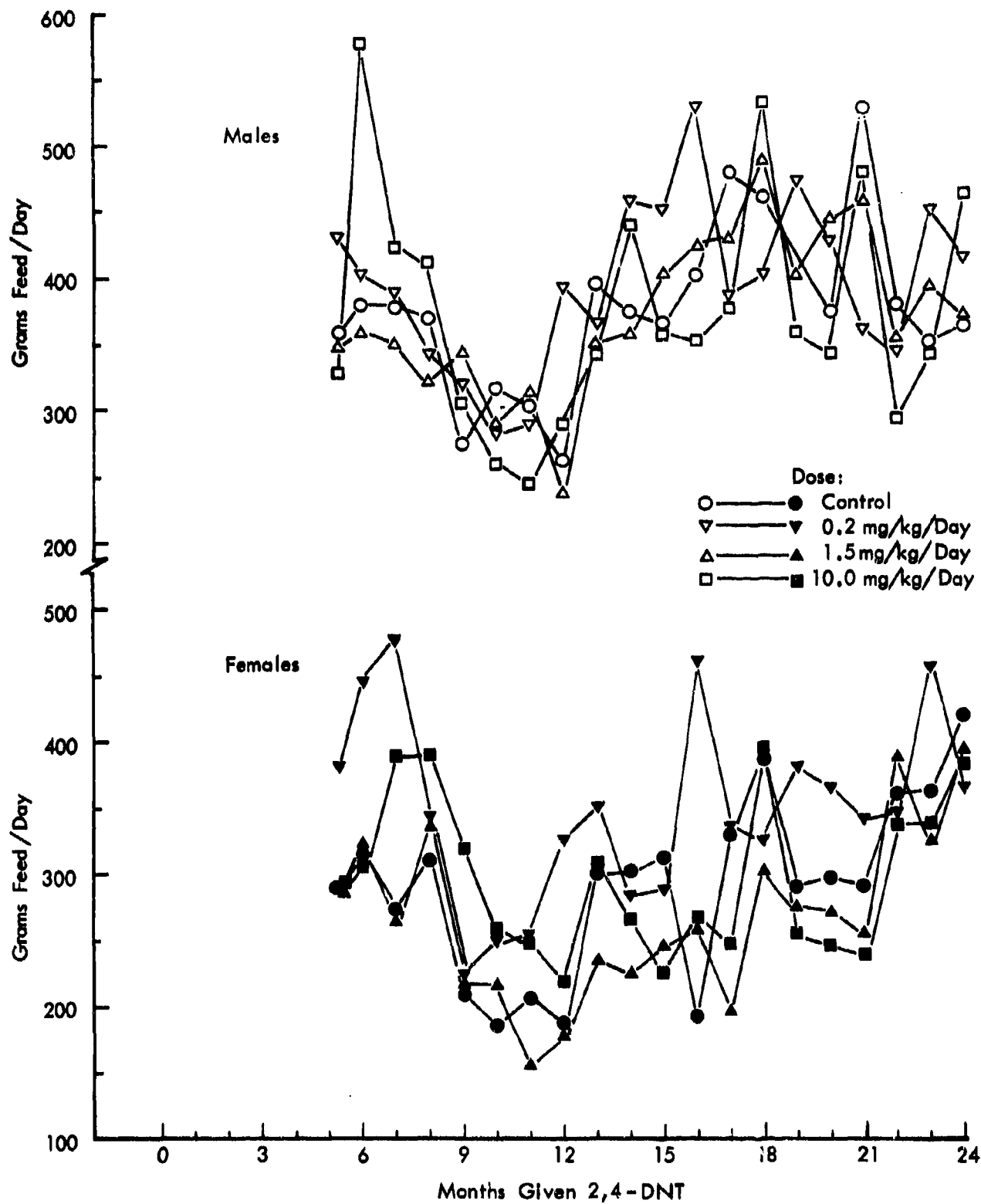


Figure 3 - Average Feed Consumption of Dogs Given 2,4-DNT

IV. RAT STUDIES

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IV. RAT STUDIES

A. Observations and Toxic Signs

1. Unscheduled Death

The first 2,4-DNT-induced effect, other than the decreased weight gain discussed below, was seen during week 21 in high dose female No. 73-596, a rat intended for the three-generation study. She was less active, much underweight, and had an odd gait, with her hind legs stiff and wide-spread. She died in week 23, as did the high dose male No. 73-434, intended for the metabolic studies. Both rats had very low body weights (181 g and 202 g, respectively) and malocclusion from extremely long incisors. Since these rats are on powdered feed, they do not wear their teeth down by gnawing. Apparently, the interaction of the 2,4-DNT and the malocclusion caused the deaths. Therefore, we increased our "preventive dentistry" program of inspecting the rats and trimming incisors with dog toenail clippers as required.

The next death was in week 27. The body weight of high dose male No. 73-331 peaked at 459 g in week 16; it slowly decreased to 437 g in week 26. This sort of weight variation is not unusual among the high dose rats. But in the next 6 days, this weight dropped to 283 g and he developed rales; there was tear pigment on his snout. He was killed for necropsy. The laboratory data showed few abnormalities except low leukocyte and platelet levels and elevated serum transaminases (Table 31). Low blood glucose and lack of body fat implied fasting, consistent with the weight loss.

In week 30, high dose male No. 73-426 developed a lump under his jaw near the right submaxillary salivary gland. From week 35, the lump rapidly grew until in week 38 it was 6 x 5 x 3 cm, hyperemic and ulcerated. He could only look left because of the tumor mass. He continued to eat and gain weight. But the ulceration was obviously causing pain; he was killed for necropsy. The lump was a well-encapsulated tumor weighing 71 g, 13% of total body weight. His liver was yellowish and weighed 28 g. Analysis of his blood revealed anemia with abnormal erythrocyte morphology (Table 31); many burr-like cells and schistocytes, several target cells, moderate polychromasia, and occasional macrocytes and teardrop cells.

The next significant unscheduled deaths were two low dosage rats (0.0015%, 2,4-DNT in feed). In week 56, male No. 71-101 was observed with paraplegia. He was isolated and weighed daily. Over the next week, he lost 12 g/day and he was killed for necropsy. He had severe anemia, elevated serum enzymes and a fasting glucose level (Table 31). He had

hepatomagaly and an enormous spleen (13.68 g) with a greenish coloration, indicating a lymphoma. Female No. 71-227 decreased from 400 g to 264 g from week 52 to week 56. She then became extremely lethargic, almost comatose. On examination, she appeared somewhat hyperexcitable. Blood analysis (Table 31) and gross necropsy were normal, except for an enlarged pituitary. The enlarged pituitary, whose pressure causes the behavioral signs, indicates pituitary adenoma, the most common naturally occurring tumor in this strain of rats in our experience and that of MacKenzie and Garner.^{15/}

By month 15, unscheduled deaths occurred fairly regularly in the high dose group, one or more per sex per month. These are illustrated by Figures 4 (Males) and 5 (Females), which show cumulative deaths among the groups of 30 male and 30 female rats intended for 24 months feeding. The lines for the low dose rats are omitted because they zigzagged around the lines for control rats. About 50% of the high dose rats had died by the end of month 20 and all high dose rats except one female died before the end of month 23. Frequent deaths did not occur in the other dose groups until after month 18, with the median death in month 23 or 24. After month 18, there were a few more deaths in the middle group than in control, but the low group did not differ significantly.

Laboratory data from selected moribund rats are shown in Table 31. The typical pattern of anemia seen in rats scheduled for routine laboratory tests as discussed later, occurred in these rats. The most significant changes were those of high dose female No. 73-280, who was killed in week 99. Although not very active, she was still alive with an erythrocyte count below a million and 26% methemoglobin. The Coulter counter gave a leukocyte count of 19,700, but correction for nucleated erythrocytes (more than three per leukocyte) reduced that to 4,800.

2. Causes of Death

There were three major causes of death: pituitary tumors, ulcerated subcutaneous tumors, and inanition.

Pituitary tumors: Pituitary tumors could be readily identified by observing the rat's behavior. The effects on motor function were pathognomonic. The most common version was one-sided ataxia or paralysis. This ataxia was seen sometimes in the hindquarters, sometimes over the entire body. In addition, many rats became hyperexcitable, showing exaggerated motor responses to stimuli. Crusts of tear pigment often occurred around the eyes. Occasionally, exophthalmos or crusts of tear pigment appeared on the snout. The body weights of these rats were followed closely during this time. When a rat lost 100 g or more within 2 weeks, he was killed for necropsy.

Ulcerated subcutaneous tumors: Any rat, that developed an ulcerated lesion, was killed to end its suffering. The usual cause was a large tumor in the subcutaneous tissues (breast or other sites). The tumor was frequently hyperemic, and usually fast-growing, as indicated by the taut skin above it. With normal movement and friction, an ulcer would start readily. These subcutaneous tumors were seen in all dosage groups, most commonly in females along the milkline, and presumably mammary tumors, although they also occurred at other sites (e.g., No. 73-426, discussed above). From month 15, these tumors began to become more and more common, especially in the high dose animals. After 18 months feeding, three quarters of the high dose males including the ones being dosed for metabolism studies, but only one other, had one or more readily apparent tumors (Table 32). Almost 90% of the high dose females, but only a third of the others, had one or more tumors. Some cases of multiple tumors are best described as grotesqueries. For example, high dose female No. 73-261, killed in week 84, had seven distinct tumors: two large ones of about 150 cm³ (one on each side), four small tumors (about 15 to 20 cm³, three on the left, one on the right), and a small perianal tumor.

Inanition: The other repeated cause of death was inanition. This was seen as a drop in body weight and inactivity without the characteristic one-sided paralysis of pituitary tumors. Odd gaits (as No. 73-596, described above) were rarely noted in high dose rats. In most cases, a cause was apparent on gross necropsy, such as the lymphoma in low dose male No. 71-101, described above. In other cases, it was due to the growth of the subcutaneous tumors, as in high dose female No. 73-261. In many instances, total body weight did not decrease, because the increase in tumor weight balanced the decrease in somatic weight. Therefore, one must detect effects more subtle than total weight. For instance, No. 73-261 was hunchbacked, with her vertebrae and ribs readily palpated dorsally, while the tumorous masses with tightly stretched, almost bald, skin were located ventrally. Some inanition deaths, especially in the last months of the study, had no obvious cause. These are ascribed to old age, possibly modified by 2,4-DNT treatment.

B. Body Weights

Average body weights of rats fed various doses of 2,4-DNT are shown in Figure 6. Control rats showed rapid weight gain from the start of the study. The rate of gain decreased as the rats matured, and did not approach plateau until about month 15 males and month 18 for females. Toward the end of the study, there were frequent lurches in the curves. This was because of the removal of rats from the study due to tumors and other old-age effects, many of which are accompanied by weight changes as discussed above, along with the usually increasing obesity of the survivors.

The growth of rats fed the low dose (0.0015% 2,4-DNT) was quite similar to that of the control rats. In fact, their average weight exceeded that of the controls more often than not. Their data has been omitted from the graph for clarity. Until about month 9, the growth of the middle-dose (0.01% 2,4-DNT) rats, too, was similar to that of the controls. Thereafter, the middle dose rats were usually of somewhat lower weight; these differences were not often statistically significant.

In striking contrast are the rats fed the high dose (0.07%). From the very first week of feeding, their weight gains were less. Their weights quickly reached a plateau, about 260 g in the females after 2 months of feeding and about 480 g in the males after 4 months of feeding. These weights were considerably below that found in the control rats, about 450 g in the females and 720 g in the males. The extremely high mass of tumors in the high dose females was obvious from the increase in total body weight to about 350 g in the later months of the study.

C. Feed Consumption and 2,4-DNT Intake

The average of the feed consumption measurements are shown in Table 33. To provide equal weighting on the time scale, the four weekly measurements in the first month were averaged to create a composite value for the month. There was a tendency for feed consumption by the males to be dose-related, but this was not statistically significant. They consumed an average of 23.25 to 25.61 g/rat/day during the entire period. No such trend was apparent in the female rats. They consumed an average of 18.30 to 19.22 g/rat/day during the study.

The average values for 2,4-DNT intake (the averages of the various monthly measurements) are shown in Table 33; the individual figures are shown in Figure 7. During the first 3 weeks, both sexes had about the same intake. As the males gained weight faster (see Figure 6), their average 2,4-DNT intake, on a per kg body weight basis, decreased faster. Their feed consumption, on a per animal basis, was relatively constant throughout the study. Therefore, the long-term trends in the 2,4-DNT intake curves reflect body weight changes, while the month-to-month variations reflect normal biological variation in feed consumption. During the entire period, the 2,4-DNT intake calculated as the average of the various monthly measurements of the low, middle and high dose males averaged 0.57, 3.9 or 34 mg/kg/day, respectively; whereas the intake of the low, middle and high dose females averaged 0.71, 5.1 or 45 mg/kg/day, respectively.

D. Laboratory Data

Baseline hematologic data for various groups of males and females are shown in Tables 34 and 35, respectively. The values for various parameters are normal, with only insignificant differences between groups.

Laboratory data after 3, 6, 9, 12, 18 and 24 months of feeding 2,4-DNT are shown in Tables 36 through 47. A fairly consistent picture of anemia, more or less compensated, was indicated by the high dose rats (0.07% 2,4-DNT in feed) at most intervals. The erythrocyte count, and, with it, the hematocrit and hemoglobin content were decreased. The reticulocyte count and sometimes the mean cell volume were increased, as the marrow responded to the anemia. Although not always statistically significant, this pattern was generally present. Occasionally, it was seen with the middle dose (0.01%) rats, but this was not consistent. Other statistically significant differences are sometimes seen, but they are not consistent. Therefore, they were considered to be normal variation rather than 2,4-DNT effects.

Laboratory data from rats fed 2,4-DNT for 12 months and allowed to recover for 1 month are shown in Tables 48 and 49. In these groups, the mild effects of anemia were not observed in the high dose rats. Laboratory data from rats fed 2,4-DNT for 24 months and allowed to recover for 1 month are shown in Tables 50 and 51. There were no high dose survivals. Laboratory data of the other groups were within normal ranges.

E. Pathology

Of the original groups of 38 rats, 32, 35, 27 and 34 males from the control, low, middle and high dose groups, respectively, and 31, 36, 34 and 33 females are included in the following data; the remainder were lost to autolysis, cannibalism, etc. Data are included from one male and seven females in the low dose group, one middle dose female, and three males and 10 females from the high dose group of the ancillary studies.

1. Feeding For 12 Months

a. Organ Weights

Absolute and relative organ weights of rats fed 2,4-DNT for 12 months are listed in Table 52. Heart weights were inadvertently omitted at the necropsies for these groups. Rats fed the high dose (0.07% 2,4-DNT) had decreased body weight, increased absolute (males) or relative (females) liver and kidney weights and decreased testis weights. The increased relative brain weights were the inverse of the decreased body weight. A constant brain weight is expected when the body weight is affected after the brain is near mature size, that is, after weaning. The decreased spleen weight was probably a reflection of the high variability of this organ's weight. When rats were allowed to recover on control feed for a month, the organ weight picture was substantially the same (Table 53).

b. Tissue Lesions

Tissue lesions from the male and female rats fed 2,4-DNT for 12 months are summarized in Tables 54 and 55, respectively; those from rats allowed to recover for a month are in Tables 56 and 57. A number of lesions were present in various tissues of these rats. Only the treatment-related lesions are discussed below. The lesions, including the pituitary adenoma and mammary tumors, were more significant after treatment for longer periods; they will be discussed later. Senile nephropathy was seen earlier in the high dose rats, but this is a normal lesion in geriatric rats.

(1) Liver

The most striking lesions after feeding for 12 months were those in the liver. There were the early stages of a progressive development of hepatocellular carcinoma, first described by Reuber^{16/} for N-2-fluorenyldiacetamide and by Newberne and Wagan^{17/} for aflatoxin B₁. Nomenclature was clarified by a National Cancer Institute-sponsored workshop,^{18/} and was followed in this report. These stages are illustrated with typical slides from this rat study.

The initial lesion was foci (smaller lesions) or areas (lesions of lobule size) of altered hepatocytes (Figure 8). These were also called "hyperplastic foci" or similar terms. The liver architecture was preserved, and there was no clear-cut demarcation between affected and non-affected cells. In rats fed 2,4-DNT for 12 months (Tables 54 and 55), a mild degree was found in nearly all the low and middle dose males; a marked or severe degree was found in about half the males and females fed the high dose. This lesion was also seen in one untreated female. In rats allowed to recover for 1 month (Tables 56 and 57), the incidence and severity of this lesion were not appreciatively changed.

The next stage was neoplastic nodules, formerly called hyperplastic nodules (Figure 9). These were spherical lesions, as large as several lobules, without the normal internal architecture. A useful criterion was the presence of a sharp boundary with compression of the normal liver tissues immediately outside the nodule. This lesion occurred in all the high dose males and females (Tables 54 and 55). Since these nodules were highly proliferative, they were probably responsible for the observed large increase in liver weight. Grossly, these were frequently apparent as white spots about pinhead size. In rats allowed to recover for 1 month (Tables 56 and 57), this lesion was also present in most of the high dose rats.

The last stage was hepatocellular carcinoma (Figure 10, gross; Figure 11, microscopic). There were several varieties with varying degrees of differentiation from almost normal liver architecture to masses

of randomly arranged cells. The cells themselves might vary from normal-appearing hepatocytes to anaplastic with variously staining cytoplasm. The workshop^{18/} concluded that all rat hepatic cell tumors had the potential for malignant behavior. Therefore, the term "hepatoma" was discarded and all tumors were labelled "hepatocellular carcinomas." In this group of rats, this stage of development occurred in only one high dose female No. 73-382 (Table 57). She was allowed to recover for 1 month. Her total liver weight (12.5 g) was not exceptionally high. But she had obvious tumors on the left (1 x 1 x 0.8 cm) and median (1.2 x 1 x 0.8 cm) lobes.

(2) Abnormal Pigmentation

Most of the high dose rats (7/8) had an excessive amount of pigmentation in the spleen (Tables 54 and 55). This was in addition to the usual hemosiderin deposits. The excess pigment physically resembled hemosiderin (brown granules) but gave very little, if any, reaction with Prussian blue, which reacted normally with the iron in the nearby hemosiderin deposits. This lesion was more pervasive in the mice and will be further discussed below. In rats allowed to recover for 1 month (Tables 56 and 57), pigmentation was also seen in most of the high dose rats (6/7) and in two of the four low dose females.

(3) Testis

All four high dose males fed 2,4-DNT for 12 months (Table 54) and two of three high dose males allowed to recover for 1 month (Table 56) had very severe atrophy of the testes (Figure 12), with almost complete lack of spermatogenesis (Figure 13). This lesion is seen in geriatric rats, but it is not normal in rats of this age. A similar effect after subchronic dosing of 2,4-DNT has been reported.^{4/}

2. Feeding For 24 Months Including Unscheduled Death

a. Organ Weights

Organ weights of rats fed 2,4-DNT for 24 months are given in Table 58. There was no high dose male surviving. As seen in the high dose males fed for 12 months, the middle dose males had decreased body weight and increased liver weight. However, the testes appeared normal. The organ weights for animals allowed to recover for 1 month (Table 59) were similar, but the unscheduled deaths decreased numbers and made interpretation difficult.

b. Tissue Lesions

Lesions in rats fed 2,4-DNT for 24 months are summarized in Tables 60 and 61 and in rats allowed to recover for 1 month are summarized in Table 62. Lesions in rats died or terminated at unscheduled times are

summarized in Tables 63 through 69. These results did not include all animals or all organs due to some rats that died at night and autolysis hindered examination. To increase the numbers available for calculating incidence we included all rats fed the same dosage mixtures used in various studies. Rats with numbers in the 400's were intended for the metabolism study; a few females in the 500's were intended for the three-generation study but not mated and continued on feed.

(1) Naturally-Occurring Lesions

A great wide variety of naturally-occurring lesions were found in these geriatric rats. The lesions were listed in the lower part of the appropriate tables. There were two classes of lesions: the degenerative lesions found in most geriatric rats and the rare lesions found in a few scattered rats. Typical degenerative lesions included mild chronic murine pneumonia (endemic among rats), mild bile duct hyperplasia, mild extramedullary hematopoiesis in the spleen, and mild retinal degeneration. The rare lesions included a variety of tumors and lesions in various tissues. The various tumors were extracted from Tables 60 through 69 and listed in Table 70. No obvious dose relationship existed among these relatively rare tumors or lesions; they were not related to the treatment.

(2) Lesions Related To Treatment

As seen in rats fed 2,4-DNT for 12 months, a number of treatment-related lesions occurred in these rats and were listed in the upper part of the appropriate tables. The incidence of these 2,4-DNT related lesions were extracted from Tables 60 through 69 including all rats fed for more than a year and one high dose male dying in month 12 and tabulated in Table 71. Statistical analysis is Chi-square tests and exact probabilities on contingency tables of these data with $p = 0.05$ considered significant.

As expected from the results after feeding for 12 months, there was a significant increase in the incidence of hepatocellular carcinoma among high dose rats, especially the females (Table 71). The increase in the males alone was not quite statistically significant ($p = 0.08$), but it is toxicologically significant when coupled with the data for females. The incidence of foci of hepatocellular alteration and hepatocellular neoplastic nodules was not apparently affected by treatment, but the severity was increased, as seen on the appropriate tables of lesions. The various stages of the development of the hepatocellular carcinoma were discussed for rats fed 2,4-DNT for 1 year.

Similarly, almost all the high dose males had severe atrophy of the seminiferous tubules (Table 71), as seen in the high dose males fed 2,4-DNT for 12 months. The incidence of testicular atrophy was about 29 to 33% in lower dose males and 16% in control males. In addition, these were generally of lesser degree.

The "lumpiness" observed in most high dose rats was the result of increased incidences of various subcutaneous mesenchymal tumors, especially in the males, and various mammary tumors (Table 71). Typical mammary tumors are illustrated in Figure 14 (gross) and 15 (microscopic). The mammary tumors were classified in Table 71. Fibroadenomas, adenomas with fibrous tissue involved, predominated in all groups. Adenocarcinoma-carcinoma and fibromas were rare. Both males with mammary tumors were fed the high dose; this may or may not be a 2,4-DNT effect. The observed subcutaneous mesenchymal tumors were classified in Table 72. The increase in incidence among treated rats was due to fibromas; the incidence of malignant tumors was similar.

The incidence of pituitary chromophobe adenoma in high dose rats was less (Table 71). This tumor is the characteristic tumor type in this strain of rat. It was the leading cause of unscheduled deaths of all rats except the high dose group. The actual percent incidence was probably somewhat lower than the tabulated figures, since a normal pituitary is so small that it could be overlooked at necropsy, lost while changing fixative, or missed in cutting the sections. A tumorous pituitary, however, was a relatively large object, usually dark from the blood within and not readily missed. The significance of the reduced incidence of pituitary chromophobe adenoma in the high dose rats is not understood.

F. Three Generation Reproduction Study

As indicated in Table 73, the mean body weights at the time of first matings for both males and females given the high dose (0.07% 2,4-DNT) were decreased. When compared with their respective control body weights, the weights for the F_0 and F_1 generation were only 77 and 75% for the males, respectively, and only 77 and 90% for the females, respectively. No treatment effects on the fertility of the males or females were apparent. However, the fertility of both males and females was reduced for all groups of the F_0 generation. This was probably due to their older age.

The absence of the F_2 parental generation for the group given 0.07% 2,4-DNT and the few animals mated in the F_1 indicated an adverse effect on reproductive performance. This adverse effect on reproduction was further clarified by the quantitative data for the individual litters given in Table 74. No treatment effects were apparent on liveborn index, weight at birth, weight at weaning or the sex ratio. Although not statistically significant, the mean litter size appeared to be reduced for F_{1a} and/or F_{1b} .

litters from dams given 2,4-DNT in the feed. This effect did not persist with subsequent parental generations. The viability and lactation indexes were also reduced for one or both litters born to the F_0 generation. With the exception of the viability index for F_0 litters born to dams receiving the high dose, these effects did not appear to be related to the treatment. This lowered viability resulted from maternal neglect and death during parturition. The incidences of deaths during parturition of first litters for the F_0 generation were 1/10, 4/13, 3/10 and 4/11 for the dams given 0, 0.0015, 0.01 and 0.07% 2,4-DNT. The incidences of deaths during parturition of the second litters for the F_0 generation were 1/5, 0/6, 0/6 and 4/5 for dams given 0, 0.0015, 0.01 and 0.07% 2,4-DNT, respectively. The deaths were associated with a prolonged parturition, excessive hemorrhage, and retention of placentas or of fetal-placental units. In some cases, the placentas were still attached to the uterus. In other cases, the placentas were free of the uterus but did not pass through the cervix. The occurrence of deaths during parturition in the control group suggests these deaths may be related to the age of the dams at first mating. However, 2,4-DNT enhanced the occurrence of these deaths. It appears that the observed adverse reproductive effects were associated with aging and/or toxicity of 2,4-DNT.

All three high dose females from F_{1b} , which were mated at 3 months of age, produced and weaned offspring as well as control dams. This performance was achieved even though one female had a large mammary tumor at the time of mating. None of the three females, however, produced second litters. The female with the tumor failed to mate. Sperm was absent in the vaginal smear of a second female with a vaginal plug. The third female failed to produce offspring.

No anomalies were detected in the offspring from any of the matings. Normal birth weights, normal postpartum survival when parturition was normal, normal weight at weaning and the lack of a teratogenic effect indicate that any 2,4-DNT received via the placenta or milk was of little consequence.

G. Mutagenesis Studies

1. Cytogenetic Study

The results of the chromosome analysis of the bone marrow and kidney cultures from rats fed 2,4-DNT for 24 months are shown in Tables 75 and 76. Results from the high dose surviving rat did not differ significantly from those of the control rats. The kidney cultures from middle dose (0.01% 2,4-DNT) rats had a statistically significant increase in tetraploid frequency, but the increase was relatively small. Furthermore, the bone marrow cultures and those from the high dose rat showed no such effect. There was not any apparent morphological aberrations of chromosomes in bone marrow or kidney cultures of rats fed 2,4-DNT for 24 months.

2. Dominant Lethal Mutation Studies

The results of the first dominant lethal mutation study are shown in the upper portion of Table 77. The decrease of the implant viability index of males fed 0.2% 2,4-DNT suggests that there is a mutagenic effect. However, the decreased fertility indexes, probably due to the effect of 2,4-DNT on spermatogenesis discussed earlier,^{4/} cast doubt on this interpretation. Therefore, additional experiments were carried out to determine if there was a dose of 2,4-DNT which would reduce implant viability without reducing fertility. The second test used males already being dosed on the chronic toxicity test. As can be seen on Table 77, no effects were observed in males fed 0.0015, 0.01 or 0.07% 2,4-DNT for 13 weeks. The third test used somewhat higher doses: 0.5% (used as the high dose in the mouse chronic toxicity study, discussed below), 0.2% (the high dose in the first test), 0.15% (a dose intermediate between 0.2% and 0.07%), and control. Only three of the 15 high dose (0.5%) males survived the 13 weeks' feeding; none mated, so they were functionally sterile. Males from the middle (0.2%) and low (0.15%) dose groups did mate (produced plug and sperm positive females), but there were no viable fetuses. About two-thirds of the plugs from middle dose males and one-third of those from low dose males had no apparent sperm. The control males mated normally. These results indicated sterility.

A fourth and last study used doses of 0.15% (sterile in third study), 0.10% (geometric mean of other two doses), 0.07% (no effect in second study) and control. Since the testicular lesions were not apparently reversible in 1 month after 1 year of feeding 0.07% 2,4-DNT (see above), we incorporated a 13 week reversibility study in the design.

Results from the last dominant lethal mutation study are shown in Table 78. All the doses of 2,4-DNT produced a dose-related decrease in weight gain. There were some decreases in feed consumption. There was a dose-related increase in spermless vaginal plugs and a dose-related decrease in fertility (pregnancies). There was only one, nonviable fetus in only one of the females mated to males fed 0.15%, 2,4-DNT. The variations in corpora lutea/dam were within normal limits. The lack of an effect on implant viability index, despite the drastic effect on implantation index, shows that there is no apparent dominant lethal mutation effect.

The microscopic evaluation of the reproductive organs of males are shown in Table 79. Nearly all males fed 0.07, 0.1 or 0.15% 2,4-DNT for 13 weeks had marked to severe atrophy or degeneration of seminiferous tubules of the testes and too few or no spermatozoa in ductules of the epididymis. After the treatment was discontinued for 13 weeks, there was no evidence of any recovery for those lesions.

H. Metabolism Studies

Metabolism results from rats fed 2,4-DNT for 3, 9 and 20 months are shown in Tables 80 through 85. The results were similar to those seen in rats not chronically fed 2,4-DNT before being given the test dose of ^{14}C -2,4-DNT.^{1/} The oral dose was well absorbed, with a large majority appearing in the urine within 24 hr; some radioactivity was found in the gastrointestinal tract and feces. Very little remained in the tissues, with the liver (organ of metabolism and biliary excretion) and kidney (organ of urinary excretion) having the highest levels of 2,4-DNT-derived radioactivity. Metabolism was extensive, as shown by chromatographic analysis of the urine. Very small amounts of 2,4-DNT itself was excreted. The major metabolites were dinitro-, aminonitro-, and diaminobenzyl alcohols, reflecting oxidation of the side chain and reduction of the nitro groups. Most of these primary metabolic products were then conjugated before excretion.

When the time came for the last study (began in month 19; completed in month 20), all the high dose males set aside for the metabolism study had died. Only three high dose females (of six) remained, but two had very large tumors and the third had inanition. These toxic effects were comparable to the other high dose as discussed above. Rather than terminate some other high dose rats, we used some extra middle dose (0.1% 2,4-DNT) rats instead. The results remained the same: no major differences between dose groups, between sexes, or between feeding periods.

I. Discussion

A general toxic effect of decreased weight gain and a shortened life span occurred in rats following chronic feeding of high dose 2,4-DNT (0.07%). There was anemia partially compensated as shown by increased reticulocyte counts. Presumably, this was a toxic hemolytic anemia of the anilism type, but there was no consistent direct evidence such as methemoglobinemia and Heinz bodies. Furthermore, no evidence of lesions in the erythropoietic system was found.

The most significant tissue lesion was the progressive development of hepatocellular carcinoma. This same progression was first reported for N-2-fluorenyldiacetamide^{16/} and aflatoxin B₁,^{17/} compounds chemically quite distinct from each other and from 2,4-DNT. This raises the hypothesis of a common mechanism which, by one means or another, all three compounds initiate. The nature of such a mechanism and its initiation are purely speculative.

Besides causing the liver tumors, 2,4-DNT greatly increased the incidence of subcutaneous tumors. In males, these were mostly fibromas. In females, they were mammary fibroadenomas, the most common type of mammary

tumor in this strain of rats. The development of these tumors contributed to the observed lethality by diverting the rat's resources and by (at times) becoming ulcerated.

Besides these tumor increases, there was a significant decrease in pituitary chromophobe adenomas, the most common background tumor in this strain.^{13/} This may be, in part, a result of the high death rate, which eliminates the rats before they could develop the pituitary tumor.

As reported earlier,^{4/} 2,4-DNT also induced atrophy of the seminiferous tubules. In severe cases, there was almost complete lack of spermatogenesis.

In a three-generation reproduction study, there were general effects, such as decreased body weight and increased parturition deaths, of the toxicity of 2,4-DNT, but no specific reproductive effects. Similarly, no significant chromosome effects were observed in the cytogenetics study. There was no dominant lethal mutation effect, although the testicular atrophy may obscure expression of a weak effect. Continual feeding of 2,4-DNT did not affect how the body absorbs, metabolized, and excretes a large oral dose of the compound.

Although occasional symptoms, such as straddling gait, were seen that suggested neuromuscular effects, these were rare and without accompanying histopathological lesions. Therefore, the nervous system is not considered a target organ of 2,4-DNT toxicity in rats in this study.

If the results of the high dose are described as devastating, the results of feeding the middle dose (0.01% 2,4-DNT) is "tantalizing." This seems to be due to the varying susceptibilities of the individual rats to 2,4-DNT. There were a few earlier deaths. Most were unaffected, except for a late decrease in body weight. Some rats had effects similar to those in the high dose group, with mild anemia, liver lesions particularly areas of hepatocellular alteration, and mammary tumors. If this dose were the highest used, we might interpret these relatively minor differences as of little significance. However, their resemblance to the high dose effects demonstrates that 2,4-DNT is toxic at this middle dose. The low dose (0.0015%) 2,4-DNT was nontoxic. These rats seem indistinguishable from controls.

J. Conclusions

The high dose, with 2,4-DNT intake of 34 mg/kg/day in males and 45 mg/kg/day in females, was quite toxic, causing decreased weight gain and shortened life span. Target organs included the blood (toxic anemia), the liver (hepatocellular carcinoma), the testis (aspermato-genesis), and

the connective tissue in males (fibromas) and the mammary tissue in females (fibroadenomas). No specific effects were seen on the reproductive process, on chromosomes, or on the metabolism of 2,4-DNT.

The middle dose, with 2,4-DNT intake of 3.9 mg/kg/day in males and 5.1 mg/kg/day in females, was somewhat toxic. It caused similar effects in some, more susceptible, individuals.

The low dose, with 2,4-DNT intake of 0.57 mg/kg/day in males and 0.71 mg/kg/day in females, had no apparent toxic effects.

TABLE 31

LABORATORY DATA OF RATS FED 2,4-DWT AND DYING AT UNSCHEDULED TIMES

Dose (Z in feed):	0.07	0.07	0.07	0.0015	0.0015	0.07	0.07	0.07	0.07	0.07	0.07	0.0015	0.07	0.07	0.07
Rat No.:	331	426	38	426	101	227	175	63	71	80	84	85	85	85	85
Week of Death:	27	38	38	38	57	57	57	57	71	80	84	85	85	85	85
Erythrocytes, $\times 10^6/\text{mm}^3$	7.23	5.10	5.10	6.83	2.96	6.83	5.18	6.64	1.82	6.01	5.59	5.53	4.51	6.57	3.09
Retz bodies, Z	0.00	0.00	0.00	0.00	0.00	0.00	—	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Reticulocytes, Z	0.22	3.54	3.54	1.73	4.65	1.73	1.08	0.80	40.11	1.28	0.66	0.04	0.55	1.61	3.34
Hematocrit, vol Z	49	32	32	40	19	40	31	41	17	40	32	30	31	40	21
Hemoglobin, gm Z	15.9	10.8	10.8	13.6	6.5	13.6	10.2	14.1	5.1	12.9	10.5	9.6	9.8	13.8	6.7
Methemoglobin, Z	6.4	7.4	7.4	0.0	0.0	0.0	—	0.0	0.0	0.0	0.0	0.0	0.0	1.4	0.0
MCV, cubic microns	67.7	62.7	62.7	58.6	64.2	58.6	59.8	61.7	93.4	66.6	56.3	54.2	68.7	60.9	68.0
MCHC, micromicrograms	22.0	21.2	22.0	19.9	22.0	19.9	19.7	21.1	28.0	21.5	18.5	17.4	21.7	21.0	21.7
MCHC, gm Z	32.4	33.8	34.2	34.0	34.2	34.0	32.9	34.4	30.0	32.3	32.8	32.0	31.6	34.5	31.9
Platelets, $\times 10^5/\text{mm}^3$	1.55	4.65	4.65	1.75	6.85	1.75	4.35	3.80	3.25	5.10	6.60	7.70	5.45	7.40	9.65
Leukocytes, $\times 10^3/\text{mm}^3$	6.4	8.3	8.3	3.8	11.1	3.8	17.0	7.7	21.2	6.2	4.0	9.0	4.2	3.5	25.1
Neutrophils, Z	57	28	28	31	11	31	44	63	40	52	55	45	24	55	64
Lymphocytes, Z	43	67	67	69	87	69	54	36	60	48	45	54	75	44	33
Bands, Z	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0
Monocytes, Z	0	2	2	0	2	0	2	0	0	0	0	0	0	0	0
Eosinophils, Z	0	1	1	0	0	0	0	1	0	0	0	0	1	1	0
Basophils, Z	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Atypical, Z	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nucleated RBC, Z	0	2	2	0	28	0	0	0	4	1	1	2	1	0	8
Glucose, mg Z	102	142	142	128	101	128	100	194	133	129	83	122	122	126	106
SGOT, IU/l	251	68	68	148	269	148	96	55	96	80	77	117	68	198	77
SGPT, IU/l	80	18	18	31	90	31	40	37	34	24	21	21	37	111	21
Alkaline phosphatase, IU/l	49	31	31	15	99	15	29	21	24	48	13	16	10	37	61
BUN, mg Z	24	16	16	12	33	12	13	16	18	67	12	35	34	15	14
IgG, IU/l	<600	800	800	—	—	—	—	—	—	800	1800	1450	1450	>5050	<500

TABLE 31 (Concluded)

Dose (Z in feed):	0	0.07	0.0015	0.07	0.01	0.07	0.07	0.07	0	0.07	0.07	0.0015	0.07	0.07
Rat No.:	018	178	474	587	133	288	289	586	021	168	189	112	99	280
Week of Death:	86	86	87	87	88	88	88	88	92	92	97	99	99	99
Erythrocytes, $\times 10^6/\text{mm}^3$	5.60	4.62	5.30	5.94	5.47	3.92	4.03	5.03	4.99	3.16	3.60	5.26	0.81	0.81
Heinz bodies, Z	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Reticulocytes, Z	0.44	5.12	1.06	1.11	0.87	1.81	2.24	1.21	0.59	17.11	3.03	2.00	31.6	31.6
Hematocrit, vol Z	43	31	40	45	38	29	30	37	33	13	24	36	7	7
Hemoglobin, gm Z	13.7	10.5	13.2	13.9	11.2	9.4	9.8	12.6	10.9	5.9	7.6	12.0	2.7	2.7
Methemoglobin, Z	0.0	0.0	0.0	6.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.2	25.9	25.9
MCV, cubic microns	76.8	67.1	75.5	75.8	69.5	74.0	74.4	73.6	66.1	60.1	66.7	68.4	86.4	86.4
MCHB, micromicrograms	24.5	22.7	24.9	23.4	24.1	24.0	24.3	25.0	21.8	18.7	21.1	22.8	33.3	33.3
MCHC, gm Z	31.9	33.9	33.0	30.9	34.7	32.4	32.7	34.1	33.0	31.1	31.7	33.3	38.6	38.6
Platelets, $\times 10^5/\text{mm}^3$	4.10	6.50	6.25	6.80	6.60	4.95	9.00	5.00	1.80	4.55	7.40	6.65	3.85	3.85
Leukocytes, $\times 10^3/\text{mm}^3$	9.4	17.5	6.1	4.5	5.6	6.5	11.9	7.1	4.0	15.6	29.9	12.5	4.8	4.8
Neutrophils, Z	68	43	48	73	56	44	65	60	38	27	45	45	84	84
Lymphocytes, Z	32	56	51	26	44	56	35	39	59	73	51	52	16	16
Bands, Z	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Monocytes, Z	0	0	1	1	0	0	0	0	1	0	2	1	0	0
Eosinophils, Z	0	0	0	0	0	0	0	1	2	0	2	2	0	0
Basophils, Z	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Atypical, Z	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nucleated RBC, Z	0	0	0	0	0	2	1	0	0	0	0	0	0	0
Glucose, mg Z	82	106	82	40	119	163	74	112	99	119	140	117	55	55
SCOT, IU/l	121	90	65	1317	114	59	77	96	72	71	68	55	334	334
SCPI, IU/l	24	43	28	245	46	21	28	34	279	37	40	28	74	74
Alkaline phosphatase, IU/l	21	89	17	26	15	9	57	17	29	131	252	52	61	61
BUN, mg Z	30	38	10	66	19	26	48	34	24	20	54	16	53	53
IgE, IU/l	1900	450	—	450	—	450	1250	1900	450	2400	<500	—	2650	2650

TABLE 32

RATS WITH APPARENT TUMORS AFTER BEING FED
2,4-DNT FOR 18 MONTHS

Dose (% in Feed)	Males		Females	
	<u>Tumor/Total</u>	<u>Percent</u>	<u>Tumor/Total</u>	<u>Percent</u>
0	1/37	3	8/29	28
0.0015	0/37	0	11/40	28
0.01	0/29	0	10/27	37
0.07	17/23	74	28/32	88

TABLE 33

FEED CONSUMPTION AND COMPOUND INTAKE OF RATS
FED 2,4-DNT FOR 24 MONTHS

Dose (% in feed)	Males		Females	
	Feed Consumption (g/rat/day)	2,4-DNT Intake (mg/kg/day)	Feed Consumption (g/rat/day)	2,4-DNT Intake (mg/kg/day)
0	25.61 $\pm$ 1.35 ^{a/}	--	18.38 $\pm$ 0.39 ^{a/}	--
0.0015	24.69 $\pm$ 0.38	0.575 $\pm$ 0.021	18.30 $\pm$ 0.40	0.706 $\pm$ 0.022
0.01	23.96 $\pm$ 0.54	3.92 $\pm$ 0.15	19.22 $\pm$ 0.74	5.14 $\pm$ 0.18
0.07	23.25 $\pm$ 0.52 ^{b/}	34.5 $\pm$ 0.8 ^{b/}	18.45 $\pm$ 0.88 ^{c/}	45.3 $\pm$ 1.4 ^{c/}

a/ Mean $\pm$ standard error of 24 measurements; the first month is the average of four measurements.

b/ Due to unscheduled deaths, only 21 measurements.

c/ Due to unscheduled deaths, only 22 measurements.

TABLE 34

LABORATORY DATA OF MALE RATS BEFORE FEEDING OF 2,4-DNT

(C.N) CONTROL

(T.N) TREATED

N = NUMBER OF RATS

DOSE: % IN FEED	0 (C, 5)	0.0015 (T, 5)	0.01 (T, 5)	0.07 (T, 5)
ERYTHROCYTES ($\times 10^6$ /MM ³)	6.20 $\pm$.12	6.17 $\pm$.12	6.43 $\pm$.04	6.00 $\pm$.12
HEINZ BODIES, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
RETICULOCYTES, %	1.57 $\pm$.45	1.74 $\pm$.47	2.23 $\pm$.57	2.74 $\pm$.45
HEMATOCRIT, VOL. %	43.2 $\pm$ 1.4	42.8 $\pm$.6	43.0 $\pm$.5	42.8 $\pm$.4
HEMOGLOBIN, GM. %	14.2 $\pm$.2	14.4 $\pm$.2	14.7 $\pm$.1	14.0 $\pm$.1
METHEMOGLOBIN, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MCV, CURIC MICRONS	69.6 $\pm$ 1.9	69.4 $\pm$.9	66.9 $\pm$.5	71.1 $\pm$ 1.3
MCHC, MICRO MICROGMS.	22.9 $\pm$.2	23.4 $\pm$.2	22.8 $\pm$.2	23.4 $\pm$.4
MCHC, GM %	33.0 $\pm$.8	33.7 $\pm$.3	34.1 $\pm$.1	32.9 $\pm$.1
PLATELETS ($\times 10^3$ /MM ³)	6.4 $\pm$.8	5.5 $\pm$.3	7.6 $\pm$.5	7.5 $\pm$.3
LEUKOCYTES ($\times 10^3$ /MM ³)	12.7 $\pm$ 1.2	13.4 $\pm$.7	14.0 $\pm$.8	14.4 $\pm$.9
NEUTROPHILS, %	9.8 $\pm$ 1.9	10.2 $\pm$ 2.5	11.0 $\pm$.4	6.8 $\pm$ 1.8
LYMPHOCYTES, %	89.2 $\pm$ 1.7	89.0 $\pm$ 2.7	88.0 $\pm$.9	92.6 $\pm$ 1.6
BANDS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
EOSINOPHILS, %	.8 $\pm$.4	.2 $\pm$.2	.6 $\pm$.4	.2 $\pm$.2
BASOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MONOCYTES, %	.4 $\pm$.4	.6 $\pm$.4	.4 $\pm$.2	.4 $\pm$.2
ATYPICAL, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
NUCLEATED PRG, %	.2 $\pm$.2	0.0 $\pm$ 0.0	.2 $\pm$.2	.2 $\pm$.2

ENTRIES ARE MEAN $\pm$ STANDARD ERROR

TABLE 35

LABORATORY DATA OF FEMALE RATS BEFORE FEEDING OF 2,4-DNT

	(C,N) CONTROL	(T,N) TREATED	N = NUMBER OF RATS	
DOSE: % IN FEED	0 (C, 3)	0.0015 (T, 3)	0.01 (T, 3)	0.07 (T, 3)
ERYTHROCYTES ($\times 10^6 / \text{MM}^3$)	6.24 $\pm$.09	6.62 $\pm$.08 ^{a/}	6.54 $\pm$.08 ^{a/}	6.35 $\pm$.06
HEINZ BODIES, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
RETICULOCYTES, %	1.54 $\pm$.27	1.40 $\pm$.14	1.32 $\pm$.32	1.49 $\pm$.30
HEMATOCRIT, VOL. %	42.0 $\pm$.9	43.2 $\pm$.4	43.0 $\pm$ 1.4	42.0 $\pm$.5
HEMOGLOBIN, GM. %	14.0 $\pm$.1	14.2 $\pm$.2	14.0 $\pm$.3	13.9 $\pm$.2
METHEMOGLOBIN, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MCV, CURIC MICRONS	67.3 $\pm$ 1.2	65.2 $\pm$.5	65.7 $\pm$ 1.7	66.2 $\pm$ 1.0
MCHM, MICRO MICROGMS.	22.4 $\pm$.3	21.4 $\pm$.2	21.4 $\pm$.3 ^{a/}	21.9 $\pm$.3
MCHMC, GM %	33.4 $\pm$.7	32.9 $\pm$.4	32.6 $\pm$.5	33.1 $\pm$.2
PLATELETS ($\times 10^3 / \text{MM}^3$)	7.2 $\pm$.9	7.6 $\pm$.4	7.1 $\pm$.2	6.8 $\pm$.7
LEUKOCYTES ($\times 10^3 / \text{MM}^3$)	11.7 $\pm$.8	9.6 $\pm$.5	11.1 $\pm$.8	14.7 $\pm$ 1.8
NEUTROPHILS, %	7.2 $\pm$ 1.2	12.8 $\pm$ 4.5	9.0 $\pm$ 1.3	9.6 $\pm$ 1.2
LYMPHOCYTES, %	92.4 $\pm$ 1.0	86.2 $\pm$ 4.7	90.8 $\pm$ 1.2	90.0 $\pm$.9
GRANULS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
EOSINOPHILS, %	.4 $\pm$.2	1.0 $\pm$.3	.2 $\pm$.2	.4 $\pm$.4
BASOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MONOCYTES, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
ATYPICAL, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
NUCLEATED WBC, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL RATS BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.ENTRIES ARE MEAN $\pm$ STANDARD ERROR.

TABLE 36

LABORATORY DATA OF MALE RATS AFTER FEEDING OF 2,4-DNT FOR 3 MONTHS

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF RATS	
	0 (C, 5)	0.0015 (T, 5)	0.01 (T, 5)	0.07 (T, 5)
DOSE: % IN FFD				
ERYTHROCYTES (X10 ⁶ /MM ³)	7.37 ± .13	7.15 ± .17	6.44 ± .16	6.11 ± .27 ^{a/}
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00 ^{a/}
RETICULOCYTES, %	.97 ± .18	1.16 ± .17	1.53 ± .18	2.07 ± .11 ^{a/}
HEMATOCRIT, VOL. %	48.4 ± 1.0	48.4 ± .8	46.8 ± 1.0	46.6 ± 1.0
HEMOGLOBIN, GM. %	15.8 ± .2	15.6 ± .2	15.5 ± .2	15.0 ± .2
METHEMOGLOBIN, %	.9 ± .4	0.0 ± 0.0	0.0 ± 0.0	1.7 ± 1.2
MCV, CURIC MICRONS	65.7 ± 1.4	67.8 ± .5	67.5 ± .7	76.7 ± 2.5 ^{a/}
MCH, MICRO MICROGMS.	21.4 ± .3	21.8 ± .3	22.3 ± .4	24.7 ± .7 ^{a/}
MCHC, GM %	32.6 ± .4	32.2 ± .7	33.1 ± .5	32.3 ± .5
PLATELETS (X10 ⁵ /MM ³)	7.0 ± .7	5.4 ± .5	6.1 ± .4	5.2 ± .3
LEUKOCYTES (X10 ³ /MM ³)	17.8 ± 1.6	21.4 ± 2.0	19.3 ± 1.4	21.9 ± 1.8
NEUTROPHILS, %	9.4 ± 1.9	17.8 ± .9 ^{a/}	9.2 ± 2.0	7.2 ± 2.0
LYMPHOCYTES, %	88.0 ± 2.3	81.8 ± 1.0	90.0 ± 1.4	92.2 ± 2.1
MONO. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EUSINOPHILS, %	1.8 ± 1.1	.4 ± .2	.8 ± .4	.4 ± .4
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.8 ± .4	0.0 ± 0.0 ^{a/}	0.0 ± 0.0 ^{a/}	.2 ± .2
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	.2 ± .2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL RATS BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

ENTRIES ARE MEAN ± STANDARD ERROR.

TABLE 37

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF 2,4-DNT FOR 3 MONTHS

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF RATS	
DOSE: % IN FEED	0 (C, 5)	0.0015 (T, 5)	0.01 (T, 5)	0.07 (T, 5)
ERYTHROCYTES ($\times 10^6 / \text{MM}^3$)	6.58 $\pm$.14	6.34 $\pm$.21	6.15 $\pm$ 0.13(4)	6.02 $\pm$.08
HEINZ BODIES, %	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
RETICULOCYTES, %	1.18 $\pm$.20	1.26 $\pm$.25	1.35 $\pm$.26	1.66 $\pm$.16
HEMATOCRIT, VOL. %	43.0 $\pm$ 1.1	42.6 $\pm$ 1.7	44.2 $\pm$ 1.2	42.8 $\pm$.4
HEMOGLOBIN, GM. %	14.4 $\pm$.3	14.3 $\pm$.4	14.1 $\pm$.4	14.0 $\pm$.1
METHEMOGLOBIN, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	1.2 $\pm$.7
MCV, CUBIC MICRONS	65.4 $\pm$ 1.6	67.2 $\pm$.8	71.2 $\pm$ 2.5(4)	71.1 $\pm$.9
MCHM, MICRO MICROGMS.	21.9 $\pm$.4	22.5 $\pm$.2	22.8 $\pm$ 0.6(4)	23.3 $\pm$.2
MCHMC, GM. %	33.4 $\pm$.3	33.6 $\pm$.5	31.9 $\pm$.4 ^{a/}	32.7 $\pm$.2
PLATELETS ($\times 10^3 / \text{MM}^3$)	6.0 $\pm$.1	5.5 $\pm$.5	4.7 $\pm$.3 ^{a/}	6.1 $\pm$.5
LEUKOCYTES ($\times 10^3 / \text{MM}^3$)	16.7 $\pm$ 1.2	14.1 $\pm$ 1.0	17.4 $\pm$.9	18.5 $\pm$ 1.1
NEUTROPHILS, %	14.8 $\pm$ 2.2	10.6 $\pm$ 2.1	10.2 $\pm$ 1.5	9.2 $\pm$ 2.2
LYMPHOCYTES, %	83.0 $\pm$ 2.7	87.6 $\pm$ 2.5	88.8 $\pm$ 1.4	90.2 $\pm$ 2.3
BANDS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
EOSINOPHILS, %	1.2 $\pm$.6	1.4 $\pm$.6	.2 $\pm$.2	1.2 $\pm$.2
BASOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MONOCYTES, %	1.0 $\pm$.3	.4 $\pm$.2	.8 $\pm$.4	.4 $\pm$.2
ATYPICAL, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
NUCLEATED PRC, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL RATS BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.ENTRIES ARE MEAN $\pm$ STANDARD ERROR.

TABLE 38

LABORATORY DATA OF MALE RATS AFTER FEEDING OF 2,4-DNT FOR 6 MONTHS

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF RATS	
DOSE: % IN FEED	0 (C, 5)	0.0015 (T, 5)	0.01 (T, 5)	0.07 (T, 3)
ERYTHROCYTES ($\times 10^6$ /MM ³)	7.06 $\pm$.14	7.01 $\pm$.17	6.59 $\pm$.31	6.62 $\pm$.21
HEINZ BODIES, %	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
RETICULOCYTES, %	1.18 $\pm$.15	1.39 $\pm$.20	1.26 $\pm$.16	1.82 $\pm$.06 ^{a/}
HEMATOCRIT, VOL. %	52.2 $\pm$ 1.4	51.2 $\pm$.8	51.0 $\pm$ 1.0	48.2 $\pm$.8 ^{a/}
HEMOGLOBIN, GM. %	16.0 $\pm$.2	15.4 $\pm$.1	15.4 $\pm$.2	15.0 $\pm$.3 ^{a/}
METHEMOGLOBIN, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MCV, CUBIC MICRONS	73.9 $\pm$ 1.1	73.2 $\pm$ 1.4	78.1 $\pm$ 4.1	73.0 $\pm$ 2.0
MCHC, MICRO MICROGMS.	22.6 $\pm$.3	22.1 $\pm$.4	23.6 $\pm$.8	22.7 $\pm$.5
MCHBC, GM. %	30.7 $\pm$.8	30.2 $\pm$.4	30.3 $\pm$.7	31.1 $\pm$.3
PLATELETS ($\times 10^3$ /MM ³)	5.6 $\pm$.3	4.9 $\pm$.2	6.4 $\pm$.2	6.1 $\pm$.5
LEUKOCYTES ($\times 10^3$ /MM ³)	18.2 $\pm$.7	19.0 $\pm$ 1.5	19.0 $\pm$ 1.3	19.9 $\pm$.8
NEUTROPHILS, %	21.6 $\pm$ 3.5	19.6 $\pm$ 7.1	19.2 $\pm$ 2.2	15.8 $\pm$ 4.3
LYMPHOCYTES, %	74.4 $\pm$ 3.4	78.2 $\pm$ 7.7	78.8 $\pm$ 2.4	81.4 $\pm$ 5.0
BANDS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
EOSINOPHILS, %	1.6 $\pm$.7	2.0 $\pm$.8	1.4 $\pm$.2	2.4 $\pm$.9
BASOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MONOCYTES, %	.4 $\pm$.4	.2 $\pm$.2	.6 $\pm$.4	.4 $\pm$.2
ATYPICAL, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
NUCLEATED PRC, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL RATS BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.ENTRIES ARE MEAN $\pm$ STANDARD ERROR.

TABLE 39

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF 2,4-DNT FOR 6 MONTHS

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF RATS	
DOSE: % IN FEED	0 (C, 5)	0.0015 (T, 5)	0.01 (T, 5)	0.07 (T, 5)
ERYTHROCYTES ($\times 10^6$ /MM ³)	6.15 $\pm$.06	5.84 $\pm$.20	6.11 $\pm$.14	5.81 $\pm$.13
HEINZ BODIES, %	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
RETICULOCYTES, %	1.46 $\pm$.20	1.56 $\pm$.25	1.33 $\pm$.08	1.98 $\pm$.26
HEMATOCRIT, VOL. %	45.0 $\pm$.3	44.4 $\pm$.7	46.2 $\pm$ 1.1	44.0 $\pm$.4
HEMOGLOBIN, GM. %	14.0 $\pm$.1	14.1 $\pm$.2	14.8 $\pm$.4	13.9 $\pm$.1
METHEMOGLOBIN, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MCV, CUBIC MICRONS	73.2 $\pm$ 1.0	76.2 $\pm$ 1.8	75.7 $\pm$ 1.1	75.8 $\pm$ 2.1
MCHM, MICRO MICROGMS.	22.8 $\pm$.4	24.3 $\pm$.6	24.2 $\pm$.4	23.9 $\pm$.5
MCHRC, GM. %	31.2 $\pm$.3	31.8 $\pm$.1	32.0 $\pm$.2	31.5 $\pm$.3
PLATELETS ($\times 10^5$ /MM ³)	7.2 $\pm$.6	5.2 $\pm$.3 ^{a/}	5.9 $\pm$.3 (4)	6.7 $\pm$.2
LEUKOCYTES ($\times 10^3$ /MM ³)	14.7 $\pm$ 1.2	13.5 $\pm$.9	16.2 $\pm$ 1.2	18.6 $\pm$ 1.2
NEUTROPHILS, %	11.8 $\pm$ 2.9	13.2 $\pm$ 4.7	12.4 $\pm$ 1.4	8.8 $\pm$ 1.9
LYMPHOCYTES, %	84.4 $\pm$ 3.2	85.6 $\pm$ 4.6	86.2 $\pm$ 1.5	89.6 $\pm$ 1.7
EOSINOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MONOCYTES, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
ATYPICAL, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
NUCLEATED PRP, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL RATS BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

ENTRIES ARE MEAN $\pm$ STANDARD ERROR.

TABLE 40

LABORATORY DATA OF MALE RATS AFTER FEEDING OF 2,4-DNT FOR 9 MONTHS

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF RATS	
DOSE: μ IN FEED	0 (C, 5)	0.0015 (T, 5)	0.01 (T, 5)	0.07 (T, 5)
ERYTHROCYTES ($\times 10^6$ /MM ³)	7.55 $\pm$.09	7.83 $\pm$.19	7.01 $\pm$.29	7.16 $\pm$.12
HEINZ BODIES, %	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
RETICULOCYTES, %	1.25 $\pm$.18	1.40 $\pm$.15	2.19 $\pm$.32 ^{a/}	2.43 $\pm$.17 ^{a/}
HEMATOCRIT, VOL. %	50.6 $\pm$.6	49.4 $\pm$.9	46.6 $\pm$.9 ^{a/}	49.4 $\pm$ 1.0
HEMOGLOBIN, GM. %	16.0 $\pm$.1	16.1 $\pm$.4	15.2 $\pm$.4	15.1 $\pm$.3
METHEMOGLOBIN, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	.5 $\pm$.3
MCV, CURIC MICRONS	67.0 $\pm$ 1.0	63.1 $\pm$.6	66.8 $\pm$ 2.2	69.0 $\pm$.9
MCH, MICRO MICROGMS.	21.2 $\pm$.2	20.8 $\pm$.2	21.8 $\pm$.4	21.2 $\pm$.4
MCH-C, GM. μ	31.6 $\pm$.3	32.7 $\pm$.7	32.6 $\pm$.6	30.7 $\pm$.9
PLATELETS ($\times 10^3$ /MM ³)	9.2 $\pm$.6	5.0 $\pm$.6	4.4 $\pm$.3	4.4 $\pm$.2
LEUKOCYTES ($\times 10^3$ /MM ³)	15.4 $\pm$.8	15.3 $\pm$.9	15.8 $\pm$ 1.2	18.1 $\pm$ 1.3
NEUTROPHILS, %	16.6 $\pm$ 2.2	15.6 $\pm$ 3.3	17.6 $\pm$ 2.8	15.8 $\pm$.7
LYMPHOCYTES, %	40.8 $\pm$ 2.1	42.4 $\pm$ 3.8	39.8 $\pm$ 2.9	41.8 $\pm$.7
MONOS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
EOSINOPHILS, %	2.2 $\pm$.7	1.6 $\pm$.4	2.0 $\pm$.7	2.0 $\pm$.7
BAZOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MONOCYTES, %	.4 $\pm$.2	.4 $\pm$.2	.6 $\pm$.4	.4 $\pm$.4
ATYPICAL, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
NUCLEATED RBC, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL RATS BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

ENTRIES ARE MEAN $\pm$ STANDARD ERROR.

TABLE 41

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF 2,4-DNT FOR 9 MONTHS

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF RATS	
DOSE: % IN FEED	0 (C, 5)	0.0015 (T, 5)	0.01 (T, 5)	0.07 (T, 5)
ERYTHROCYTES ($\times 10^6 / \text{MM}^3$)	6.87 $\pm$.19	6.95 $\pm$.21	6.79 $\pm$.14	6.13 $\pm$.06 ^{a/}
HEINZ BODIES, %	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
RETICULOCYTES, %	1.30 $\pm$.28	1.38 $\pm$.08	1.46 $\pm$.25	1.49 $\pm$.12
HEMATOCRIT, VOL. %	45.4 $\pm$.5	43.4 $\pm$.9	45.2 $\pm$ 1.0	41.8 $\pm$.9 ^{a/}
HEMOGLOBIN, GM. %	14.4 $\pm$.2	13.9 $\pm$.5	14.8 $\pm$.3	14.2 $\pm$.2
METHEMOGLOBIN, %	3.0 $\pm$ 1.4	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0 ^{a/}
MCV, CUBIC MICRONS	66.3 $\pm$ 2.1	66.3 $\pm$ 1.0	66.6 $\pm$ 1.2	68.1 $\pm$ 1.0
MCHM, MICRO MICROGRAMS.	21.1 $\pm$.3	21.2 $\pm$.7	21.8 $\pm$.4	23.1 $\pm$.1 ^{a/}
MCHMC, GM. %	31.8 $\pm$.5	32.0 $\pm$.7	32.8 $\pm$.3	33.9 $\pm$.4 ^{a/}
PLATELETS ($\times 10^5 / \text{MM}^3$)	5.0 $\pm$.4	4.8 $\pm$.4	4.7 $\pm$.3	4.3 $\pm$.1
LEUKOCYTES ($\times 10^3 / \text{MM}^3$)	12.7 $\pm$ 1.1	13.2 $\pm$ 1.2	13.9 $\pm$ 1.1	14.0 $\pm$ 1.0
NEUTROPHILS, %	14.6 $\pm$ 2.2	15.2 $\pm$ 2.0	12.2 $\pm$ 1.4	12.4 $\pm$ 2.2
LYMPHOCYTES, %	83.6 $\pm$ 1.9	82.6 $\pm$ 2.3	86.0 $\pm$ 1.6	86.4 $\pm$ 2.5
EOSINOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
BASOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MONOCYTES, %	.4 $\pm$.2	.4 $\pm$.2	.6 $\pm$.4	.2 $\pm$.2
ATYPICAL, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
NUCLEATED RBC, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL RATS BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.ENTRIES ARE MEAN $\pm$ STANDARD ERROR.

TABLE 42

LABORATORY DATA OF MALE RATS AFTER FEEDING OF 2,4-DNT FOR 12 MONTHS

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF RATS	
DOSE: g IN FEED	0 (C, 5)	0.0015 (T, 5)	0.01 (T, 5)	0.07 (T, 5)
ERYTHROCYTES ($\times 10^6$ /MM ³)	7.45 $\pm$.20	7.10 $\pm$.07	6.70 $\pm$.22 ^{a/}	6.14 $\pm$.25 ^{a/}
HEINZ BODIES, %	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
RETICULOCYTES, %	.93 $\pm$.24 (4)	.46 $\pm$.20	1.41 $\pm$.29 (4)	1.60 $\pm$.31
HEMATOCRIT, VOL. %	51.8 $\pm$.9 (4)	50.4 $\pm$.7	48.2 $\pm$.9	46.4 $\pm$ 1.3 ^{a/}
HEMOGLOBIN, GM. %	16.0 $\pm$.2	15.0 $\pm$.2	15.1 $\pm$.3	14.3 $\pm$.5 ^{a/}
METHEMOGLOBIN, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MCV, CURIC MICRONS	69.5 $\pm$ 1.9 (4)	71.0 $\pm$ 1.2	72.1 $\pm$ 1.4	75.3 $\pm$ 2.0
MCHC, MICRO MICROGMS.	21.5 $\pm$.5	21.1 $\pm$.3	22.6 $\pm$.5	23.2 $\pm$.6
MCHC, GM %	31.1 $\pm$.4 (4)	29.8 $\pm$.3	31.4 $\pm$.3	30.8 $\pm$.9
PLATELETS ($\times 10^3$ /MM ³)	5.7 $\pm$.6	5.2 $\pm$.6	6.5 $\pm$.8	6.1 $\pm$.5
LEUKOCYTES ($\times 10^3$ /MM ³)	13.1 $\pm$.5	13.5 $\pm$ 1.2	13.7 $\pm$ 1.4	15.4 $\pm$ 2.2
NEUTROPHILS, %	16.6 $\pm$.7	18.2 $\pm$ 3.4	15.8 $\pm$ 2.0	24.8 $\pm$ 5.9
LYMPHOCYTES, %	80.6 $\pm$.7	80.4 $\pm$ 3.9	81.2 $\pm$ 1.9	73.6 $\pm$ 5.9
BANDS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
EOSINOPHILS, %	1.8 $\pm$.4	.4 $\pm$.2	1.0 $\pm$.4	.4 $\pm$.4
BASOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MONOCYTES, %	1.0 $\pm$.5	.8 $\pm$.4	2.0 $\pm$.9	1.0 $\pm$.5
ATYPICAL, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
NUCLEATED RBC, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
GLUCOSE (FASTING), MG %	133.3 $\pm$ 13.0	134.0 $\pm$ 9.9	114.5 $\pm$ 2.6	155.0 $\pm$ 31.0
SGOT, IU/L	130 $\pm$ 24	75 $\pm$ 11	83 $\pm$ 11	46 $\pm$ 42 ^{a/}
SGPT, IU/L	51.5 $\pm$ 20.0	36.0 $\pm$ 5.1	25.3 $\pm$ 3.4	26.5 $\pm$ 2.5
ALK. PHOS., IU/L	34 $\pm$ 6	41 $\pm$ 4	29 $\pm$ 3	49 $\pm$ 14
ALB, MG %	15.0 $\pm$ 1.1	15.0 $\pm$ 1.6	14.0 $\pm$ 4.4	24.8 $\pm$ 5.2
IMMUNOGLOBULIN E, IU/ML	1825 $\pm$ 498			650 $\pm$ 106

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL RATS BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

ENTRIES ARE MEAN $\pm$ STANDARD ERROR.

TABLE 43

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF 2,4-DNT FOR 12 MONTHS

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF RATS	
DOSE: % IN FEED	0 (C, 5)	0.0015 (T, 5)	0.01 (T, 5)	0.07 (T, 5)
ERYTHROCYTES ($\times 10^6$ /MM ³)	6.45 $\pm$.30	6.22 $\pm$.22	6.49 $\pm$.13	5.61 $\pm$.10 ^{a/}
HEINZ BODIES, %	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
RETICULOCYTES, %	1.09 $\pm$.23	1.06 $\pm$.12	.77 $\pm$.14	1.40 $\pm$.22
HEMATOCRIT, VOL. %	47.2 $\pm$ 1.2	46.6 $\pm$ 1.0	47.8 $\pm$.6	43.0 $\pm$.4 ^{a/}
HEMOGLOBIN, GM. %	14.2 $\pm$.5	13.8 $\pm$.4	14.8 $\pm$.2	13.1 $\pm$.3
METHEMOGLOBIN, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MCV, CURIC MICRONS	73.5 $\pm$ 1.7	75.1 $\pm$ 1.6	71.6 $\pm$ 1.2	76.7 $\pm$ 1.7
MCHM, MICRO MICROGMS.	22.1 $\pm$.4	22.3 $\pm$.6	22.2 $\pm$.2	23.4 $\pm$.3
MCHMC, GM %	30.1 $\pm$.4	29.7 $\pm$.5	31.0 $\pm$.2	30.6 $\pm$.4
PLATELETS ($\times 10^5$ /MM ³)	6.0 $\pm$.3 (4)	5.2 $\pm$.1	5.2 $\pm$.6	6.3 $\pm$.3
LEUKOCYTES ($\times 10^3$ /MM ³)	12.6 $\pm$ 1.0	11.7 $\pm$.7	12.4 $\pm$ 1.1	13.2 $\pm$.9
NEUTROPHILS, %	13.8 $\pm$ 2.3	15.4 $\pm$ 2.2	12.6 $\pm$ 3.4	14.2 $\pm$ 2.7
LYMPHOCYTES, %	85.6 $\pm$ 2.7	83.4 $\pm$ 2.0	86.8 $\pm$ 3.5	85.2 $\pm$ 2.8
BANDS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
EOSINOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	.6 $\pm$.2 ^{a/}	.2 $\pm$.2
BASOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MONOCYTES, %	.6 $\pm$.4	1.2 $\pm$.4	0.0 $\pm$ 0.0	.4 $\pm$.2
ATYPICAL, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
NUCLEATED RBC, %	0.0 $\pm$ 0.0	.2 $\pm$.2	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
GLUCOSE (FASTING), MG %	136.3 $\pm$ 15.2	121.3 $\pm$ 4.8	132.0 $\pm$ 7.8	128.3 $\pm$ 6.8
SUOT, IU/L	78.5 $\pm$ 9.0	71.0 $\pm$ 9.2	63.3 $\pm$ 5.3	82.5 $\pm$ 6.7
SGPT, IU/L	24.5 $\pm$ 5.0	23.0 $\pm$ 3.1	23.8 $\pm$ 2.5	30.8 $\pm$ 3.4
ALK. PHOS., IU/L	12 $\pm$ 1	14 $\pm$ 1	16 $\pm$ 2	10 $\pm$ 2
BUN, MG %	13.8 $\pm$.8	14.5 $\pm$.6	16.0 $\pm$.9	19.0 $\pm$.9 ^{a/}
IMMUNOGLOBULIN E, IU/ML	900 $\pm$ 211			775 $\pm$ 179

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL RATS BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.ENTRIES ARE MEAN $\pm$ STANDARD ERROR.

TABLE 44

LABORATORY DATA OF MALE RATS AFTER FEEDING OF 2,4-DNT FOR 18 MONTHS

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF RATS	
DOSE: % IN FEED	0 (C, 5)	0.0015 (T, 5)	0.01 (T, 5)	0.07 (T, 5)
ERYTHROCYTES ($\times 10^6$ /MM ³)	7.52 $\pm$.15	7.44 $\pm$.13	6.80 $\pm$.34	6.07 $\pm$.27 ^{a/}
HEINZ BODIES, %	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
RETICULOCYTES, %	.37 $\pm$.12	.75 $\pm$.19	.69 $\pm$.20	1.27 $\pm$.34 ^{a/}
HEMATOCRIT, VOL. %	44.4 $\pm$.6	48.8 $\pm$.7	47.0 $\pm$.8	45.2 $\pm$ 1.6 ^{a/}
HEMUGLOBIN, GM. %	15.2 $\pm$.2	15.4 $\pm$.2	14.5 $\pm$.3	12.9 $\pm$.5 ^{a/}
METHEMOGLOBIN, %	.6 $\pm$.6	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MCV, CUBIC MICRONS	65.8 $\pm$.6	65.7 $\pm$ 1.6	69.8 $\pm$ 3.5	74.7 $\pm$ 2.4 ^{a/}
MCHB, MICRO MICROGMS.	20.2 $\pm$.1	20.7 $\pm$.4	21.6 $\pm$ 1.0	21.3 $\pm$.4
MCHBC, GM %	30.7 $\pm$.3	31.5 $\pm$.3	30.9 $\pm$.4	28.6 $\pm$.7 ^{a/}
PLATELETS ($\times 10^5$ /MM ³)	4.2 $\pm$.2	4.7 $\pm$.3	5.5 $\pm$.5	7.1 $\pm$.9 ^{a/}
LEUKOCYTES ($\times 10^3$ /MM ³)	15.4 $\pm$ 1.6	15.0 $\pm$ 1.5	15.3 $\pm$ 2.2	18.7 $\pm$ 3.9
NEUTROPHILS, %	18.8 $\pm$ 1.4	21.8 $\pm$ 3.2	21.6 $\pm$ 4.4	25.0 $\pm$ 2.3
LYMPHOCYTES, %	79.2 $\pm$ 1.4	75.2 $\pm$ 3.3	75.2 $\pm$ 4.5	73.8 $\pm$ 2.4
BANDS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
EUSINOPHILS, %	1.4 $\pm$.2	2.2 $\pm$.8	2.2 $\pm$.7	.4 $\pm$.2
BASOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MONOCYTES, %	.6 $\pm$.2	.8 $\pm$.4	1.0 $\pm$.5	.8 $\pm$.4
ATYPICAL, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
NUCLEATED RBC, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL RATS BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.ENTRIES ARE MEAN $\pm$ STANDARD ERROR.

TABLE 45

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF 2,4-DNT FOR 18 MONTHS

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF RATS	
DOSE: % IN FEED	0 (C, 5)	0.0015 (T, 5)	0.01 (T, 5)	0.07 (T, 5)
ERYTHROCYTES ($\times 10^6$ /MM ³)	6.87 $\pm$.28	6.40 $\pm$.16	6.75 $\pm$.21 (4)	5.57 $\pm$.28 ^{a/}
HEINZ BODIES, %	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
RETICULOCYTES, %	.81 $\pm$.14	1.04 $\pm$.22	.77 $\pm$.21 (3)	2.23 $\pm$.36 ^{a/}
HEMATOCRIT, VOL. %	45.4 $\pm$ 1.5	44.6 $\pm$ 1.3	45.7 $\pm$ 2.3 (3)	40.4 $\pm$ 2.2
HEMOGLOBIN, GM. %	13.9 $\pm$.4	12.8 $\pm$.1	13.8 $\pm$.4 (4)	12.0 $\pm$.5 ^{a/}
METHEMOGLOBIN, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MCV, CUBIC MICRONS	66.2 $\pm$ 1.1	69.7 $\pm$ 1.7	67.5 $\pm$ 2.0 (3)	72.5 $\pm$ 1.0 ^{a/}
MCHC, MICRO MICROGMS.	20.3 $\pm$.4	20.1 $\pm$.5	20.4 $\pm$.3 (4)	21.5 $\pm$.2
MCHBC, GM %	30.7 $\pm$.4	28.9 $\pm$.8 ^{a/}	30.1 $\pm$.4 (3)	29.8 $\pm$.4
PLATELETS ($\times 10^5$ /MM ³)	3.8 $\pm$.3	4.3 $\pm$.2	3.6 $\pm$.3	4.7 $\pm$.3
LEUKOCYTES ($\times 10^3$ /MM ³)	10.4 $\pm$ 1.1	14.1 $\pm$ 1.0	12.6 $\pm$.4 (4)	13.5 $\pm$ 1.5
NEUTROPHILS, %	25.2 $\pm$ 5.8	20.2 $\pm$ 2.2	16.2 $\pm$ 1.9	19.2 $\pm$ 4.6
LYMPHOCYTES, %	72.8 $\pm$ 5.6	79.2 $\pm$ 2.4	83.2 $\pm$ 1.9	78.8 $\pm$ 4.4
BANDS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
EUSINOPHILS, %	1.4 $\pm$.5	.4 $\pm$.2	.4 $\pm$.2	1.4 $\pm$.4
BASOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MONOCYTES, %	.6 $\pm$.4	.2 $\pm$.2	.2 $\pm$.2	.6 $\pm$.2
ATYPICAL, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
NUCLEATED RBC, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL RATS BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

ENTRIES ARE MEAN $\pm$ STANDARD ERROR.

TABLE 46

LABORATORY DATA OF MALE RATS AFTER FEEDING 2,4-DNT FOR 36 MONTHS

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF RATS
DOSE: MG/KG/DAY	0.00 (C, 4)	0.0015 (T, 4)	.01 (T, 4)
ERYTHROCYTES ($\times 10^6$ /MM ³)	6.29 $\pm$.41	6.04 $\pm$.40	5.66 $\pm$.47
HEINZ BODIES, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
RETICULOCYTES, %	.72 $\pm$.04	1.17 $\pm$.15	4.45 $\pm$ 2.32
HEMATOCRIT, VOL. %	44.0 $\pm$ 2.4	43.3 $\pm$ 2.4	44.0 $\pm$ 2.7
HEMOGLOBIN, GM. %	13.6 $\pm$.8	13.8 $\pm$.9	13.3 $\pm$.8
METHEMOGLOBIN, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MCV, CUBIC MICRONS	70.2 $\pm$ 1.8	71.8 $\pm$ 1.4	78.4 $\pm$ 3.1
MCH, MICRO MICROGMS.	21.6 $\pm$.5	22.8 $\pm$.6	23.7 $\pm$.6
MCHC, GM %	30.8 $\pm$.5	31.8 $\pm$.3	30.3 $\pm$.8
PLATELETS ($\times 10^5$ /MM ³)	4.2 $\pm$.4	3.8 $\pm$.4	3.9 $\pm$.4
LEUKOCYTES ($\times 10^3$ /MM ³)	22.3 $\pm$ 1.5	17.7 $\pm$ 4.1	18.2 $\pm$ 2.9
NEUTROPHILS, %	31.8 $\pm$ 4.3	30.8 $\pm$ 6.8	32.3 $\pm$ 5.9
LYMPHOCYTES, %	65.5 $\pm$ 4.1	68.8 $\pm$ 6.7	66.3 $\pm$ 5.9
BANDS, %	.4 $\pm$.2	0.0 $\pm$ 0.0	.5 $\pm$.4
EOSINOPHILS, %	.9 $\pm$.5	.3 $\pm$.2	.3 $\pm$.2
BASOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MONOCYTES, %	.1 $\pm$.1	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
ATYPICAL, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
NUCLEATED RBC, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
GLUCOSE (FASTING), MG %	127.0 $\pm$ 4.7	113.3 $\pm$ 6.8	129.3 $\pm$ 8.9
SGOT, IU/L	45 $\pm$ 2	62 $\pm$ 10	156 $\pm$ 78
SGPT, IU/L	17.3 $\pm$ 1.4	19.8 $\pm$ 3.3	59.0 $\pm$ 27.8
ALK. PHOS., IU/L	32 $\pm$ 3	28 $\pm$ 4	41 $\pm$ 9
BUN, MG %	12.5 $\pm$.9	13.3 $\pm$ 2.3	21.3 $\pm$ 3.6
IMMUNOGLOBULIN E, IU/ML	1400 $\pm$ 246		

ENTRIES ARE MEAN $\pm$ STANDARD ERROR

TABLE 47

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF 2,4-DNT FOR 24 MONTHS

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF RATS	
DOSE: MG/KG/DAY	0 (C, 4)	0.0015 (T, 4)	0.01 (T, 4)	0.07 (T, 1)
ERYTHROCYTES (X10 ⁶ /MM ³)	6.00 ± .47	6.09 ± .19	5.44 ± .61	.89
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
RETICULOCYTES, %	1.34 ± .34	1.41 ± .18	3.02 ± 1.31	9.56
HEMATOCRIT, VOL. %	43.0 ± 2.0	43.5 ± 1.0	40.5 ± 3.0	33.0
HEMOGLOBIN, GM. %	14.2 ± .7	14.1 ± .5	12.8 ± 1.1	9.4
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
MCV, CURIC MICRONS	72.2 ± 2.5	71.5 ± 1.7	75.5 ± 3.5	370.8
MCH, MICRO MICROGMS.	23.8 ± .8	23.2 ± .6	23.8 ± .4	105.8
MCHC, GM %	33.0 ± .1	32.5 ± .7	31.6 ± .5	28.5
PLATELETS (X10 ⁵ /MM ³)	4.0 ± .2	5.2 ± .6	4.1 ± .2	4.6
LEUKOCYTES (X10 ³ /MM ³)	10.8 ± .4	14.5 ± 2.9	11.1 ± 1.7	14.8
NEUTROPHILS, %	23.8 ± 1.8	50.8 ± 2.1	36.3 ± 4.7	66.0
LYMPHOCYTES, %	74.3 ± 1.4	49.0 ± 2.0	62.5 ± 5.2	33.0
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
EOSINOPHILS, %	1.0 ± .4	0.0 ± 0.0	.7 ± .4	1.0
BASEOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
MONOCYTES, %	0.0 ± 0.0	.1 ± .1	0.0 ± 0.0	0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	.1 ± .1	6.0
GLUCOSE (FASTING), MG %	114.0 ± 10.7	109.5 ± 3.8	109.0 ± 10.6 (3)	89.0
SGOT, IU/L	66 ± 5	80 ± 13	108 ± 48 (3)	513
SGPT, IU/L	20.3 ± .4	26.0 ± 5.6	79.0 ± 58.0 (3)	65.0
ALK. PHOS., IU/L	13 ± 2	15 ± 2	21 ± 10 (3)	21
BUN, MG %	12.8 ± 1.7	13.8 ± 1.5	12.1 ± 1.5 (3)	24.0
IMMUNOGLOBULIN E, IU/ML	1863 ± 405			2200

±/ SIGNIFICANTLY DIFFERENT FROM CONTROL RATS BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

ENTRIES ARE MEAN ± STANDARD ERROR.

TABLE 48

LABORATORY DATA OF MALE RATS AFTER FEEDING 2,4-DNT FOR 12 MONTHS AND ALLOWING TO RECOVER FOR 1 MONTH

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF RATS	
DOSE: % IN FEED	0 (C, 3)	0.0015 (T, 4)	0.10 (T, 4)	0.07 (T, 3)
ERYTHROCYTES ($\times 10^6$ /MM ³)	7.42 $\pm$.15 (2)	7.23 $\pm$.22	8.06 $\pm$.28 (3)	7.50 $\pm$.38
HEINZ BODIES, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
RETICULOCYTES, %	.76 $\pm$.06 (2)	.81 $\pm$.09	.98 $\pm$.01 (3)	.67 $\pm$.12
HEMATOCRIT, VOL. %	42.0 $\pm$ 1.0 (2)	42.3 $\pm$.5	43.0 $\pm$ 1.0 (3)	39.3 $\pm$.9
HEMOGLOBIN, GM. %	14.5 $\pm$.2 (2)	14.1 $\pm$.4	14.2 $\pm$.5 (3)	13.4 $\pm$.5
METHEMOGLOBIN, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MCV, CUBIC MICRONS	55.2 $\pm$ 2.4 (2)	56.5 $\pm$ 1.6	53.4 $\pm$ 1.1 (3)	52.7 $\pm$ 3.2
MCHM, MICRO MICROGMS.	19.1 $\pm$.2 (2)	19.5 $\pm$.3	17.6 $\pm$.3 (3) ^{a/}	17.4 $\pm$.2
MCHC, GM. %	34.7 $\pm$ 1.2 (2)	33.3 $\pm$.4	33.0 $\pm$.3 (3)	34.1 $\pm$ 1.4
PLATELETS ($\times 10^3$ /MM ³)	4.7 $\pm$.5 (2)	4.7 $\pm$.3	4.6 $\pm$.2 (3)	4.8 $\pm$.4
LEUKOCYTES ($\times 10^3$ /MM ³)	8.8 $\pm$.7 (2)	9.5 $\pm$.4	9.1 $\pm$.2 (3)	7.6 $\pm$ 3.8
NEUTROPHILS, %	20.3 $\pm$ 7.2	22.5 $\pm$ 8.5	24.0 $\pm$ 3.5	25.0 $\pm$ 4.4
LYMPHOCYTES, %	76.3 $\pm$ 4.9	76.0 $\pm$ 8.3	74.8 $\pm$ 4.7	74.3 $\pm$ 4.9
BANDS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
EOSINOPHILS, %	2.3 $\pm$.9	.8 $\pm$.5	.8 $\pm$.5	.7 $\pm$.7
MASOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MONOCYTES, %	1.0 $\pm$.4	.8 $\pm$.3	.5 $\pm$.2	0.0 $\pm$ 0.0
ATYPICAL, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
NUCLEATED RBC, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
GLUCOSE (FASTING), MG %	123.0 $\pm$ 9.8	119.0 $\pm$ 4.8	140.8 $\pm$ 9.1	135.0 $\pm$ 22.4
SGOT, IU/L	75.0 $\pm$ 2.4	64.0 $\pm$ 4.4	63.3 $\pm$ 6.5	73.0 $\pm$ 16.6
SGPT, IU/L	27.7 $\pm$ 3.8	23.5 $\pm$ 1.7	28.5 $\pm$ 4.0	29.7 $\pm$ 3.1
ALP, PHOS., IU/L	41 $\pm$ 0	32 $\pm$ 2	43 $\pm$ 2	48 $\pm$ 12
BUN, MG %	15.3 $\pm$ 1.2	27.5 $\pm$ 9.7	12.5 $\pm$ 1.0	14.7 $\pm$ 1.9
IMMUNOGLOBULIN E, IU/ML	767 $\pm$ 196			2833 $\pm$ 722

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL RATS BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

ENTRIES ARE MEAN $\pm$ STANDARD ERROR.

TABLE 49

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF 2,4-DNT FOR 12 MONTHS AND ALLOWING TO RECOVER FOR 1 MONTH

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF RATS	
DOSE: % IN FEED	0 (C. 4)	0.0015 (T. 4)	0.01 (T. 4)	0.07 (T. 4)
ERYTHROCYTES (X10 ⁶ /MM ³)	7.04 ± .13	6.52 ± .18	6.70 ± .20	6.29 ± .21 (3) ^{a/}
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	1.07 ± .15	1.03 ± .16	1.27 ± .33	1.00 ± .11 (3)
HEMATOCRIT, VOL. %	45.8 ± .9	44.8 ± 1.5	42.8 ± 1.3	42.7 ± .9 (3)
HEMOGLOBIN, GM. %	14.1 ± .0	13.6 ± .1	13.3 ± .4	13.2 ± .4 (3)
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	.4 ± .4	0.0 ± 0.0
MCV, CUBIC MICRONS	69.1 ± 2.1	68.8 ± 3.5	63.9 ± 2.2	68.0 ± 3.4 (3)
MCH, MICRO MICROGMS.	20.0 ± .3	21.0 ± .6	19.9 ± .6	21.0 ± .1 (3)
MCHC, GM %	30.8 ± .7	30.6 ± 1.0	31.2 ± .3	31.1 ± 1.4 (3)
PLATELETS (X10 ³ /MM ³)	5.1 ± .3	4.2 ± .2	3.5 ± .3 ^{a/}	5.8 ± .1 (3)
LEUKOCYTES (X10 ³ /MM ³)	5.3 ± .4	4.9 ± .7	8.6 ± 1.3	7.4 ± 1.8 (3)
NEUTROPHILS, %	21.0 ± 3.4	27.5 ± 5.1	19.3 ± 3.7	17.5 ± 5.0
LYMPHOCYTES, %	77.3 ± 3.4	70.8 ± 5.8	79.8 ± 3.3	81.8 ± 5.1
MONOS, %	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0
EOSINOPHILS, %	.3 ± .3	1.0 ± .4	0.0 ± 0.0	.5 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.4 ± .3	.8 ± .5	.8 ± .3	.3 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED PRC, %	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
GLUCOSE (FASTING), MG %	123.0 ± 10.9	120.0 ± 14.2	125.5 ± 4.4	116.3 ± 4.3
SGOT, IU/L	78.0 ± 9.2	69.5 ± 4.5	70.3 ± 4.1	42.3 ± 4.2
SGPT, IU/L	25.8 ± 4.8	28.5 ± 3.6	22.0 ± 3.3	24.3 ± 1.4
ALK. PHOS., IU/L	14 ± 1	10 ± 1	13 ± 2	13 ± 1
BUN, MG %	12.3 ± .6	11.8 ± .5	13.8 ± .5	18.3 ± 1.2 ^{a/}
IMMUNOGLOBULIN E, IU/ML	2650 ± 818			650 ± 0

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL RATS BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

ENTRIES ARE MEAN ± STANDARD ERROR.

TABLE 50

LABORATORY DATA OF MALE RATS AFTER FEEDING OF 2,4-DNT FOR 24 MONTHS AND ALLOWING TO RECOVER FOR 1 MONTH

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF RATS
DOSE: MG/KG/DAY	0 (C. 4)	0.0015 (T. 3)	.01 (T. 1)
ERYTHROCYTES (X10 ⁶ /MM ³)	5.61 ± 1.26	6.02 ± .43	6.80
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00
RETICULOCYTES, %	3.79 ± 2.74	1.81 ± .53	.68
HEMATOCRIT, VOL. %	34.0 ± 6.8	36.7 ± 2.4	42.0
HEMOGLOBIN, GM. %	11.2 ± 2.3	12.1 ± .4	13.4
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0
MCV, CUBIC MICRONS	62.7 ± 3.2	61.0 ± 2.3	61.8
MCH, MICRO MICROGMS.	20.6 ± 1.1	20.1 ± .4	20.0
MCHC, GM %	32.8 ± .4	32.9 ± .3	32.4
PLATELETS (X10 ⁵ /MM ³)	4.6 ± .3	5.1 ± .9	8.1
LEUKOCYTES (X10 ³ /MM ³)	10.8 ± .7	10.3 ± 3.0	15.2
NEUTROPHILS, %	44.3 ± 7.0	31.3 ± 3.5	22.0
LYMPHOCYTES, %	54.5 ± 6.7	68.0 ± 3.2	78.0
RANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0
EOSINOPHILS, %	.8 ± .5	.7 ± .3	0.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0
MONOCYTES, %	.5 ± .5	0.0 ± 0.0	0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0
GLUCOSE (FASTING), MG %	97.8 ± 18.6	105.7 ± 12.0	95.0
SGOT, IU/L	58.5 ± 7.7	52.3 ± 14.1	65.0
SGPT, IU/L	21.3 ± 3.5	32.3 ± 8.3	55.0
ALK. PHOS., IU/L	59 ± 23	34 ± 2	37
BUN, MG %	34.5 ± 13.2	21.0 ± 9.0	42.0
IMMUNOGLOBULIN E, IU/ML	450 ± 0		450

ENTRIES ARE MEAN ± STANDARD ERROR

TABLE 51

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF 2,4-DNT FOR 24 MONTHS AND ALLOWING TO RECOVER FOR 1 MONTH

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF RATS
DOSE: MG/KG/DAY	0.00 (C. 4)	0.0015 (T. 2)	.01 (T. 4)
ERYTHROCYTES ($\times 10^6$ /MM ³)	6.06 $\pm$.67	5.66 $\pm$.24	5.55 $\pm$.63
HEINZ BODIES, %	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
RETICULOCYTES, %	1.87 $\pm$.87	1.03 $\pm$.34	2.06 $\pm$ 1.01
HEMATOCRIT, VOL. %	37.8 $\pm$ 2.7	35.5 $\pm$.5	35.0 $\pm$ 2.7
HEMOGLOBIN, GM. %	12.5 $\pm$ 1.1	11.6 $\pm$.4	11.4 $\pm$ 1.1
METHEMOGLOBIN, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MCV, CUBIC MICRONS	63.4 $\pm$ 3.6	62.7 $\pm$ 1.8	63.9 $\pm$ 3.0
MCH, MICRO MICROGRAMS	20.8 $\pm$.9	20.6 $\pm$.3	20.7 $\pm$.5
MCHC, GM %	32.9 $\pm$.5	32.8 $\pm$.5	32.5 $\pm$.7
PLATELETS ($\times 10^3$ /MM ³)	5.1 $\pm$.3	6.2 $\pm$.5	5.0 $\pm$.2
LEUKOCYTES ($\times 10^3$ /MM ³)	5.7 $\pm$.6	10.7 $\pm$.1 ^{a/}	5.8 $\pm$ 1.1
NEUTROPHILS, %	43.5 $\pm$ 4.5	67.5 $\pm$ 7.5 ^{a/}	37.3 $\pm$ 1.4
LYMPHOCYTES, %	55.5 $\pm$ 4.5	32.5 $\pm$ 7.5 ^{a/}	61.3 $\pm$.9
MONOS. %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
EOSINOPHILS, %	.8 $\pm$.5	0.0 $\pm$ 0.0	1.5 $\pm$ 1.2
BASOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
PLASMOCYTES, %	.3 $\pm$.3	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
ATYPICAL, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
NUCLEATED RBC, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
GLUCOSE (FASTING), MG %	99.0 $\pm$ 8.4	78.5 $\pm$ 5.5	101.0 $\pm$ 7.9
SGOT, IU/L	64.8 $\pm$ 5.3	97.5 $\pm$ 26.5	56.3 $\pm$ 1.7
SGPT, IU/L	26.5 $\pm$ 3.6	35.0 $\pm$ 14.0	21.3 $\pm$ 2.9
ALK. PHOS., IU/L	11 $\pm$ 2	37 $\pm$ 14	25 $\pm$ 13
HUN, MG %	12.3 $\pm$ 1.0	17.5 $\pm$ 5.5	15.5 $\pm$.6
IMMUNOGLOBULIN E, IU/ML	450 $\pm$ 0		450 $\pm$ 0

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL RATS BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.ENTRIES ARE MEAN $\pm$ STANDARD ERROR.

TABLE 52

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF RATS FED 2,4-DNT FOR 12 MONTHS

Sex	Dose (% in feed)	Terminal Body Weight (g)	Absolute Organ Weight (g)					Ovary
			Brain	Liver	Kidney	Spleen	Testis	
Male	0	626 ± 14 ^{a/}	2.28 ± 0.04	16.5 ± 1.5	3.5 ± 0.1	0.96 ± 0.09	3.7 ± 0.1	
	0.0015	649 ± 28	2.26 ± 0.08	16.2 ± 0.4	4.1 ± 0.3	1.00 ± 0.05	3.6 ± 0.1	
	0.01	600 ± 31	2.30 ± 0.07	14.9 ± 1.7	3.9 ± 0.3	1.04 ± 0.11	3.4 ± 0.2	
	0.07	438 ± 11 ^{b/}	2.21 ± 0.07	21.8 ± 2.0 ^{b/}	5.6 ± 0.4 ^{b/}	0.79 ± 0.09	2.1 ± 0.2 ^{b/}	
Female	0	363 ± 19	1.98 ± 0.02	9.5 ± 0.4	2.3 ± 0.1	0.61 ± 0.04		0.149 ± 0.021
	0.0015	421 ± 19	2.07 ± 0.05	10.1 ± 0.7	2.5 ± 0.2	0.74 ± 0.06		0.194 ± 0.027
	0.01	373 ± 41	2.03 ± 0.08	9.4 ± 0.6	2.3 ± 0.1	0.65 ± 0.04		0.183 ± 0.015
	0.07	255 ± 11 ^{b/}	1.90 ± 0.09	11.9 ± 0.8	2.7 ± 0.2	0.31 ± 0.02 ^{b/}		0.164 ± 0.009

Sex	Dose (% in feed)	Relative Organ Weight (g/100 g body weight)					Ovary
		Brain	Liver	Kidney	Spleen	Testis	
Male	0	0.36 ± 0.00	2.63 ± 0.19	0.56 ± 0.01	0.154 ± 0.013	0.59 ± 0.02	
	0.0015	0.35 ± 0.01	2.51 ± 0.10	0.64 ± 0.05	0.154 ± 0.008	0.55 ± 0.02	
	0.01	0.39 ± 0.02	2.46 ± 0.20	0.67 ± 0.07	0.175 ± 0.022	0.58 ± 0.03	
	0.07	0.51 ± 0.02 ^{b/}	4.95 ± 0.35 ^{b/}	1.28 ± 0.09 ^{b/}	0.182 ± 0.023	0.48 ± 0.09	
Female	0	0.55 ± 0.03	2.65 ± 0.18	0.64 ± 0.05	0.171 ± 0.015		0.041 ± 0.006
	0.0015	0.50 ± 0.03	2.43 ± 0.29	0.61 ± 0.08	0.178 ± 0.020		0.047 ± 0.008
	0.01	0.57 ± 0.07	2.59 ± 0.19	0.62 ± 0.06	0.179 ± 0.012		0.050 ± 0.003 ^{b/}
	0.07	0.75 ± 0.01 ^{b/}	4.69 ± 0.38 ^{b/}	1.07 ± 0.02 ^{b/}	0.123 ± 0.011		0.065 ± 0.004 ^{b/}

Sex	Dose (% in feed)	Relative Organ Weight (g/g brain weight)				Ovary
		Liver	Kidney	Spleen	Testis	
Male	0	7.24 ± 0.60	1.55 ± 0.05	0.422 ± 0.038	1.62 ± 0.07	
	0.015	7.21 ± 0.36	1.85 ± 0.18	0.442 ± 0.015	1.59 ± 0.08	
	0.01	6.47 ± 0.69	1.72 ± 0.10 ^{b/}	0.451 ± 0.040	1.49 ± 0.06 ^{b/}	
	0.07	9.88 ± 1.04	2.53 ± 0.17 ^{b/}	0.356 ± 0.033	0.94 ± 0.16 ^{b/}	
Female	0	4.81 ± 0.24	1.16 ± 0.02	0.310 ± 0.018		0.076 ± 0.011
	0.0015	4.87 ± 0.28	1.21 ± 0.07	0.358 ± 0.028		0.093 ± 0.012
	0.01	4.68 ± 0.38 ^{b/}	1.11 ± 0.04 ^{b/}	0.322 ± 0.016 ^{b/}		0.091 ± 0.007
	0.07	6.29 ± 0.54 ^{b/}	1.43 ± 0.03 ^{b/}	0.165 ± 0.016 ^{b/}		0.087 ± 0.005

^{a/} Mean ± standard error of four rats.^{b/} Significantly different from control by Dunnett's multiple comparison procedure.

TABLE 53

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF RATS FED 2,4-DNT FOR 12 MONTHS AND ALLOWED TO RECOVER FOR 1 MONTH

Sex	Dose (% in feed)	Terminal	Absolute Organ Weight (g)					
		Body Weight (g)	Brain	Liver	Kidney	Spleen	Testis	Ovary
Male	0	678 ± 36 ^{a/}	2.02 ± 0.08	16.1 ± 0.3	3.7 ± 0.1	1.11 ± 0.10	3.7 ± 0.4	
	0.0015	650 ± 46 ^{b/}	2.11 ± 0.08	17.5 ± 1.3	4.1 ± 0.5	1.13 ± 0.10	3.8 ± 0.3	
	0.01	675 ± 47 ^{b/}	2.31 ± 0.06	16.6 ± 0.8	3.8 ± 0.2	0.84 ± 0.04	3.5 ± 0.1	
	0.07	471 ± 62 ^{a/}	2.11 ± 0.07	26.1 ± 0.7 ^{c/}	4.2 ± 0.0	0.73 ± 0.10 ^{a/}	2.1 ± 0.7	
Female	0	467 ± 37 ^{b/}	1.96 ± 0.06	11.1 ± 1.3	2.4 ± 0.1	0.66 ± 0.07		0.229 ± 0.017
	0.0015	446 ± 33 ^{b/}	1.95 ± 0.06	11.3 ± 0.7	2.9 ± 0.2 ^{a/}	0.77 ± 0.02		0.224 ± 0.062
	0.01	427 ± 21 ^{b/}	1.88 ± 0.05	11.8 ± 0.7	2.3 ± 0.1	0.69 ± 0.07		0.190 ± 0.015
	0.07	266 ± 5 ^{a/}	1.85 ± 0.09	11.3 ± 0.6	2.3 ± 0.1	0.31 ± 0.02 ^{a/}		0.153 ± 0.017
Sex	Dose (% in feed)	Relative Organ Weight (g/100 g body weight)						
		Brain	Liver	Kidney	Spleen	Testis	Ovary	
Male	0	0.30 ± 0.00	2.39 ± 0.16	0.55 ± 0.05	0.164 ± 0.017	0.54 ± 0.03		
	0.0015	0.33 ± 0.02	2.76 ± 0.36	0.64 ± 0.10	0.178 ± 0.026	0.60 ± 0.06		
	0.01	0.35 ± 0.02	2.49 ± 0.18 ^{a/}	0.56 ± 0.03	0.125 ± 0.008	0.52 ± 0.02		
	0.07	0.46 ± 0.04 ^{a/}	5.75 ± 0.80 ^{a/}	0.97 ± 0.11 ^{a/}	0.156 ± 0.006	0.43 ± 0.09		
Female	0	0.43 ± 0.03	2.37 ± 0.10	0.52 ± 0.05	0.142 ± 0.010		0.049 ± 0.003	
	0.0015	0.44 ± 0.03	2.55 ± 0.06	0.65 ± 0.06	0.174 ± 0.008		0.052 ± 0.016	
	0.01	0.44 ± 0.03	2.76 ± 0.09 ^{a/}	0.55 ± 0.03 ^{a/}	0.163 ± 0.019		0.045 ± 0.003	
	0.07	0.70 ± 0.03 ^{a/}	4.28 ± 0.27 ^{a/}	0.86 ± 0.03 ^{a/}	0.119 ± 0.009		0.057 ± 0.006	
Sex	Dose (% in feed)	Relative Organ Weight (g/g brain weight)						
		Liver	Kidney	Spleen	Testis	Ovary		
Male	0	7.98 ± 0.44	1.83 ± 0.13	0.549 ± 0.051	1.81 ± 0.13			
	0.0015	8.38 ± 0.94	1.96 ± 0.31	0.541 ± 0.069	1.80 ± 0.08			
	0.01	7.21 ± 0.37	1.64 ± 0.08	0.364 ± 0.019	1.51 ± 0.01			
	0.07	12.43 ± 0.66 ^{a/}	2.12 ± 0.07	0.347 ± 0.038	0.99 ± 0.28 ^{a/}			
Female	0	5.69 ± 0.66	1.21 ± 0.05	0.339 ± 0.033		0.117 ± 0.008		
	0.0015	5.83 ± 0.35	1.47 ± 0.10 ^{a/}	0.395 ± 0.013		0.113 ± 0.028		
	0.01	6.30 ± 0.54	1.24 ± 0.02	0.367 ± 0.036 ^{a/}		0.102 ± 0.010		
	0.07	6.18 ± 0.50	1.24 ± 0.06	0.171 ± 0.014 ^{a/}		0.084 ± 0.012		

a/ Mean ± standard error of three rats.

b/ Mean ± standard error of four rats.

c/ Significantly different from control by Dunnett's multiple comparison procedure.

TABLE 54

SUMMARY OF LESIONS IN MALE RATS FED 2,4-DNT FOR 12 MONTHS

Dose (% in feed):	0				0.0015				0.01				0.07			
Rat No.:	301	302	303	304	309	310	311	312	317	318	319	320	325	326	327	328
<u>Treatment-Related Lesions</u>																
Pituitary																
Chromophobe adenoma										X						
Liver																
Foci or areas of hepato-																
cellular alteration			±	±	1	1	1	1	1	1	1		4	4		
Hepatocellular neoplastic																
nodules													X	X	X	X
Spleen																
Excessive pigmentation													1	1	1	1
Testis																
Atrophy of seminiferous																
tubules													4	4	4	4
Epididymis																
Depletion of spermatozoa													2	2		
in ductulus																
Kidney																
Benign nephropathy									4			1	4	3	4	
<u>Other Lesions</u>																
Adrenal Gland																
Cystic degeneration						1					1		1	1		
Fatty change										1						
Pituitary																
Cyst formation						2									1	
Trachea																
Tracheitis		2		1		1	2	2			2	2				
Lung																
Chronic murine																
pneumonia		1	1	1	1	1	2	1	1	2	1	2		1		
Heart																
Focal myocarditis or																
fibrosis		1	1				1		1	1			1	1		1
Liver																
Bile duct hyperplasia		1	1	1		2	1	1	2		1					
Portal inflammation or																
granuloma		1							1	1	1	1				
Extramedullary hemato-																
poiesis														1		
Focal necrosis				1												
Spleen																
Extramedullary hemato-																
poiesis														1		

TABLE 54 (concluded)

Dose (% in feed):	0				0.0015				0.01				0.07			
Rat No.:	301	302	303	304	309	310	311	312	317	318	319	320	325	326	327	328
<u>Other Lesions (concluded)</u>																
Testis																
Seminoma				X												
Degeneration											1	1				
Epididymis																
Interstitial inflammation													1			
Spermatic granuloma																1
Prostate																
Prostatitis															4	
Seminal Vesicle																
Vasculitis									1				2			
Interstitial edema															1	1
Pancreas																
Focal acinar atrophy						1										
Kidney																
Minor lymphocytic infiltration		1		1					1							
Hydronephrosis																1
Bone Marrow																
N/E ratio	0	0	2.2	0	2.0	1.6	1.0	7.2	1.9	1.8	1.6	2.3	2.0	1.7	2.1	2.7

Tissues not listed were normal.

g/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; ± = questionable; X = present;
0 = tissue missing or unreadable.

TABLE 55

SUMMARY OF LESIONS IN FEMALE RATS FED 2,4-DNT FOR 12 MONTHS

Dose (% in feed):	0				0.0015				0.01				0.07			
Rat No.:	351	352	353	354	359	360	361	362	367	368	369	370	375	376	377	378
<u>Treatment-Related Lesions^{a/}</u>																
Pituitary																
Chromophobe adenoma										X	X					
Liver																
Foci or areas of hepatocellular alteration			1										4		3	3
Hepatocellular neoplastic nodules													X	X	X	X
Spleen																
Excessive pigmentation													1	1		1
Mammary Gland																
Adenoma															4	
Kidney																
Senile nephropathy		1					2						4	3	4	
<u>Other Lesions</u>																
Adrenal Gland																
Cystic degeneration		1	1	1	1				2	1	1	1		1		
Lung																
Chronic murine pneumonia			1	2	1		1	1	1	1	1	1	1			
Heart																
Focal myocarditis or fibrosis			1			1							1	1	1	
Liver																
Bile duct hyperplasia							1							2		
Portal inflammation or granuloma			1				1	1	1	1	1			1		
Fatty change			1	1		1										
Extramammary hemato-																
-oma													1			
Spleen																
Extramammary hemato-																
-oma																2
Ovary																
Ovarian cyst																1
Kidney																
Minor lymphocytic infiltration									1	1		1				
-oma			2								1					
Bone Marrow																
M/E Ratio	0	0	1.1	2.0	1.2	2.3	2.1	2.1	1.2	1.8	1.2	0.8	1.2	1.6	1.8	

Tissues not listed were normal.

^{a/} Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; ± = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 56

SUMMARY OF LESIONS IN MALE RATS FED 2,4-DNT FOR 12 MONTHS AND ALLOWED TO RECOVER FOR 1 MONTH

Dose (% in feed):	0				0.0015				0.01				0.07		
Rat No.:	305	306	308	313	314	315	316	321	322	323	324	329	330	332	
<u>Treatment-Related Lesions^{a/}</u>															
Liver															
Foci or areas of hepatocellular alteration				1		1	1	1		1	2	4	4	1	
Hepatocellular neoplastic nodules					1							X	X		
Spleen															
Excessive pigmentation													1	1	
Testis															
Atrophy of seminiferous tubules												4	4		
Epididymus															
Depletion of spermatozoa in ductules												4	4		
Kidney															
Senile nephropathy		1	1	1	2	1	3			1	1	4	3		
<u>Other Lesions</u>															
Adrenal Gland															
Cystic degeneration					1										
Fatty change								1						1	
Pituitary															
Cyst formation													1		
Trachea															
Tracheitis			2			3		1	1						
Lung															
Chronic murine pneumonia		2	2	1	1	3	1	3	2	1	1	1		1	
Heart															
Focal myocarditis or fibrosis			1		1			1	1			1	1		
Liver															
Bile duct hyperplasia		1		1				1			1			1	
Portal inflammation or granuloma			1				1							1	
Fatty change						1									
Extra medullary hematopoiesis					1										
Focal necrosis		1								1					

TABLE 56 (concluded)

Dose (% in feed):	0				0.0015				0.01				0.07		
Rat No.:	305	306	308		313	314	315	316	321	322	323	324	329	330	332
<u>Other Lesions (concluded)</u>															
Spleen															
-- Extramedullary hematopoiesis													1	1	1
Testis															
-- Calcified tubules													1		
Epididymis															
-- Vascular cuffs							1								
-- Vacuolization of epithelium															1
Prostate															
-- Mononuclear cells foci										1					
Seminal Vesicle															
-- Vesiculitis													4		
Pancreas															
-- Focal fibrosis of islet						1								1	
Intestine															
-- Enteritis															1
Kidney															
-- Minor focal lymphocytic infiltration		1			1					1		1			
-- Microcalculi			2												
Eye															
-- Corneal epithelial degeneration														1	
Bone Marrow															
-- M/E ratio	1.0	0.9	1.2		1.9	2.0	0.9	1.2	1.3	2.0		1.5	1.3	1.5	1.7

Tissues not listed were normal.

g/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked, 4 = severe; ± = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 57

SUMMARY OF LESIONS OF FEMALE RATS FED 2,4-DNT FOR 12 MONTHS AND ALLOWED TO RECOVER FOR 1 MONTH

Dose (% in feed):	0				0.0015				0.01				0.07			
Rat No.:	355	356	357	358	363	364	365	366	371	372	373	374	379	380	381	382
<u>Treatment-Related Lesions^{a/}</u>																
Liver																
Foci or areas of hepatocellular alteration								1	1				4	2	1	
Hepatocellular neoplastic nodules														X	X	X
Hepatocellular carcinoma																X
Spleen																
Excessive pigmentation						1		1					1	1	1	1
Mammary Gland																
Fibroadenoma											X					
Kidney																
Senile nephropathy				1		2	1	1	1							
<u>Other Lesions</u>																
Adrenal Gland																
Cystic degeneration		2	2		2		1		2	2	1	2	2	2	2	2
Pituitary																
Mononuclear cells foci							1									
Trachea																
Tracheitis			1								1					
Lung																
Chronic murine pneumonia		1	1	2	1	2		1	1	1	1	1	1	1	1	1
Heart																
Focal myocarditis or fibrosis			1		1				1		1	1				
Liver																
Bile duct hyperplasia		1	1		1			1					1	1		
Portal inflammation or granuloma			1				1	1		1	1					
Fatty change				2	1		1		1		2					
Megakaryohepatocytes						1										
Ovary																
Ovarian cyst													1			
Uterus																
Endometritis															1	
Pancreas																
Focal fibrosis of islet											1					
Focal acinus atrophy					1		1				1					
Interstitial mononuclear cell infiltration					1		1				1					
Intestine																
Enteritis									1							
Kidney																
Minor focal lymphocytic infiltration			1									1	1		1	
Microcalculi						1	1					2				
Pyelitis								1								
Urinary Bladder																
Hydropic degeneration of epithelial															1	
Bone Marrow																
M/E ratio	2.0	0.9	1.5	1.8	1.7	1.2	1.2	1.8	0.9	1.9	1.5	1.7	1.8	2.2	2.7	1.7

Tissues not listed were normal.

^{a/} Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; ± = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 58

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF RATS FED 2,4-DNT FOR 24 MO. WS

Sex	Dose (% in feed)	Terminal Body Weight (g)	Absolute Organ Weight (g)					
			Brain	Heart	Liver	Kidney	Spleen	Testis
Male	0	725 ± 20 ^{a/}	2.03 ± 0.18	1.85 ± 0.18	14.9 ± 2.3	6.1 ± 1.7	1.63 ± 0.61	3.6 ± 0.5
	0.0015	719 ± 29 ^{b/}	2.28 ± 0.05	1.90 ± 0.08	15.6 ± 1.1	4.2 ± 0.2	0.99 ± 0.10	3.7 ± 0.4
	0.01	603 ± 40 ^{c,d/}	2.70 ± 0.06	1.84 ± 0.08	18.3 ± 0.9	5.0 ± 0.1	1.20 ± 0.08	3.5 ± 0.2
	0.007	339 ^{f/}	1.91 ± 0.08	1.22 ± 0.17	13.0 ± 1.0	2.7 ± 0.2	0.48 ± 0.06	0.303 ± 0.110
Female	0	478 ± 25 ^{e/}	1.97 ± 0.03	1.40 ± 0.07	11.4 ± 0.6	2.8 ± 0.2	0.67 ± 0.05	0.190 ± 0.022
	0.0015	457 ± 49 ^{e/}	2.00 ± 0.06	1.38 ± 0.07	17.8 ± 4.1	3.0 ± 0.2	0.71 ± 0.10	0.166 ± 0.057
	0.01	339 ^{f/}	1.81	1.13	9.6	2.4	0.47	0.031
	0.007							
Sex	Dose (% in feed)		Relative Organ Weight (g/100 g. body weight)					
			Brain	Heart	Liver	Kidney	Spleen	Testis
Male	0	0.29 ± 0.02	0.22 ± 0.04	2.06 ± 0.36	0.56 ± 0.09	0.127 ± 0.019	0.49 ± 0.08	
	0.0015	0.32 ± 0.01	0.27 ± 0.01	2.21 ± 0.20	0.59 ± 0.04	0.141 ± 0.015	0.53 ± 0.07	
	0.01	0.37 ± 0.02 ^{a/}	0.31 ± 0.02	3.07 ± 0.16	0.84 ± 0.04 ^{a/}	0.201 ± 0.012 ^{a/}	0.59 ± 0.04	
	0.007							
Female	0	0.42 ± 0.02	0.26 ± 0.03	2.80 ± 0.12	0.58 ± 0.04	0.105 ± 0.016	0.070 ± 0.029	
	0.0015	0.42 ± 0.02	0.30 ± 0.01	2.41 ± 0.08	0.59 ± 0.04	0.143 ± 0.013	0.040 ± 0.004	
	0.01	0.46 ± 0.04	0.31 ± 0.03	3.72 ± 0.45 ^{a/}	0.69 ± 0.06	0.155 ± 0.012	0.032 ± 0.013	
	0.007	0.53 ±	0.33	2.84	0.71	0.130	0.009	
Sex	Dose (% in feed)		Relative Organ Weight (g/g. brain weight)					
			Heart	Liver	Kidney	Spleen	Testis	Ovary
Male	0	0.86 ± 0.04	8.43 ± 0.72	2.39 ± 0.38	0.532 ± 0.065	2.08 ± 0.30		
	0.0015	0.84 ± 0.04	6.89 ± 0.48	1.86 ± 0.10	0.437 ± 0.042	1.63 ± 0.17		
	0.01	0.83 ± 0.03	6.31 ± 0.25	2.28 ± 0.02	0.544 ± 0.026	1.58 ± 0.06		
	0.007							
Female	0	0.62 ± 0.07	6.85 ± 0.59	1.38 ± 0.07	0.256 ± 0.040		0.174 ± 0.077	
	0.0015	0.71 ± 0.03	5.80 ± 0.26	1.42 ± 0.08	0.339 ± 0.025		0.096 ± 0.011	
	0.01	0.69 ± 0.02	8.90 ± 2.14	1.52 ± 0.07	0.353 ± 0.044		0.070 ± 0.026	
	0.07	0.62	5.31	1.34	0.260		0.017	

a/ Mean ± standard error of eight rats, except brain weight on only seven.

b/ Mean ± standard error of nine rats.

c/ Mean ± standard error of five rats.

d/ Mean ± standard error of six rats.

e/ Mean ± standard error of 10 rats.

f/ One surviving rat.

g/ Significantly different from control by Dunnett's multiple comparison procedure.

TABLE 59

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF RATS FED 2,4-DNT FOR 24 MONTHS AND ALLOWED TO RECOVER FOR 1 MONTH

Sex	Dose (% in feed)	Terminal Body Weight (g)	Absolute Organ Weight (g)					Testis	Ovary
			Brain	Heart	Liver	Kidney	Spleen		
Male	0	596 ± 58 ^{a/}	2.29 ± 0.05	2.00 ± 0.08	15.4 ± 1.2	6.2 ± 1.4	0.95 ± 0.05	3.4 ± 0.8	
	0.0015	715 ± 62 ^{b/}	2.27 ± 0.06	2.00 ± 0.05	18.3 ± 1.1	7.8 ± 3.6	1.72 ± 0.75	2.8 ± 0.2	
	0.01	512 ^{c/}	2.27	2.05	43.1	6.7	0.82	1.7	
Female	0	496 ± 43 ^{a/}	1.97 ± 0.02	1.51 ± 0.05	13.7 ± 2.2	3.1 ± 0.2	0.62 ± 0.08		0.215 ± 0.014
	0.0015	414 ± 47 ^{d/}	1.98 ± 0.10	1.29 ± 0.01	10.8 ± 0.3	2.6 ± 0.1	0.56 ± 0.13		0.177 ± 0.032
	0.01	421 ± 53 ^{b/}	1.91 ± 0.05	1.54 ± 0.12	12.6 ± 2.4	2.7 ± 0.1	0.49 ± 0.04		0.218 ± 0.012

Sex	Dose (% in feed)	Relative Organ Weight (g/100 g body weight)					Testis	Ovary
		Brain	Heart	Liver	Kidney	Spleen		
Male	0	0.40 ± 0.05	0.35 ± 0.04	2.70 ± 0.44	1.127 ± 0.33	0.165 ± 0.018	0.54 ± 0.12	
	0.0015	0.32 ± 0.02	0.28 ± 0.03	2.61 ± 0.35	1.20 ± 0.66	0.234 ± 0.087	0.41 ± 0.07	
	0.01	0.46	0.40	8.42	1.31	0.160	0.34	
Female	0	0.41 ± 0.04	0.31 ± 0.03	2.72 ± 0.26	0.64 ± 0.06	0.124 ± 0.012		0.044 ± 0.002
	0.0015	0.32 ± 0.03	0.32 ± 0.04	2.63 ± 0.22	0.65 ± 0.09	0.134 ± 0.016		0.047 ± 0.003
	0.01	0.46 ± 0.04	0.37 ± 0.03	2.95 ± 0.19	0.65 ± 0.08	0.119 ± 0.006		0.053 ± 0.004

Sex	Dose (% in feed)	Relative Organ Weight (g/g brain weight)					Testis	Ovary
		Heart	Liver	Kidney	Spleen			
Male	0	0.87 ± 0.02	6.69 ± 0.54	2.68 ± 0.56	0.415 ± 0.016	1.45 ± 0.35		
	0.0015	0.88 ± 0.04	8.09 ± 0.69	3.51 ± 1.72	0.756 ± 0.324	1.25 ± 0.14		
	0.01	0.86	18.19	2.84	0.346	0.73		
Female	0	0.77 ± 0.02	6.97 ± 1.12	1.59 ± 0.08	0.314 ± 0.044		0.109 ± 0.007	
	0.0015	0.66 ± 0.04	5.44 ± 0.11	0.34 ± 0.11	0.280 ± 0.052		0.089 ± 0.012	
	0.01	0.81 ± 0.06	6.57 ± 1.12	1.40 ± 0.07	0.238 ± 0.013		0.114 ± 0.003	

a/ Mean ± standard error of four rats.

b/ Mean ± standard error of three rats.

c/ One surviving rat.

d/ Mean ± standard error of two rats.

e/ Significantly different from control by Dunnett's multiple comparison procedure.

TABLE 60

SUMMARY OF LESIONS IN MALE RATS FED 2,4-DNT FOR 24 MONTHS

Dose (Z in feed): Rat No.:	0										0.0015										0.01									
	014	015	016	017	019	026	029	030	109	114	117	118	120	122	124	125	129	148	151	154	158	160								
<u>Treatment-Related Lesions^{a/}</u>																														
Pituitary				X			X	X			X	X	X							X	X									
Chromophobe adenoma																														
Liver																														
Foci or areas of hepatocellular alteration			1	3	2		1				1	2		1		1	2		1	1	1	1								
Hepatocellular neoplastic nodules				X			+																							
Testis																														
Atrophy of seminiferous tubules				4			2	2	2	1		4						1												
Skin																														
Lipoma										X																				
<u>Other Lesions</u>																														
Adrenal Gland																														
Cystic degeneration			1			1				1					1				1		1									
Neurochromocytoma																		X												
Cortical atrophy								3																						
Thyroid																														
Follicular epithelium adenoma																														
Hypertrophy of parathyroid																						1								
Trachea																														
Tracheitis			1	1	3		4		1				3			3	3			3										
Lung																														
Chronic murine pneumonia		1	1	1		1	1		1	2	2		1	1	1	1	1	3	1	1	1	2								
Abscess																	2													
Heart																														
Myocardial fibrosis/degeneration (focal)			1	1	1		1			1	1	1	1	1	1	1		1	1	1	1	1								
Dilated ventricle																														
Arteriosclerosis																														
Liver																														
Bile duct hyperplasia		1		1	1	1	2	1	1	2	1		1	1	1	1	1	1	1	1	2	1								
Partial inflammation		1		1	1	1			1	1	1		1	1	1	1	1			1										
Focal necrosis																														
Fatty change																														
Cystic degeneration		1					1												1		1	1								

TABLE 60 (concluded)

Dose (% in feed): Bat No.:	0										0.0015										0.01				
	014	015	016	017	019	026	029	030	109	114	117	118	120	122	124	125	129	148	151	154	158	160			
<u>Other Lesions (concluded)</u>																									
Spleen																									
Extramedullary hematopoiesis																									
Excessive hemosiderin																									
Lymphoid depletion																									
Testis																									
Interstitial cell tumor																									
Periarteritis nodosa																									
Epididymis																									
Epithelial vacuolization																									
Too few spermatozoa																									
Foci of mononuclear cells																									
Prostate																									
Prostatitis																									
Atrophy																									
Pancreas																									
Acinar atrophy (focal)																									
Islet cell tumor																									
Lymph Node																									
Lymphoid hyperplasia																									
Salivary Gland																									
Fibrosis (interstitial)																									
Stomach																									
Gastritis																									
Dilated crypts																									
Calcification of mucosa																									
Intestine																									
Mucosal calcification																									
Enteritis																									
Kidney																									
Senile chronic nephropathy																									
Calculi																									
Hydronephrosis																									
Foci of mononuclear cells																									
Urinary Bladder																									
Foci of mononuclear cells																									
Rib																									
Hypocellularity of bone marrow																									
Eye																									
Keratitis																									
Retinal atrophy																									

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; + = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 61

SUMMARY OF LESIONS IN FEMALE RATS FED 2.4-DNT FOR 24 MONTHS

Dose (% in feed): Rat No.:	0								0.0015								0.01								0.07			
	066	068	070	074	076	078	080	214	216	218	221	222	224	225	226	228	230	244	251	254	256	258	260	284				
<u>Treatment-Related Lesions^{a/}</u>																												
Pituitary																												
Chromophobe adenoma	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0	X	X	X	X	X	X	X	X					
Liver																												
Foci or areas of hepatocellular alteration								1	2	1	1			1	1	1	1	1		3	1	1	1					
Hepatocellular neoplastic nodules										X										X								
Hepatocellular carcinoma																												
Mammary Gland																												
Adenoma										X														X				
Fibroadenoma										X	X					X		X				X	X					
Adenocarcinoma-carcinoma												X			X							X	X					
Fibroma																							X					
<u>Other Lesions</u>																												
Adrenal Gland																												
Cystic degeneration	3	4	1	2	2	3	4	2	3	3	1	3	2	2	1	1	3	2	2	3	2	1	1					
Phaeochromocytoma												X					X											
Cortical hyperplasia																												
Necrosis																												
Cortical atrophy																												
Thyroid																												
C cell tumor (adenoma)																												
Follicular epithelium adenoma																												
Squamous metaplastic follicle																												
Trachea																												
Tracheitis											2																	
Lung																												
Chronic murine pneumonia																												
Granuloma																												
Heart																												
Myocardial fibrosis/degeneration (focal)																												

TABLE 61 (concluded)

Dose (% in feed): Rat No.:	0										0.0015										0.01										0.07																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
	066	068	070	074	076	078	080	214	216	218	221	222	224	225	226	228	230	244	251	254	256	258	260	284																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																								
Other Lesions (concluded)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
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Tissues not listed were normal.

a/ Severity of lesions: 1 - mild; 2 = moderate; 3 = marked; 4 = severe; ± = questionable; X = present; 0 = tissue missing or unobtainable.

TABLE 62

SUMMARY OF LESIONS OF RATS FED 2,4-DNT FOR 24 MONTHS
AND ALLOWED TO RECOVER FOR 1 MONTH

Dose (% in feed):	0								0.0015				0.01					
Sex:	Male				Female				Male		Female		Male	Female				
Lot No.:	002	006	008	012	054	057	058	062	104	105	106	207	210	138	234	236	237	242
<u>Treatment-Related Lesions^{a/}</u>																		
Pituitary	0			0	0									0				
Chromophobe Adenoma		X					X	X	X	X		X	X			X	X	±
Liver																		
Foci or areas of hepatocellular alteration	1		1		1	1	1	1	1		1				1	1		3
Hepatocellular neoplastic nodules													X					
Hepatocellular carcinoma														X				
Testis																		
Atrophy of seminiferous tubules		3							4		4			3				
Skin																		
Subcutaneous mesenchymal tumor														X				
Mammary Gland																		
Adenoma												X			X	X		X
Fibroadenoma					X	X		X				X			X	X	X	X
Adenocarcinoma-carcinoma																X		
Fibroma													X					
<u>Other Lesions</u>																		
Adrenal Gland																		
Cystic degeneration	1		1	1	1	2	3	3			3	2	2		1	2	3	1
Cortical tumor				X														
Pheochromocytoma	X		X								X	X						X
Cortical hyperplasia							X							X		X		
Thyroid																		
C cell tumor (adenoma)										X								
Parathyroid hyperplasia		3																
Squamous metaplasia follicle				1		1												
Trachea																		
Tracheitis	1				1					X								1
Lung																		
Chronic murine pneumonia	1	1	1	1	1	1	1	1	1		1	2	1	1	1	1	1	1
Calcification of alveolar wall		2																
Heart																		
Myocardial fibrosis/degeneration	1	1			1	1	1	1				1	1		1			
Calcification of vascular wall		1																
Dilated ventricle			1															
Liver																		
Bile duct hyperplasia	1	1	1	1	1		1		1		1	1			1			1
Portal inflammation	1	1	1	1	1	1	1	1			2	1	1	1				
Fatty change		1			1	1		1						1				
Cystic degeneration	1																	

TABLE 62 (concluded)

Dose (% in feed):

Sex:

Rat No.:

Doses (% in feed):	0								0.0015				0.01					
Sex:	Male				Female				Male		Female		Male		Female			
Rat No.:	002	004	008	012	034	037	038	062	104	105	106	207	210	138	234	236	237	242
Other Lesions (concluded)																		
Spleen																		
Extramedullary hematopoiesis	1	2		1		1		1	1	2				1	3	1		
Excessive hemosiderin												1					1	
Testis																		
Interstitial cell tumor				X	X													
Periarteritis nodosa			3							3								
Epididymis																		
Atrophy of ductules		1																
Too few spermatozoa in																		
ductules					2									1				
Prostate																		
Atrophy	1	1	1							2				1				
Seminal Vesicle				0														
Ovary																		
Ovarian cyst					X		X	X				X			X	X		
Uterus																		
Endometritis												1			1	2		
Pancreas																		
Acinar atrophy (focal)														1				
Acinar cell tumor											X							
Islet cell tumor										X								
Islet cell hyperplasia						X										X		
Periarteritis nodosa				1														
Focal necrosis									1									
Lymph node																		
Lymphoid hyperplasia			1						1	2								
Malignant lymphoma												X						
Salivary Gland																		
Salivadenitis									4									
Stomach																		
Dilated crypts														1				
Calcification of mucosa		3										1		1				
Periarteritis nodosa (quantum)				1														
Intestine																		
Calcification of vascular wall																		
(mesentery)		2																
Kidney																		
Chronic senile nephropathy	2	4	4	2					1	4	1			4				1
Calculi					1							1	1			1	1	1
Pyelitis	1				1	1	1											
Hydronephrosis										3								
Yeast of mononuclear cells						1	1					1						
Urinary Bladder																		
Distended lumen										1								
Skin																		
Cellulitis													1					
Eye																		
Keratitis or corneal calci-																		
fication				1		1						1	1					
Retinal atrophy	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Tissues not listed were normal.

g/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; ± = questionable; X = present; D = tissue missing or unreadable.

TABLE 63

SUMMARY OF LESIONS IN CONTROL RATS BEING AT UNSCHEDULED TIMES

Sex:	Male										Female									
	013	011	020	018	005	021	003	024	023	010	027	028	067	071	049	073	077	052	065	072
Week of Death:	70	80	85	86	91	91	92	94	96	97	100	103	57	76	85	87	89	90	91	97
Treatment-related lesions ^{a/}																				
Pituitary			0																	
Adipose																				
Liver																				
Foci or areas of hepatocellular alteration																				
Hepatocellular carcinoma																				
Skin																				
Squamous cell carcinoma																				
Basal cell tumor																				
Carcinoma																				
Thyroid Gland																				
Adenoma																				
Fluoradenoma																				
Adenocarcinoma																				
Other Lesions																				
Adrenal Glands																				
Cystic degeneration																				
Neuritis																				
Thyroid																				
C cell adenoma																				
Squamous metaplasia follicle																				
Trachea																				
Tracheitis																				
Lung																				
Chronic murine pneumonia																				
Malignant lymphoma																				
Heart																				
Myocardial degeneration/fibrosis																				
Bilateral ventricle																				
Bladder																				
Bile duct hyperplasia																				
Portal inflammation																				
Fatty change																				
Tuberculosis																				
Cystic degeneration																				
Malignant lymphoma																				
Spleen																				
Extramedullary hematopoiesis																				
Excessive humeralitis																				
Lymphoid depletion																				
Malignant lymphoma																				
Testis																				
Interstitial tumor																				
Peritonitis nodosa																				
Degeneration of seminiferous tubules																				
Ovaries																				
Too few spermatocytes in ducts																				
Foci of mononuclear cells																				
Prostate																				
Atrophy																				

TABLE 63 (continued)

Sex:	Male														Female															
	013	011	020	018	005	021	003	024	022	023	010	027	028	067	071	059	073	077	052	065	072	069	090	091	097	069	079	056	053	
Week of Death:	70	80	85	86	91	91	92	94	96	97	102	103	103	57	76	85	87	89	90	91	97	98	100	100	102					
Other Lesions (concluded)																														
Seminal Vesicle																														
Abscessation																														
Ovary																														
Ovarian Cyst																														
Uterus																														
Endometrial hyperplasia																														
Pancreas																														
Acinar atrophy (focal)																														
Lymph Node																														
Lymphoid hyperplasia																														
Malignant lymphoma																														
Stomach																														
Dilated crypts																														
Intestine																														
Enteritis																														
Kidney																														
Senile chronic nephropathy																														
Microcalculi in pelvis																														
Pyelitis																														
Hydronephrosis																														
Foci of mononuclear cells																														
Malignant lymphoma																														
Urinary Bladder																														
Foci of mononuclear cells																														
in submucosa																														
Skin																														
Abscessation																														
Rib																														
Hypocellularity of bone marrow																														
Eye																														
Keratitis																														
Retinal dystrophy																														
Uveitis																														

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; + = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 64

SUMMARY OF LESIONS IN MALE RATS FED 0.0015% OF 2,4 DDT AND DYING AT UNSPECIFIED TIMES

Rat No.:	123	101	130	106	110	111	115	119	116	127	112	121	102	103	107
Week of Death:	53	57	71	78	81	84	85	92	97	90	99	99	102	102	104
<u>Treatment-related lesions:</u>															
Pituitary		0	0	0	0										0
- Chromophobe adenoma						X	X	X	X	X		X	X	X	
Liver															
Foci or areas of hepatocellular alterations															
- Hepatocellular carcinoma											2			2	2
- Testis											X			X	
- Atrophy of seminiferous tubules															
Skin				4				1					4		
- Subcutaneous mesenchymal tumor															
Other Lesions				X									X	X	
Adrenal Gland															
Cystic degeneration		1	1	0	0										
- Pheochromocytoma												X			0
Thyroid				0	0										
Squamous metaplastic follicle								1							
- Follicular hyperplasia															3
Trachea															
- Tracheitis		2	3				4	3							
Lung															
Chronic murine pneumonia															2
Lympho-epithelioid disease															
- Abscess		1	1	1		1	1	1	1	1	1	1	1	1	1
- Calcification of alveolar wall															1
Heart															2
- Myocardial degeneration/fibrosis															
- Arteriosclerosis				1	2	1			1	1	1		1		
Liver															
Bile duct hyperplasia															2
Portal inflammation															
Focal necrosis				2					2	1	2	1	1	1	1
Fatty changes															
- Telangiectasis															
Cystic degeneration					1				1	2	3	1	1		
- Lympho-epithelioid disease															1

TABLE 64 (continued)

Rat No.:	123	101	130	108	110	111	115	418	119	116	127	112	121	102	103	107
Week of Death:	53	57	71	79	81	84	85	92	92	97	90	99	99	102	102	104
Other Lesions (continued)																
Spleen			i					1					1	1	2	
Extramedullary hematopoiesis								3	1	1						
Excessive hemosiderin							1		1							
Lymphoid depletion																
Lymphomyploproliferative disease	x	x														
Testis																
Periarteritis nodosa	0				0									2		3
Epididymis														1		
Atrophy							1									
Foci of mononuclear cells						1										
Prostate	0				0	0	0	0								
Atrophy														1		
Seminal Vesicle	0		0	0	0	0	0	0								
Atrophy						2										
Seminal vesiculitis		3														
Pancreas	0			0	0											
Acinar atrophy (focal)								1								
Islet cell tumor												x				
Periarteritis nodosa				0	0									1		
Lymph Node	0															
Lymphoid hyperplasia	0													1		
Stomach					1											
Calcification of mucosa					0											3
Kidney			1	1												
Senile chronic nephropathy																
Microcalculi in pelvis			1											2	4	4
Pyelitis			1	1												
Hydrocephrosis							2									
Myeloproliferative disease	x															
Skin																
Abscession				x												
Brain				0	0											
Myelomalacia			1													
Astrocystoma																
Rib	0			0	0											
Osteodystrophic fibrosis																1

TABLE 64 (concluded)

Rat No.:	123	101	130	108	110	111	115	418	119	116	127	112	121	102	103	107
Week of Death:	53	57	71	79	81	84	85	92	92	97	90	99	99	102	102	104
<u>Other Lesions (concluded)</u>																
Eye																
Keratitis									1	1	1		3			1
Retinal atrophy																
Abdominal Cavity					1											
Lipomas									X							
Peritonitis												3				

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; + = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 65

SUMMARY OF LESIONS IN FEMALE RATS FED 0.0015% 2,4-DNT AND DYING AT UNSCHEDULED TIMES

Rat No.:	202	473	227	219	208	472	205	215	469	201	474	468	470	471	229	209	213	217	204	223	220	211 ^{b/}
Week of Death:	55	57	57	71	74	80	82	84	84	85	87	91	91	92	93	96	98	100	102	102	103	107
<u>Treatment Related Lesions^{a/}</u>																						
Pituitary	0	0					0	0						0		X	X	X	X	X	X	X
Chromophobe adenoma																						
Liver																						
Foci or areas of hepatocellular alteration			1			1	1	1				1	2				1	1	3	1		2
Hepatocellular neoplastic nodules																						
Skin																						
Subcutaneous mesenchymal tumor						X	X												X			
Mammary Gland																						
Fibroadenoma																X				X		
Adenocarcinoma-carcinoma																				X		
<u>Other Lesions</u>																						
Adrenal	0		2	2	1		0	0		3	3	3	2	1		3	3	2	1	4	3	2
Cystic degeneration																						
Cortical tumor																						
Thyroid	0																					
C cell adenoma																						4
Follicular epithelium adenoma									X													
Parathyroid hyperplasia																						
Trachea																						
Tracheitis						1										1				1		
Lung																						
Chronic murine pneumonia	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Heart																						
Myocardial degeneration/fibrosis							1	1														
Liver																						
Bile duct hyperplasia			2			1	1			2	1		1	1	1	1	1	1	1		1	1
Portal inflammation						1																
Focal necrosis																						
Fatty changes																						
Spleen	0																					
Extramedullary hematopoiesis																						
Excessive hemosiderin																					1	4
Lymphoid depletion																						

TABLE 65 (concluded)

Rat No.:	202	473	227	219	208	472	203	204	215	469	201	474	87	91	468	470	91	92	471	229	209	213	217	204	223	220	211	b/	
Week of Death:	55	57	57	71	74	80	82	82	84	84	85	87	87	91	91	91	91	92	92	93	96	98	100	102	102	102	103	107	
Other Lesions (concluded)																													
Ovary			0																										
Ovarian cyst						1				1													1		1	1	1		
Sarcoma									0																				
Uterus	0	0							0																				
Endometritis				1																									
Endometrial hyperplasia																					1								
Pancreas	0																												
Acinar atrophy (focal)																					2								
Islet cell tumor																													
Islet cell hyperplasia																													
Lymph Node																													
Lymphoid hyperplasia			2						4																				
Mediastinum																													
Fibrosarcoma																													
Stomach			0																										
Dilated crypts																													
Calcification of mucosa									1																				
Intestine			0																										
Enteritis									3																				
Kidney			0																										
Senile chronic nephropathy																													
Microcalculi in pelvis																													
Foci of mononuclear cells																													
Eye	0	0							0																				
Keratitis																													
Retinal atrophy																													
Abdominal cavity																													
Fibroma																													

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; + = questionable; X = present; 0 = tissue missing or unreadable.

b/ Died during third week of recovery after 2 1/2 months feeding.

TABLE 66

SUMMARY OF LESIONS IN MALE RATS FED 0.01% 2,4-DNT AND DYING AT UNSCHEDULED TIMES

Rat No.	132	142	152	164	133	136	137	150	135	132	156	147	137 ^b
Week of Death:	79	83	84	85	88	88	92	98	98	98	101	104	107
<u>Treatment-Related Lesions^a</u>													
Pituitary	0	0							0	0			0
- Chromophobe adenoma			X		X		X	X			X		
Liver													
Foci or areas of hepatocellular alteration									2		1	3	1
Hepatocellular carcinoma										X			
Testis													
Atrophy of seminiferous tubules							4	4	4	4			
Skin													
Subcutaneous mesenchymal tumor								X					X
<u>Other Lesions</u>													
Adrenal Gland	0	0											
Cystic degeneration											1		1
Pheochromocytoma				X				X				X	
Thyroid	0	0											
C cell adenoma							X						
Thyroiditis								3					
Parathyroid hyperplasia									X				
Trachea													
Tracheitis						1		4			1	1	1
Lung													
Chronic murine pneumonia			1	1	1		1		1	1	1	1	1
Pseudotuberculosis	4												
Calcification of alveolar wall										1			
Heart													
Myocardial degeneration/fibrosis			1					1		1	1	3	2
Dilated ventricle													
Liver													
Bile duct hyperplasia					1	1	3				1	1	
Portal inflammation							1	1	1		1		1
Focal necrosis			2			2							
Fatty change							1	1		1			
Cystic degeneration											1		1
Spleen													
Extramedullary hematopoiesis													1
Excessive hemosiderin							1						
Fibrosarcoma										X			
Testis	0												
Interstitial cell tumor			X							X			
Periarteritis nodosa								3					
Aspermiosgenesis			2	2									

TABLE 66 (concluded)

Rat No.	132	142	152	144	133	136	137	150	133	132	136	147	137 ^b
Week of Death:	79	83	84	85	88	88	92	98	98	98	101	104	107
<u>Other Lesions (concluded)</u>													
Epididymis	0	0											
Atrophy			1	1			1	3	1				
Too few spermatozoa in ductuli			4				4	4					
Prostate	0	0	0							0			
Prostatitis								3					
Atrophy				1			1						
Seminal Vesicle	0	0	0				1			0			
Atrophy				2									
Vasculitis								1					
Pancreas	0	0											
Acinar atrophy (focal)								1					1
Islet cell tumor						X						X	
Lymph Node	0	0											
Lymphoid hyperplasia						1	2						
Stomach	0	0											
Calcification of mucosa				2		1		2		4			
Intestine													
Carcinoma												X	
Kidney		0											
Senile chronic nephropathy			3	4	3		2	4	2	4	1	3	1
Nephritis											4		
Hydronephrosis			2			2							
Urinary Bladder	0	0											
Cystitis				1									
Papilloma										X			
Brain	0	0											
Abscessation											2		
Rib													
Osteodystrophia fibrosa										1			
Eye	0	0	0										
Keratitis													1
Retinal atrophy				1	1			1			1	1	
Squamous cell carcinoma									X				

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; ± = questionable; X = present; 0 = tissue missing or unreadable.

b/ Died during third week of recovery after 24 months feeding.

TABLE 67

SUMMARY OF LESIONS IN FEMALE RATS FED 0.01% 2,4-DNT AND DYING AT UNSCHEDULED TIMES

Rat No.:	235	247	241	245	253	231	233	270	249	257	248	238	232	243	250	252	259
Week of Death:	57	59	69	76	78	86	86	86	88	91	92	92	95	95	95	98	103
<u>Treatment-Related Lesions^a</u>																	
Pituitary	0												0	0			
Chromophobe adenoma		X	X	X	X	X	X		X	X	X	X			X	X	X
Liver																	
Foci or areas of hepatocellular alteration		1	1			2		2	2	3	2		2	2	2		2
Hepatocellular neoplastic nodule														X			
Hepatocellular carcinoma						X											
Mammary Gland																	
Adenoma														X			
Fibroadenoma		X			X	X	X	X					X	X	X	X	
<u>Other Lesions</u>																	
Adrenal Gland																	
Cystic degeneration	4	1	3	3		3	3	3	3			1	3	2	3	3	
Cortical tumor																	X
Phaeochromocytoma										X							
Thyroid	0			0													0
C cell adenoma										X							
Trachea																	
Tracheitis							1	2		1			2				
Lung																	
Chronic murine pneumonia		1	1			1		1		1	1		1	1	2	2	1
Heart																	
Myocardial degeneration/fibrosis	1					1				1			1				1
Liver																	
Bile duct hyperplasia							1			1			1			1	1
Portal inflammation		1		1			1										1
Focal necrosis	3										3						
Fatty change			1	1	1	1			1	3				1			
Telangiectasis														1			
Extramedullary hematopoiesis								1			3			2	2		3
Spleen																	
Extramedullary hematopoiesis			1			3		4			4			3	2		3
Excessive hemosiderin	1															1	
Lymphoid depletion	1								2			3					

TABLE 67 (concluded)

Rat No.:	235	247	241	245	253	231	233	270	249	257	248	238	232	243	250	252	259
Week of Death:	57	59	69	76	78	86	86	86	88	91	92	92	95	95	95	98	103
<u>Other Lesions (concluded)</u>																	
Ovary																	
- Ovarian cyst							1		1								1
Uterus					0												0
- Endometritis																1	
Pancreas	0	0															
- Acinar atrophy (focal)						1											
- Islet cell hyperplasia														1		1	1
Lymph Node																	
- Lymphoid hyperplasia											3						
Stomach																	
- Dilated crypt							1										
Kidney																	
- Senile chronic nephropathy				1		1											1
- Microcalculi in pelvis			1			1	1	1	1	1				1	1	1	1
- RBC-pigment in epithelium																	1
- Foci of mononuclear cells															1	1	
Eye																	
- Keratitis						3		1									
- Retinal atrophy								1		1		1		1	1	1	1
Peritoneum Mesothelium												X					

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; ± = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 68

SUMMARY OF LESIONS OF MALE RATS FED 0.07% 2,4-DT AND DYING AT UNSCHEDULED TIMES

Rat No.:	426	179	181	175	167	163	183	162	188	173	182	186	174	176	169	164	170	161	455	178	432	190	187	172	165	168	180	171	184	189
Week of death:	48	59	59	63	68	68	70	73	82	82	82	83	83	83	83	83	84	84	85	84	86	89	89	90	90	97	96	96	96	97
Treatment-Related Lesions ^{a/}																														
Pituitary	0	0	0	0	0	0	0	0	0	0																				
Chromophobe adenoma																														
Liver																														
Foci or areas of hepatocellular alteration																														
Hepatocellular neoplastic nodules	3	1	3	2	1																									
Hepatocellular carcinoma																														
Testis																														
Atrophy of seminiferous tubules	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Intestine	0	0	0	0	0	0	0	0	0	0																				
Parasites																														
Skin																														
Subcutaneous mesenchymal tumor																														
Thymus Gland																														
Adenoma-papilloma																														
Fibroadenoma																														
Other Lesions																														
Adrenal Gland	0	0	0	0	0	0	0	0	0	0																				
Cystic degeneration																														
Neurochromocytoma																														
Cortical hyperplasia																														
Thyroid	0	0	0	0	0	0	0	0	0	0																				
Follicular epithelium adenoma																														
Squamous metaplastic follicle																														
Trachea	0	0	0	0	0	0	0	0	0	0																				
Tracheitis																														
Lung																														
Chronic murine pneumonia	1	0	1	1	1	1	1	1	1	1																				
Malignant lymphoma																														
Heart																														
Myocardial fibrosis/degeneration	1	1	1	1	1	1	1	1	1	1																				
Liver																														
Bile duct hyperplasia																														
Portal inflammation																														
Focal necrosis																														
Fatty change																														
Telangiectasis																														
Cystic degeneration																														
Extramedullary hematopoiesis																														

426	179	181	175	167	163	183	162	108	173	182	186	174	176	169	164	170	161	455	178	432	190	187	172	165	168	180	171	184	189
48	59	59	63	68	68	70	73	82	82	82	83	83	83	83	83	84	84	85	86	86	89	89	90	90	92	96	96	96	97

[illegible]

Times not listed are normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; + = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 69

SUMMARY OF LESIONS IN FEMALE RATS FED 0.07% 2,4-DNT AND DYING AT UNSCHEDULED TIMES

Est No.:	269	267	276	274	263	279	266	261	270	275	265	480	481	587	288	595	278	289	586	262	285	282	272	271	281	483	593	594	482	592	290	286	280	
Week of death:	54	62	69	70	74	78	82	83	83	83	84	84	86	87	88	90	91	92	92	92	94	94	95	96	96	96	96	96	96	98	98	98	99	
Treatment-Related Lesions																																		
Pituitary	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Chromophobe adenoma	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Liver	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Foci or areas of hepatocellular alteration	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Hepatocellular neoplastic nodules	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Hepatocellular carcinoma	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Intestine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Parasites	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Skin	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Subcutaneous mesenchymal tumor	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Mammary Gland	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Adenoma-papilloma	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Fibroadenoma	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Fibroma	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Other Lesions																																		
Adrenal Gland	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Cystic degeneration	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Cortical tumor	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Phenochromocytoma	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Parathyroid	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Adenoma	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Thyroid	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
C cell adenoma	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Squamous metaplastic follicle	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Trachea	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Tracheitis	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Lung	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Chronic serous pneumonia	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Bronchopneumonia	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Malignant lymphoma	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Heart	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Myocardial fibrosis/degeneration	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Malignant lymphoma	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Liver	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Bile duct hyperplasia	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Portal inflammation	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Focal necrosis	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Telangiectasis	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Cystic degeneration	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Extramedullary hematopoiesis	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Myelocytic proliferative disease	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	

TABLE 69 (continued)

Pat. No.: Week of death:	244	267	276	274	263	279	266	261	270	275	265	480	481	567	288	595	278	289	586	262	285	282	283	272	277	281	483	593	594	482	592	290	286	280
54	62	69	70	74	78	82	83	83	85	86	86	86	87	88	90	91	92	92	92	92	94	94	94	96	96	96	96	96	96	96	96	96	98	99
Other Lesions (concluded)																																		
Spleen																																		
Extramedullary hematopoiesis																																		
Excessive pigment																																		
Lymphoid depletion																																		
Myeloproliferating/lymphoproliferating disease																																		
Ovary																																		
Ovarian cyst																																		
Cremulous cell tumor																																		
Uterus																																		
Endometrial hyperplasia																																		
Pancreas																																		
Acinar atrophy (focal)																																		
Acinar cell tumor																																		
Islet cell tumor																																		
Islet cell hyperplasia																																		
Lymph Node																																		
Lymphoid hyperplasia																																		
Malignant lymphoma																																		
Stomach																																		
Unlabeled crypts																																		
Malignant lymphoma																																		
Kidney																																		
Severe chronic nephropathy																																		
Calculi in pelvis																																		
Pyelitis																																		
Pigment in epithelium																																		
Hydronephrosis																																		
Foci of mononuclear cells																																		
Malignant lymphoma																																		
Papilloma																																		
Adenoma																																		
Urinary bladder																																		
Papilloma																																		
Eye																																		
Keratitis																																		
Respiratory																																		
Chondroma																																		

Tissues not listed are normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; $\frac{1}{2}$ = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 70

NON-2,4-DNT-RELATED TUMORS IN RATS FED 2,4-DNT MORE THAN 12 MONTHS

Dose (% in feed):	0		0.0015		0.01		0.07	
Sex:	Male	Female	Male	Female	Male	Female	Male	Female
<u>Tumors</u> ^{a/}								
Adrenal Gland								
Cortical tumor	1		1		1		1	
Pheochromocytoma	2	1	2	3	4	2	2	3
Thyroid								
C cell tumor (adenoma)	1	1	1		1	2		2
Follicular epithelium adenoma	1	2		1			1	
Pancreas								
Acinar cell tumor				1			1	5
Islet cell tumor			3	1	2	1		2
Intestine								
Carcinoma					1			
Testis								
Interstitial cell tumor	5		2		2		5	
Ovary								
Sarcoma				2				
Spleen								
Fibrosarcoma					1			
Lymphoma								
Single site				1			1	1
Multiple sites		1						1
Thoracic/Abdominal Cavities								
Fibroma			1					
Lipoma			1					
Mesothelioma						1		
Fibrosarcoma				1				
Bone								
Chondroma								1
Kidney								
Adenoma								1
Papilloma								1
Urinary Bladder								
Papilloma					1			1
Brain								
Astrocytoma			1					

^{a/} Number of rats.

TABLE 71

INCIDENCE OF 2,4-DNT-RELATED LESIONS IN RATS FED 2,4-DNT MORE THAN 12 MONTHS

Dose (% in feed): Sex:	0		0.0015		0.01		0.07	
	Male	Female	Male	Female	Male	Female	Male	Female
Lesions ^{a/}								
Pituitary								
Chromophobe adenoma	9/22 (41) ^{a/}	18/23 (78)	14/23 (61)	24/30 (80)	7/14 (50)	20/24 (83)	2/20 (10)	7/23 (30)
Liver								
Foci or areas of hepatocellular alteration	9/25 (36)	7/23 (30)	10/28 (36)	18/35 (51)	9/19 (47)	19/27 (70)	16/29 (55)	13/34 (38)
Hepatocellular neoplastic nodule	1/25 (4)	0/23	2/28 (7)	3/35 (9)	1/19 (5)	2/27 (7)	2/29 (7)	6/34 (18)
Hepatocellular carcinoma	1/25 (4)	0/23	0/28	0/35	1/19 (5)	1/27 (4)	6/29 (21)	18/34 (53)
Testis								
Atrophy of seminiferous tubules	4/25 (16)	--	8/28 (29)	--	6/18 (33)	--	25/29 (86)	--
Skin								
Subcutaneous mesenchymal and epithelial tumors	2/25 (8)	1/22 (5)	4/28 (14)	3/35 (9)	3/19 (16)	0/27	17/30 (57)	6/35 (17)
Mammary Gland								
Tumors (any type)	0/25	11/23 (48)	0/28	12/35 (34)	0/19	17/27 (63)	2/30 (7)	33/35 (94)
Adenoma-papilloma	--	1/11 (9) ^{b/}	--	5/12 (42)	--	4/17 (24)	1/2 (50)	6/33 (18)
Fibroadenoma	--	9/11 (81)	--	7/12 (58)	--	16/17 (94)	1/2 (50)	32/33 (97)
Adenocarcinoma-carcinoma	--	3/11 (27)	--	2/12 (17)	--	3/17 (18)	--	0/33
Fibroma	--	0/11	--	1/12 (8)	--	1/17 (6)	--	2/33 (6)

^{a/} Rats with lesion/rats with readable slides (percent incidence).^{b/} Figures for rats with mammary tumors.

TABLE 72

CLASSIFICATION OF EPITHELIAL AND SUBCUTANEOUS
MESENCHYMAL TUMORS OF SKIN

Dose (% in feed):		0		0.0015		0.01	0.07	
	Sex:	<u>Male</u>	<u>Female</u>	<u>Male</u>	<u>Female</u>	<u>Male</u>	<u>Male</u>	<u>Female</u>
<u>Type</u>								
Fibroma				2	2	2	13	5
Lipoma				1			1	
Basal cell tumor	1 ^a /							
Sarcoma (undifferentiated)				1		1	2	1
Fibrosarcoma					1		1	
Carcinosarcoma			1					
Squamous cell carcinoma	1							

a/ Number of rats with tumors.

TABLE 73

AGE, WEIGHT AND FERTILITY OF THREE GENERATIONS OF RATS GIVEN 2,4-DNT

2,4-DNT (% in feed)	Generation	Age at First Mating (months)	Mating Ratio	Pregnancy Ratio	Males		Females		Duration of Gestation (days)
					Fertile Mated	Weight (g) at First Mating	Fertile Mated	Weight (g) at First Mating	
0	F0	8	30/40 ^{a/}	17/30 ^{b/}	7/10	601±12 ^{c/}	13/22	333±7 ^{c/}	23
	F1	3	38/38	38/38	15/15	475±9	19/19	276±3	22
	F2	3	39/40	38/39	20/20	438±11	20/20	265±5	22
0.0015	F0	8	32/38	20/32	8/10	618±12	15/21	342±9	23
	F1	3	29/32	24/29	14/16	489±9	14/16	272±6	22
	F2	3	39/40	38/39	19/20	451±9	20/20	260±5	22
0.01	F0	8	29/38	17/29	7/10	593±18	12/20	311±5	23
	F1	3	39/40	36/39	19/20	469±6	20/20	267±4	22
	F2	3	38/40	38/38	20/20	456±9	20/20	264±5	22
0.07	F0	8	33/38	16/33	8/10	464±13 ^{d/}	12/21	255±6 ^{d/}	23
	F1	3	4/6	3/4	3/3	355±10 ^{d/}	3/3	249±5	22

^{a/} Number of copulations detected by vaginal smear to the number of male-female pairings.^{b/} Number of confirmed pregnancies to the number of copulations.^{c/} Mean ± S.E.^{d/} Significantly different from the mean value of the respective control generation (Dummett's multiple comparison procedure).

TABLE 74

REPRODUCTIVE PERFORMANCE OF FEMALE RATS GIVEN 2,4-DNT IN A THREE-GENERATION STUDY

2,4-DNT (% in Feed)	Litter No.	Litter Size	Live-born Index	Weight at Birth	Viability Index	Lactation Index	Weight at Weaning	Sex Ratio Males:Total
0	F1a	6.9±1.1(10) ^{a/}	85±7	7.2±0.3	70±15	61±15	45±8(7)	23:38
	F1b	12.7±1.8(5)	91±5	7.0±0.5	100	91±6	55±7(4)	13:35
	F2a	12.7±0.6(19)	98±1	7.0±0.2	98±2	96±3	43±2(19)	111:222
	F2b	14.0±0.3(19)	96±2	7.2±0.1	98±1	92±6	42±1(18)	122:253
	F3a	11.3±0.4(23)	97±2	7.1±0.1	96±2	95±3	39±1(23)	123:249
	F3b	12.2±0.6(18)	99±1	6.4±0.1	94±2	94±3	40±1(18)	93:216
0.0015	F1a	6.8±1.0(13)	83±9	7.1±0.2	69±13	91±6	53±2(9)	28:56
	F1b	8.2±1.9(6)	100	7.2±0.8	99±1	70±6	45±9(6)	16:32
	F2a	13.9±0.4(14)	97±1	6.6±0.1	99±1	95±3	41±1(14)	92:178
	F2b	15.4±0.6(10)	95±2	6.8±0.3	96±4	83±6	41±2(10)	58:140
	F3a	12.2±0.6(23)	95±2	7.1±0.2	96±1	93±2	36±1(23)	95:266
	F3b	13.2±0.4(19)	98±1	6.4±0.2	95±3	87±6	35±1(18) ^{b/}	93:242
0.01	F1a	4.9±1.1(10)	89±10	7.4±0.1	60±16	61±18	38±10(7)	22:35
	F1b	9.7±1.1(6)	88±6	7.5±0.4	83±17	83±17	44±13(6)	24:44
	F2a	13.8±0.4(18)	99±1	6.9±0.2	94±6	95±5	42±1(18)	119:245
	F2b	14.2±0.8(18)	97±1	7.1±0.2	94±6	97±1	41±2(17)	108:242
	F3a	12.6±0.5(20)	98±2	6.9±0.1	94±2	87±5	35±1(20)	105:243
	F3b	13.6±0.5(20)	98±2	6.5±0.1	97±1	91±3	35±2(20) ^{b/}	123:265
0.07	F1a	4.5±1.1(11)	94±4	7.5±0.4	64±15	90±10	53±6(7)	14:29
	F1b	7.4±1.2(5)	90±5	7.0±0.3	20±20 ^{b/}	78	44(1)	4:7
	F2a	11.0±1.0(3)	100	6.4±0.2	89±11	100	35±1(3)	19:29

^{a/} Mean ± S.E. and in parentheses the number of litters included in the mean.

^{b/} Significantly different from the mean value of the respective control litters (Tukey's omega procedure).

TABLE 75

CHROMOSOMES DERIVED FROM RATS FED 2,4-DNT FOR 24 MONTHS

Dose (% in feed)	Tissue Cultured	Number of Rats	Chromosome Frequency					Tetraploids per 100 cells
			<u>≤40</u>	<u>41</u>	<u>42</u>	<u>43</u>	<u>≥44</u>	
0	Bone marrow	4	<u>1^{b/}</u>	2	46	1	0	$0.25 \pm 0.14c/$
0	Kidney	4	4	4	40	1	1	0.50 ± 0.35
0.01	Bone marrow	5	2	6	38	3	1	0.40 ± 0.40
0.01	Kidney	6	5	8	33	3	1	$1.50 \pm 0.22d/$
0.07	Bone marrow	<u>1^{a/}</u>	2	9	35	4	0	0
0.07	Kidney	1	2	11	33	4	0	0.5

a/ Only surviving rat.b/ Mean.c/ Mean $\pm$ standard error.d/ Significantly different from control by "t" test.

TABLE 76

MORPHOLOGICAL ABERRATIONS OF CHROMOSOMES DERIVED FROM RATS FED 2,4-DNT FOR 24 MONTHS

Dose (% in feed)	Tissue Cultured	Number of Rats	Chromatid Breaks and Gaps per 50 cells	Translocations Per 50 cells	Total Aberrations per 50 cells
0	Bone marrow	4	0.2 ± 0.2^b	0	0.2 ± 0.2
0	Kidney	4	0.3 ± 0.2	0	0.3 ± 0.2
0.01	Bone marrow	5	0.2 ± 0.2	0	0.2 ± 0.2
0.01	Kidney	6	0.3 ± 0.2	0	0.3 ± 0.2
0.07	Bone marrow	1 ^a	1	0	1
0.07	Kidney	1	0	0	0

^a/ Only surviving rat.

^b/ Mean $\pm$ standard error.

TABLE 77

OCCURRENCE OF DOMINANT LETHAL MUTATIONS IN
RATS FED 2,4-DNT

<u>Dose</u> <u>(% of feed)</u>	<u>Number of</u> <u>Males</u>	<u>Fertility Index^{a/}</u>	<u>Implant Viability.</u> <u>Index^{b/}</u>
0	4	92 ± 8 ^{c/}	92 ± 1
0.02	4	67 ± 14	62 ± 24
0.2	5	20 ± 13 ^{d/}	0 ^{d/}
0	8	96 ± 4	96 ± 1
0.0015	9	89 ± 6	94 ± 1
0.01	7	100 ± 0	97 ± 1
0.07	10	93 ± 7	94 ± 1

a/ Confirmed pregnancies/sperm positive females x 100.

b/ Viable fetuses/implants x 100.

c/ Mean ± standard error.

d/ Significantly different from control ("t" test).

TABLE 78

BODY WEIGHT, FEED CONSUMPTION, 2,4-DNT INTAKE AND REPRODUCTIVE PERFORMANCE
FOR RATS FROM THE LAST DOMINANT LETHAL STUDY

Dose (% in Feed):	<u>0</u>	<u>0.07</u>	<u>0.10</u>	<u>0.15</u>
<u>Males</u>				
Body weight (gm)				
Initial	166 $\pm$ 3 ^{a/}	164 $\pm$ 2	165 $\pm$ 2	164 $\pm$ 2
Week 4	341 $\pm$ 9	341 $\pm$ 6	322 $\pm$ 6	306 $\pm$ 5 ^{b/}
Week 8	449 $\pm$ 10	419 $\pm$ 6 ^{b/}	384 $\pm$ 6 ^{b/}	354 $\pm$ 7 ^{b/}
Week 13	510 $\pm$ 9	432 $\pm$ 9 ^{b/}	400 $\pm$ 7 ^{b/}	366 $\pm$ 10 ^{b/}
Feed consumption (gm/rat/day) ^{c/}	23.8 $\pm$ 0.8	22.0 $\pm$ 0.6	22.2 $\pm$ 1.5	19.8 $\pm$ 0.6
2,4-DNT intake (mg/kg/day) ^{c/}	0	45	65	98
Mated ^{d/}	21	22	24	23
Sperm ^{e/}	21	22	18	11
Plugs, but no sperm	0	0	4	7
Fertile ^{f/}	20	15	2	1
<u>Females Mated with Treated Males</u>				
Corpora lutea/dam	15.7 $\pm$ 0.6	14.2 $\pm$ 0.5 ^{b/}	14.8 $\pm$ 0.6	13.3 $\pm$ 0.5 ^{b/}
Total implants/dam	12.9 $\pm$ 0.7	10.9 $\pm$ 1.1	15.0 $\pm$ 1.1	0
Viable implants/dam	12.4 $\pm$ 0.7	10.5 $\pm$ 1.1	14.0 $\pm$ 0.9	0
<u>Indexes</u>				
Fertility ^{g/}	90(76-97)	53(38-69)	11(3-26)	2(0-12)
Gestation ^{h/}	100(90-100)	100(85-100)	100(40-100)	0
Implant viability ^{i/}	96 $\pm$ 1	93 $\pm$ 4	94 $\pm$ 4	0
Implantation ^{j/}	73 $\pm$ 5	38 $\pm$ 6 ^{k/}	9 $\pm$ 4 ^{k/}	0

a/ Mean or mean $\pm$ standard error.

b/ Significantly different from control (Dunnett's multiple comparison procedure).

c/ Average of the 13 weekly means. 2,4-DNT intake based on mean body weight for each week.

d/ Exposed to females.

e/ Sperm found in the vaginal smear of at least one female.

f/ Evidence of conception found in at least one female.

g/ Confirmed pregnancies/plug positive females x 100 (95% confidence limits).

h/ Pregnancies with viable embryos/confirmed pregnancies x 100 (95% confidence limits).

i/ Viable embryos/implants x 100. Mean $\pm$ S.E.

j/ Implants/corpora lutea x 100. Mean $\pm$ S.E.

k/ Significantly different from control (two-sample rank test).

**LESIONS IN MALE GENITAL ORGANS IN MALES
FED 2,4-DNT FOR 13 WEEKS**

a/ Severe => 50% of seminiferous tubules involved.
Marked = 30-50% of seminiferous tubules involved.
Moderate = 10-30% of seminiferous tubules involved.
Mild = <10% of seminiferous tubules involved.

TABLE 80

DISTRIBUTION AND EXCRETION OF RADIOACTIVITY IN RATS 24 HR AFTER
ORAL ADMINISTRATION OF 2,4-DNT (RING-UL-¹⁴C) FOLLOWING
3 MONTHS OF 2,4-DNT IN FEED

	<u>% of Administered Dose</u>			
	<u>Control</u>		<u>High Dose</u>	
	<u>Males</u>	<u>Females</u>	<u>(0.07% 2,4-DNT)</u> <u>Males</u>	<u>Females</u>
G.I. Tract and Contents	14.0±6.8 ^{c/}	12.8±4.9	5.5±1.9	4.2±2.9
Feces	7.3±3.8	10.5±3.5	20.7±5.3	14.4±2.6
Urine	68.0±12.1	74.5±2.2	68.4±5.0	77.2±6.6
Blood ^{a/}	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
Spleen	< 0.1	< 0.1	< 0.1	< 0.1
Liver	2.0±1.6	0.3±0.0	0.2±0.0	0.3±0.0
Kidney	< 0.1	0.1 0.0	< 0.1	< 0.1
Brain	< 0.1	< 0.1	< 0.1	< 0.1
Lungs	< 0.1	< 0.1	< 0.1	< 0.1
Muscle ^{b/}	1.2±0.9	0.4±0.1	0.3±0.0	0.2±0.0
Gonads	< 0.1	< 0.1	< 0.1	< 0.1
Recovery	92.7±5.4	98.6±3.7	95.4±4.2	96.4±2.9

^{a/} Based on 7% of body weight.

^{b/} Based on 40% of body weight.

^{c/} Mean ± S.E. of three rats.

TABLE 81

METABOLITES OF 2,4-DNT IN RAT URINE 24 HR AFTER
ORAL ADMINISTRATION OF 2,4-DNT-(RING-UL-¹⁴C)
FOLLOWING 3 MONTHS OF 2,4-DNT IN FEED

	Male			Female		
	Fresh		Hydrolyzed ^{b/}	Fresh		Hydrolyzed ^{b/}
	Control	High Dose		Control	High Dose	
2,4-DNT	0.02 ^{b/}	0.01	1.3	0	0	0.2
4NH ₂ -2NT 2NH ₂ -4NT	0.5	0.3	3.2	0.1	0.1	5.9
2,4-DNBA ^{c/}	1.1	4.8	19.3	3.1	4.9	18.1
2,4-DINH ₂ BA MononH ₂ -NBA	1.4	2.1	25.1	1.6	0.6	23.8
2,4-DINH ₂ T	4.9	4.5	7.8	5.3	2.8	7.6
Conjugate and Others	92.1	88.3	39.1	89.9	91.5	42.6
			40.1			42.0

^{a/} Mean of three rats, expressed as percent of total radioactivity.

^{b/} Hydrolyzed by equal volume of 5N HCl for 1 hr in 100°C water bath.

^{c/} 2,4-Dinitrobenzyl alcohol.

TABLE 82

DISTRIBUTION AND EXCRETION OF RADIOACTIVITY IN RATS 24 HR AFTER
ORAL ADMINISTRATION OF 2,4-DNT (RING-UL-¹⁴C) FOLLOWING
9 MONTHS OF 2,4-DNT IN FEED

	<u>% of Administered Dose</u>			
	<u>Control</u>		<u>High Dose</u>	
	<u>Males</u>	<u>Females</u>	<u>(0.07% 2,4-DNT)</u> <u>Males</u>	<u>Females</u>
G.I. Tract and Contents	8.4±2.2 ^{a/}	11.6±1.9	5.8±0.8	4.2±0.9
Feces	3.4±2.3	7.5±3.2	8.0±2.6	8.6±3.2
Urine	82.4±2.6	80.2±4.7	76.2±7.9	87.8±3.1
Blood ^{a/}	0.1±0.0	0.1±0.0	<0.1	<0.1
Spleen	<0.1	<0.1	<0.1	<0.1
Liver	0.2±0.1	0.1±0.0	0.2±0.0	0.1±0.0
Kidney	0.1±0.0	0.1±0.1	0.1±0.1	0.5±0.5
Brain	<0.1	<0.1	<0.1	<0.1
Lungs	<0.1	<0.1	<0.1	<0.1
Muscle ^{b/}	0.3±0.0	0.5±0.4	<0.1	0.1±0.0
Gonads	<0.1	<0.1	<0.1	<0.1
Recovery	94.7±5.7	100.0±0.0	90.4±9.6	101.5±0.8

^{a/} Based on 7% of body weight.

^{b/} Based on 40% of body weight.

^{c/} Mean ± S.E. of three rats.

TABLE 83

METABOLITES OF 2,4-DNT IN RAT URINE 24 HOURS AFTER
ORAL ADMINISTRATION OF 2,4-DNT-(RING-UL-¹⁴C)
FOLLOWING 9 MONTHS OF 2,4-DNT IN FEED

	Male			Female		
	Fresh		Hydrolyzed ^{c/}	Fresh		Hydrolyzed ^{c/}
	Control	High Dose		Control	High Dose	
2,4-DNT	< 0.1 ^{b/}	0.1	0.7	0.1	< 0.1	0.7
4NH ₂ -2NT						
2NH ₂ -4NT	0.3	1.2	7.4	0.3	0.1	5.2
2,4-DNRA ^{s/}	13.0	16.7	28.7	7.6	2.6	35.5
2,4-DiNH ₂ BA						
MonoNH ₂ -NBA	0.9	0.9	8.2	0.2	0.8	10.2
2,4-DiNH ₂ T	0.1	0.4	2.2	0.5	1.2	3.4
2,4-DNB Acid	25.2	22.6	3.1	31.1	28.0	3.2
Conjugate and Others	60.5	58.1	49.6	60.3	67.2	41.9
			34.8			63.7

a/ 2,4-Dinitrobenzyl alcohol.

b/ Mean of three rats, expressed as percent of total radioactivity.

c/ Hydrolyzed by equal volume of 5N HCl for 1 hour in 100°C water bath.

TABLE 84

DISTRIBUTION AND EXCRETION OF RADIOACTIVITY IN RATS 24 HR AFTER
ORAL ADMINISTRATION OF 2,4-DNT (RING-UL-¹⁴C) FOLLOWING
20 MONTHS OF 2,4-DNT IN FEED

	<u>% of Administered Dose</u>			
	<u>Control</u>		<u>Mid Dose</u> <u>(0.01% 2,4-DNT)</u>	
	<u>Males</u>	<u>Females</u>	<u>Males</u>	<u>Females</u>
G.I. Tract and Contents	15.9±0.9 ^{c/}	15.4±4.3	5.9±1.7	6.0±1.5
Feces	2.8±1.1	4.8±3.2	14.0±5.4	13.7±0.5
Urine	70.1±5.6	42.2±10.4	72.9±9.9	71.5±8.6
Blood ^{a/}	0.1±0.0	0.4±0.3	0.5±0.3	0.5±0.2
Spleen	<0.1	<0.1	<0.1	<0.1
Liver	0.2±0.0	0.2±0.0	0.4±0.2	0.3±0.0
Kidney	0.5±0.4	0.2±0.1	0.2±0.1	<0.1
Brain	<0.1	<0.1	<0.1	<0.1
Lungs	<0.1	<0.1	<0.1	<0.1
Muscle ^{b/}	0.9±0.0	2.36±0.5	5.7±2.4	4.4±1.8
Gonads	<0.1	<0.1	<0.1	<0.1
Recovery	90.5±6.3	65.8±9.6	99.9±0.2	99.6±9.7

^{a/} Based on 1% of body weight.

^{b/} Based on 40% of body weight.

^{c/} Mean ± S.E. of three rats.

METABOLITES OF 2,4-DNT IN RAT URINE 24 HOURS AFTER ORAL ADMINISTRATION OF 2,4-DNT-(RING-UL-¹⁴C) FOLLOWING 20 MONTHS OF 2,4-DNT IN FEED

	Male				Female			
	Fresh		Hydrolyzed ^{c/}		Fresh		Hydrolyzed ^{c/}	
	Control	Mid Dose	Control	Mid Dose	Control	Mid Dose	Control	Mid Dose
2,4-DNT	<0.1 ^{b/}	0	0.4	0.3	<0.1	<0.1	0.6	1.0
4NH ₂ -2NT								
2NH ₂ -4NT	0.3	0.2	6.5	5.6	0.1	0.2	7.2	4.0
2,4-DNRA ^{a/}	9.3	2.0	15.2	13.2	8.3	2.6	14.8	15.0
2,4-DiNH ₂ RA Mono NH ₂ -NRA	0.6	1.7	3.3	3.7	0.3	1.4	4.1	2.8
2,4-DiNH ₂ T	0.1	0.1	1.6	0.9	0.1	0.1	1.2	1.4
2,4-DNB Acid	17.7	12.5	9.7	9.9	18.4	21.1	16.2	16.0
Conjugate and Others	72.0	83.5	63.3	66.4	71.8	74.6	55.9	59.8

b/ Mean of three rats, expressed as percent of total radioactivity.

c/ Hydrolyzed by equal volume of 5N HCl for one hour in 100°C water bath.

CUMULATIVE DEATHS AMONG MALE RATS FED 2,4-DNT

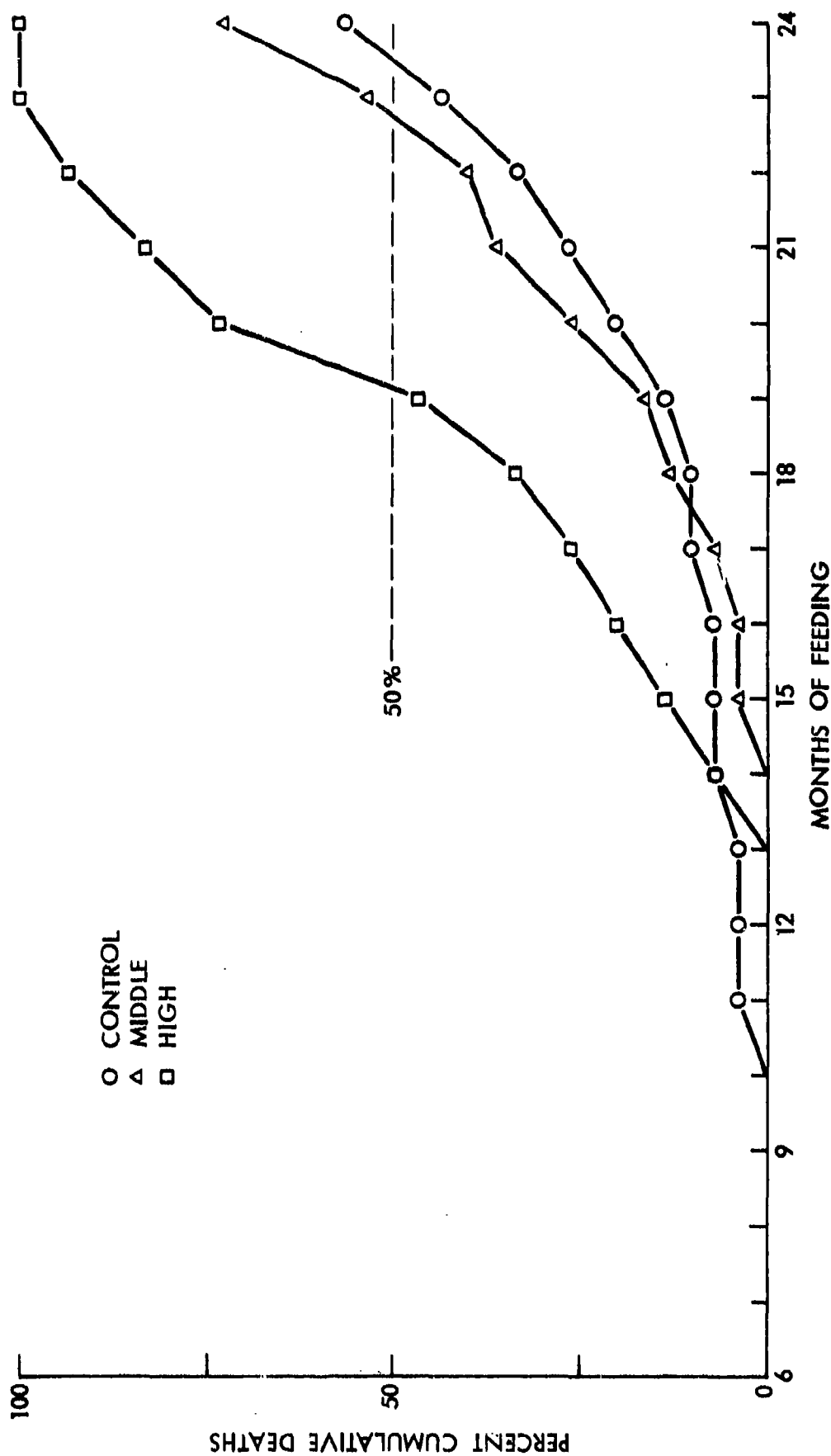


Figure 4 - Cumulative Deaths Among Male Rats Fed 2,4-DNT

CUMULATIVE DEATHS AMONG FEMALE RATS FED 2,4-DNT

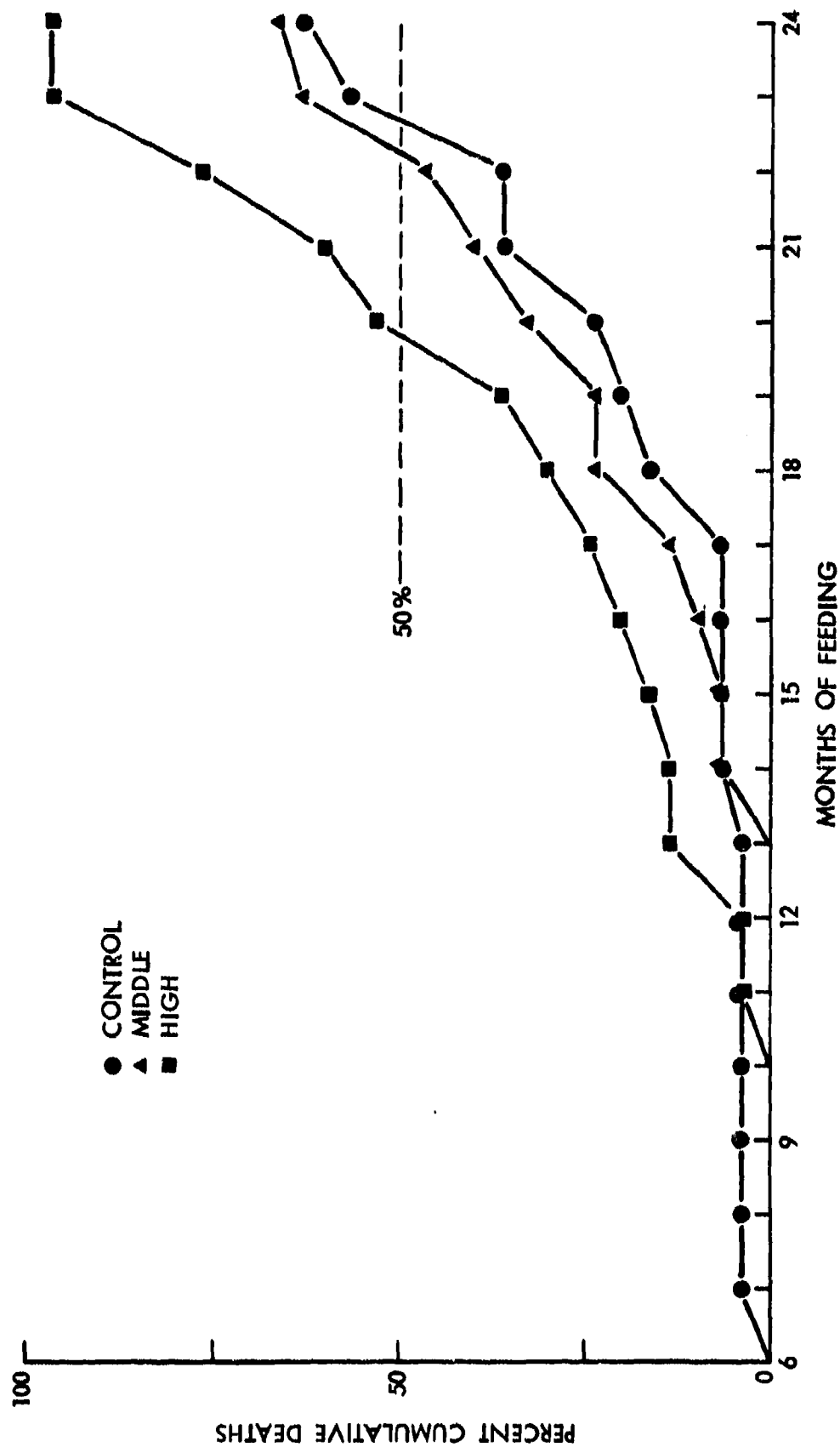


Figure 5 - Cumulative Deaths Among Female Rats Fed 2,4-DNT

BODY WEIGHTS OF RATS FED 2,4-DNT

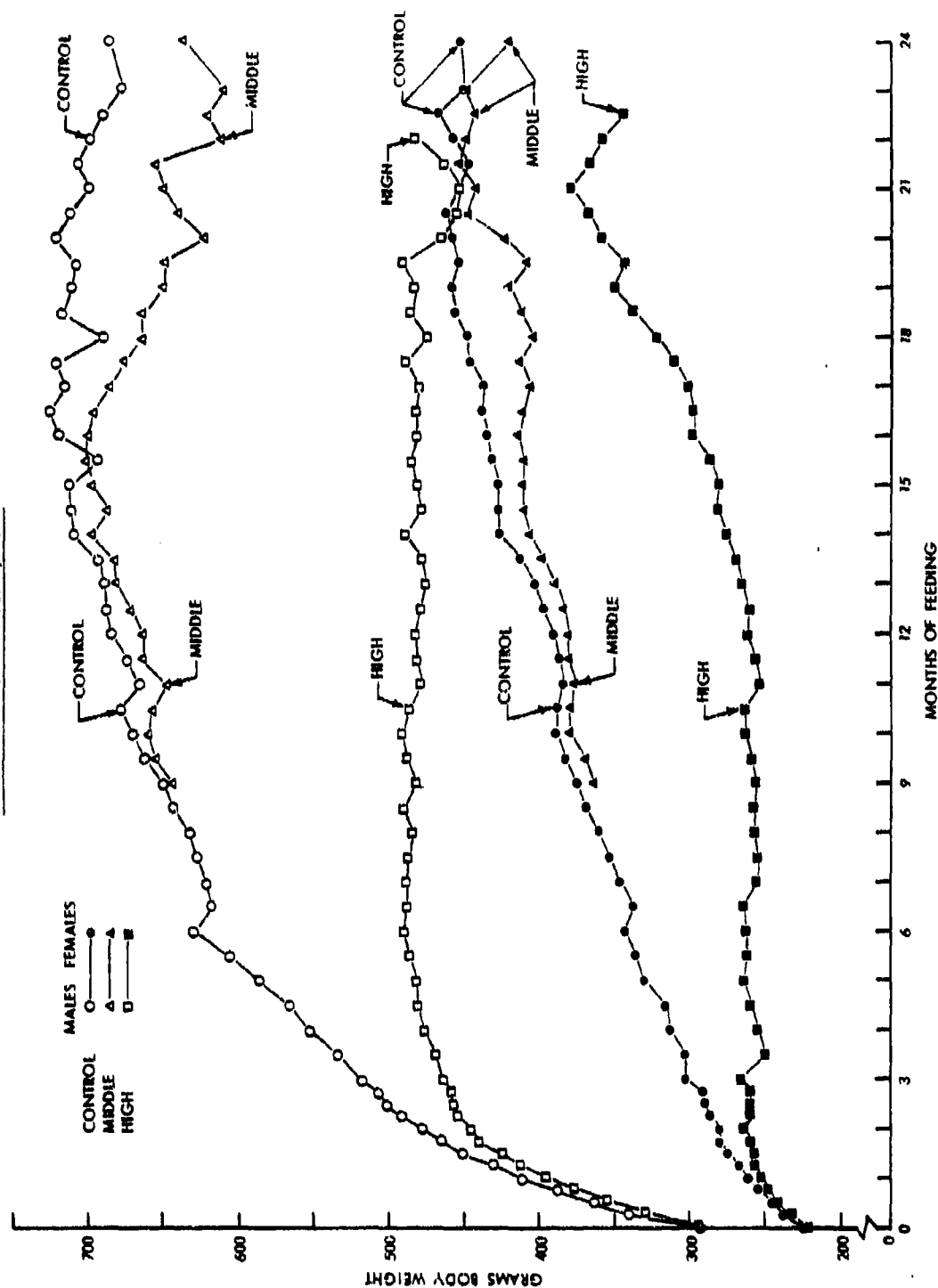


Figure 6 - Body Weights of Rats Fed Various Doses of 2,4-DNT

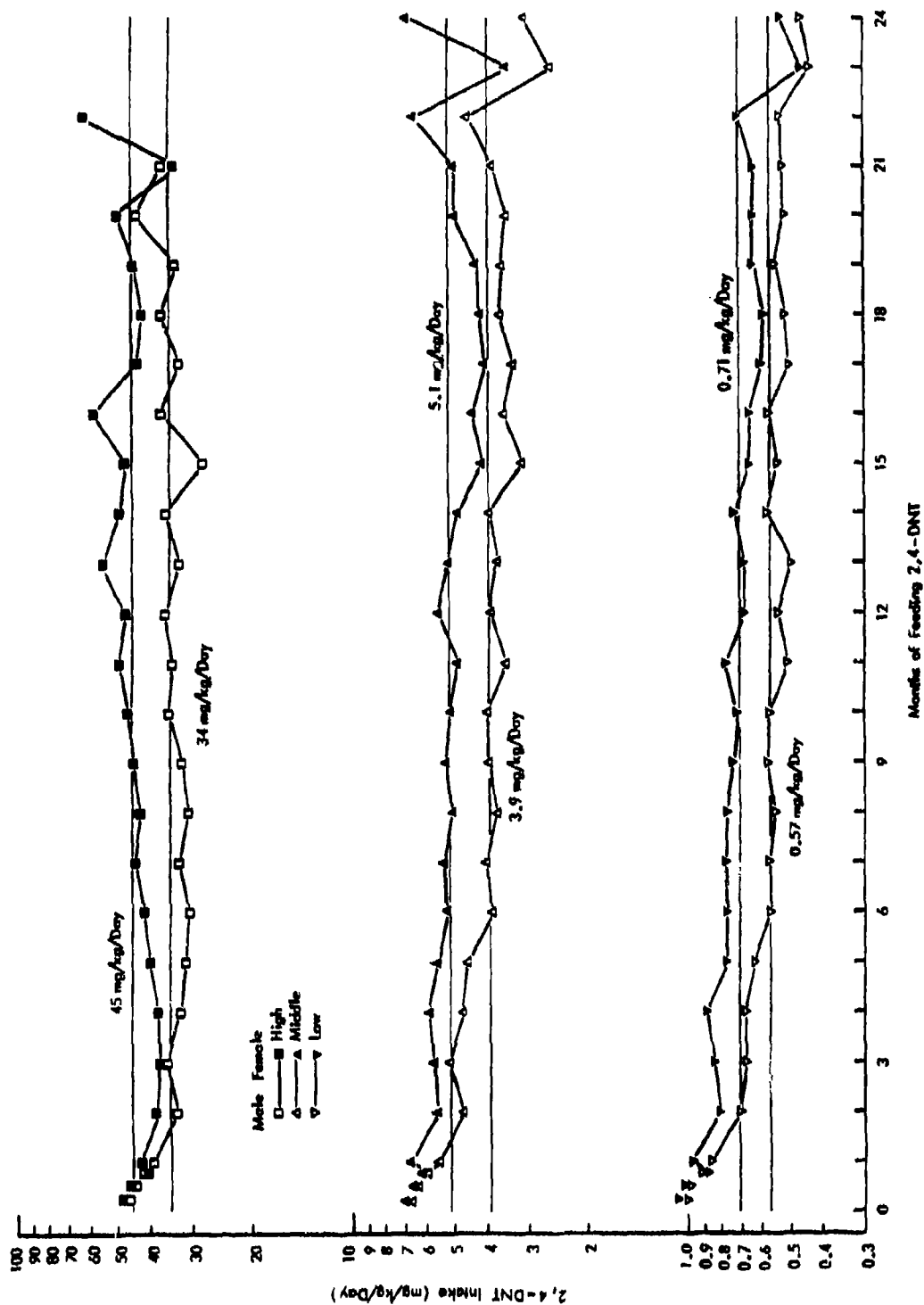
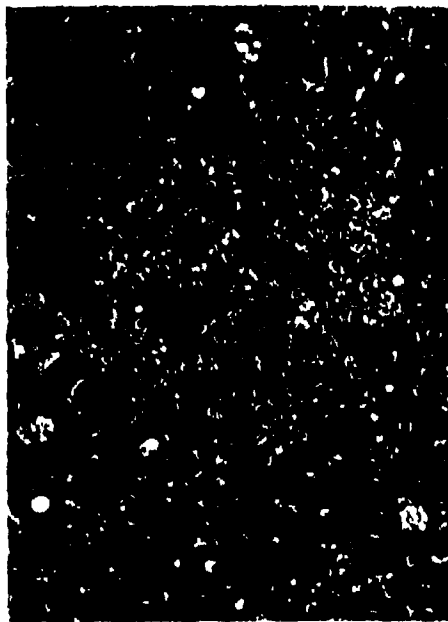


Figure 7 - Intake of 2,4-DNT by Rats Fed 0.07% (high), 0.01% (middle) or 0.0015% (low) 2,4-DNT. Horizontal lines are average intakes for males (lower line of pair) and females.



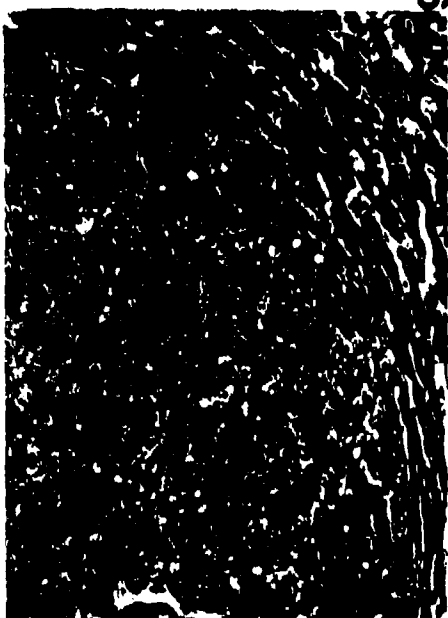
8

Figure 8 - Photomicrograph of Liver from Rat No. 73-326 Fed 0.07% 2,4-DNT for 12 Months. Note the focus of ground glass (eosinophilic) hepatocellular alteration (hyperplastic focus). H and E stain, 100 x.



10

Figure 10 - Photograph of Abdominal Viscera from Rat No. 73-463 Fed 0.07% 2,4-DNT for 96 Weeks. Note the nodular new growth of the liver--hepatocellular carcinoma.



9

Figure 9 - Photomicrograph of Liver from Rat No. 73-380 Fed 0.07% 2,4-DNT for 12 Months. Note the hyperplastic nodule. H and E stain, 100 x.



11

Figure 11 - Photomicrograph of Liver from Rat No. 73-284 Fed 0.07% 2,4-DNT for 26 Months. Note the pseudoacinar arrangement of liver cells--hepatocellular carcinoma. H and E stain, 100 x.



12

Figure 12 - Photograph of Testes from a Control and a High Dose Rat Fed 0.07% 2,4-DNT for 12 Months. Note the difference in size between two pairs of testes--testicular atrophy.



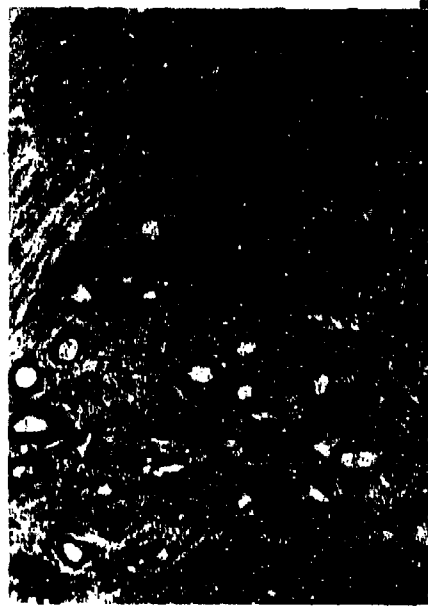
14

Figure 14 - Photograph of a High Dose Rat Fed 2,4-DNT. Note the multiple mammary gland masses--mammary gland tumor.



13

Figure 13 - Photomicrograph of Atrophic Testis from a High Dose Rat Fed 0.07% 2,4-DNT for 12 Months. Note the disappearance of all spermatozoic cells in seminiferous tubules and relative prominent interstitial tissue. H and E stain, 100 x.



15

Figure 15 - Photomicrograph of Mammary Gland. Note the typical architecture of fibroadenoma. H and E stain, 100 x.

V. MOUSE STUDIES

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V. MOUSE STUDIES

A. Observations and Toxic Signs

During the first 5 weeks of the study, five mice (one female, four males) were found dead. The death of the female is unexplained; the males had trauma from fighting. These deaths were judged unrelated to the test and the animals replaced by extra mice fed the appropriate dosage mixtures. Fighting was controlled by removing the aggressive individuals to separate cages.

During week 28, we noted that high dose (0.5% 2,4-DNT in feed) male No. 73-608 had orange stains, presumably from his urine, on his fur and proximal tail. His weight had dropped from 29 g to 23 g in 2 weeks. He died in the beginning of week 29, with a terminal weight of 19 g. From this time, we began to have frequent deaths in the high dose animals, as well as the sporadic unscheduled deaths in the other groups, as shown in Figures 16 and 17. Some of these deaths occurred at night without premonitory signs; the tissues were lost to autolysis. This occurs much more quickly in mice than in larger animals. Cannibalization by cage mates also destroyed tissues before histopathological analysis.

During months 8 through 12, when most of the high dose mice died, we often saw a characteristic syndrome. The mice had low body weight, often with recent loss, and red-orange stains, apparently from urine, as seen earlier with No. 73-608. They were hunchbacked and relatively inactive, often resting with their feet tucked in and hair erect as if chilled. When stimulated, the mice became hyperactive, running around the cage with a peculiar, stiff-legged gait. Often their eyes appeared dark and sunken.

B. Body Weights

Body weights of male and female mice fed 2,4-DNT are shown in Figures 18A and 18B, respectively. The control mice gained weight quickly, then leveled off near 45 g (male) or 36 g (female) after 6 months. Thereafter, the weights fluctuated with a slight downward drift. Many of the oscillations in the later months were caused by individual mice losing weight shortly before death.

Male mice fed the low dose (0.01% 2,4-DNT) had consistently lower body weights from month 12 to the end of the study. The averages for the low dose females varied about the control values. The first parts of these curves are omitted for clarity.

Male mice fed the middle dose (0.07% 2,4-DNT) weighed less than the control mice from month 3. The corresponding females were so similar to the control mice that the curve is omitted for clarity.

The high dose mice (0.5% 2,4-DNT) had greatly decreased weight gain; some mice even went below their starting weights. They did reach relatively constant average of 31 g (male) or 28 g (females).

C. Feed Consumption and Compound Intake

The averages of the monthly feed consumption measurements from throughout the entire study, as shown in Table 86, were quite similar in all dose groups. However, there was a pronounced dose effect in the first few weeks of the study with the high dose mice eating distinctly less (Table 87). During the later months, the relatively high consumptions of the few surviving high dose mice contributed to raise the overall averages. Average intakes of 2,4-DNT, calculated from the various monthly measures of feed consumption and body weight during the entire study are listed in Table 86. The monthly individual data are shown in Figure 19. There were small differences in intake between sexes, and relatively small oscillations around the average values of about 13.5, 95 and 900 mg/kg/day for the low, middle and high dose groups, respectively.

D. Laboratory Data

Laboratory data from mice dying at unscheduled times are listed in Table 88. Most noteworthy were the groups of high dose mice (0.5% 2,4-DNT) culled in week 40. The picture was anemia, with decreased erythrocytes and hemoglobin. The body compensated by increasing production; many immature erythrocytes (reticulocytes) with their characteristically large cell volumes (MCV) were seen. The cause of the anemia is apparent from the occasional methemoglobinemia and the high level of Heinz bodies, sometimes affecting nearly half of the erythrocytes. In later months, similar effects are seen in some middle dose (0.07% 2,4-DNT) mice, although the occurrence of Heinz bodies is more irregular.

Laboratory data from male and female mice after feeding of 2,4-DNT for 12 months are shown in Tables 89 and 90, respectively. Results in the high dose mice were similar to those seen in the moribund mice shown in Table 88. There was a toxic anemia, evidenced by some methemoglobin, many Heinz bodies and reticulocytes, as well as decreased erythrocyte count and hemoglobin. As usual, the increased proportion of reticulocytes raised the mean cell volume and hemoglobin, but decreased mean cell hemoglobin concentration. The increased BUN in the males might be toxicologically significant, but it was not seen in the females. The extremely

high average SGPT value in the middle dose males was due to No. 72-404's level of 775 IU/liter. Although this was the highest value seen, most groups had one mouse with an elevated SGPT. This high value is presumably not related to 2,4-DNT.

Results after feeding for 24 months are shown in Tables 91 and 92. The middle dose males had a low grade anemia, but the middle dose females were similar to controls. This represents a minor effect in the males, despite the lack of statistical significance. As discussed above, anemic effects were seen in high dose rats and some moribund middle dose rats (Table 88).

Results from the 1 month recovery studies after feeding of 2,4-DNT for 12 or 24 months are shown in Tables 93 through 96. Partial recovery from the toxic anemia had occurred.

E. Pathology

Of the original groups of 58 mice, histopathology is available on 33, 33, 29 and 40 males of the control, low, middle and high dose groups, respectively, and 31, 29, 31 and 33 females.

1. Feeding For 12 Months

a. Organ Weights

Average organ weights of mice after feeding of 2,4-DNT for 12 months are given in Table 97. Heart weights and some brain weights were accidentally omitted. Some mice had an unfortunate practice of dying the night before weighing; there were no organ weights on some. The high dose mice had decreased body weights and increased liver weights relative to body weights. The males have decreased testis weights. The variation in spleen weight may not be toxicologically important, since this organ normally shows much variation in size. The trend in the changes of body weight, liver weight and testis weight in the mice allowed to recover for 1 month remained (Table 98). However, the changes were usually not statistically significant. The significance of decrease in kidney weight of the mice allowed to recover for 1 month is not known.

b. Tissue Lesions

The lesions in mice fed 2,4-DNT for 12 months are summarized in Tables 99 and 100. The major lesions associated with the feeding of 2,4-DNT were testicular atrophy in all the high dose males and one middle dose male, pigmentation at many sites of both sexes (particularly spleen and liver, but including adrenal gland, brain, bone marrow, eye and lymph

node). Pinworms were absent in high dose mice, although they were found in seven of the other 24 mice. In addition, one high dose male and one high dose female had benign liver cell tumors and one high dose male had a renal carcinoma. There was increased incidence and greater severity of hepatocellular dysplasia and nephropathy in the high dose mice. These lesions were typical of those found in mice fed 2,4-DNT for 24 months and in mice that died at unscheduled times. The lesions will be more fully described below. A variety of non-treatment related lesions were found. Some lesions, such as hepatic inflammation and renal perivascular cuffing, were found in most mice. Other lesions, particularly various degenerative lesions, were found scattered throughout the various groups.

The lesions of mice fed 2,4-DNT for 12 months and allowed to recover for 1 month are summarized in Tables 101 and 102. The treatment related lesions in the testis, liver, kidney, intestine and other tissues seen in mice not allowed to recover (Tables 98 and 99), were also seen in these mice allowed to recover for 1 month (Tables 101 and 102). The severity of these lesions, except the pigmentation in various tissues, was often less.

2. Feeding For 24 Months Including Unscheduled Deaths

a. Organ Weights

The organ weights from mice after feeding for 24 months are given in Table 103. With all the high dose mice dead, there were no significant differences among the various organ weights. The organ weights from mice allowed to recover for 1 month after feeding for 24 months are listed in Table 104. There were statistically significant differences in spleen weights. The high average and large variation in kidney weights of the middle males were due to No. 72-423, whose right kidney weighed 3.03 g, due to a cystic carcinoma (Table 106). The large variation in ovary weight was due to the sporadic incidence of cysts in these geriatric mice; a typical example was No. 72-520, whose right ovary weighed 0.87 g and left ovary a normal 0.05 g.

b. Tissue Lesions

Lesions from mice fed 2,4-DNT for 24 months are summarized in Tables 105 through 107. The lesions were similar to those from the high dose mice died at unscheduled times. The most striking effect was the kidney tumors in the middle dose males (0.07%); many of the kidneys were grossly cystic. No mice from the high dose group survived at 24 months. Lesions from mice allowed to recover for 1 month are summarized in Table 108 and were generally similar to those from mice not allowed to recover. Lesions from mice dying at unscheduled times, including most of the high dose mice, are summarized in Tables 109 through 113. Incidence of treatment related lesions is summarized in Table 114.

(1) Liver

The liver was much more affected in males than in females (Table 114). Even the low dose (0.01% 2,4-DNT) males showed a significant increase in what we have termed "liver dysplasia," a lesion which took several forms. The usual form was various hyperplastic and degenerative changes including great variability of nuclear configuration, focal hepatocyte necrosis, swelling, slight fatty change, hepatocyte regeneration and hyperplasia with some tendency to nodular regeneration. A few mice had a zonal pattern of necrosis, affecting the centrolobular area only. These effects were more intense in the high dose mice, which had greatly enlarged hepatocytes with varying intensity of staining. Some had coagulation necrosis or coarse-droplet fatty change, rare lesions in the liver. There were some signs of recovery (hypertrophy of uniformly staining hepatocytes with rich ergastoplasm, suggesting increased protein synthesis) in three of the male mice fed the low dose for 24 months and allowed to recover for 1 month.

This liver dysplasia did not correlate well with the incidence of liver cell tumors. However, these tumors were seen earlier in the high dose mice. The tumors were probably hepatomas, but, in view of results on rats, some might be classified as hepatocellular carcinomas.

(2) Kidney

Very unusual lesions were found in the kidneys of many males, which were grossly cystic. Some kidneys were difficult to identify, except by their anatomical location and connections (Figure 21). The middle dose mice had high incidence of renal tumors (Table 114) including cystic papillary adenomas (Figure 22), solid renal cell carcinomas (Figure 23), and cystic papillary carcinomas. High dose mice had a few, presumably because they died before the lesions developed. Low dose males also had a few tumors.

More widespread than the frank tumors was a "toxic nephropathy" in both high dose males and high dose females (Table 114). This consisted of the presence of many cysts lined with cobblestone-like tubular epithelium, appearing much like the multilocular cysts seen in man. They were readily distinguished from urinary retention cysts, which were smaller and lined with flattened epithelium. Many high dose male mice also had atypical epithelium lining the cysts and occasionally elsewhere in the kidney (Table 114). These atypical epithelial cells were quite irregular in size, shape and staining characteristics. Some cells were polyploid or heteropoloid. Histology alone could not consider these cells malignant, but this could be a premalignant metaplasia.

(3) Abnormal Pigmentation

One striking effect in the liver and other organs of mice fed 2,4-DNT was the presence of an unusual pigment especially in the high dose mice (Table 114). The pigment occurred in golden brown to blackish brown coarse granules (Figure 20). The amount of pigment was proportional to dose and period of dosing. Its chemical identity is unknown; the primary possible sources are 2,4-DNT metabolites and heme-derived pigments. It was earlier described as hemosiderin,^{4/} but gave little if any reaction to Prussian blue, indicating the absence of iron, although normal splenic hemosiderin reacted intensely. There was a slight reaction to the Schiff procedure,^{19/} indicating the presence of aldehyde groups, found in 2,4-DNT metabolites and in protein metabolites. There was no reaction to stains for bile and mucopolysaccharides. Heaviest deposits were found in the reticulo-endothelial system, the liver, spleen, kidney, zona intermedia of the adrenal, and the medullary cords and reticulo-endothelial cells of the lymph nodes. The pituitary, thyroid and pancreas were generally pigment-free. Pulmonary macrophages had much pigment, but not cardiac macrophages. Some pigment apparently crossed the blood-brain barrier, for it was found in the cytoplasm of some neurons and glial cells. The greatest neural accumulation was in the neurons of a peri-adrenal sympathetic ganglion. These neuronal deposits resemble the lipofuscin ("brown atrophy pigment") seen in elderly humans; perhaps this is an effect of debilitation. Pigment within the kidneys was seen in the tubular epithelium, in tubular casts and within macrophages, but rarely in glomeruli. The same substance was probably responsible for both the kidney pigment and the urine stains on the fur. Possible excretion was also seen in the serous epithelium of the submandibular salivary gland, but not the mucinous epithelium. Despite this widespread accumulation of pigment, there was no evidence that it had any pathological consequence. No degeneration, necrosis or inflammation was seen in the areas of pigment accumulation. Despite massive accumulation of the pigment in macrophages of the spleen, lymph node and bone marrow, there were no apparent effects even on the sensitive cells of the lympho- and hematopoietic series.

(4) Gonads

There were similar 2,4-DNT related effects in the gonads of both sexes. In males, there was atrophy and aspermatogenesis of the testes. This resembles the usual senile effect, but it was seen in most high dose males (Table 114) and it was more severe in the males fed 2,4-DNT for 12 months (Tables 99 and 101). In more than half the high dose females, there was an analogous lesion; non-functioning follicles with lacking of corpora lutea (Table 114), implying a cessation of oogenesis.

(5) Intestinal Parasites

One possible effect of 2,4-DNT could be considered beneficial; a decrease in pinworms (only *Aspicularis tetraptera* was identified) from about 40% in control, low and middle dose mice to less than 10% in high dose mice. Although occasional pigment was seen in the intestinal lumen and mucosa, the pinworms never ingested it.

(6) Lesions Not Related to 2,4-DNT

A large variety of other lesions were seen in these geriatric mice. The occasional incidence of tumors is summarized in Table 115. Middle and high dose males had a lower incidence of bronchoalveolar adenoma, but this seems to be within normal variation. The other tumors showed a very sporadic incidence.

Other lesions (Tables 99-102 and 105-113) included a high incidence of "aging changes" in various organs. This term is used to cover the minor and non-specific degenerative lesions found in geriatric animals. These lesions were sporadic and occasionally seen. Mice tend to have a high incidence of amyloid deposits. In the milder cases, these are found in the intestine, especially the ileum. A "generalized amyloidosis" with involvement of most visceral organs was found in mice fed 2,4-DNT.

F. Discussion

The major target organs of 2,4-DNT toxicity in the mouse are the hemoglobin of the blood, the kidney, the liver and the gonads. Males were more affected than females. The blood effects were those of toxic methemoglobinemia leading to Heinz bodies, reticulocytosis and a more or less compensated anemia, the same picture seen with larger species. The kidney effects (cysts, metaplasia and tumors) appeared to be species specific, while the liver effects were a milder analog of those in rats. The non-functioning gonads in most males and many females in the high dose group were quite serious. The combination of these apparent effects and the general toxicity, including lower feed consumption and weight gain, produced a considerably shortened life-span in the high dose mice and a suggestion of such effects in the middle dose mice. The pigment deposits, whatever their composition, did not seem to be "pathologic" in the strict sense. The decrease in intestinal pinworms is presumably beneficial.

Because of the presence of a few kidney tumors in the low dose males, but not the control males, we cannot consider the low dose a "no effect dose." However, it is probably very close to the actual no-effect dose. If the feed concentrations in this study were the same as in the rat, rather than one step higher, we would probably have found that the 0.0015% 2,4-DNT in mice was a no-effect dose.

G. Conclusions

The low dose, with 2,4-DNT intake of about 13.5 mg/kg/day in both male and female mice, was slightly toxic, with effects in the kidneys to cause cystic dysplasia and tumors. The middle and high doses with 2,4-DNT intake of about 95 or 900 mg/kg/day, also produced decreased feed consumption, decreased weight gain, shortened life span, behavioral effects (depression with hyperexcitability), toxic methemoglobinemia, liver dysplasia, non-functioning gonads, pigment deposits of undefined origin, and a decrease in intestinal pinworms. Males were more seriously affected than females.

TABLE 86

FEED CONSUMPTION AND COMPOUND INTAKE OF MICE
FED 2,4-DNT FOR 24 MONTHS

<u>Dose</u> <u>(% in feed)</u>	<u>Males</u>		<u>Females</u>	
	<u>Feed</u> <u>Consumption</u> <u>(g/mouse/day)</u>	<u>2,4-DNT</u> <u>Intake</u> <u>(mg/kg/day)</u>	<u>Feed</u> <u>Consumption</u> <u>(g/mouse/day)</u>	<u>2,4-DNT</u> <u>Intake</u> <u>(mg/kg/day)</u>
0	5.10 ± 0.13 ^{a/}	--	4.62 ± 0.21	--
0.01	5.26 ± 0.12	13.3 ± 0.3	4.59 ± 0.11	13.7 ± 0.4
0.07	5.22 ± 0.11	96.9 ± 2.1	4.43 ± 0.09	93.8 ± 2.6
0.5	5.22 ± 0.19 ^{b/}	885 ± 26 ^{b/}	4.66 ± 0.26 ^{c/}	911 ± 25 ^{c/}

^{a/} Mean ± standard error of 24 measurements; the 1st month is the average of four measurements.

^{b/} Due to unscheduled deaths, only 15 measurements.

^{c/} Due to unscheduled deaths, only 20 measurements.

TABLE 87

FEED CONSUMPTION IN THE 1ST MONTH OF FEEDING 2,4-DNT

<u>Dose</u> <u>(% in feed)</u>	<u>Feed Consumption (g/mouse/day)</u>	
	<u>Males</u>	<u>Females</u>
0	4.83 $\pm$ 0.28 ^{a/}	4.75 $\pm$ 0.26
0.01	4.85 $\pm$ 0.28	4.55 $\pm$ 0.22
0.07	4.78 $\pm$ 0.22	4.33 $\pm$ 0.25
0.5	3.68 $\pm$ 0.42	3.65 $\pm$ 0.27 ^{b/}

^{a/} Mean $\pm$ standard error of four weekly measurements.

^{b/} Significantly different from control by Dunnett's multiple comparison procedure.

TABLE 88

LABORATORY DATA OF MICE FED 2,4-DNT AND DYING AT UNSCHEDULED TIMES

Dose (Z in feed):	0.5	0.5	0.5	0.5	0.5
Mouse No.:	738	731	a/	d/	e/
Week of Death:	36	38	40	40	44
Erythrocytes, $\times 10^6/\text{mm}^3$	6.81	7.04	5.03 $\pm$ 0.39 (2.76-6.87)	5.62 $\pm$ 0.25 (5.13-6.46)	5.61 $\pm$ 0.3
Heinz bodies, %	5.41	12.96	33.78 $\pm$ 2.39 (21.39-48.33) b/	5.51 $\pm$ 1.33 (2.67-10.56)	21.51 $\pm$ 4.89
Reticulocytes, %	1.85	3.30	2.23 $\pm$ 0.78 (0.33-8.75)	2.05 $\pm$ 0.31 (1.41-3.20)	4.78 $\pm$ 1.46
Hematocrit, vol %	40.0	42.0	37 $\pm$ 2 (24-49)	39 $\pm$ 1 (37-41)	40 $\pm$ 0
Hemoglobin, g %	13.2	13.8	11.1 $\pm$ 0.7 (6.9-15.1)	11.3 $\pm$ 0.3 (10.7-12.5)	12.1 $\pm$ 0.3
Methemoglobin, %	9.1	1.5	1.1 $\pm$ 0.6 (0-5.0) c/	0	0.5 $\pm$ 0.5
MCV, cubic microns	58.7	59.7	74.8 $\pm$ 3.4 (60.7-97.8)	70.0 $\pm$ 1.8 (63.5-73.6)	72.2 $\pm$ 2.9
MCBH, micromicrograms	19.4	19.6	22.7 $\pm$ 1.2 (18.5-30.8)	20.2 $\pm$ 0.4 (19.3-21.2)	21.6 $\pm$ 0.6
MCHBC, g %	33.0	32.9	30.3 $\pm$ 0.4 (28.3-32.8)	29.0 $\pm$ 0.5 (27.9-30.5)	29.9 $\pm$ 0.6
Platelets, $\times 10^5/\text{mm}^3$	--	11.30	4.43 $\pm$ 0.51 (2.35-8.45)	3.97 $\pm$ 0.80 (2.35-6.45)	19.68 f/
Leukocytes, $\times 10^3/\text{mm}^3$	2.5	97.0	2.7 $\pm$ 0.3 (1.0-4.3)	2.9 $\pm$ 0.2 (2.4-3.6)	4.4 $\pm$ 0.2
Neutrophils, %	45	73	48 $\pm$ 3 (37-71)	35 $\pm$ 2 (30-41)	37 $\pm$ 6
Lymphocytes, %	54	19	52 $\pm$ 3 (29-63)	64 $\pm$ 2 (58-70)	61 $\pm$ 6
Bands, %	0	8	0	0	0.3 $\pm$ 0.3
Monocytes, %	1	0	0	0.4 $\pm$ 0.4 (0-2)	2 $\pm$ 1
Eosinophils, %	0	0	0	0.4 $\pm$ 0.2 (0-1)	0.3 $\pm$ 0.3
Basophils, %	0	0	0	0	0
Atypical, %	0	0	0	0	0
Nucleated RBC, %	0	0	0	0	0
SGPT, IU/L	--	86	213 $\pm$ 22 (114-362) b/	115 $\pm$ 17 (90-164)	182 $\pm$ 46
BUN, mg %	--	49	62 $\pm$ 8 (40-131)	31 $\pm$ 4 (19-40)	45 $\pm$ 6

TABLE 88 (continued)

Dose (% in feed)	0.01	0.5	0	0.5	0.01	0.5	0.07	0.07	0	0.07	0.07
Mouse No.:	342	646	135	628	338	747	421	529	145	530	437
Week of Death:	48	48	49	49	64	70	74	74	85	85	93
Erythrocytes, $\times 10^6/\text{mm}^3$	2.44	4.42	3.65	4.80	2.65	6.79	2.77	6.14	4.55	5.88	2.94
Heinz bodies, %	0	2.70	0	10.16	0	5.33	0	95.00	--	--	0
Reticulocytes, %	0.85	3.05	2.92	2.98	1.33	1.48	0.67	5.00	2.55	2.32	15.5
Hematocrit, vol %	17	30	23	34	--	39	18	--	29	39	24
Hemoglobin, g %	5.7	9.6	7.3	11.5	5.9	12.8	6.5	11.9	8.9	11.8	7.9
Methemoglobin, %	0	25	0	5.2	8.5	9.4	0	10.5	--	--	6.3
MCV, cubic microns	69.7	67.9	63.0	70.8	--	57.4	65.0	--	63.7	66.3	81.6
MCHB, micromicrograms	23.4	21.7	20.0	24.0	22.3	18.9	23.5	19.4	19.6	20.1	26.9
MCHBC, g %	33.5	32.0	31.7	33.8	--	32.8	36.1	--	30.7	30.3	32.9
Platelets, $\times 10^5/\text{mm}^3$	3.15	7.70	3.25	16.95	3.70	3.35	2.60	12.40	4.75	4.20	2.25
Leukocytes, $\times 10^3/\text{mm}^3$	2.9	3.6	2.0	3.5	93.8	1.7	2.4	3.4	2.8	3.4	11.4
Neutrophils, %	16	19	35	55	74	12	46	14	14	34	46
Lymphocytes, %	84	80	65	44	17	86	54	84	85	65	48
Bands	0	0	0	0	7	0	0	0	0	0	0
Monocytes, %	0	0	0	0	2	2	0	1	0	0	0
Eosinophils, %	0	1	0	0	0	0	0	1	1	1	6
Basophils, %	0	0	0	1	0	0	0	0	0	0	0
Atypical, %	0	0	0	0	0	0	0	0	0	0	0
Nucleated RBC, %	0	1	0	0	0	0	0	0	0	0	0
SGPT, IU/l	--	--	62	46	--	99	--	28	179	62	83
BUN, mg %	--	--	25	48	--	21	--	--	80	80	70

TABLE 88 (concluded)

Dose (Z in feed):	0.07	0.01	0.07	0.07
Mouse No.:	514	245	419	432
Week of Death:	98	100	100	103
Erythrocytes, $\times 10^6/\text{mm}^3$	5.63	6.60	3.75	6.73
Heinz bodies, %	0.37	0	0	0
Reticulocytes, %	3.31	6.00	15.00	1.65
Hematocrit, vol %	38	39	23	39
Hemoglobin, g %	13.4	13.5	7.7	13.3
Methemoglobin, %	7.2	0	0	0
MCV, cubic micron	67.5	59.1	61.3	57.9
MCHB, micromicrograms	23.8	20.5	20.5	19.8
MCHBC, g %	35.3	34.6	33.5	34.1
Platelets, $\times 10^5/\text{mm}^3$	3.10	2.85	2.05	5.55
Leukocytes, $\times 10^3/\text{mm}^3$	2.2	1.6	2.6	1.9
Neutrophils, %	70	40	62	73
Lymphocytes, %	30	60	38	27
Bands, %	0	0	0	0
Monocytes, %	0	0	0	0
Eosinophils, %	0	0	0	0
Basophils, %	0	0	0	0
Atypical, %	0	0	0	0
Nucleated RBC, %	0	0	0	1
SGPT, IU/l	—	48	120	291
BUN, mg %	—	96	—	41

a/ Mean $\pm$ standard error (range) of four males and seven females.

b/ Data of 10 mice.

c/ Data of 10 mice; three non-zero mice were 3.8 ± 0.6 .d/ Mean $\pm$ standard error (range) of one male and four females.e/ Mean $\pm$ standard error of three males.

f/ Mean of two mice.

TABLE 89

LABORATORY DATA OF MALE MICE FED 2,4-DNT FOR 12 MONTHS

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

DOSE: % IN FEED	0.0 (C. 4)	0.01 (T. 4)	0.07 (T. 4)	0.5 (T. 4)
ERYTHROCYTES (X10 ⁶ /MM ³)	6.23 ± .28	6.63 ± .40	7.06 ± .20 (3)	5.28 ± .25 (3)
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	7.79 ± 2.79 ^{a/}
RETICULOCYTES, %	1.46 ± .22 (3)	1.06 ± .08	1.87 ± .14 (3)	4.10 ± .81 (3) ^{a/}
HEMATOCRIT, VOL. %	40.3 ± 2.1	41.0 ± 1.6	45.0 ± 1.2 (3)	36.0 ± 2.0 (3)
HEMOGLOBIN, GM. %	13.5 ± .6	13.0 ± .6	13.6 ± .7 (3)	11.5 ± .5 (3)
METHENOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	4.2 ± 1.7 ^{a/}
MCV, CUBIC MICRONS	64.7 ± 2.4	62.2 ± 1.9	63.8 ± 1.5 (3)	68.1 ± .7 (3)
MCHC, MICRO MICROGMS.	21.7 ± .9	19.8 ± .5	19.2 ± .7 (3)	21.8 ± 1.4 (3)
MCHBC, GM %	33.6 ± .6	31.8 ± .3	30.1 ± .8 (3)	32.0 ± 2.1 (3)
PLATELETS (X10 ⁵ /MM ³)	5.0 ± .5	7.1 ± .8	6.8 ± .5 (3)	11.6 ± 2.6 (3) ^{a/}
LEUKOCYTES (X10 ³ /MM ³)	3.6 ± .5	3.8 ± .7	5.0 ± .8 (3)	3.6 ± .9 (3)
NEUTROPHILS, %	31.8 ± 9.0	18.5 ± 2.3	29.7 ± 3.8 (3)	30.8 ± 9.0
LYMPHOCYTES, %	66.3 ± 9.3	81.5 ± 2.3	69.7 ± 3.8 (3)	68.3 ± 8.9
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	2.0 ± .4	.3 ± .3 ^{a/}	.5 ± .3	1.0 ± .4
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
SGPT, IU/L	128 ± 53 (3)	86 ± 16 (3)	315 ± 230 (3)	134 ± 14 (3)
BUN, MG %	23.0 ± 1.2	19.3 ± 2.3 (3)	37.0 ± 8.9 (3)	54.3 ± 14.9 (3) ^{a/}

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL MICE BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

ENTRIES ARE MEAN ± STANDARD ERROR.

TABLE 90

LABORATORY DATA OF FEMALE MICE FED 2,4-DNT FOR 12 MONTHS

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF MICE	
DOSE: % IN FEED	0.0 (C. 4)	0.01 (T. 4)	0.07 (T. 4)	0.5 (T. 4)
ERYTHROCYTES (X10 ⁶ /MM ³)	6.61 ± .19	7.32 ± .38 (3)	7.22 ± .12	5.18 ± .19 (2) ^{a/}
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	5.88 ± 3.41
RETICULOCYTES, %	1.08 ± .16	1.19 ± .23 (3)	1.17 ± .11	3.46 ± .70 (3) ^{a/}
HEMATOCRIT, VOL. %	41.3 ± 1.3	43.7 ± 2.6 (3)	42.3 ± 1.7	44.7 ± .9 (3)
HEMOGLOBIN, GM. %	12.5 ± .3	13.1 ± .7 (3)	13.3 ± .2	11.8 ± .3 (2)
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.2 ± 2.1
MCV, CUBIC MICRONS	62.4 ± .4	59.6 ± .4 (3)	58.6 ± 2.9	87.9 ± 2.3 (2) ^{a/}
MCHC, MICRO MICROGMS.	18.9 ± .2	17.9 ± .1 (3) ^{a/}	18.4 ± .1	22.8 ± .3 (2) ^{a/}
MCHBC, GM %	30.3 ± .3	30.0 ± .4 (3)	31.6 ± 1.5	25.9 ± .4 (2) ^{a/}
PLATELETS (X10 ⁵ /MM ³)	5.3 ± .1	5.4 ± .5 (3)	7.6 ± .9	15.6 ± 4.8 (3)
LEUKOCYTES (X10 ³ /MM ³)	2.7 ± .3	3.8 ± .8 (3)	4.0 ± .7	3.5 ± .0 (2)
NEUTROPHILS, %	23.5 ± 2.8	34.7 ± 5.5 (3)	26.0 ± 1.6	23.0 ± 3.2 (3)
LYMPHOCYTES, %	73.5 ± 3.4	64.0 ± 5.0 (3)	72.0 ± 2.5	76.7 ± 3.2 (3)
BANDS, %	.3 ± .3	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0
EOSINOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	2.8 ± .6	1.0 ± .6	1.5 ± 1.2	.3 ± .3
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
SGPT, IU/L	111 ± 17	142 ± 36 (3)	70 ± 13	40 ± 6 (2)
BUN, MG %	24.5 ± 3.2	25.7 ± 3.2 (3)	27.3 ± 2.8	30.5 ± 4.5 (2)

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL MICE BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

ENTRIES ARE MEAN ± STANDARD ERROR.

TABLE 91

LABORATORY DATA OF MALE MICE AFTER FEEDING 2,4-DNT FOR 24 MONTHS

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF MICE
DOSE: % IN FEED	0.0 (C, 4)	0.01 (T, 4)	0.07 (T, 4)
ERYTHROCYTES ($\times 10^6$ /MM ³)	6.86 $\pm$.73	7.09 $\pm$.20	6.11 $\pm$.63
HEINZ BODIES, %	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
RETICULOCYTES, %	1.28 $\pm$.38	1.25 $\pm$.32	2.03 $\pm$.70
HEMATOCRIT, VOL. %	37.8 $\pm$ 3.3	39.3 $\pm$ 1.2	35.5 $\pm$ 3.0
HEMOGLOBIN, GM. %	12.2 $\pm$ 1.2	12.8 $\pm$.4	11.7 $\pm$.9
METHEMOGLOBIN, %	0.0 $\pm$ 0.0	1.4 $\pm$ 1.4	0.0 $\pm$ 0.0
MCV, CUBIC MICRONS	55.4 $\pm$ 1.4	55.4 $\pm$ 1.0	58.5 $\pm$ 1.7
MCHB, MICRO MICROGMS.	17.9 $\pm$.3	18.1 $\pm$.4	19.3 $\pm$.7
MCHBC, GM %	32.2 $\pm$.4	32.6 $\pm$.1	33.0 $\pm$.4
PLATELETS ($\times 10^3$ /MM ³)	2.8 $\pm$.4	5.9 $\pm$.5 ^{a/}	4.8 $\pm$.5 ^{a/}
LEUKOCYTES ($\times 10^3$ /MM ³)	2.9 $\pm$.9	2.6 $\pm$.5	3.7 $\pm$.8
NEUTROPHILS, %	31.8 $\pm$ 4.5	27.5 $\pm$ 2.8	32.3 $\pm$ 2.3
LYMPHOCYTES, %	68.0 $\pm$ 4.5	72.3 $\pm$ 3.0	67.8 $\pm$ 2.3
BANDS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
EOSINOPHILS, %	0.0 $\pm$ 0.0	.3 $\pm$.3	0.0 $\pm$ 0.0
BASOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MONOCYTES, %	.3 $\pm$.3	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
ATYPICAL, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
NUCLEATED RBC, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
SGOT, IU/L	180 $\pm$ 66	121 $\pm$ 18	143 $\pm$ 26 (3)
SGPT, IU/L	68.0 $\pm$ 28.1	38.0 $\pm$ 5.6 (3)	71.0 $\pm$ 14.3 (3)
HUN, MG %	40.8 $\pm$ 3.8	37.0 $\pm$ 6.5 (3)	54.3 $\pm$ 16.3 (3)

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL MICE BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

ENTRIES ARE MEAN $\pm$ STANDARD ERROR.

TABLE 92

LABORATORY DATA OF FEMALE MICE AFTER FEEDING 2,4-DNT FOR 24 MONTHS

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF MICE
DOSE: % IN FEED	0.0 (C. 4)	0.01 (T. 4)	0.07 (T. 4)
ERYTHROCYTES (X10 ⁶ /MM ³)	7.67 ± .28	7.43 ± .30	7.90 ± .19
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	1.07 ± .13	1.42 ± .27	1.00 ± .11
HEMATOCRIT, VOL. %	43.0 ± 1.1	41.5 ± 1.0	44.3 ± 1.7
HEMOGLOBIN, GM. %	13.9 ± .4	13.4 ± .5	14.5 ± .5
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	56.1 ± 1.3	56.0 ± 1.4	56.0 ± 1.2
MCHC, MICRO MICROGMS.	18.1 ± .5	18.1 ± .2	18.3 ± .3
MCHC, GM %	32.3 ± .3	32.4 ± .4	32.7 ± .2
PLATELETS (X10 ⁵ /MM ³)	6.2 ± 1.0	5.9 ± .3	6.0 ± .3
LEUKOCYTES (X10 ³ /MM ³)	3.7 ± .8	2.5 ± .5	3.0 ± .4
NEUTROPHILS, %	37.0 ± 5.4	29.5 ± 5.2	35.0 ± 4.4
LYMPHOCYTES, %	62.8 ± 5.7	70.3 ± 5.4	64.8 ± 4.5
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.3 ± .3	0.0 ± 0.0	.3 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0
SGOT, IU/L	261 ± 109	102 ± 7	170 ± 9
SGPT, IU/L	109 ± 37	60 ± 11	127 ± 38
BUN, MG %	28.3 ± 7.1	25.0 ± 1.4	24.0 ± 1.0

±/ SIGNIFICANTLY DIFFERENT FROM CONTROL MICE BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

ENTRIES ARE MEAN + STANDARD ERROR.

TABLE 93

LABORATORY DATA OF MALE MICE FED 2,4-DNT FOR 12 MONTHS
AND ALLOWED TO RECOVER FOR 1 MONTH

	(C.N) CONTROL		(T.N) TREATED		N = NUMBER OF MICE	
DISE: % IN FEED	0.0 (C. 4)		0.01 (T. 4)		0.07 (T. 4)	
ERYTHROCYTES (X10 ⁶ /MM ³)	4.76 ± .49		4.80 ± .91		5.37 ± .22	5.59 ± .58 (3)
HEINZ BODIES, %	0.00 ± 0.00		0.00 ± 0.00		0.00 ± 0.00	.34 ± .06 ^{a/}
RETICULOCYTES, %	.71 ± .12		1.31 ± .17 (3)		.90 ± .02	1.05 ± .36 (3)
HEMATOCRIT, VOL. %	44.0 ± 1.2		40.0 ± 3.5		45.0 ± .9	38.0 (1)
HEMOGLOBIN, GM. %	13.7 ± .3		12.6 ± .7		13.8 ± .3	12.5 ± .5 (3)
METHEMOGLOBIN, %	0.0 ± 0.0		0.0 ± 0.0		0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	94.5 ± 7.1		87.7 ± 8.5		84.3 ± 3.9	81.9 (1)
MCHB, MICRO MICROGMS.	29.4 ± 2.4		28.1 ± 3.8		25.8 ± 1.5	22.7 ± 2.0 (3)
MCHBC, GM %	31.1 ± .5		31.7 ± 1.2		30.6 ± .5	32.6 (1)
PLATELETS (X10 ⁵ /MM ³)	3.8 ± .7		3.1 ± .2		2.8 ± .1	2.4 ± .1 (3)
LEUKOCYTES (X10 ³ /MM ³)	4.5 ± .2		5.2 ± .4		4.6 ± .9	3.5 ± .7 (3)
NEUTROPHILS, %	13.8 ± 1.9		13.8 ± 6.8		16.0 ± 3.7	27.5 ± 3.9
LYMPHOCYTES, %	85.0 ± 2.0		83.8 ± 7.1		83.0 ± 3.6	72.0 ± 4.2
BANDS, %	0.0 ± 0.0		.3 ± .3		0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	0.0 ± 0.0		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
MONOCYTES, %	1.3 ± .5		2.3 ± .6		1.0 ± .7	.3 ± .3
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
SPT, IU/L	19 ± 2 (3)		51 ± 21 (3)		21 ± 4	119 ± 11 (2)
BUN, MG %	22.0 ± 2.1		28.8 ± 5.6		29.3 ± 1.9	37.0 ± 1.0 (2)

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL MICE BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

ENTRIES ARE MEAN ± STANDARD ERROR.

TABLE 94

LABORATORY DATA OF FEMALE MICE FED 2,4-DNT FOR 12 MONTHS AND ALLOWED TO RECOVER FOR 1 MONTH

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF MICE	
DOSE: % IN FEED	0.0 (C, 4)	0.01 (T, 4)	0.07 (T, 4)	0.5 (T, 4)
ERYTHROCYTES ($\times 10^6$ /MM ³)	5.75 $\pm$.24	5.13 $\pm$.26	6.24 $\pm$.43	6.61 $\pm$.27
HEINZ BODIES, %	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
RETICULOCYTES, %	.79 $\pm$.11	1.05 $\pm$.13 (3)	.77 $\pm$.11	1.23 $\pm$.26
HEMATOCRIT, VOL. %	45.5 $\pm$.5	46.0 $\pm$ 1.0 (3)	44.3 $\pm$.6	42.0 $\pm$ 1.5
HEMOGLOBIN, GM. %	14.1 $\pm$.1	13.7 $\pm$.3	13.4 $\pm$.6	12.8 $\pm$.6
METHEMOGLOBIN, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MCV, CUBIC MICRONS	79.6 $\pm$ 3.5	85.5 $\pm$ 2.7 (3)	71.7 $\pm$ 3.6	63.6 $\pm$ 1.5 ^{a/}
MCHB, MICRO MICROGMS.	24.7 $\pm$ 1.0	26.9 $\pm$ 1.2	21.6 $\pm$.9	19.4 $\pm$.2 ^{a/}
MCHBC, GM %	31.1 $\pm$.2	36.1 $\pm$.1 (3)	30.3 $\pm$ 1.0	30.5 $\pm$.6
PLATELETS ($\times 10^3$ /MM ³)	4.0 $\pm$.7	2.6 $\pm$.2	2.3 $\pm$.1 ^{a/}	3.1 $\pm$.3
LEUKOCYTES ($\times 10^3$ /MM ³)	5.2 $\pm$.3	4.6 $\pm$.2	4.0 $\pm$.6	6.7 $\pm$ 2.0
NEUTROPHILS, %	18.8 $\pm$ 1.4	31.5 $\pm$ 6.7	35.5 $\pm$ 5.6	32.8 $\pm$ 5.1
LYMPHOCYTES, %	77.5 $\pm$ 1.2	66.0 $\pm$ 7.9	62.5 $\pm$ 4.9	65.3 $\pm$ 5.0
BANDS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
EOSINOPHILS, %	2.5 $\pm$.5	2.0 $\pm$ 1.1	2.0 $\pm$ 1.2	.5 $\pm$.3
BASOPHILS, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
MONOCYTES, %	1.3 $\pm$.3	.5 $\pm$.5	0.0 $\pm$ 0.0	1.8 $\pm$.9
ATYPICAL, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
NUCLEATED RBC, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
SGPT, IU/L	36.0 $\pm$ 9.8	18.5 $\pm$ 9.5 (2)	30.5 $\pm$ 6.5 (2)	34.7 $\pm$ 6.4 (3)
BUN, MG %	22.0 $\pm$ 1.5	23.5 $\pm$ 3.5 (2)	24.7 $\pm$ 3.8 (3)	49.7 $\pm$ 8.5 (3) ^{a/}

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL MICE BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.ENTRIES ARE MEAN $\pm$ STANDARD ERROR.

TABLE 95

LABORATORY DATA OF MALE MICE AFTER FEEDING 2,4-DNT FOR 24 MONTHS AND ALLOWING TO RECOVER FOR 1 MONTH

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF MICE
DOSE: % IN FEED 6 3	0.0 (C, 3)	0.01 (T, 5)	0.07 (T, 3)
ERYTHROCYTES (X10 /MM)	6.97 ± .41	7.73 ± .75	6.75 ± .32
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	.31 ± .17 ^{a/}
RETICULOCYTES, %	1.84 ± .34	1.53 ± .46	3.32 ± 1.43 (2)
HEMATOCRIT, VOL. %	40.0 ± 2.5	44.0 ± 4.2	40.0 ± .6
HEMOGLOBIN, GM. %	13.5 ± .6	15.2 ± 1.5	13.8 ± .1
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	.6 ± .6
MCV, CUBIC MICRONS	57.4 ± .4	57.0 ± 1.4	59.5 ± 2.8
MCH, MICRO MICROGMS.	19.4 ± .5	19.6 ± .4	20.5 ± .9
MCHC, GM %	33.8 ± .9	34.4 ± .4	34.4 ± .5
PLATELETS (X10 /MM) 5 3	4.8 ± 1.1	5.5 ± .5	6.3 ± 1.3
LEUKOCYTES (X10 /MM) 3 3	2.6 ± .1	4.3 ± .8	1.7 ± 1.1
NEUTROPHILS, %	37.3 ± 6.2	40.8 ± 6.0	33.3 ± 4.4
LYMPHOCYTES, %	61.7 ± 6.2	57.6 ± 5.6	66.3 ± 4.7
HANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.0 ± .6	1.6 ± 1.6	.3 ± .3
BASOPHILS, %	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
ATYPICAL, %	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
SGOT, IU/L	176 ± 71	153 ± 35 (4)	257 ± 83
SGPT, IU/L	38.5 ± 10.5 (2)	84.8 ± 11.3 (4)	63.5 ± 26.5 (2)
BUN, MG %	22.0 ± 4.0 (2)	29.6 ± 5.1	47.5 ± 17.5 (2)

^{a/} SIGNIFICANTLY DIFFERENT FROM CONTROL MICE BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

ENTRIES ARE MEAN ± STANDARD ERROR.

TABLE 96

LABORATORY DATA OF FEMALE MICE AFTER FEEDING 2,4-DNT FOR 24 MONTHS AND ALLOWING TO RECOVER FOR 1 MONTH

	(C.N) CONTROL	(T.N) TREATED	N = NUMBER OF MICE
DOSE: % IN FEED	0.0 (C, 3)	0.01 (T, 2)	0.07 (T, 4)
ERYTHROCYTES (X10 ⁶ /MM ³)	6.48 ± .53	5.06 ± 1.36	5.42 ± .72
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	.19 ± .11
RETICULOCYTES, %	2.23 ± .58	3.24 ± 1.93	1.96 ± .10 (2)
HEMATOCRIT, VOL. %	37.3 ± 2.9	30.5 ± 7.5	32.5 ± 4.6
HEMOGLOBIN, GM. %	12.2 ± 1.0	10.9 ± 2.0	11.5 ± 1.3
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	1.1 ± .7
MCV, CUBIC MICRONS	57.6 ± .4	60.7 ± 1.5	60.1 ± 4.7
MCHB, MICRO MICROGMS.	18.9 ± .2	22.2 ± 1.9	21.4 ± 1.3
MCHBC, GM %	32.8 ± .5	36.5 ± 2.2	35.7 ± 1.2
PLATELETS (X10 ⁵ /MM ³)	4.6 ± .8	2.4 ± 1.2	4.6 ± 1.1
LEUKOCYTES (X10 ³ /MM ³)	3.1 ± 1.3	4.8 ± 3.2	1.2 ± .2
NEUTROPHILS, %	23.0 ± 10.4	24.0 ± 0.0	28.3 ± 10.4
LYMPHOCYTES, %	69.7 ± 6.2	76.0 ± 0.0	71.8 ± 10.4
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
BASOPHILS, %	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
ATYPICAL, %	7.3 ± 7.3	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
SGOT, IU/L	107 ± 10	142 ± 31	246 ± 51
SGPT, IU/L	38.0 ± 5.1	64.5 ± 9.5	44.5 ± 4.5 (2)
BUN, MG %	43.0 ± 11.2	82.5 ± 37.5	133.5 ± 83.5 (2)

±/ SIGNIFICANTLY DIFFERENT FROM CONTROL MICE BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

ENTRIES ARE MEAN ± STANDARD ERROR.

TABLE 97

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MICE FED 2,4-DNT FOR 12 MONTHS

Sex	Dose (% in feed)	Terminal Body Weight (gm)	Absolute Organ Weight (gm)					Ovary
			Brain	Liver	Kidney	Spleen	Testis	
Male	0	41 ± 1 ^{a/}	0.45 ± 0.02	1.60 ± 0.08	0.65 ± 0.01	0.13 ± 0.03	0.32 ± 0.06	
	0.01	38 ± 2 ^{a/}	0.42 ± 0.01	1.63 ± 0.25	0.63 ± 0.02	0.11 ± 0.03	0.26 ± 0.01	
	0.07	45 ± 1 ^{b/}	0.56	1.89 ± 0.33	0.63 ± 0.11	0.14 ± 0.02	0.35 ± 0.05	
	0.5	28 ± 2 ^{a,f/}	0.46 ± 0.05	1.72 ± 0.13	0.56 ± 0.08	0.16 ± 0.03	0.11 ± 0.01 ^{f/}	
Female	0	34 ± 2 ^{a/}	0.49 ± 0.02	1.43 ± 0.13	0.50 ± 0.06	0.10 ± 0.00		0.14 ± 0.08
	0.01	37 ± 3 ^{c/}	0.48 ± 0.05	1.55 ± 0.13	0.58 ± 0.06	0.14 ± 0.01		0.09 ± 0.03
	0.07	34 ± 1 ^{d/}	0.56 ± 0.17	1.62 ± 0.06	0.50 ± 0.04	0.20 ± 0.03 ^{f/}		0.17 ± 0.09
	0.5	29 ± 3 ^{e/}	0.46 ± 0.07	2.07 ± 0.37	0.33 ± 0.01	0.25 ± 0.06 ^{f/}		0.04 ± 0.02
Relative Organ Weight (gm/100 gm body weight)								
Male	0		1.10 ± 0.06	3.94 ± 0.22	1.60 ± 0.07	0.31 ± 0.08	0.80 ± 0.16	
	0.01		1.12 ± 0.05	4.25 ± 0.45	1.66 ± 0.12	0.30 ± 0.09	0.70 ± 0.04	
	0.07		1.24	4.23 ± 0.82	1.41 ± 0.28	0.32 ± 0.04	0.78 ± 0.12	
	0.5		1.72 ± 0.29	6.33 ± 0.69 ^{f/}	2.12 ± 0.43	0.57 ± 0.08	0.41 ± 0.05	
Female	0		1.48 ± 0.13	4.28 ± 0.38	1.49 ± 0.13	0.31 ± 0.03		0.39 ± 0.23
	0.01		1.20 ± 0.15	3.86 ± 0.41	1.45 ± 0.12	0.36 ± 0.03		0.22 ± 0.10
	0.07		1.86 ± 0.46	4.79 ± 0.19	1.48 ± 0.12	0.57 ± 0.06		0.48 ± 0.25
	0.5		1.57 ± 0.01	7.00 ± 0.17 ^{f/}	1.15 ± 0.14	0.82 ± 0.08 ^{f/}		0.12 ± 0.05
Relative Organ Weight (gm/gm brain weight)								
Male	0			3.61 ± 0.31	1.46 ± 0.05	0.30 ± 0.08	0.73 ± 0.15	
	0.01			3.89 ± 0.62	1.49 ± 0.06	0.27 ± 0.08	0.63 ± 0.05	
	0.07			4.08	1.36	0.29	0.73	
	0.5			3.80 ± 0.35	1.23 ± 0.16	0.36 ± 0.09	0.25 ± 0.03 ^{f/}	
Female	0			2.92 ± 0.24	1.02 ± 0.08	0.21 ± 0.01		0.28 ± 0.17
	0.01			3.26 ± 0.28	1.25 ± 0.21	0.30 ± 0.02		0.17 ± 0.05
	0.07			2.81 ± 0.55	0.92 ± 0.23	0.35 ± 0.04 ^{f/}		0.27 ± 0.10
	0.5			4.47 ± 0.13	0.74 ± 0.08	0.52 ± 0.06 ^{f/}		0.08 ± 0.03

a/ Mean ± standard error of four mice.

b/ Mean ± standard error of two mice, except only one brain weight.

c/ Mean ± standard error of three mice.

d/ Mean ± standard error of four mice, except brain weight for only three mice.

e/ Mean ± standard error of two mice.

f/ Significantly different from control mice by Dunnett's multiple comparison procedure.

TABLE 98

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MICE FED 2,4-DNT FOR 12 MONTHS
AND ALLOWED TO RECOVER FOR 1 MONTH

Sex	Dose (% in feed)	Terminal Body Weight (gm)	Absolute Organ Weight (gm)					Ovary
			Brain	Heart	Liver	Kidney	Spleen	Testis
Male	0	37 ± 2 ^{a/}	0.45 ± 0.02	0.18 ± 0.02	1.42 ± 0.09	0.66 ± 0.05	0.09 ± 0.02	0.24 ± 0.01
	0.01	46 ± 4 ^{b/}	0.47 ± 0.02	0.22 ± 0.02	1.99 ± 0.31	0.70 ± 0.06	0.16 ± 0.02	0.75 ± 0.04
	0.07	43 ± 1	0.50 ± 0.02	0.21 ± 0.01	1.65 ± 0.09	0.56 ± 0.04	0.15 ± 0.01	0.29 ± 0.01
	0.5	32 ± 1	0.41 ± 0.01	0.21 ± 0.05	1.47 ± 0.22	0.42 ± 0.03 ^{b/}	0.15 ± 0.04	0.18 ± 0.01
Female	0	35 ± 2	0.49 ± 0.02	0.15 ± 0.01	1.42 ± 0.12	0.51 ± 0.05	0.14 ± 0.02	0.05 ± 0.01
	0.01	38 ± 2	0.49 ± 0.02	0.14 ± 0.01	1.45 ± 0.08	0.44 ± 0.03	0.13 ± 0.02	0.12 ± 0.04
	0.07	36 ± 1	0.50 ± 0.03	0.18 ± 0.03	1.55 ± 0.05	0.48 ± 0.03	0.16 ± 0.00	0.09 ± 0.03
	0.5	29 ± 1	0.45 ± 0.01	0.17 ± 0.02	1.44 ± 0.11	0.32 ± 0.02 ^{b/}	0.17 ± 0.03	0.06 ± 0.03
Relative Organ Weight (gm/100 gm body weight)								
Male	0		1.22 ± 0.02	0.50 ± 0.04	3.82 ± 0.10	1.77 ± 0.11	0.24 ± 0.03	0.65 ± 0.02
	0.01		1.04 ± 0.08	0.48 ± 0.04	4.35 ± 0.65	1.54 ± 0.20	0.34 ± 0.04	0.55 ± 0.08
	0.07		1.16 ± 0.05	0.49 ± 0.02	3.81 ± 0.14	1.29 ± 0.06	0.35 ± 0.03	0.67 ± 0.04
	0.5		1.29 ± 0.05	0.69 ± 0.13	4.57 ± 0.47	1.31 ± 0.07	0.46 ± 0.10 ^{b/}	0.58 ± 0.04
Female	0		1.41 ± 0.09	0.41 ± 0.01	4.04 ± 0.12	1.44 ± 0.05	0.38 ± 0.05	0.13 ± 0.03
	0.01		1.32 ± 0.07	0.37 ± 0.01	3.85 ± 0.18	1.18 ± 0.08	0.35 ± 0.07	0.33 ± 0.11
	0.07		1.41 ± 0.06	0.49 ± 0.06 ^{b/}	4.34 ± 0.18	1.35 ± 0.06	0.43 ± 0.01	0.24 ± 0.09
	0.5		1.57 ± 0.05	0.58 ± 0.06 ^{b/}	4.99 ± 0.38 ^{b/}	1.12 ± 0.11 ^{b/}	0.59 ± 0.10	0.22 ± 0.09
Relative Organ Weight (gm/gm brain weight)								
Male	0		0.41 ± 0.04	0.14 ± 0.13	1.45 ± 0.13	0.20 ± 0.03	0.53 ± 0.01	
	0.01		0.47 ± 0.03	0.25 ± 0.73	1.47 ± 0.08 ^{b/}	0.34 ± 0.05	0.53 ± 0.08	
	0.07		0.42 ± 0.01	0.31 ± 0.16	1.12 ± 0.07 ^{b/}	0.31 ± 0.02	0.58 ± 0.02	
	0.5		0.53 ± 0.12	0.59 ± 0.48	1.03 ± 0.09 ^{b/}	0.37 ± 0.09	0.45 ± 0.04	
Female	0		0.30 ± 0.02	0.29 ± 0.25	1.04 ± 0.10	0.28 ± 0.04	0.10 ± 0.03	
	0.01		0.28 ± 0.01	0.24 ± 0.20	0.90 ± 0.07	0.26 ± 0.04	0.24 ± 0.08	
	0.07		0.35 ± 0.04	0.31 ± 0.25	0.96 ± 0.00	0.31 ± 0.02	0.17 ± 0.06	
	0.5		0.37 ± 0.05	0.37 ± 0.23	0.71 ± 0.07 ^{b/}	0.38 ± 0.06	0.14 ± 0.06	

a/ Mean ± standard error of four mice.

b/ Significantly different from control mice by Dunnett's multiple comparison procedure.

TABLE 99

SUMMARY OF LESIONS OF BALB/NICE FED 2,4-DNT FOR 12 MONTHS

Dose (% in feed): House No.:	0					0.01				0.07				0.5				
	001	002	003	005		201	202	203	204	401	403	404	405	602	604	605	609	
<u>Treatment-Related Lesions^{a/}</u>																		
Liver																		
Liver cell tumor																		
Dysplasia																		
Kidney																		
Carcinoma																		
Nephropathy																		
Testis																		
Atrophy																		
Multiple Sites																		
Pigmentation																		
Intestine																		
Pituitary																		
<u>Other Lesions</u>																		
Adrenal Gland																		
Amyloidosis																		
Dysplasia																		
Ceroid degeneration																		
Lung																		
Peribronchiolar cuffings																		
Acute pneumonia																		
Bronchoalveolar adenoma																		
Heart																		
Myocardial degeneration																		
Liver																		
Portal inflammation																		
Focal necrosis																		
Spleen																		
Excessive extramedullary hematopoiesis																		
Testis																		
Hypertrophy of interstitial cell																		
Amyloidosis																		
Intestine																		
Amyloidosis																		
Salivary gland																		
Perivascular cuffings																		
Pancreas																		
Perivascular cuffings																		
Kidney																		
Tubular necrosis																		
Amyloidosis																		
Perivascular cuffings																		
Eye																		
Retinal atrophy																		
Bone Marrow Sarcoma																		
M/E ratio	1.5	2.4	1.5	2.0		1.3	1.7	1.8	1.7	0	1.3	1.6	1.3	2.1	2.1	2.7	1.5	

Tumors not listed were normal.

^{a/} Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; ? = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 300

SUMMARY OF LESIONS OF FEMALE MICE FED 2,4-DUT FOR 12 MONTHS

Dose (g in feed): Mouse No.:	0				0.01				0.07				0.5			
	102	103	104	105	101	102	103	104	101	103	104	105	105	107	111	112
<u>Treatment-Related Lesions^{a/}</u>																
Liver																
Liver cell tumor													X	X	X	X
Dysplasia																
Kidney																
Nephropathy																
Multiple Sites																
Pigmentation																
Intestine																
Minors																
Ovary																
Atrophy																
Other Lesions																
Adrenal Gland																
Amyloidosis																
Dysplasia																
Thyroid																
Lung																
Peribronchiolar cuffings																
Acute pneumonia																
Bronchoalveolar adenoma																
Liver																
Portal inflammation																
Focal necrosis																
Intestine																
Amyloidosis																
Lymphoid hyperplasia																
Salivary Gland																
Perivascular cuffings																
Pancreas																
Perivascular cuffings																
Kidney																
Tubular necrosis																
Amyloidosis																
Perivascular cuffing																
Urinary Bladder																
Foci of megakaryocytic cells																
Ovary																
Ovarian cyst																
Amyloidosis																
Uterus																
Endometritis																
Hydrometra																
Eye																
Retinal atrophy																
Bone marrow necrot																
W/E ratio																

Lesions not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; ± = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 101

SUMMARY OF LESIONS OF MALE MICE FED 2,4-DNT FOR 12 MONTHS
AND ALLOWED TO RECOVER FOR 1 MONTH

Dose (X in feed): House No.:	0			0.01			0.07			0.5		
	006	007	008	009	205	206	207	208	405	406	407	408
<u>Treatment-Related Lesions^{a/}</u>												
Liver												
Liver cell tumor												
Dysplasia												
Kidney												
Adenoma												
Nephropathy	X							X				
Testis												
Atrophy												
Multiple Sites												
Pigmentation												
Intestine												
Plethora	X	X	X	X				X				
<u>Other Lesions</u>												
Adrenal Gland												
Amyloidosis												
Dysplasia												
Cystoid degeneration												
Lung												
Peribronchiolar cuffings												
Bronchioloalveolar adenoma												
Heart												
Myocardial degeneration												
Liver												
Portal inflammation												
Focal necrosis												
Amyloidosis												
Spleen												
Excessive extramedullary hematopoiesis												
Intestine												
Amyloidosis												
Lymphoid hypertrophy												
Salivary Gland												
Perivascular cuffings												
Stomach												
Gastritis												
Kidney												
Amyloidosis												
Perivascular cuffings												
Lymph node												
Focal necrosis												
Eye												
Cataract												
Retinal atrophy or detachment												
Bone Marrow												
M/E ratio	1.5	2.1	1.7	1.1	1.6	2.7	1.1	1.0	1.5	1.9	2.2	1.9
Tissues not listed were normal.												
^{a/} Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; ? = questionable; X = present; 0 = tissue missing or unreadable.												

TABLE 102

SUMMARY OF LESIONS OF FEMALE MICE FED 2,4-DNT FOR 12 MONTHS
AND ALLOWED TO RECOVER FOR 1 MONTH

Dose (2 in feed):	0				0.01				0.07				0.5			
Mouse No.:	106	107	108	110	305	306	307	308	309	309	310	311	713	714	715	719
<u>Treatment-Related Lesions^{a/}</u>																
Liver																
Hyperplasia																X
Multiple sites																
Pigmentation													1	1	1	1
Intestine																
Parasites	X											X				
Ovary																
Atrophy													1	1	1	1
<u>Other Lesions</u>																
Adrenal Gland																
Amyloidosis							1									
Desmoplasia			1		1	1	1		1		1	1	1	1		
Coroid degeneration			1					1		1						
Thyroid																
Amyloidosis									1							
Thyroiditis											1					
Lung																
Peribronchiolar cuffings	1	1		1	1	1	1				1	1	1	1	1	1
Bronchioloalveolar adenoma								X								
Heart																
Myocardial degeneration													1			
Liver																
Portal inflammation	1	2	1	1	1		1	1		1	1	2	1	1	1	
Focal necrosis		2		1								1				
Amyloidosis									2						1	
Spleen																
Amyloidosis													1			
Intestine																
Amyloidosis				3			4		3	3	1		4	4	1	
Colon hyperplasia			1												1	
Enteritis																
Salivary Gland																
Perivascular cuffings	1	1			1		1		1	1	1	1				
Stomach																
Gastritis				1			1			2						
Amyloidosis							1						2			
Pancreas																
Perivascular cuffings	1												1			
Amyloidosis																
Kidney																
Amyloidosis		1	1	1			2		3	1			3	2		1
Perivascular cuffings	1	2	2	1	1	1	1		1	1	2	1		1	2	1
Chronic nephritis													4			
Urinary bladder																
Nonneoplastic cells foci								1					1			
Ovary																
Ovarian cyst		1				1	1			1			1		1	2
Amyloidosis				2			4		4			1	4	2		
Uterus																
Cystic endometrial hyperplasia	1	1					1			2			1			
Lymph Node																
Amyloidosis							1									
Focal necrosis										1					1	
Eye																
Retinal atrophy or detachment															1	
Bone Marrow																
M/E ratio	0	1.5	1.3	0	1.1	0	1.1	2.0	0	1.4	1.6	1.6	2.2	1.0	2.0	0

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; ± = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 103

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MICE FED 2,4-DNT FOR 24 MONTHS

Sex	Dose (% in feed)	Terminal Body Weight (gm)	Absolute Organ Weight (gm)					Testis	Ovary
			Brain	Heart	Liver	Kidney	Spleen		
Male	0	39 ± 1 ^{a/}	0.46 ± 0.01	0.23 ± 0.01	2.06 ± 0.31	0.93 ± 0.16	0.09 ± 0.01	0.23 ± 0.01	
	0.01	37 ± 1 ^{a/}	0.44 ± 0.01	0.23 ± 0.01	2.74 ± 0.52	0.68 ± 0.03	0.08 ± 0.01	0.21 ± 0.01	
	0.07	37 ± 1 ^{b/}	0.44 ± 0.02	0.21 ± 0.02	2.09 ± 0.13	0.81 ± 0.20	0.10 ± 0.04	0.21 ± 0.01	
Female	0	36 ± 2 ^{b/}	0.48 ± 0.03	0.23 ± 0.05	1.72 ± 0.14	0.52 ± 0.07	0.20 ± 0.03		0.24 ± 0.11
	0.01	37 ± 1 ^{c/}	0.49 ± 0.01	0.20 ± 0.01	1.60 ± 0.08	0.51 ± 0.03	0.17 ± 0.03		0.19 ± 0.10
	0.07	34 ± 1 ^{c/}	0.47 ± 0.01	0.18 ± 0.02	1.88 ± 0.19	0.46 ± 0.04	0.24 ± 0.05		0.48 ± 0.22
Relative Organ Weight (gm/100 gm body weight)									
Male	0		1.19 ± 0.03	0.58 ± 0.02	5.32 ± 0.80	2.40 ± 0.44	0.24 ± 0.03	0.59 ± 0.03	
	0.01		1.20 ± 0.05	0.62 ± 0.05	7.59 ± 1.53	1.81 ± 0.06	0.22 ± 0.03	0.55 ± 0.03	
	0.07		1.18 ± 0.08	0.55 ± 0.05	5.61 ± 0.36	2.21 ± 0.57	0.26 ± 0.11	0.56 ± 0.02	
Female	0		1.34 ± 0.02	0.64 ± 0.12	4.82 ± 0.35	1.45 ± 0.17	0.56 ± 0.08		0.66 ± 0.30
	0.01		1.34 ± 0.06	0.56 ± 0.05	4.36 ± 0.18	1.37 ± 0.06	0.44 ± 0.07		0.51 ± 0.26
	0.07		1.37 ± 0.04	0.52 ± 0.04	5.49 ± 0.51	1.35 ± 0.10	0.70 ± 0.13		1.38 ± 0.64
Relative Organ Weight (gm/gm brain weight)									
Male	0		0.49 ± 0.02	0.53 ± 0.72	2.04 ± 0.37	0.20 ± 0.02	0.50 ± 0.02		
	0.01		0.51 ± 0.02	6.14 ± 1.15	1.53 ± 0.08	0.18 ± 0.03	0.47 ± 0.03		
	0.07		0.48 ± 0.07	4.82 ± 0.39	1.83 ± 0.37	0.21 ± 0.08	0.48 ± 0.04		
Female	0		0.48 ± 0.09	3.61 ± 0.32	1.09 ± 0.14	0.42 ± 0.06			0.50 ± 0.24
	0.01		0.42 ± 0.03	3.30 ± 0.21	1.05 ± 0.08	0.34 ± 0.06			0.39 ± 0.21
	0.07		0.38 ± 0.03	4.02 ± 0.40	0.98 ± 0.08	0.52 ± 0.10			1.03 ± 0.49

a/ Mean ± standard error of 12 mice.

b/ Mean ± standard error of four mice.

c/ Mean ± standard error of seven mice.

TABLE 104

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MICE FED 2,4-DNT FOR 24 MONTHS
AND ALLOWED TO RECOVER FOR 1 MONTH

Sex	Dose (% in feed)	Terminal Body Weight (gm)	Absolute Organ Weight (gm)					Testis	Ovary	
			Brain	Heart	Liver	Kidney	Spleen			
Male	0	36 ± 2 ^{a/}	0.46 ± 0.01	0.24 ± 0.02	3.16 ± 1.71	0.86 ± 0.16	0.14 ± 0.00	0.25 ± 0.04		
	0.01	35 ± 2 ^{b/}	0.48 ± 0.01	0.25 ± 0.02	2.29 ± 0.40	0.71 ± 0.12	0.10 ± 0.01 ^{e/}	0.23 ± 0.01		
	0.07	37 ± 1 ^{a/}	0.45 ± 0.01	0.22 ± 0.02	1.93 ± 0.25	2.14 ± 0.64	0.09 ± 0.01 ^{e/}	0.20 ± 0.01		
Female	0	35 ± 1 ^{a/}	0.48 ± 0.02	0.21 ± 0.02	1.71 ± 0.22	0.59 ± 0.08	0.25 ± 0.05		0.31 ± 0.13	
	0.01	36 ± 3 ^{c/}	0.48 ± 0.01	0.19 ± 0.02	1.93 ± 0.14	0.51 ± 0.03	0.69 ± 0.14 ^{e/}		0.11 ± 0.03	
	0.07	30 ± 1 ^{d,e/}	0.46 ± 0.02	0.20 ± 0.02	1.45 ± 0.08	0.52 ± 0.05	0.17 ± 0.04		0.33 ± 0.20	
Relative Organ Weight (gm/100 gm body weight)										
Male	0		1.21 ± 0.04	0.63 ± 0.02	8.42 ± 3.49	2.22 ± 0.27	0.38 ± 0.01	0.65 ± 0.07		
	0.01		1.36 ± 0.04	0.69 ± 0.05	6.43 ± 1.02	1.98 ± 0.23	0.27 ± 0.02 ^{e/}	0.65 ± 0.01		
	0.07		1.24 ± 0.03	0.59 ± 0.06	5.27 ± 0.69	5.95 ± 1.92	0.24 ± 0.02 ^{e/}	0.55 ± 0.04		
Female	0		1.37 ± 0.05	0.60 ± 0.03	4.94 ± 0.81	1.68 ± 0.23	0.71 ± 0.12		0.86 ± 0.35	
	0.01		1.31 ± 0.10	0.51 ± 0.01	5.34 ± 0.74	1.39 ± 0.01	1.92 ± 0.51		0.30 ± 0.10	
	0.07		1.58 ± 0.10	0.66 ± 0.04	4.93 ± 0.33	1.76 ± 0.16	0.60 ± 0.14		1.08 ± 0.69	
Relative Organ Weight (gm/gm brain weight)										
Male	0		0.52 ± 0.03	6.88 ± 2.73	1.85 ± 0.28		0.31 ± 0.00 ^{e/}	0.54 ± 0.07		
	0.01		0.52 ± 0.04	4.82 ± 0.86	1.48 ± 0.21		0.20 ± 0.01 ^{e/}	0.48 ± 0.01		
	0.07		0.48 ± 0.04	4.29 ± 0.65	4.77 ± 1.44 ^{e/}		0.19 ± 0.02 ^{e/}	0.44 ± 0.04		
Female	0		0.44 ± 0.04	3.56 ± 0.53	1.21 ± 0.13		0.52 ± 0.11		0.64 ± 0.27	
	0.01		0.39 ± 0.04	4.21 ± 0.09	1.07 ± 0.07		1.73 ± 0.26 ^{e/}		0.23 ± 0.06	
	0.07		0.43 ± 0.05	3.12 ± 0.10	1.13 ± 0.11		0.38 ± 0.07		0.77 ± 0.51	

a/ Mean ± standard error of three mice.

b/ Mean ± standard error of five mice.

c/ Mean ± standard error of two mice.

d/ Mean ± standard error of four mice.

e/ Significantly different from control mice by Dunnett's multiple comparison procedure.

TABLE 105

SUMMARY OF TISSUE LESIONS IN CONTROL MICE FOR MICE FED 2,4-DNT FOR 24 MONTHS

	Sex:	Male												Female						
House No.:		25	27	28	30	33	34	35	41	42	46	49	50	52	115	131	132	146	159	160
<u>Treatment-Related Lesions</u> ^{a/}																				
Liver																				
Liver cell tumor		X			X		X	X			X						X		X	
Dysplasia																	X		X	
Testis																				
Atrophy			1		2	1					1	1								
Intestine																				
Aspicularis tetraptera			X		X	X			0	0			X				X			
<u>Other Lesions</u>																				
Eye																				
Retinal degeneration			X		X		X	X	X		0	X	X	X		X	X	X	X	X
Heart																				
Myocardial infarct															X		X			
Lung																				
Bronchoalveolar adenoma		X	X		X					X	X	X							X	
Papillary hyperplasia						X														
Bronchial epithelium hyperplasia							X													
Chronic pneumonia hyperplasia												2								
Salivary Glands																				
Degeneration		1	1	2	2	2	1	1	1	1	1	1	1	1	1	0	1	1	1	1
Liver																				
Aging changes		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X
Fatty change		1				1				1										
Kidney																				
Aging changes		2	1	1	1	1	1	1	1	1	1	1			1	1	1	1	1	1
Metastatic hepatic carcinoma													X							
Amyloidosis															3		3	3		
Focal pyelonephritis		2																		
Cortical cysts							2	2	1					2	2					
Ovary																				
Cysts															X		X	X		
Uterus																				
Leiomyoma																	X			
Benign neoplastic cyst															X					
Testis																				
Focal vasculitis		1										1								
Calcification					2	1														
Generalized Amyloidosis			X	X										X	X	X	X	X		X
Rib																				
Chondroma								X												
Adrenal																				
Aging changes		0	X	X	X	X	X	X	X	X	X	X	X	X	0	X	X	X	0	X
Pituitary																				
Adenoma		X																		
Spleen																				
Hemangioma																		X		
Bone Marrow																				
Hypoplasia		2			2	2	2													
Undetermined Site																				
Hemangioma, cavernous		X																		

Tissues not listed were normal.

^{a/} Severity of lesions: 1 - mild, 2 - moderate, 3 - severe, ± - minimal or questionable, X - present, 0 - tissue missing or could not be read.

TABLE 106

SUMMARY OF TISSUE LESIONS IN MALE MICE FED 2,4-DNT FOR 24 MONTHS

Treatment-Related Lesions ^{a/}	Dose (% in feed):				0.01%								0.07%							
	Mouse No.:				230	233	234	235	236	237	238	239	242	247	248	249	443	446	449	459
Liver																				
Liver cell tumor							X	X	X						X	X	X	X	X	X
Dysplasia							X	X			X	X								X
Kidney																				
Cyst with metaplastic epithelium									X											
Cystic papillary adenoma																		X		X
Cystic papillary carcinoma																	X			
Solid cortical adenoma																X				
Solid renal cell carcinoma							X												X	X
Toxic nephropathy								X	X											
Testis																				
Atrophy												1			1		2			
Multiple Sites																				
Pigmentation							1	±	±	±	±				±	±		±	±	1
Intestine																				
Aspicularis tetrastere							X	X	0	X				0	0	0				0
Other lesions																				
Eye																				
Retinal degeneration							X	X		X	X	X			X	0				
Lung																				
Bronchoalveolar adenomas							X	X			X	X			X				X	
Salivary Glands																				
Degeneration							1	1	0	1	1	1	1	1	1	2	1	1	1	1
Stomach																				
Polyps							X					X								
Metastatic tumor (renal?)							X													
Liver																				
Aging changes							X	X		X			X	X	X	X	X	X	X	X
Fatty change																		X		
Cystic focal necrosis										X										
Kidney																				
Chronic interstitial nephritis												1								
Focal calcification																X				
Pigmented casts																				
Cortical retention cyst							1			1										
Aging changes							1					1	±	±	1	±	1			1
Testis																				
Calcification of seminiferous tubules																X				
Prostate																				
Carcinoma							X													
Adrenal																				
Aging changes							X	X	X	X	X	X	X	X	X	X	X	X	X	X
Thyroid																				
Aging changes							0	X	0	X	X	0	0	0	X	0	X	0	X	X

Tissues not listed were normal.

^{a/} Severity of lesions: 1 - mild, 2 - moderate, 3 - severe, ± - minimal or questionable, X - present, 0 - tissue missing or could not be read.

SUMMARY OF TISSUE LESIONS IN FEMALE MICE FED 2.4-DNT FOR 24 MONTHS

Dose (% in feed):		0.01%								0.07%							
Mouse No.:		322	323	330	331	332	334	351	354	526	527	531	547	548	552	557	558
<u>Treatment-Related Lesions^{a/}</u>																	
Liver																	
Liver cell tumor								X		X	X			X			
Dysplasia			X		X												
Kidney																	
Toxic nephropathy			X														
Multiple Sites																	
Pigmentation			±	±	±	±	1	±	±	1	1	1		1	±	±	±
Intestine																	
Aspicularis tetrapara			0			X		X	0				X				X
<u>Other Lesions</u>																	
Eye																	
Retinal degeneration				X				X	X								
Breast																	
Carcinoma																	X
Salivary Gland																	
Degeneration			1	1	1	1	1	1	1								
Liver																	
Aging changes				X	X	X	X	X	X	X	X	X	X	X	X	X	X
Inflammatory infiltrate														X			
Focal granulomas						X											
Focal necrosis									X								
Kidney																	
Chronic interstitial nephritis																	
Cortical retention cysts					X												
Amyloidosis			X		X			X	X		X	X		X			
Aging changes			1		1	1	1	1	1	1						1	1
Ovary																	
Cysts						X				X	X			0	X		
Serous cystadenoma					X										X		
Mucinous cystadenoma										X	X						
Follicular cell tumor																X	
Surface papilloma				X													
Myxoma, subcutaneous									X								
General Amyloidosis			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adrenal																	
Aging changes			X	X	X	X	X	X	X	X	X	X	0	X	X	X	X
Thyroid																	
Aging changes			0	X	X	X	X	0	X	X	0	0	X	X	X	X	0
Follicular adenoma						X											
Pituitary																	
Adenoma										X							
Lymph Node																	
Hemangioma									X								

Tissues not listed were normal.

a/ Severity of lesions: 1 - mild, 2 - moderate, 3 - severe, ± - minimal or questionable, X - present,
0 - tissue missing or could not be read.

TABLE 103

SUMMARY OF TISSUE LESIONS IN MICE FED 2,4-DMT FOR 24 MONTHS
AND ALLOWED TO RECOVER FOR 1 MONTH

Sex:	Male					Female				
	Dose (2 in feed):					Dose (2 in feed):				
Mouse No.:	0	11	23	26	43	0	119	122	128	0.07
		217	226	227	228	254				
Treatment-Related Lesions ^{a/}										
Liver										
Liver cell tumor				X	X	X				
Dysplasia				X	X	X				
Kidney				X	X	X				
Cystic papillary adenoma				X						
Cystic papillary carcinoma										
Solid renal cell carcinoma										
Toxic nephropathy										
Testis										
Atrophy										
Ovary										
Nonfunctioning follicles										
Multiple Sites										
Pigmentation										
Intestine										
Amphicellular teratoma										
Other Lesions										
Eye										
Retinal degeneration										
Lung										
Bronchoalveolar adenoma										
Salivary Gland										
Degeneration										
Stomach										
Polyps										
Liver										
Aging changes										
Fatty change										
Focal necrosis										
Pericolangiolitis										
Hemangioma										
Kidney										
Aging changes										
Amyloidosis										
Ovary										
Calcification										

TABLE 108 (Concluded)

Sex: Dose (Z in feed): Mouse No.:	Male										Female																		
	0					0.01					0.07					0					0.01					0.07			
	11	23	26	43		217	226	227	228	234	415	423	442		119	122	128	311	318	319	519	520	522	523					
General Amyloidosis																													
Adrenal					X	X												X	X	X	X	X	X	X					
Aging changes																													
Thyroid					X	X	X	X		+	X	X	X		X	X	X	X	X	X	X	X	X	X					
Aging changes																													
Spleen					X	X	X	X		0	X	X			X	X		X	X	0	X	0	X	X					
Hemangioma																													
Bone Marrow															0					X									
Erythrocytic hypoplasia					X																								

Tissues not listed were normal.

a/ Severity of lesions: 1 - mild, 2 - moderate, 3 - severe, f - minimal or questionable, X - present, 0 - tissue missing or could not be read.

TABLE 109

SUMMARY OF TISSUE LESIONS IN CONTROL MICE FOR MICE FED 2,4-DNT DYING AT UNSCHEDULED TIMES

	Male								Female													
Sex:																						
Mouse No.:	48	4	31	32	18	37	38	47	134	130	101	102	135	136	116	134	117	145	121	129	142	130
Week of Death:	13	41	44	44	46	80	80	95	41	43	46	46	48	48	68	73	82	84	90	93	94	101
Treatment-Related Lesions^{a/}																						
Testis																						
- Atrophy	0								1													
Intestine																						
- Aspiculex isoraphe	X	X	X	X	0	X			0	X	X							X	X	X		
Other Lesions																						
Eye																						
- Retinal degeneration	0	0	X			0	X	X	0	X			0	X			X	X	X	X	X	X
Lung																						
- Bronchoalveolar adenoma					X					X												
- Metastatic mammary																						
- Adenocarcinoma																						
Salivary Gland																						
- Degeneration	0	0	0			1		1	1	0	0			0	1	0	1	1	0	1	1	1
Liver																						
- Aging changes									1													
- Faty changes			1																			
- Starvation effects		2							1	1	1	1										
- Hemangioma																						
Pancreas																						
- Exocrine adenoma					X																	
Kidney																						
- Aging changes							X								X			X	X	X	X	
- Amyloidosis																						
Ovary																						
- Benign cyst																						
Uterus																						
- Hemangioma																						
Mammary Gland																						
- Adenocarcinoma																						
- Generalized Amyloidosis									X			0			X	X		X	X	X	X	X
Multiple Sites																						
- Endothelioma																						
- Inflammation									X													
- Myelocytosis																						
Adrenal																						
- Aging changes	0	0	0	X	X	X	X	X	X		X	0	X	X	X	X	X	X	0	X	0	X
Thyroid																						
- Aging changes	0	0	0	0	0	0	0	0	0	0	X	0	0	X	X	X	X	X	0	X	0	0
Bone Marrow																						
- Hypogonadism									X	X	X											

Tissues not listed were normal.

^{a/} Severity of lesions: 1 - mild, 2 - moderate, 3 - severe, ± - minimal or questionable, X - present, 0 - tissue missing or could not be read.

TABLE 110

SUMMARY OF TISSUE LESIONS IN MALE MICE FED 0.012 OR 0.071 2,4-DNT AND DYING AT UNSCHEDULED TIMES

Dose (2 in feed):	0.012										0.071									
	240	213	225	258	260	210	253	265			427	424	438	416	425	431	421	458	433	437
Mouse No.:	18	19	33	46	56	72	80	100			9	35	46	50	63	69	74	78	90	93
Week of Death:																				
Treatment-Related Lesions ^{g/}																				
Liver																				
Liver cell tumor																				
Dysplasia	X	X				X									X	X	X	X	X	X
Kidney																				
Cystic papillary adenoma																				
Cystic papillary carcinoma																				
Solid renal cell carcinoma																				
Toxic nephropathy																				
Testes																				
Atrophy																				
Multiple Sites																				
Pigmentation																				
Intestine																				
Aspicularis tetraptera	0	0	0	X	0	0	0	0			0	0	0	X	X	X	X	0	X	0
Other Lesions																				
Eye																				
Retinal degeneration	X	0	X	0	0	X	X				0	0	0	0						
Lung																				
Bronchioloalveolar adenoma																				
Salivary glands																				
Degeneration																				
Liver																				
Aging changes																				
Fatty change																				
Zonal necrosis																				
Focal hepatitis																				
Pericholangitis																				
Kidney																				
Aging change																				
Cortical cysts																				
Angiodosis																				
Generalized Angiodosis																				
Multiple Sites																				
Spindle cell sarcoma																				
Reticulum cell tumor																				
Leucosis																				
Adrenal																				
Aging change																				
Cortical atrophy																				
Thyroid																				
Aging change																				
Lymph Node																				
Lymphosarcoma																				

Tissues not listed were normal.

g/ Severity of lesions: 1 - mild, 2 - moderate, 3 - severe, ± - minimal or questionable, X - present, 0 - tissue missing or could not be read.

TABLE III PART A

SUMMARY OF TISSUE LESIONS IN MALE MICE FED 0.5% 2,4-DNT
AND DYING AT UNSCHEDULED TIMES

Mouse No.:	612	618	657	603	651	653	636	637	641	644	606	632	634	638	640	643
Week of Death:	35	35	35	36	36	36	39	39	39	39	40	40	40	40	40	40
Treatment-Related Lesions ^{a/}																
Liver																
- Dysplasia	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Kidney																
- Toxic nephropathy	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
- Atypical epithelium					X	X	X	X	X	X	X	X	X	X	X	X
- Cortical cyst with metaplastic epithelium						X		X								
Testis																
- Atrophy	0		1	1	2	1	2	2		2	1	2	2	1	2	2
- Multiple Sites																
- Pigmentation	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
- Intestine																
- Aspicularis tetraptera					X	0	X			X						
- Brain																
- Pigment granules			0		0	0	±	X	0	0	X	±				
Other Lesions																
Eye																
- Retinal degeneration	0	0	0		0	0	0	0	X	X				0		
Heart																
- Focal myocarditis				±		±			±	±	±	±	±	0	±	X
Lung																
- Bronchoalveolar adenoma												X				
Salivary Glands																
- Degeneration	1	0	1		1	±	1		0	0	0		±	0		
Ileum																
- Amyloid						X										
Kidney																
- Benign cysts											X					
Multiple Sites																
- Lymphosarcoma																
Thyroid																
- Aging changes																
Adrenal																
- Aging changes																

Tissues not listed were normal.

a/ Severity of lesions: 1 - mild, 2 - moderate, 3 - severe, ± - minimal or questionable, X - present, 0 - tissue missing or could not be read.

TABLE 111 PART B

SUMMARY OF TISSUE LESIONS IN MALE MICE FED 0.5% 2,4-DNT
AND DYING AT UNSCHEDULED TIMES

Mouse No.:	601	632	622	630	631	647	653	621	646	619	628	607	610	626	650	649
Week of Death:	41	41	43	43	43	43	43	44	46	48	48	49	50	51	51	69
<u>Treatment-Related Lesions^{a/}</u>																
Liver																
Liver cell tumor												X				X
Dysplasia	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Kidney																
Cystic papillary carcinoma					X											
Toxic nephropathy	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Atypical epithelium			X	X	X	X		X	X		X		X	X		X
Cortical cyst with metaplastic epithelium			X	X				X			X		X	X		
Testis																
Atrophy			2	1	2	1	1	2	2	2	2	2	2	2	3	4
Multiple Sites																
Pigmentation	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Intestine																
Aspicularis retraction									0	X	0					
Brain		0							0						0	
Pigment granules in neurons, etc.			X	X		X	X	X	X	X			X	X		
<u>Other Lesions</u>																
Eye																
Retinal Regeneration		X		0		0	0	0	0	0	0	0	0	0	0	1
Heart																
Focal myocarditis			X		0	0	±		±	±	±	±			X	0
Salivary Gland																
Regeneration		±	0			0						2	0		0	
Kidney																
Benign cysts								X						X		
Brain		0							0						0	
Focal calcification	X							X								
Thyroid																
Anisomorphism	0		0	X	0	0	0	X	X	X	0		0	0	0	

Tissues not listed were normal.

^{a/} Severity of lesions: 1 - mild, 2 - moderate, 3 - severe, ± - minimal or questionable, X - present, 0 - tissue missing or could not be read.

TABLE 112

SUMMARY OF TISSUE LESIONS IN FEMALE MICE FED 0.01Z OR 0.07Z 2,4-DNT
AND DYING AT UNSCHEDULED TIMES

Dose (Z in feed): Mouse No.: Week of Death:	0.01Z										0.07Z									
	341	342	135	346	333	338	349	328	340	352	517	502	505	507	512	518	540	529	530	514
	48	48	53	53	59	62	77	82	88	100	34	46	47	47	47	73	73	74	84	98
<u>Treatment-Related Lesions:</u> ^{a/}																				
Liver																				
Dysplasia							X											X		
Kidney																				
Toxic nephropathy					X											X		X		
Multiple Sites																				
Pigmentation										X						X	X		X	X
Intestine																				
Aspicularis tetraptera	0	X		X	0	X		0		0		X		0	0	X	X	X	X	X
<u>Other Lesions</u>																				
Eye																				
Retinal degeneration	0	X	+	0	0	X				X		0		0	0					
Lung																				
Bronchoalveolar adenoma																			X	X
Salivary Glands																				
Degeneration	0	1	0	1	0	1	1	1	1	1	0	1	0	0	0	1	1	1	1	1
Liver																				
Aging changes				X	X		0	X	X	X		X	X	X	X	X	X	0	X	X
Zonal necrosis							X					X								
Focal hepatitis																		X		
Starvation effect																				
Kidney	X	X	X	X																
Aging changes				X	X		X		X	X								X		
Amyloidosis				X			X										X		X	X
Pyelonephritis										3										
Ovary																				
Calcified corpora albicantia	0	0		0	X		0		X			0		0	0					
Uterus																				
Leiomyoma				X																
Leiomyosarcoma									X											
Mammary Gland																				
Carcinoma							X													

TABLE 112 (Concluded)

Dose (2 in feed): Mouse No.: Week of Death:	0.012										0.072									
	341	342	335	346	333	338	349	328	340	352	517	502	505	507	512	518	540	530	514	541
	48	48	53	53	59	62	77	82	88	100	34	46	47	47	47	73	73	84	98	107
General Amlodosis																				
Multiple Sites																				
Myelocytic leucosis																				
Squamous cell carcinoma																				
Lymphosarcoma																				
Adrenal																				
Aging changes																				
Thyroid																				
Aging changes																				
Bone Marrow																				
Hypoplasia																				

Tissues not listed were normal.

a/ Severity of lesions: 1 - mild, 2 - moderate, 3 - severe, ± - minimal or questionable, X - present, 0 - tissue missing or could not be used.

TABLE 113

SUMMARY OF TISSUE LESIONS IN FEMALE RICE FED 0.5% 2,4-DNT
AND DYING AT UNSCHEDULED TIMES

Treatment-Related Lesions ^a	Week of Death:										Week of Death:										Week of Death:									
	753	736	736	736	750	751	718	722	740	701	708	710	721	725	726	727	728	735	749	756	757	760	739	746	747	732	732	732	732	732
Liver																														
- Dysplasia																														
- Kidney																														
- Toxic nephropathy	0																													
- Cyst with metaplastic epithelium	0																													
- Ovary																														
- Hypofunctioning follicles	0																													
- Multiple Sites																														
- Pigmentation																														
- Brain																														
- Pigment granules in neurons, glia																														
- Other Lesion																														
Eye																														
- Retinal Degeneration	0																													
- Heart																														
- Focal myocarditis																														
- Lung																														
- Bronchoalveolar edema																														
- Salivary Gland																														
- Degeneration	0																													
- Liver																														
- Lymphatics																														
- Licens																														
- Angiogenesis																														
- Ovary																														
- Serous cystadenoma																														
- Mucinous cystadenoma																														
- Brain																														
- Fresh microhemorrhages																														
- Focal neuronal necrosis																														
- Adrenal																														
- Aging change	0																													
- Thyroid																														
- Aging change	0																													

Tissues not listed were normal.

g/ Severity of lesions: 1 - mild, 2 - moderate, 3 - severe, + - minimal or questionable, X - present, 0 - tissue missing or could not be read.

TABLE 114

INCIDENCE OF TREATMENT-RELATED LESIONS IN MICE FED 2,4-DNT

	Dos: (% in feed):		0		0.01		0.07		0.5	
	Sex:									
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Liver										
Liver cell tumor	7/33 ^a	2/31	9/33	1/29	8/28	3/31	5/40	1/33		
Dysplasia	2/33	5/31	14/33	3/29	12/28	5/31	40/40	29/33		
Kidney										
Tumors	0/33	0/31	5/33	0/29	16/28	0/31	3/40	1/32		
Toxic Nephropathy (TN)	0/33	0/31	3/33	3/29	3/28	2/31	32/40	10/32		
TN with Atypical Epithelium	0/33	0/31	0/33	0/29	0/28	0/31	18/40	1/32		
Abnormal Pigmentation	0/33	0/31	2/33	4/29	4/29	8/31	38/40	27/33		
Testis										
Atrophy	7/32	--	4/33	--	11/28	--	34/39	--		
Ovary										
Non-functioning Follicles	--	1/28	--	2/23	--	0/27	--	15/24		
Intestine										
Pinworms	16/30	8/28	10/25	8/22	9/23	11/30	4/38	0/30		

a/ Mice with lesion/mice with readable slide.

TABLE 115

NON-TREATMENT-RELATED TUMORS IN MICE FED 2,4-DNT

Dose (% in feed): Sex:	0		0.01		0.07		0.5	
	Male	Female	Male	Female	Male	Female	Male	Female
<u>Site, Tumors</u>								
Lung								
- Bronchoalveolar adenoma	2 ^{a/}	3	10	2	1	2	2	2
Pancreas								
- Exocrine adenoma	1							
Prostate								
- Carcinoma			1					
Ovary								
- Serous cystadenoma				1		1		1
- Mucinous cystadenoma						2		1
- Follicular cell tumor						1		
- Surface papilloma				1				
Uterus								
- Benign neoplastic cyst		1						
- Leiomyoma		1		1				
- Leiomyosarcoma				1				
Mammary Gland								
- Carcinoma				1		1		
- Adenocarcinoma		1						
Connective Tissue								
- Hemangioma	1	3		1		1		
- Myxoma				1				
- Reticulum cell tumor						1		
- Endothelioma		1						
- Spindle cell sarcoma						1		
- Squamous cell carcinoma				1				
Rib								
- Chondroma	1							
Pituitary								
- Adenoma	1							
Lymph Node								
- Lymphangioma								1
- Lymphosarcoma							1	

^{a/} Number of mice with the lesions.

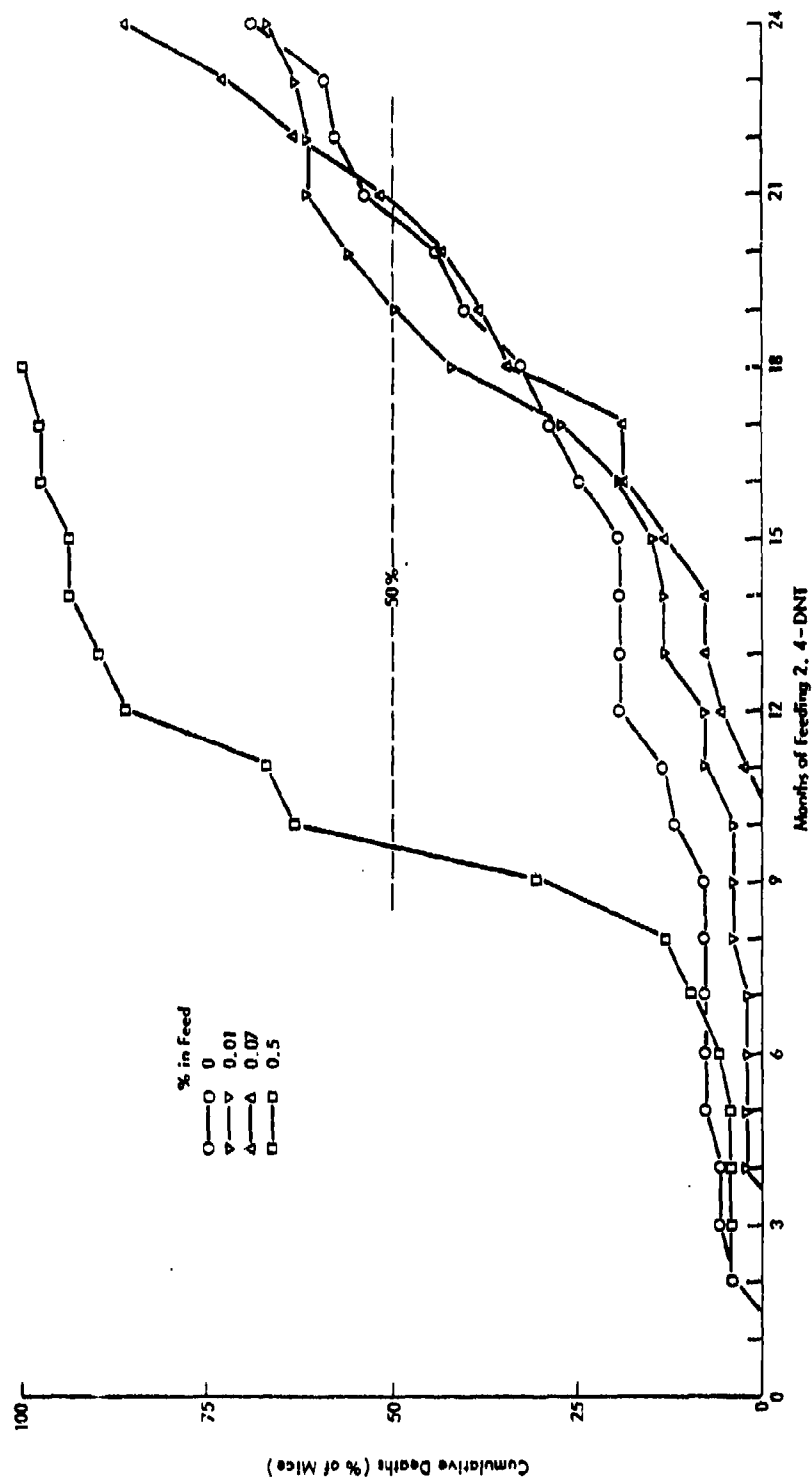


Figure 16 - Cumulative Unscheduled Deaths Among Male Mice Fed 2,4-DNT

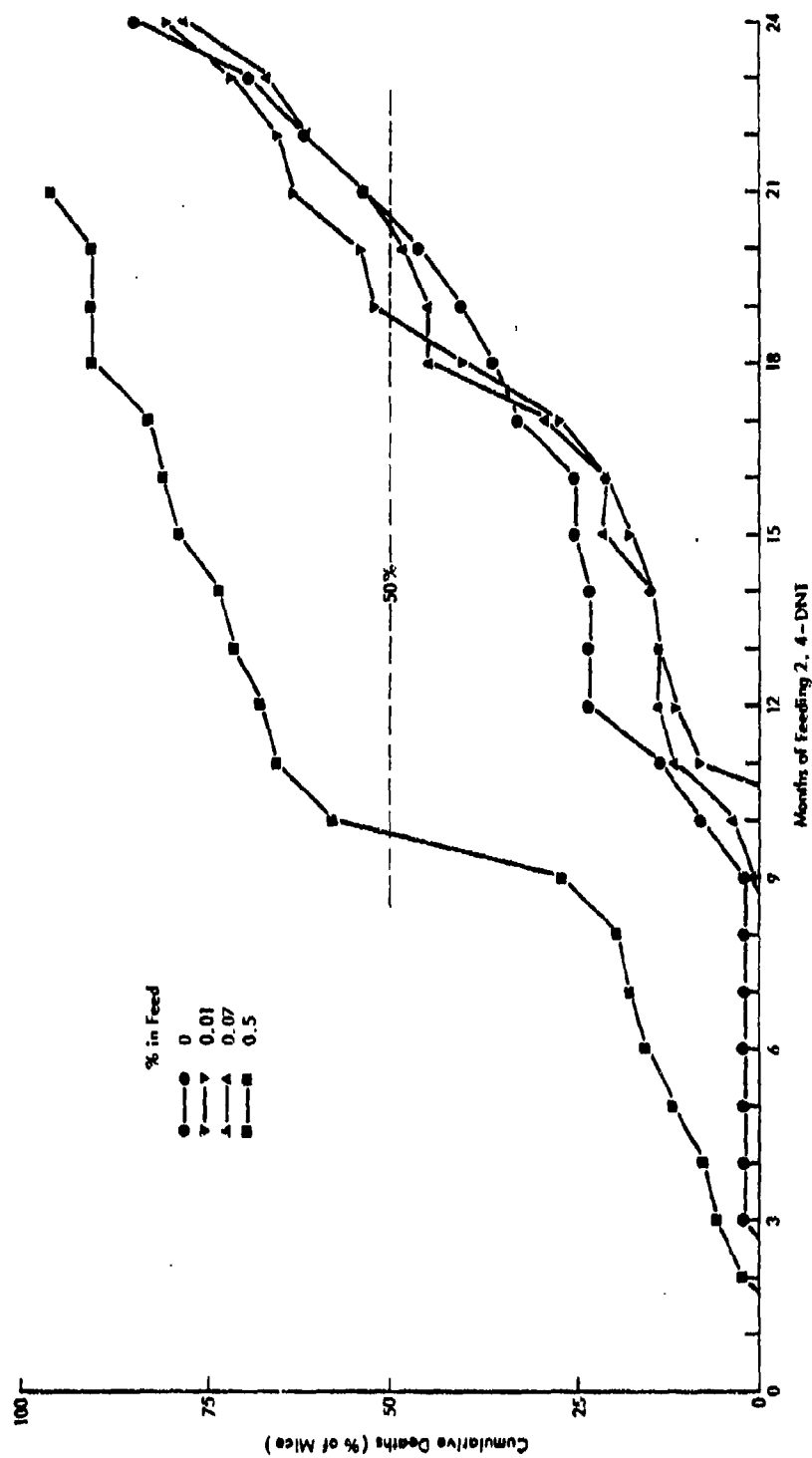


Figure 17 - Cumulative Unscheduled Deaths Among Female Mice Fed 2,4-DNT

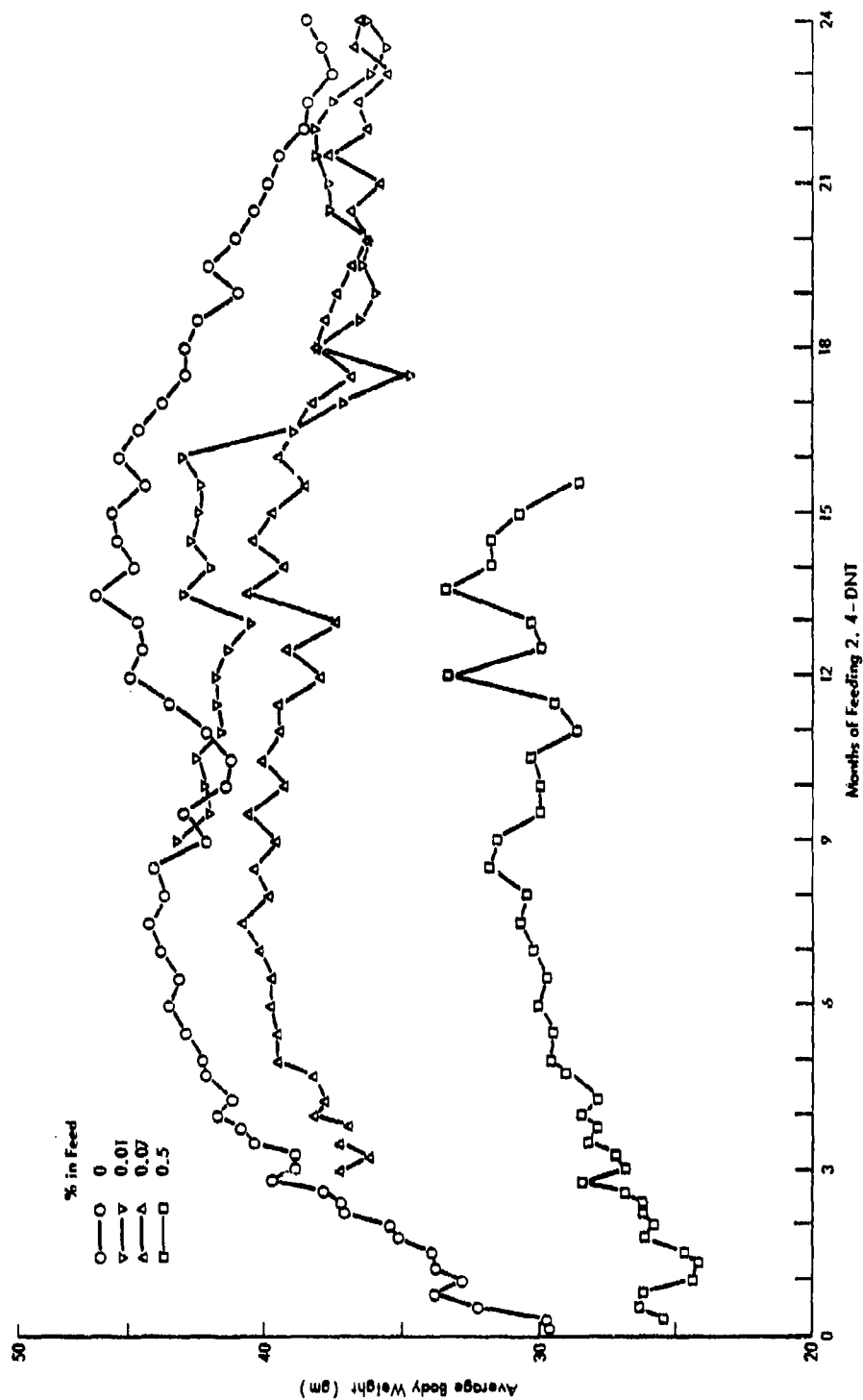


Figure 18A - Average Body Weights of Male Mice Fed 2,4-DNT

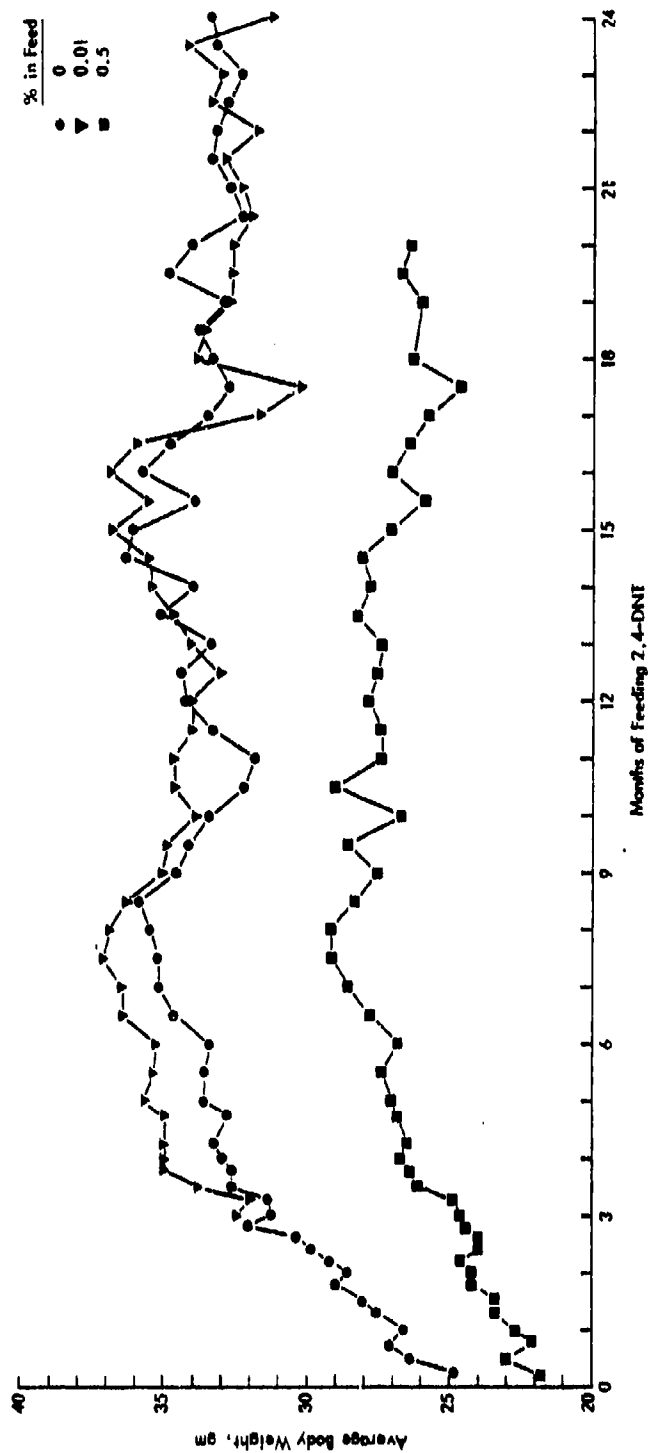


Figure 18B - Average Body Weights of Female Mice Fed 2,4-DNT

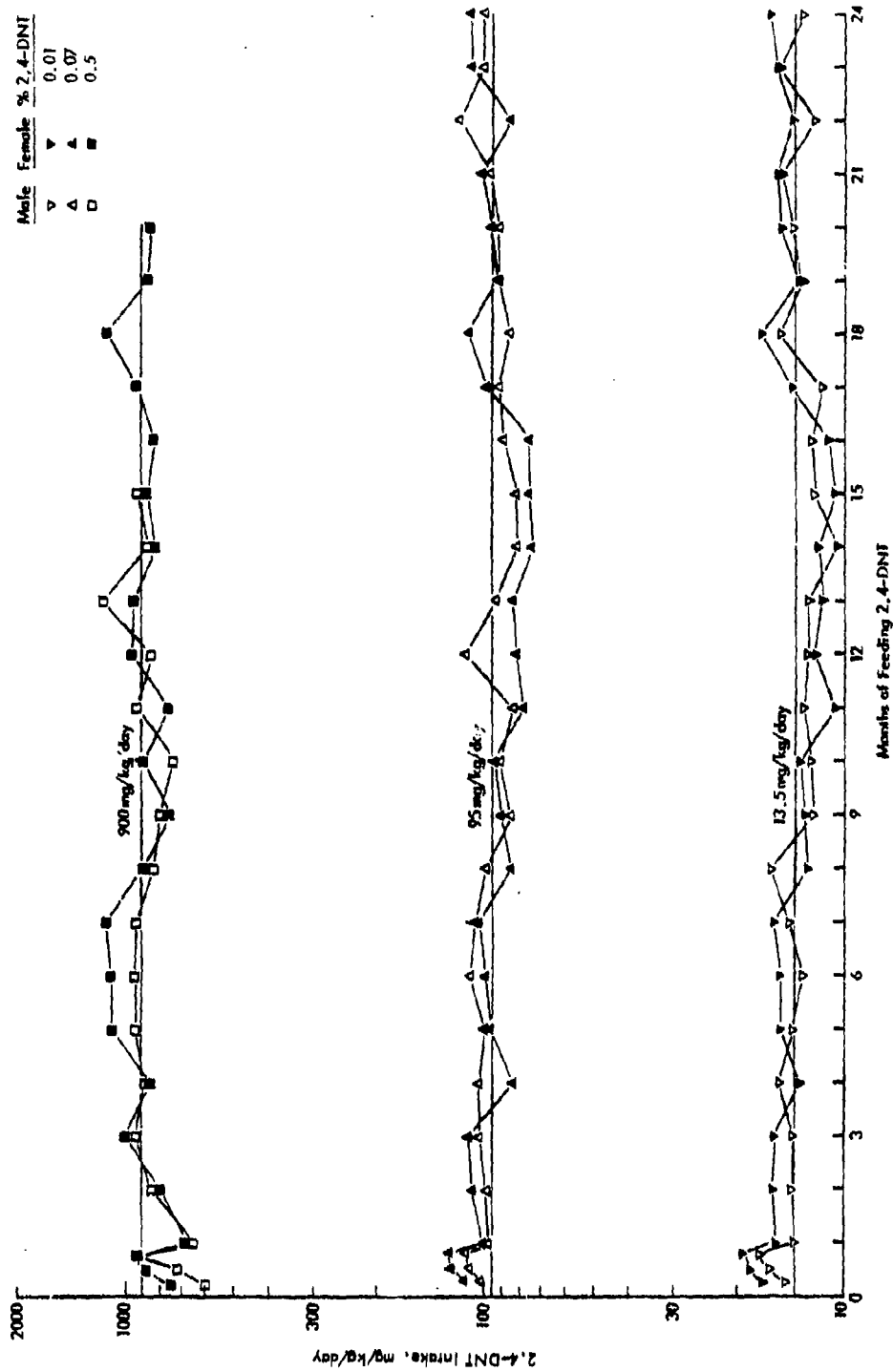


Figure 19 - Average Compound Intake by Mice Fed 2,4-DNT



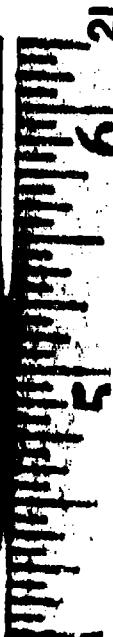
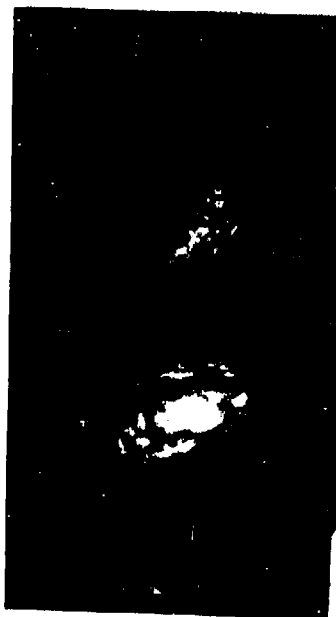
20

Figure 20 - Photomicrograph of Liver from Mouse No. 73-646 Fed 0.5% 2,4-DNT for 47 Weeks. Note the heavy deposition of pigment in portal area. H and E stain, 250 X.



22

Figure 22 - Photomicrograph of kidney from Mouse No. 72-419 Fed 0.07% 2,4-DNT for 100 Weeks. Note the cystic proliferation of renal tubular epithelium--cystic papillary adenoma. H and E stain, 25 X.



21

Figure 21 - Photograph of Kidney from Mouse No. 72-431 Fed 0.07% 2,4-DNT for 69 Weeks. Note the enlargement and distorted contour of kidney.



23

Figure 23 - Photomicrograph of Kidney from Mouse No. 72-433 Fed 0.07% 2,4-DNT for 90 Weeks. Note the compact arrangement of proliferation of renal tubular epithelium--solid carcinoma. H and E stain, 100 X.

VI. GENERAL DISCUSSION AND CONCLUSIONS

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VI. GENERAL DISCUSSION AND CONCLUSIONS

A. Toxic Doses

There was a large variation in doses of 2,4-DNT required for a given toxic effect in the three species studied.

In dogs, the low dose, 0.2 mg/kg/day, had no apparent effects; 1.5 mg/kg/day was toxic to some; and 10 mg/kg/day was toxic to all and lethal to some.

In rats, the low dose with intake of 0.57 or 0.71 mg/kg/day in the feed for the males and females, respectively, had no apparent effects. The middle dose with intake of 3.9 or 5.1 mg/kg/day for the males and females, respectively, was toxic to some. The high dose with intake of 34 or 45 mg/kg/day for the males and females, respectively, was toxic to all and shortened the life span.

In mice, the low dose with intake of 13.5 mg/kg/day in the feed for both males and females was slightly toxic to some. The middle dose with intake of 95 mg/kg/day was toxic to all. The high dose with intake of 900 mg/kg/day halved the life span.

B. Target Organs

In addition to its nonspecific effects on body weight and life span, 2,4-DNT caused toxicities on a number of target organs in the three species studied. The target organs included the blood (methemoglobinemia and sequelae), central nerve system (incoordination, sometimes including ataxia and paralysis), liver (degenerative and hyperplastic lesions including hepatocellular carcinoma), kidney (cystic changes and tumors), gonads (atrophy and aspermatogenesis in the male, nonfunctional follicles in the female), subcutaneous and mammary gland tumors (fibromas and fibroadenomas, respectively), abnormal pigmentation (macrophages and other cells in various tissues), and possibly intestinal pinworms.

There were no significant specific effects seen in the various special studies. These studies included assay for immunoglobulin E, three generation reproduction, mutagenesis and metabolism.

1. Non-Specific Effects

As with most chemically toxic compounds, large doses of 2,4-DNT produced a decrease in weight gain accompanied by a small decrease in feed consumption and a decrease in life span. These effects were quite

pronounced in the high dose rodents, but not obvious in dogs, where the unscheduled deaths were due to the nervous system effects.

The symptoms reported in human occupational exposure^{20,21/} include headache, weakness and lassitude, inappetence, nausea and vomiting, vertigo, pain or paresthesia in extremities and upper abdominal discomfort. Most of these effects would be associated with no obvious pathological lesion in animals, but could produce the observed non-specific effects.

2. The Blood

Of the many effects of 2,4-DNT, the effect on the blood is the most thoroughly studied.^{22/} This effect is produced by aromatic amines, hence the term "anilinism," and most organic and inorganic nitrates and nitrites. These compounds, or, probably, the nitrosamine and hydroxylamine derivatives, oxidize the iron in hemoglobin, producing methemoglobin. Within limits, the body can correct this. Inborn deficiencies in metabolism, such as glucose-6-phosphate dehydrogenase deficiency,^{14/} or high levels of the poison can overwhelm the normal protective measures, producing numerous secondary effects.

While 2,4-DNT does produce methemoglobin, levels rapidly decrease.^{23/} Thus, measurable methemoglobin was seldom found. The task was rendered more difficult by the available assay method, which involves measuring the difference in absorption at 630 nm before and after conversion to cyanmethemoglobin (Appendix I). Because of this subtraction, even control blood can have some apparent methemoglobin. Low levels up to 4 to 5% of the total hemoglobin may be artifactual.

The presence of methemoglobin in erythrocytes leads to the formation of aggregates of ill-defined degradation products, called Heinz bodies.^{14/} These have been seen after human poisoning with dinitrotoluene and 2,4-DNT was probably the major isomer present.^{24/} These are readily detected, even if less than 0.1% of erythrocytes are so affected, by the use of appropriate stain. Thus, the presence of Heinz bodies seems to be the most sensitive indicator of this blood toxicity, and a reasonably useful indication of degree of toxicity.

High levels of methemoglobin are removed by catabolism. Therefore, anemia develops. Within limits, the homeostatic mechanisms of body can compensate by increasing erythrocyte production. This can be detected by the increased proportion of immature erythrocytes, reticulocytes, in the blood. Since reticulocytes are larger, with a relatively low hemoglobin concentration, changes in the Wintrobe indexes can occur. If the toxic dose is not too severe, these mechanisms suffice. Thus, "compensated anemia" may exist, normal erythrocyte levels with reticulocytosis. In other cases, frank anemia may be present.

This effect was found in the high dose animals of all species, and in some individual middle dose animals. As the studies continued into the second year, there was a decrease in the amount of this "anilinism" seen. This was probably due to the adaptation of the animals, either producing less methemoglobin or more erythrocytes, although no evidence of changes in the erythropoietic system were found. It could be due to the earlier deaths of the more susceptible animals. However, methemoglobin did occur in the dogs surviving during the second year.

3. Central Nervous System

Behavioral changes indicative of central nervous system effects were prominent in the dog, fairly common in the mouse and rare in the rat. In the dog, there was incoordination, sometimes leading to paralysis. Two of the most severe cases were accompanied by degenerative lesions in the cerebellum, which provides motor coordination. In the mouse, there was depression with hyperexcitability, but no lesions were found (except rare pigmentation). A few rats showed some minor signs, possibly indicating incoordination. Many of the signs reported^{20,21/} in humans suffering from DNT toxicity, including unpleasant taste in mouth, headache, weakness, nausea, vertigo, pain or paresthesia in extremities, could be interpreted as similar mild manifestations of the same biochemical lesion(s) seen in these animal studies, but that is mere speculation. There are no data on the biochemical events involved in the CNS toxicity.

4. Liver

Some hepatic lesions were seen in dogs and mice. But the most severe hepatotoxicity was found in rats, where the progressive development of hepatocellular carcinoma occurred. This progression is known for several compounds,^{16,17/} as discussed above.

Occasional tenderness of the liver^{21/} and rare jaundice^{20/} were found in workers poisoned by DNT. Furthermore, eight of 22 deaths from trinitrotoluene toxicity were reported due to "toxic hepatitis" with 13 other deaths ascribed to aplastic anemia and one to a combination of the two syndromes.^{28/} There were no deaths reported from DNT toxicity.^{25/}

5. The Kidney

Serious renal effects were seen only in the mouse. There were characteristic cystic degeneration, anaplastic epithelium, possibly pre-neoplastic, and a variety of tumors, generally cystic. These effects were rare in the high dose mice, but most of them died before the end of the first year of study. The effects were much more pronounced in the male mice. No such effects were seen in the NCI bioassay.^{26/} However, they used a different strain of mouse (B6C3F1, rather than CD-1®) and used a high dose (0.04% 2,4-DNT in feed) intermediate between our low (0.01%) and middle (0.07%) doses.

6. The Gonads

2,4-DNT caused atrophy of the testes with, in sufficient dose, a complete cessation of spermatogenesis. The severely affected tubules become empty, merely epithelium. This effect has not been reported in humans, but there is no indication that it has been sought. For instance, the massive report on occupational disease in U.S. government owned explosives plants^{25/} contains no mention of sterility. The NCI Bioassay Report^{26/} reports no increase in testicular atrophy.

The mechanism of this effect is unknown. However, 2,4-DNT has a deleterious effect on frog sperm in vitro.^{27/} Since an analogous effect, non-functional follicles with lacking of corpora lutea, was seen in the high dose female mice, it is an interesting speculation that these effects may have a common mechanism, perhaps a derangement of the pituitary hormonal systems, with no apparent lesions.

7. Subcutaneous Tumors

In rats, 2,4-DNT caused major increases in the incidence of naturally-occurring subcutaneous fibromas in males and mammary fibroadenomas in females. These tumors contributed to the death rate. The NCI Bioassay^{26/} found similar increases of the same tumors in rats of a different strain (Fischer 344, rather than CD[®]) fed a lower dose (0.02% 2,4-DNT) for 18 months, followed by 6 months recovery. The only mammary tumors that occurred in male rats from the present study were in two high dose rats; this may be a 2,4-DNT induced effect.

8. Pigmentation

A peculiar pigment was found in the mice, dogs and a few rats. The composition of this pigment is unknown. However, it did not appear to be toxicologically significant, since it was not associated with any lesions. Because the metabolism of 2,4-DNT produces many compounds, generally orange to red,^{4/} it seems likely that some metabolites are a part of the pigment. It is also possible that degraded hemoglobin is part of the pigment.

9. Pituitary Adenomas

A decrease in pituitary adenomas occurred in rats fed high dose of 2,4-DNT. This pituitary adenoma is the most common spontaneous tumor in the strain of rat used in the study,^{15/} and was the primary cause of unscheduled deaths in the control, low and middle dose groups. The increased death rate in the high dose rats may have contributed to the decreased incidence of the pituitary adenomas. However, even those high dose rats surviving beyond month 20 had an abnormally low incidence of pituitary adenomas.

10. Intestinal Parasites

Despite all the adverse effects, there was one possible beneficial effect of 2,4-DNT. Pinworm infestation was decreased in the high dose mice. A similar effect was seen in rats killed after 12 month's dosing, but this was probably due to the small sample, because it was not seen in rats living longer.

C. Carcinogenesis Mechanism

The NCI bioassay on 2,4-DNT^{26/} found results similar to ours with respect to the increased subcutaneous tumors, but not to other effects. However, 2,4-DNT can be and is, in part, metabolized to 2,4-diaminotoluene,^{4/} which has recently been bioassayed.^{29/} Their rat doses bracketed our middle dose (0.01%); their mouse doses were our low dose (0.01%) and 0.02%. They found in their rats neoplastic nodules and hepatocellular carcinomas, mammary adenomas and carcinomas, and (in males), subcutaneous fibromas, just as we found in our rats given 2,4-DNT. Also, their female mice had an increased incidence of hepatocellular carcinomas. They noted decreased survival and lesions indicating hepatonephrotoxicity, although details were not yet available.

The similar effects of 2,4-diaminotoluene in Fischer 344 rats at doses lower than those of 2,4-DNT we gave CD[®] rats implies that at least part of the carcinogenic mechanism of 2,4-DNT involves conversion to 2,4-diaminotoluene or that both compounds are converted to the same toxic metabolite. Their results in B6C3F1 mice are less similar to ours in CD-1[®] mice. Both strains have similar low absorption of 2,4-DNT.^{4/} We found kidney tumors, they found liver tumors and we both found hepatonephrotoxicity. Common mechanisms may still be at work in mice, but they are not as striking as in rats.

D. Medical Surveillance

From the results of these studies and the available literature, the most useful objective parameters for estimating an individual's exposure to toxic doses of 2,4-DNT are Heinz bodies and the reticulocyte count. Heinz bodies persist and are easily detected. An increase in the reticulocyte count would indicate increased destruction of erythrocytes before obvious anemia develops. Methemoglobinemia is apparently too transient to be useful, especially for low-levels of exposure. The other effects observed developed later than these blood effects. In addition, a hand-eye coordination test might be useful for determining if a neuromuscular incoordination effect exists.

E. Water Quality Criterion

1. Rationale

Water quality criteria are used to estimate the amounts of noxious compounds in ambient water which will not be hazardous to the human population. The EPA has developed methodology^{30/} for the determination of these criteria. We will use our data on 2,4-DNT to assess the risk to humans. Of the effects of 2,4-DNT discussed above, the critical one is carcinogenicity.

As a matter of policy, the EPA uses the "one-hit model" to extrapolate animal carcinogenic data to man. This model, expressed mathematically as:

$$P = 1 - e^{-BD},$$

assumes that one molecule of a carcinogen delivered to the proper active site is adequate to initiate the irreversible process of carcinogenicity. Therefore, the probability (P) of an individual developing a tumor is a function of the dose (D) and the slope (B) of the dose-response curve, a measure of the potency of the subject carcinogen.

From the above chronic studies, the following data are available: nt, the number of animals exposed to the lowest dose that produced tumors at a level significantly higher than controls, using the Fisher exact test at the $p < 0.05$ level of significance; d, average dose per unit of time (mg/kg/day) during administration of the chemical; NT, the total number of animals exposed to the selected dose level; NC, the total number of control animals; nc, the number of control animals with the tumor type studied; Le, the maximum lifespan for the test animal (i.e., 6 weeks from birth to start of dosing, then 2 years of dosing); le, the actual maximum time of exposure for test animals; w, average weight of test animals in kilograms. These data are then converted to parameters applicable to humans using the expanded model:

$$P_t = P_c + (1 - P_c) \cdot (1 - e^{-t^3 BD})$$

where P_t and P_c are the proportion of tumors in treated and control animals, respectively, and t is the ratio of test animal lifespan to human lifespan. The human dose is considered to come from direct consumption of 2 liters of contaminated water each day and from the consumption of 0.0187 kg/day of fish (T) from the contaminated water. The 2,4-DNT intake from the fish is derived from the bioconcentration factor (R) calculated by Mr. J. G. Pearson.^{31/} From these data we can calculate the dose associated with a given P_t .

2. Calculations

The first step is determining the lowest dose group with a statistically significant tumor increase. These are listed in Table 116. Since we have several tumor types, both between groups (species, sex) and even within female rats, we calculate the criteria from the data set providing the highest potency factor (steepest slope, B). The data used are summarized in Table 117. The potency factors and the calculated criterion levels are shown in Table 118. The largest is that of mammary tumors in female rats.

3. Conclusion

Because 2,4-DNT has carcinogenic effects, an ambient water concentration of zero is necessary for maximum protection of human health. However, exposure to 1.152 µg/liter for a lifetime produces an estimated risk of 10^{-5} (1 in 100,000) that a tumor will develop in man. A tenfold decrease in dose would produce a tenfold decrease in the estimated risk. Because of the similarities between the isomeric DNTs, this limit for 2,4-DNT is appropriate for a normal mixture of DNTs.

TABLE 116

SIGNIFICANT TUMOR INCIDENCES IN ANIMALS GIVEN 2,4-DNT

<u>Species, Sex</u>	<u>2,4-DNT Intake (mg/kg/day)</u>	<u>Tumor Type</u>	<u>Tumor Incidence</u>		<u>Probability^{a/}</u>
			<u>Control</u>	<u>Treated</u>	
Rat, Female	45.3	Hepatocellular carcinoma	0/23	18/34	0.000 007 1
Rat, Male	34.5	Subcutaneous tumors	2/25	15/30	0.000 53
Rat, Female	45.3	Mammary tumors	11/23	33/35	0.000 083
Mouse, Male	13.3	Renal tumors	0/33	5/33	0.027

a/ Fisher's exact test for contingency tables.

TABLE 117

DATA FOR CALCULATING WATER QUALITY CRITERIA

<u>Tumor Type</u>	<u>Female Rats Hepatocellular Carcinoma</u>	<u>Male Rats Subcutaneous Tumors</u>	<u>Female Rats Mammary Tumors</u>	<u>Male Mice Renal Tumors</u>
nt	18	15	33	5
NT	34	30	35	33
nc	0	2	11	0
NC	23	25	23	33
Le (wk)	110	110	110	110
le (wk)	104	104	104	104
d (mg/kg/day)	45.3	34.5	45.3	13.3
W (kg)	0.285	0.470	0.285	0.040
L (wk)	104	104	104	90
R	18.8	18.8	18.8	18.8
T (kg)	0.0187	0.0187	0.0187	0.0187

TABLE 118

WATER QUALITY CRITERIA LEVELS ($\mu\text{g}/\text{l}$; ppb) FOR RISK TO MAN

<u>Tumor Type</u>	<u>Slope (B)</u>	<u>Risk Level</u>		
		<u>10^{-5}</u>	<u>10^{-6}</u>	<u>10^{-7}</u>
Hepatocellular carcinoma, female rats	0.08807	3.380	0.338	0.034
Subcutaneous tumors, male rats	0.07918	3.760	0.376	0.038
Mammary tumors, female rats	0.25841	1.152	0.115	0.016
Renal tumors, male mice	0.08156	3.650	0.365	0.036

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APPENDIX I

MANUAL FOR

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

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January 1977

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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:

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

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

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

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

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

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 $\pm$ 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 $\pm$ 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 $\pm$ 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/}

	<u>No. of Determinations</u>	<u>Mean $\pm$ S.D.</u>	<u>Range</u>
Erythrocytes ($\times 10^6/\text{mm}^3$)			
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 ($\times 10^3/\text{mm}^3$)			
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 (mg %)	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/}

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

<u>Unknowns</u>	<u>MRI Results</u>	<u>PTS Results</u>	<u>Participating Laboratories (10-90 Percentiles)</u>		<u>Acceptable Performance^{b/}</u>
			<u>Median</u>	<u>Mean</u>	
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		Observed Results	
	Number Studied	Body Weight (kg) Mean $\pm$ S.D.	Mean $\pm$ S.D.	Range
Erythrocytes ($\times 10^6/\text{mm}^3$)	108	3.74 $\pm$ 0.50	5.51 $\pm$ 0.45	3.75 - 6.61
Reticulocytes (%)	108	3.74 $\pm$ 0.50	0.97 $\pm$ 0.82	0.07 - 2.41
Hematocrit (vol %)	108	3.74 $\pm$ 0.50	43.0 $\pm$ 2.6	37.0 - 50.0
Hemoglobin (gm %)	108	3.74 $\pm$ 0.50	13.4 $\pm$ 0.8	10.8 - 15.4
MCV (μ^3)	108	3.74 $\pm$ 0.50	77.8 $\pm$ 7.0	69.6 - 117.3
MCHC (μg)	108	3.74 $\pm$ 0.50	24.4 $\pm$ 1.8	21.0 - 33.6
Platelets ($\times 10^5/\text{mm}^3$)	99	3.74 $\pm$ 0.50	31.4 $\pm$ 1.3	27.2 - 34.1
Leukocytes ($\times 10^3/\text{mm}^3$)	108	3.74 $\pm$ 0.50	3.08 $\pm$ 0.45	0.80 - 7.10
Neutrophils I (%)	108	3.74 $\pm$ 0.50	10.4 $\pm$ 4.9	3.8 - 30.1
Neutrophils II (%)	108	3.74 $\pm$ 0.50	0.18 $\pm$ 0.45	0 - 2
Lymphocytes (%)	108	3.74 $\pm$ 0.50	39.30 $\pm$ 17.72	10 - 83
Eosinophils (%)	108	3.74 $\pm$ 0.50	56.83 $\pm$ 17.74	13 - 84
Monophils (%)	108	3.74 $\pm$ 0.50	1.91 $\pm$ 2.42	0 - 13
Basophils (%)	108	3.74 $\pm$ 0.50	1.37 $\pm$ 1.58	0 - 7
Atypical cells (%)	108	3.74 $\pm$ 0.50	0.04 $\pm$ 0.20	0 - 2
Nucleated RBC (%)	108	3.74 $\pm$ 0.50	0.00 $\pm$ 0.00	0 - 0
Fasting Glucose (mg %)	100	3.74 $\pm$ 0.50	0.00 $\pm$ 0.00	0 - 0
SGOT (IU/l)	100	3.76 $\pm$ 0.51	96.9 $\pm$ 15.2	59 - 127
SGPT (IU/l)	100	3.76 $\pm$ 0.51	33.7 $\pm$ 9.2	20 - 60
Alkaline Phosphatase (IU/l)	100	3.76 $\pm$ 0.51	31.3 $\pm$ 7.8	15 - 46
BUN (mg %)	100	3.76 $\pm$ 0.51	360.0 $\pm$ 116.0	143 - 501
Proth. Time (sec)	62	3.91 $\pm$ 0.44	19.5 $\pm$ 7.5	12 - 65
Serum Creat. (mg %)	100	3.76 $\pm$ 0.51	10.2 $\pm$ 0.7	9.3 - 11.9
Bilirubin	62	3.91 $\pm$ 0.44	1.1 $\pm$ 0.3	0.6 - 1.8
Total (mg %)	62	3.91 $\pm$ 0.44	0.1 $\pm$ 0.2	0.0 - 0.8
Direct (mg %)	62	3.91 $\pm$ 0.44	0.0 $\pm$ 0.0	0.0 - 0.0
BSP 15 min (2 ret.)	62	3.91 $\pm$ 0.44	18.0 $\pm$ 7.4	2 - 34
Ka (mEq/l)	62	3.91 $\pm$ 0.44	154.0 $\pm$ 19.1	144 - 179
K (mEq/l)	62	3.91 $\pm$ 0.44	4.8 $\pm$ 0.6	3.9 - 5.7
Cl (mEq/l)	62	3.91 $\pm$ 0.44	109.0 $\pm$ 6.4	93 - 118
Ca (mEq/l)	62	3.91 $\pm$ 0.44	5.2 $\pm$ 0.4	4.2 - 6.3
Mg (mEq/l)	62	3.91 $\pm$ 0.44	1.6 $\pm$ 0.1	1.2 - 1.8
Bone Marrow				
Myeloid/erythroid ratio	15	3.65 $\pm$ 0.41	1.5 $\pm$ 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 RHEUS MONKEYS^{a/}

	Female Rhesus Monkeys		Observed Results	
	Number Studied	Body Weight (kg) Mean $\pm$ S.D.	Range	
			Mean $\pm$ S.D.	
Erythrocytes ($\times 10^6/\text{mm}^3$)	81	3.51 $\pm$ 0.48	5.33 $\pm$ 0.40	4.25 - 6.03
Reticulocytes (Z)	81	3.51 $\pm$ 0.48	1.07 $\pm$ 0.54	0.35 - 3.31
Hematocrit (vol Z)	81	3.51 $\pm$ 0.48	41.5 $\pm$ 2.8	30.0 - 46.0
Hemoglobin (gm Z)	81	3.51 $\pm$ 0.48	13.1 $\pm$ 1.0	7.9 - 14.1
MCV (μ^3)	81	3.51 $\pm$ 0.48	77.7 $\pm$ 5.3	66.5 - 95.2
MCH (mg)	81	3.51 $\pm$ 0.48	24.6 $\pm$ 1.7	17.6 - 29.7
MCHC (mg Z)	81	3.51 $\pm$ 0.48	31.6 $\pm$ 1.4	26.6 - 34.2
Platelets ($\times 10^5/\text{mm}^3$)	81	3.51 $\pm$ 0.48	3.11 $\pm$ 1.23	1.85 - 7.90
Leukocytes ($\times 10^3/\text{mm}^3$)	81	3.51 $\pm$ 0.48	9.5 $\pm$ 3.9	3.2 - 24.8
Neutrophils I (Z)	81	3.51 $\pm$ 0.48	0.10 $\pm$ 0.43	0 - 3
Neutrophils II (Z)	81	3.51 $\pm$ 0.48	36.41 $\pm$ 13.32	13 - 56
Lymphocytes (Z)	81	3.51 $\pm$ 0.48	60.38 $\pm$ 13.26	41 - 79
Eosinophils (Z)	81	3.51 $\pm$ 0.48	2.28 $\pm$ 3.10	0 - 18
Monophils (Z)	81	3.51 $\pm$ 0.48	0.75 $\pm$ 0.98	0 - 4
Basophils (Z)	81	3.51 $\pm$ 0.48	0.05 $\pm$ 0.22	0 - 1
Atypical cells (Z)	81	3.51 $\pm$ 0.48	0.00 $\pm$ 0.00	0 - 0
Nucleated RBC (Z)	74	3.56 $\pm$ 0.50	0.00 $\pm$ 0.00	0 - 0
Fasting Glucose (mg Z)	81	3.51 $\pm$ 0.48	92.1 $\pm$ 15.3	57 - 116
SGOT (IU/l)	81	3.51 $\pm$ 0.48	32.1 $\pm$ 7.6	20 - 70
SGPT (IU/l)	81	3.51 $\pm$ 0.48	30.1 $\pm$ 7.6	12 - 39
Alkaline Phosphatase (IU/l)	81	3.51 $\pm$ 0.48	149.9 $\pm$ 112.3	148 - 572
BUN (mg Z)	81	3.51 $\pm$ 0.48	17.3 $\pm$ 4.2	13 - 29
Froth. Time (sec)	59	3.56 $\pm$ 0.43	10.5 $\pm$ 0.9	9.7 - 12.3
Serum Creat. (mg Z)	81	3.51 $\pm$ 0.48	1.1 $\pm$ 0.3	0.6 - 1.7
Bilirubin				
Total (mg Z)	81	3.51 $\pm$ 0.48	0.1 $\pm$ 0.1	0.0 - 0.8
Direct (mg Z)	81	3.51 $\pm$ 0.48	0.0 $\pm$ 0.0	0.0 - 0.0
ASP 15 min (Z ret.)	59	3.56 $\pm$ 0.43	16.4 $\pm$ 8.3	5 - 34
Na (mEq/l)	59	3.56 $\pm$ 0.43	158.2 $\pm$ 6.5	147 - 174
K (mEq/l)	59	3.56 $\pm$ 0.43	4.8 $\pm$ 0.7	3.9 - 6.2
Cl (mEq/l)	59	3.56 $\pm$ 0.43	109.0 $\pm$ 6.1	95 - 113
Ca (mEq/l)	59	3.56 $\pm$ 0.43	5.3 $\pm$ 0.5	4.3 - 6.3
Mg (mEq/l)	59	3.56 $\pm$ 0.43	1.6 $\pm$ 0.2	1.3 - 2.0
Bone Marrow				
Myeloid/erythroid ratio	11	3.49 $\pm$ 0.62	1.4 $\pm$ 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/}

<u>Organ Weight</u>	<u>Absolute</u>	
	<u>Mean $\pm$ S.D.</u>	<u>Range</u>
Liver (gm)	82 $\pm$ 17	64 - 122
Spleen (gm)	4.6 $\pm$ 1.8	2.0 - 9.3
Kidneys (gm)	15.1 $\pm$ 3.8	8.0 - 22.0
Adrenals (gm)	0.73 $\pm$ 0.15	0.45 - 0.86
Thyroids (gm)	0.57 $\pm$ 1.30	0.37 - 0.81
Testes (gm)	1.29 $\pm$ 0.67	0.53 - 3.30
 <u>Relative (per kg body weight)</u>		
	<u>Mean $\pm$ S.D.</u>	<u>Range</u>
Liver (gm)	23.4 $\pm$ 2.5	18.8 - 30.4
Spleen (gm)	1.25 $\pm$ 0.47	0.57 - 2.38
Kidneys (gm)	4.13 $\pm$ 0.92	2.20 - 6.43
Adrenals (mg)	201 $\pm$ 44	129 - 254
Thyroids (mg)	154 $\pm$ 42	86 - 250
Testes (gm)	0.34 $\pm$ 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/}

<u>Organ Weight</u>	<u>Absolute</u>	
	<u>Mean $\pm$ S.D.</u>	<u>Range</u>
Liver (gm)	83 $\pm$ 17	64 - 122
Spleen (gm)	3.8 $\pm$ 1.4	2.0 - 6.0
Kidneys (gm)	14.5 $\pm$ 2.8	11.0 - 20.0
Adrenals (gm)	0.68 $\pm$ 0.16	0.53 - 1.14
Thyroids (gm)	0.60 $\pm$ 0.20	0.37 - 1.11
Ovaries (gm)	0.28 $\pm$ 0.10	0.14 - 0.45
 <u>Relative (per kg body weight)</u>		
	<u>Mean $\pm$ S.D.</u>	<u>Range</u>
Liver (gm)	25.4 $\pm$ 5.8	19.2 - 37.4
Spleen (gm)	1.16 $\pm$ 0.49	0.60 - 1.89
Kidneys (gm)	4.40 $\pm$ 0.86	3.20 - 6.25
Adrenals (mg)	212 $\pm$ 80	138 - 438
Thyroids (mg)	173 $\pm$ 66	97 - 346
Ovaries (mg)	82 $\pm$ 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			Observed Results	
	Number Studied	Age (months)	Body Weight (kg) Mean $\pm$ S.D.	Mean $\pm$ S.D.	
				Mean $\pm$ S.D.	Range
Erythrocytes ($\times 10^6/\text{mm}^3$)	276	4 - 7	8.3 $\pm$ 1.7	5.55 $\pm$ 0.73	3.62 - 7.60
Reticulocytes (%)	284	4 - 7	8.3 $\pm$ 1.7	0.72 $\pm$ 0.46	0.04 - 4.35
Hematocrit (vol %)	276	4 - 7	8.3 $\pm$ 1.7	41.6 $\pm$ 3.5	31 - 50
Hemoglobin (gm %)	276	4 - 7	8.3 $\pm$ 1.7	13.5 $\pm$ 1.4	10.0 - 16.9
MCV (μ^3)	276	4 - 7	8.3 $\pm$ 1.7	75.6 $\pm$ 8.3	56.7 - 127.1
MCHb (μg)	276	4 - 7	8.3 $\pm$ 1.7	24.6 $\pm$ 3.0	17.1 - 41.7
MCHbC (mg %)	276	4 - 7	8.3 $\pm$ 1.7	32.5 $\pm$ 1.5	28.1 - 40.3
Platelets ($\times 10^5/\text{mm}^3$)	270	4 - 7	8.4 $\pm$ 1.7	2.91 $\pm$ 1.02	0.93 - 6.35
Leukocytes ($\times 10^3/\text{mm}^3$)	284	4 - 7	8.3 $\pm$ 1.7	11.9 $\pm$ 3.5	4.6 - 24.6
Neutrophils I (%)	284	4 - 7	8.3 $\pm$ 1.7	0.55 $\pm$ 1.06	0 - 6
Neutrophils M (%)	284	4 - 7	8.3 $\pm$ 1.7	56.81 $\pm$ 9.47	22 - 80
Lymphocytes (%)	284	4 - 7	8.3 $\pm$ 1.7	37.94 $\pm$ 9.26	13 - 71
Eosinophils (%)	284	4 - 7	8.3 $\pm$ 1.7	2.76 $\pm$ 2.93	0 - 16
Monophils (%)	284	4 - 7	8.3 $\pm$ 1.7	1.78 $\pm$ 1.84	0 - 11
Basophils (%)	284	4 - 7	8.3 $\pm$ 1.7	0.01 $\pm$ 0.10	0 - 2
Atypical cells (%)	284	4 - 7	8.3 $\pm$ 1.7	0.11 $\pm$ 0.37	0 - 2
Nucleated RBC (%)	284	4 - 7	8.3 $\pm$ 1.7	0.02 $\pm$ 0.10	0 - 2
Fasting Glucose (mg %)	284	4 - 7	8.3 $\pm$ 1.7	100.9 $\pm$ 12.6	66 - 134
SGOT (IU/l)	276	4 - 7	8.3 $\pm$ 1.7	23.2 $\pm$ 7.4	11 - 59
SGPT (IU/l)	276	4 - 7	8.3 $\pm$ 1.7	25.7 $\pm$ 7.9	8 - 46
Alkaline Phosphatase (IU/l)	276	4 - 7	8.3 $\pm$ 1.7	73.3 $\pm$ 18.5	21 - 133
BUN (mg %)	284	4 - 7	8.3 $\pm$ 1.7	12.1 $\pm$ 3.3	4 - 23
Bone Marrow					
Myeloid/erythroid ratio	34	5 - 9	9.4 $\pm$ 1.6	1.6 $\pm$ 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			Observed Results	
	Number Studied	Age (months)	Body Weight (kg) Mean $\pm$ S.D.	Mean $\pm$ S.D.	Range
Erythrocytes ($\times 10^6/\text{mm}^3$)	257	4 - 7	6.9 $\pm$ 1.3	5.59 $\pm$ 0.73	3.27 - 7.75
Reticulocytes (%)	265	4 - 7	6.9 $\pm$ 1.3	0.74 $\pm$ 0.52	0.04 - 5.05
Hematocrit (vol %)	257	4 - 7	6.9 $\pm$ 1.3	42.3 $\pm$ 3.5	32 - 51
Hemoglobin (gm %)	257	4 - 7	6.9 $\pm$ 1.3	13.7 $\pm$ 1.3	11.0 - 18.6
MCV (μ^3)	257	4 - 7	6.9 $\pm$ 1.3	76.7 $\pm$ 9.7	55.8 - 128.4
MCHb (μg)	257	4 - 7	6.9 $\pm$ 1.3	24.8 $\pm$ 3.3	17.1 - 41.6
MCHbC (mg %)	257	4 - 7	6.9 $\pm$ 1.3	32.3 $\pm$ 1.6	28.7 - 40.4
Platelets ($\times 10^5/\text{mm}^3$)	227	4 - 7	6.9 $\pm$ 1.3	3.08 $\pm$ 1.15	1.08 - 7.95
Leukocytes ($\times 10^3/\text{mm}^3$)	265	4 - 7	6.9 $\pm$ 1.3	10.9 $\pm$ 3.4	3.8 - 26.9
Neutrophils I (%)	265	4 - 7	6.9 $\pm$ 1.3	0.54 $\pm$ 1.16	0 - 7
Neutrophils M (%)	265	4 - 7	6.9 $\pm$ 1.3	57.08 $\pm$ 10.10	31 - 85
Lymphocytes (%)	265	4 - 7	6.9 $\pm$ 1.3	37.15 $\pm$ 10.46	10 - 61
Eosinophils (%)	265	4 - 7	6.9 $\pm$ 1.3	2.37 $\pm$ 2.25	0 - 13
Monophils (%)	265	4 - 7	6.9 $\pm$ 1.3	1.94 $\pm$ 2.01	0 - 9
Basophils (%)	265	4 - 7	6.9 $\pm$ 1.3	0.01 $\pm$ 0.09	0 - 1
Atypical cells (%)	265	4 - 7	6.9 $\pm$ 1.3	0.11 $\pm$ 0.43	0 - 4
Nucleated RBC (%)	265	4 - 7	6.9 $\pm$ 1.3	0.03 $\pm$ 0.17	0 - 2
Fasting Glucose (mg %)	248	4 - 7	6.9 $\pm$ 1.3	99.6 $\pm$ 14.4	55 - 130
SGOT (IU/l)	257	4 - 7	6.9 $\pm$ 1.3	23.5 $\pm$ 7.2	6 - 52
SGPT (IU/l)	257	4 - 7	6.9 $\pm$ 1.3	15.3 $\pm$ 7.0	8 - 49
Alkaline Phosphatase (IU/l)	257	4 - 7	6.9 $\pm$ 1.3	13.5 $\pm$ 19.2	30 - 146
BUN (mg %)	265	4 - 7	6.9 $\pm$ 1.3	12.4 $\pm$ 3.3	4 - 26
Bone Marrow					
Myeloid/erythroid ratio	34	5 - 9	7.8 $\pm$ 1.4	1.4 $\pm$ 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/}

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

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

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

TABLE I

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

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

a/ Data collected between September 1971 and December 1976 from 139 rats, weighing 352 $\pm$ 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 98 ^{c/}	615 ^{b/}	112 565 ^{c/}	84 ^{b/}	18 56 ^{d/}
No. of Collections:	141		615		84	
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:	<u>Monkeys</u>		<u>Dogs</u>	
No. of Animals:	<u>44^{a/}</u>	8	<u>118^{a/}</u>	30
No. of Collections:	<u>44</u>	<u>48^{b/}</u>	<u>118</u>	<u>156^{b/}</u>
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).

APPENDIX II

MANUAL FOR

STUDY OF DEVELOPMENTAL TOXICITY

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June 1975

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STUDY OF DEVELOPMENTAL TOXICITY

I. INTRODUCTION

The thalidomide catastrophe provides an unfortunate example of the need for reliable information concerning the effects of agents on human development. Prospective and retrospective epidemiological studies are the only ethical procedures currently available to obtain this information in humans. There are, however, a variety of protocols available to obtain preliminary developmental toxicity information in animals. This preliminary animal information can be used to form the basis from which it is possible to evaluate the risk of exposing the human population to potentially toxic agents.

The purpose of this manual is to describe the protocol used in our laboratory to obtain developmental toxicity information. Sections, in addition, are included which discuss both the statistical analysis and interpretation of the data. A working definition of common anomalies is presented. These studies are based on "The Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use" distributed by the U.S. Food and Drug Administration, 1966, and "The Testing of Chemicals for Carcinogenicity, Mutagenicity, and Teratogenicity" distributed by the Ministry of Health and Welfare, Canada, 1973.

II. PROTOCOL FOR STUDY OF DEVELOPMENTAL TOXICITY

A. Fertility and General Reproductive Performance Study

1. Objectives

The emphasis in this phase is placed on determining the effect of an agent on gonadal function, estrus cycle, mating behavior, conception rates, and the early stages of development. This study serves as an overall pilot screening of the agent on the entire reproductive process including organogenesis, late stages of gestation, parturition, and lactation. The results obtained from this phase serve as a guide for conducting subsequent studies in greater depth.

2. Method

The rat is the animal generally used for this study and both males and females are used to provide an adequate study of fertility. Male rats,

at least 40 days of age, are treated for 60 to 80 days prior to mating to determine if the agent affects spermatogenesis. Male animals from subacute or chronic toxicity studies may be used and each male, from a group of at least 10 animals, is bred with two non-treated females. Each male is exposed overnight to females in proestrus or early estrus until (1) a male mates with two females or (2) a male is exposed, on at least three different occasions, to a total of at least five receptive females. A female is considered receptive if there is an estrous vaginal smear the morning following exposure. This procedure minimizes attributing male infertility to sexual inexperience.

Sexually mature female rats are treated for at least 14 days prior to mating with untreated males. The stages of the estrous cycle are determined by vaginal smears to verify that the animals cycle normally and to detect possible treatment effects on the duration of the estrous cycle. The occurrence of copulation is established by daily vaginal inspection for the presence of sperm. The day on which evidence of copulation is discovered is identified as being day 0 of gestation. Confirmation of pregnancy, however, is not obtained until the animal is sacrificed on day 13 of gestation or delivers a litter at the end of gestation. Females treated prior to mating are continued on the same treatment schedule until the time of sacrifice.

Half of the females from each group are sacrificed on day 13 of gestation. The dams are examined for number of corpora lutea and implantation sites, number and distribution of embryos in each uterine horn, presence of empty implantation sites, embryos undergoing resorption, and any abnormal conditions. The following parameters are determined:

- a. Number of viable litters (litters with one or more viable implants)
- b. Corpora lutea/dam
- c. Total implants/dam
- d. Viable implants/dam
- e. Indexes of
 - (1) Fertility: confirmed pregnancies/sperm positive females
 - (2) Gestation: confirmed pregnancies with viable fetuses/confirmed pregnancies
 - (3) Implantation: implants/corpora lutea
 - (4) Implant viability: viable fetuses/implants

The remaining dams are allowed to deliver and the litters are examined at birth, day 4, and day 21. The litters are examined for number, weight, mortality, and abnormalities of the pups. The following parameters are determined:

- a. Number of viable litters (litters with one or more viable pups)
- b. Pups/dam
- c. Weight of pups
- d. Indexes of
 - (1) Fertility: confirmed pregnancies/sperm positive females
 - (2) Gestation: confirmed pregnancies with viable fetuses/confirmed pregnancies
 - (3) Implant viability: viable pups/implants
 - (4) Viability: pups alive at day 4/pups alive at birth
 - (5) Lactation: pups alive at day 21/pups alive at day 4

B. Teratology Study

1. Objectives

The objective of this phase is to determine if an agent has a potential for producing embryotoxicity and/or teratogenicity. Treatment, therefore, is restricted to the period of organogenesis. Dosage may be high during this brief treatment period in order to obtain results concerning teratogenic potential and risk.

2. Method

Two species of animals are employed in this test. The species most frequently used are the mouse, rat, and rabbit. Drug treatment covers the period of organogenesis which is day 6 through 15 of gestation for the mouse and rat and day 6 through 18 for the rabbit.

Sexually mature virgin mice are obtained from reputable suppliers and conditioned in our animal quarters for 10 days. The conditioning period permits the animals to stabilize and establish regular estrus cycles of 4 to 5 days in duration. Females are placed overnight with a non-treated proven male breeder and examined the next morning for evidence of copulation. Successful mating is identified by the presence of a vaginal copulatory plug. The day that plugs are discovered is identified as day 0 of gestation. Mice are sacrificed on day 18 of gestation for fetal examination.

Sexually mature virgin female rats are obtained and conditioned as previously described for mice. Females are examined by vaginal lavage late in the afternoon for signs of proestrus (75-90% of nucleated epithelial cells). Females in proestrus are placed overnight with an experienced male. The following morning, females are examined for sperm or the presence of a vaginal

plug. The plug, however, is not as reliable an indicator of successful mating in rats as it is in mice. Rats are sacrificed on day 20 of gestation and examined for fetal anomalies.

Virgin female rabbits, 6 to 8 months of age, are obtained from commercial sources and are conditioned for 18 days in our animal quarters. Ovulation is induced by the intravenous administration of 1 mg/kg pituitary lutenizing hormone. Females are artificially inseminated within 1 hour by the method of Gibson, et al.^{1/} Fetuses are delivered by cesarean section on days 27 to 28 of pregnancy and examined for anomalies.

Mouse, rat and rabbit dams are sacrificed by CO₂ anesthesia prior to delivery since many animals tend to cannabilize their defective offspring. A laparotomy is performed and the uterine horns are exposed. The number of corpora lutea and number and position of live, dead, and resorbed fetuses is recorded. The umbilical cord is clamped and severed distally in order to prevent blood loss. Fetuses are removed, weighed and immediately examined by experienced personnel for external anomalies as fully described by Wilson.^{2/}

One-half of the rodent fetuses from each litter are dissected and examined for soft tissue anomalies by the free-hand slicing method of Wilson.^{2/} Each fetus is fixed in 20 to 25 ml of Bouin's fluid for 2 weeks. The hardened fetuses are examined for external anomalies and serially cut from the head through the trunk into 1 mm thick sections using a sharp razor blade. No slices are made beyond the kidneys and the intestines are carefully removed from the pelvic cavity. The cross sections of the fetuses and the genito-urinary organs on the pelvic floor are carefully examined by experienced personnel. The remaining fetuses from each litter are processed for skeletal examination. Fetuses are fixed in 70% alcohol for 2 weeks and eviscerated. The fetuses are stored in 1% KOH for 2 days and then stained with alizarin red.^{3/} After differential decolorization, the skeletons are examined by experienced personnel for anomalies. For rabbits, all fetuses are examined for both soft tissue and skeletal defects.

C. Perinatal and Postnatal Study

1. Objectives

The purpose of this phase of the protocol is to determine the effect of drugs administered during the last third of pregnancy and the period of lactation. The specific areas of study are the drug effects on late fetal development, labor and delivery, lactation, neonatal viability, and growth of the newborn.

2. Method

The conditioning, mating, and establishment of pregnancy in rats and mice are as previously described. The drug is administered to the dam during the final one-third of gestation and continued throughout lactation to weaning. The test compound is incorporated into the diet and a pair-fed control group, whose food intake is limited to the least amount of food consumed by the treated group, is included in the study. Treatment in rats and mice is initiated on day 16 of gestation and continued until the pups are weaned at 21 days of age. Labor and delivery are observed whenever possible and any signs of abnormal, prolonged, or delayed labor are carefully noted. The duration of gestation is calculated for each mother in all groups. The litters are examined as soon as possible after delivery, and at 4 and 21 days of age. The examination of the pups is conducted with a minimum disturbance of the mother. The following information is recorded for all the litters in each group:

- a. Litter size
- b. Number of stillborn and live born
- c. Anomalies of dead and live pups
- d. Number and weight of pups at 4 and 21 days of age
- e. Indexes of
 - (1) Fertility: confirmed pregnancies/sperm positive females
 - (2) Gestation: confirmed pregnancies with viable fetuses/confirmed pregnancies
 - (3) Viability: pups alive at day 4/pups alive at birth
 - (4) Lactation: pups alive at day 21/pup alive at day 4

III. STATISTICAL ANALYSIS OF DATA

Two important considerations in performing a valid statistical analysis are the determination of the sample size and the selection of appropriate statistical tests. In a study of developmental toxicity the sample size is determined by the selection of experimental units. The litter, rather than individual fetuses, is considered to be the unit of observation for our studies since the dam is the unit of treatment and the fetal response is dependent, to some degree, on maternal influences.

The data collected fall into two categories. The first category is enumeration or discontinuous data. Examples of discontinuous data are the number of sperm positive animals with evidence of conception, mortality, and indexes of fertility and gestation. The Fisher Exact Probability Test^{4/}

is the test of choice to evaluate the significance. Such enumeration data are reported with the exact 95% confidence limits.

The second category is quantitative or continuous data. Examples of continuous data are body weight, food consumption, and the remaining indexes. Such quantitative data are reported as the mean $\pm$ the standard error (S.E.). These data are analyzed by Bartlett's test^{5/} for homogeneity. The tests of significance for homogeneous data are either Dunnnett's procedure (one control group) or Tukey's omega procedure (more than one control group). If heterogeneity is indicated, then significance is based on multiple comparisons with the nonparametric rank test.^{6/} The level of significance is selected as $P < 0.05$.

IV. INTERPRETATION OF DATA

A. Phases of Fetal Development

The development of an adult organism from a single cell may be divided into six phases.^{7/} The first phase of development, gametogenesis, involves the growth and maturation of the egg and sperm. The gametes fuse during the second phase of development and the quiescent egg is activated to continue its developmental program. Cleavage, the third phase of development, encompasses a period of rapid cell division without a significant change in embryonic size or cellular differentiation. The embryo, at the end of cleavage, is referred to as a blastula and consists of a layer of cells, the blastoderm, surrounding a cavity, the blastocoele. The embryo attaches to the uterine wall and begins the process of placentation at the blastula stage. Gastrulation is the fourth developmental phase and involves the formation of germinal layers from the blastoderm. Primary organ rudiments are derived from the germinal layers during organogenesis, the fifth phase of development. The sixth phase of development is a period of growth and histological differentiation. The organ rudiments grow during this period and acquire the structure and biochemical properties characteristic of adult tissues. Organs grow by increasing both the number and size of cells. Tissue specific characteristics are established by a differential expression of the genetic information.

Treatments may affect the various phases of both animal and human development. The protocol used in our laboratory is designed to determine the developmental toxicity of a treatment in laboratory animals. The various parameters used to measure developmental toxicity help to determine if an agent affects any of the six developmental phases previously described. Since it is not practical to study each phase of development separately, the various phases are combined into periods of study. The units of study are the pre-implantation period (phases 1-3), post-implantation period (phases 3-5), and the period of differentiation (phase 6).

The dam and developing animal represent an integrated unit during the time of treatment. Effects which are observed in the developing animal, therefore, may be due to toxicity of the treatment in either the dam or developing animal. As development progresses it becomes more difficult to attribute an effect to a single period of study or treatment.

B. Fertility and General Reproductive Performance Study

The fertility and general reproductive performance study involves treating females during all six phases of development and treating males only during the period of gametogenesis. The effect of the treatment in females is studied at mid-gestation and after delivery. Males, on the other hand, are mated with normal females and treatment effects are studied in these females at mid-gestation and after delivery.

Some females are examined at mid-gestation and the various parameters previously described are recorded. The number of corpora lutea are counted by gross inspection and this value provides a measure of the ova released during ovulation. The number of implantations is used as a measure of the fertilized ova that developed to a stage where an attachment to the uterine wall is obvious at the time of inspection. The observations are summarized in the form of indexes. The fertility index is the percentage of mated females that are pregnant. A reduction in this index reflects pre-implantation losses. The implantation index is the percentage of ova that implant and it also provides a measure of pre-implantation losses. The implant viability index is the percentage of implants which appear normal at the time of examination. A reduction in this index serves as an indication of post-implantation losses. The gestation index is the percentage of pregnant females with one or more viable embryos and provides a measure of post-implantation survival.

Some females are examined after birth and the growth and development of the pups is recorded as previously described. Effects observed at this time may have been produced at any of the six developmental phases. The observations are summarized in the form of indexes. The fertility index provides a measure of pre-implantation losses. The gestation and implant viability indexes calculated on the basis of pups rather than embryos, provide an indication of post-implantation losses. The viability index is the percentage of live-born pups which survive to day 4. A reduction in this index reflects an effect at the post-implantation or differentiation period since normal pups can survive for brief periods without maternal care. The lactation index is the percentage of pups alive on day 4 which survive to day 21 and is a measure of effects occurring during the period of treatment. A reduction in this index reflects an impaired ability of the mother to nourish the young, the passage of toxic material to the young through the milk, and/or the manifestation of a developmental defect.

Effects observed at the mid-gestation or postnatal examination in females mated with treated males are indicative of toxicity produced during spermatogenesis, the first phase of development. Abnormalities in sperm may be manifested at any of the developmental stages beginning with fertilization. The previously described parameters are used to identify these effects.

C. Teratology Study

The teratology study involves treating pregnant females during the period of organogenesis and observing fetuses prior to term in order to identify possible effects on development. Treatment of rodents from day 6 through 15 of gestation roughly corresponds to developmental stages 3 to 5 which are in the post-implantation period. If evidence of toxicity is observed during fetal examination, then a primary effect was produced at any of these stages. The primary effect may be compounded into a series of secondary effects as development progresses.

Malformations may fall into three groups.⁸⁷ The first group is common variations and includes retarded ossifications. The second group is minor anomalies and refers to effects such as malformed sternabrae, wavy ribs, and supernumerary ribs. The third group is major malformations and includes anomalies which seriously affect the growth and survival of the offspring. Malformations are not equally significant or useful in interpreting or extrapolating animal experimental studies to man. Anomalies such as supernumerary ribs and decreased or abnormal sternal ossification patterns, for example, might be of little importance both to the animal and to attempts at predicting toxicity in humans. Malformations of doubtful significance include curly tail, straight legs, malrotated limbs and paws, wrist drop, protruding tongue, enlarged atria and/or ventricles, abnormal renal pelvic development and translucent skin.

The defects are reported as an anomaly index. The percent of the fetuses with a given defect is calculated for each litter and these values are then averaged and presented as the mean $\pm$ standard error (S.E.). The mean value provides a measure of the affected fetuses per litter for the group and the standard error provides an estimation of the distribution of the effect between litters within the group.

D. Perinatal and Postnatal Study

This study involves treating the dam during both the later portion of developmental phase 5 and most of phase 6. The growth and development of the pups is observed to monitor possible developmental toxicity. The various

indexes which are used to summarize these observations are discussed above in the section on Interpretation of the Fertility and General Reproductive Performance Study.

V. DISCUSSION OF PROTOCOL

A variety of experimental protocols are available to obtain information concerning the effects of agents on reproduction and development. An aim of these animal studies is to provide information concerning the risk of exposing the human population to chemical agents. The procedure used in our laboratory to obtain this information complies with the FDA guidelines for general reproduction, teratology, and perinatal and postnatal studies. There are problems associated with conducting and evaluating the results.

A. Problems Conducting Protocol

1. Selection of Test Animal

The ideal test animal should (1) absorb, metabolize and eliminate the test substance the same way as humans, (2) transmit the substance and its metabolites to the developing animal at the same rate as humans, and (3) have embryos, fetuses, and neonates with the same development schedules and metabolic pathways as the developing human. The existing comparative data is insufficient to determine which animal species is most like man in any of these characteristics. The currently available information, however, indicates that no presently used species, including simian primates, is like man in all of these respects.^{9/} The degree of similarity to man that a given species exhibits may vary from one test substance to another. The above criteria for an ideal test animal should be considered, as far as the available information permits, in the selection of test species. The advantages and disadvantages of species commonly used for these tests are:

a. Mouse: The mouse is inexpensive to maintain in large breeding colonies and its embryology is well documented. Small size with a limited supply of tissues and body fluids is a disadvantage in the examination of fetuses for defects and in studies on absorption, metabolism, and excretion of chemical agents. Mice respond to some substances that have limited teratogenicity in other animals and have earned the reputation for unusual sensitivity to teratogens.^{9/}

b. Rat: The rat has a convenient size for evaluation and analytical purposes, high fecundity, and a low incidence of spontaneous malformations. There is, however, no adequate single source of information on rat embryology, although this information is covered in numerous research papers.

c. Rabbit: The large size of this species permits the collection of large amounts of body fluids and tissues for analysis. Disease and parasites present obstacles to high reproductive performance in some laboratories and good stocks of rabbits are not universally available. The embryology is not fully documented for rabbits but is adequate for most purposes. Since this species was among the first animals to respond teratogenically to thalidomide, rabbits have been credited with greater similarity in teratogenic sensitivity to man than is warranted. There is no reason to regard the rabbit rather than the various species of rodents, which are their close relatives, as a more valid test animal for evaluating the teratogenic risk of agents in humans.

2. Selection of Dosage

Problems associated with selecting the dosage are the route, amount and duration of treatment. The practice of administering test substances to animals by the same route that will be used clinically is sound. If animals are treated orally for a short period of time, as in teratology studies, then gastric gavage is preferred to incorporating the agent into the diet. A stomach tube permits the accurate administration of a dose and eliminates the variables of food wastage and possible chemical change as a result of exposure to air, light, and other dietary ingredients. Prolonged treatment of animals by gastric gavage is not practical, however, due to the increased risk of trauma and expense associated with daily animal treatments. Agents incorporated into an animal's diet may alter the normal food intake as a result of an effect on appetite or a disagreeable odor or smell. Pair feeding, therefore, is required to determine the effect of reduced feed consumption on growth and development.

The dose levels should include a dose which produces maternal toxicity. The rationale for selecting this dose is to ensure that a maternal response is produced. Maternal toxicity may be measured in terms of lethality, weight loss or any other parameter that is related to treatment. If development is disrupted at doses which produce maternal toxicity, then lower doses should be studied in order to identify a dose below which no effect is observed on development. The identification of a dose which produces neither adult nor developmental toxicity is of value in estimating a safe dose for humans.

Animals are treated throughout various phases of development in this protocol to determine the effect of the agent on development. A treatment schedule which involves prolonged drug exposure presents three basic problems which affect the actual level of drug exposure and the detection of developmental toxicity. First, prolonged drug exposure may increase the activity of the drug metabolizing enzymes which are responsible for the biotransformation of chemicals. The metabolism of the test compound, therefore, is increased; maternal blood levels of the parent compound are decreased;

and maternal exposure to metabolites may be increased. Second, prolonged drug exposure may produce liver and/or kidney damage. A reduction in the functional capability of the liver reduces the biotransformation of the test compound while impaired kidney function may reduce the elimination of the drug from the body. Third, if a compound is administered during the early portion of gestation, then implantation and early embryonic survival may be impaired. The presence of small litter size and a high degree of resorption prevents the detection of teratogenic effects.

The length of gestation in most experimental animals is short compared to that of humans. Treating experimental animals during gestation may not produce tissue levels which could occur from more prolonged drug exposure as in human pregnancy. This difficulty can, in some cases, be overcome by increasing the dose, but problems may arise if the drug is poorly absorbed or degraded prior to absorption.

3. Determination of Feed Consumption

Animals may be treated during developmental toxicity studies by incorporating the test compound(s) into their diet. The compounds may represent either a fixed or variable percent of the diet. Since feed consumption varies during gestation and lactation, it is advisable to administer the drug as a variable percent of the diet in order to administer a constant amount of drug. The drug intake can be calculated from the percent of the drug in the diet and the amount of feed consumed. Thus, an accurate estimation of feed intake is imperative.

Accurate measurement of feed intake in laboratory animals, especially in rodents, is difficult due to spillage. Feed can be given to rats in stainless steel diet feeders (Model HB-69, Hoeltge, Cincinnati, Ohio) and to mice in stainless steel compartment feeders (Lab Products Inc., Garfield, New Jersey) which are designed to eliminate spillage. In most cases, these feeders are spill-proof; however, animals occasionally acquire the necessary skill to defeat the feeder. When feed consumption is high and the spillage can be measured, then the true feed consumption is calculated. If, on the other hand, the spillage can not be reasonably estimated, the result is omitted.

B. Problems Interpreting the Data

The ultimate goal of testing drugs in animals is to obtain information for making predictive statements concerning a drug's effect in humans. There are problems inherent to animal experiments, particularly in reproduction and teratology studies which make this extrapolation especially difficult. After the data from a developmental toxicity study have been collected

and analyzed statistically, it is necessary to determine both the significance of defects on normal adult animals and the relevance of the defects to humans.

When developing animals are examined at various times after treatment, evidence of deviant development, as demonstrated by growth retardation, malformations, intrauterine death and functional defects, may be apparent. These observations, however, do not provide information concerning the consequences of these effects in the adult. Growth retardation, for example, may be present in fetuses during a teratology study but may be absent in the adult as a result of maturation and compensatory growth processes. A delayed ossification of bones and the presence of extra ribs are examples of defects which may be corrected during growth or present a problem of questionable significance to the adult. The relevance of these defects to normal growth are, in some cases, difficult to assess experimentally.

The variation between species in response to agents presents the major obstacle to achieving the ultimate goal of any drug testing program. These unique responses of species to agents may be due to metabolic and pharmacokinetic factors.^{10/} The complexity of the animal system and degree of interspecies variability increases during development as a result of the formation of a placenta and its influence on drug transport, and a changing embryonic sensitivity to drugs. The ability to demonstrate developmental toxicity, therefore, depends on biotransformation of the drug by the mother, placenta, or embryo; pharmacokinetic properties of the drug in the mother and embryo; and embryonic sensitivity at the time of treatment.

VI. TERMINOLOGY OF ANOMALIES

A. Gross Anomalies

1. General

edematous - abnormal accumulation of clear fluid under the skin
hematoma - a localized mass of extravasated blood that is relatively or completely confined within an organ or tissue; not as a result of cesarean section handling
immature skin - skin is sticky with a shiny appearance

2. Head

anophthalmia - absence of one or both eyes
brachygnathia - abnormal shortness or recession of the mandibles
cranium, domed - excessively domed cranium suggestive of hydrocephalus

exencephalus - skull defective, the brain is exposed or extruded
eye, open - eyeball exposed with lids absent or withdrawn
lip, cleft - fissure in the lip, usually causing conjunction
of nasal passage and mouth
meningocele - skin intact, translucent, and elevated by a
fluid filled vesicle of meninges which protrude through a
midline defect in the cranium
meningoencephalocele - meninges and part of brain protruding
through a cranial defect to cause an irregular mass beneath
the skin
microphthalmia - small or rudimentary eyes
palate, cleft - fissure in hard palate, due to a failure of
the palatine shelves to unite
platycephaly - flatness of the skull

3. Trunk

anus, closed - (imperforate anus) anus closed by a membrane
so as to prevent the normal passage of intestinal contents
gastroschisis - protrusion of intestines and other abdominal
viscera through a ventral midline defect
kyphosis - convexity backward, dorsal-ventral curvature of
the spine
myelomeningocele - absence of the vertebral arches through
which the spinal cord and its membranes protrude, denoted
by a bubble-like bulge along the dorsal midline
rachischisis - congenital fissure of the spinal column with
failure of the skin and vertebral column to close
spina bifida - absence of the vertebral arches through which
the spinal membranes, with or without the spinal-cord tissue,
protrude, denoted by a raw, usually bloody depression
umbilical hernia - protrusion of intestines through a small
ventral midline defect

4. Extremities

acaudate - no tail
adactyly - absence of digits
club foot - abnormal flexion of the foot
micromelia - rudimentary limbs
oligodactyly - fewer than five digits
polydactyly - more than five digits
syndactyly - fused or webbed digits
tail, short - tail is less than half the normal length

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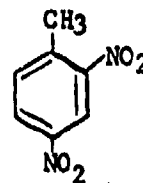
APPENDIX III

ASSAY REPORTS

MIDWEST RESEARCH INSTITUTE
Contract No. DAMD17-74-C-4073
18 November 1974

Data on: 2,4-Dinitrotoluene

Submitted by: James Dilley



Supplier: K & K Laboratories
Lot No.: 2,4-DNT-1

I. Identity

A. Capillary Melting Point

Observed - 70-72.5°
Reported^{1/} - 71°

B. Spectra

1. Infrared: The IR spectrum (KBr wafer) of the sample was compatible with the compound's structure and identical to the reported spectrum.^{2/}

2. Nuclear Magnetic Resonance: The NMR spectrum (solvent CDCl₃) of the sample was compatible with the compound's structure and identical to the reported spectrum.^{3/}

3. Ultraviolet: The sample in cyclohexane exhibited the same absorption maximum (233 nm) as in the reported spectrum.^{4/}

- ^{1/} Handbook of Chemistry and Physics, 50th edition, p. C-518.
^{2/} Sadtler Standard Spectra. Infrared No. 175.
^{3/} Sadtler Standard Spectra. NMR No. 3229.
^{4/} Sadtler Standard Spectra. UV No. 2550.

II. Assay

A. Elemental Analysis

<u>Element</u>	<u>C</u>	<u>H</u>	<u>N</u>
% calculated	46.11	3.30	15.37
% observed	46.36	3.32	15.32

B. Thin-Layer Chromatography

1. Plate: Brinkmann silica gel NF
2. Solvent System: ethyl acetate/petroleum ether (15:85)
3. Material Spotted: 100 µg Lot 2,4-DNT-1
10 µg 2,3-dinitrotoluene
10 µg 2,5-dinitrotoluene
10 µg 2,6-dinitrotoluene
10 µg 3,4-dinitrotoluene
10 µg 3,5-dinitrotoluene
4. Detection: 5% diphenylamine in ethanol spray
5. Results: Lot 2,4-DNT-1 moved as a single spot with a $R_f = 0.52$.

C. Gas Chromatography

The sample was studied using the following system:

Gas chromatograph: Varian 200

Detector: Flame ionization

Column: 6 ft x 1/8 in., aluminum
1.5% DC LSX-3-0295
1.5% GE XE-60
on Gas chrom Q

Injector T° : 150°

Column T° : 150°

Detector T° : 200°

Flow rate: 40 cc N_2 /min

This work indicated 2,6-dinitrotoluene as an impurity in a concentration of 1.7%.

III. Conclusions

Lot 2,4-DNT-1 contains 98% 2,4-dinitrotoluene and about 2% 2,6-dinitrotoluene.

MIDWEST RESEARCH INSTITUTE

Sandra Reich

Sandra Reich
Assistant Chemist

Nita West

Nita West
Assistant Chemist

M. Harris

Mike Harris
Assistant Chemist

Approved:

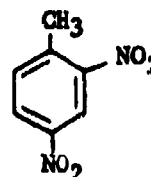
Dan Helton

Danny O. Helton
Associate Chemist

MIDWEST RESEARCH INSTITUTE
Contract No. DAMD17-74-C-4073
12 June 1975

Data on: 2,4-Dinitrotoluene

Submitted by: James Dilley



Supplier: K & K Laboratories
Lot No.: 2,4-DNT-3

I. Identity

A. Capillary Melting Point

Observed - 67 to 70°
Reported^{1/} - 71°

B. Spectra

1. Infrared: The IR spectrum (KBr wafer) of the sample was compatible with the compound's structure and identical to the reported spectrum.^{2/}

2. Nuclear Magnetic Resonance: The NMR spectrum (solvent CDCl₃) of the sample was compatible with the compound's structure and identical to the reported spectrum.^{3/}

3. Ultraviolet: The sample in cyclohexane exhibited the same absorption maximum (233 nm) as in the reported spectrum.^{4/} An ϵ_{233} of 17,400 was observed. Our previous Lot 2,4-DNT-1 gave an ϵ_{233} of 16,900.

^{1/} Handbook of Chemistry and Physics, 50th edition, p. C-518.

^{2/} Sadtler Standard Spectra. Infrared No. 175.

^{3/} Sadtler Standard Spectra. NMR No. 3229.

^{4/} Sadtler Standard Spectra. UV No. 2550.

II. Assay

A. Elemental Analysis

<u>Element</u>	<u>C</u>	<u>H</u>	<u>N</u>
% calculated	46.11	3.30	15.37
% observed	46.08	3.33	15.33

B. Thin-Layer Chromatography

1. Plate: Brinkmann silica gel F
2. Solvent System: Ethyl acetate/petroleum ether (15:85)
3. Material Spotted: 100 µg Lot 2,4-DNT-3
10 µg 2,3-dinitrotoluene
10 µg 2,6-dinitrotoluene
10 µg 3,4-dinitrotoluene
10 µg 3,5-dinitrotoluene
10 µg 2,4-dinitrotoluene
4. Detection: UV (254 nm)
5. Results: Lot 2,4-DNT-3 showed a major spot at R_f 0.60 and a trace spot at the R_f of 2,6-DNT.

C. Gas Chromatography

The sample was studied using the following system:

Gas chromatograph: Bendix 2500

Detector: Flame ionization

Column: 6 ft x 1/4 in. glass
1.5% DC LSX-3-0295
1.5% GE XE-60
on Gas chrom Q

Injector T° : 170°

Column T° : 150°

Detector T° : 200°

Flow rate: 50 cc N_2 /min

This work indicates 2,6-dinitrotoluene to be present in a concentration of 1.3%

III. Conclusions

Lot 2,4-DNT-3 contains 2,4-dinitrotoluene in a purity of $98.5 \pm 0.5\%$ and $1.5 \pm 0.5\%$ 2,6-dinitrotoluene.

MIDWEST RESEARCH INSTITUTE

Bernadette Chipko
Assistant Chemist

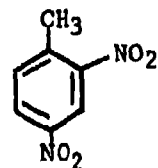
Approved:

Danny O. Helton
Associate Chemist

MIDWEST RESEARCH INSTITUTE
Contract No. DAMD17-74-C-4073
September 16, 1975

Data on: 2,4-Dinitrotoluene

Submitted by: Harry Ellis, III



Supplier: K & K Laboratories
Lot No.: 2,4-DNT-4

I. Identity

A. Capillary Melting Point

Observed - 69-71°
Reported^{1/} - 71°

B. Spectra

1. Infrared: The IR spectrum (KBr wafer) of the sample was compatible with the compounds structure and identical to the reported spectrum.^{2/}

2. Nuclear magnetic resonance: The NMR spectrum (solvent CDCl₃) of the sample was compatible with the compound's structure and identical to the reported spectrum.^{3/}

3. Ultraviolet: The sample in cyclohexane exhibited the same absorption maximum (233 nm) as in the reported spectrum.^{4/}

II. Assay

<u>Element</u>	<u>C</u>	<u>H</u>	<u>N</u>	<u>O</u>
% Calculated	46.11	3.30	15.37	35.22
% Observed	46.30	3.46	15.46	35.03

^{1/} Handbook of Chemistry and Physics, 50th edition, p. C-518.

^{2/} Sadtler Standard Spectra, Infrared No. 175.

^{3/} Sadtler Standard Spectra, NMR No. 3229.

^{4/} Sadtler Standard Spectra, UV No. 2550.

B. Thin-Layer Chromatography

1. Plate: Brinkmann silica gel NF
2. Solvent system: Ethyl acetate/petroleum ether (15:85)
3. Material spotted: 100 µg Lot 2,4-DNT-1
10 µg 2,3-dinitrotoluene
10 µg 2,6-dinitrotoluene
10 µg 3,4-dinitrotoluene
10 µg 3,5-dinitrotoluene
4. Detection: 5% diphenylamine in ethanol spray
5. Results: Lot 2,4-DNT-1 moved as single spot with a $R_f = 0.224$

C. Gas Chromatography

The sample was studied using the following system:

Gas chromatograph: Bendix 2500

Detector: Flame ionization

Column: 6 ft x 1/8 in. glass
1.5% DC LSX-3-0295
1.5% GE XE-60 on gas chrom Q

Injector T° : 150°

Column T° : 150°

Detector T° : 220°

Flow rate: 50 cc N_2 /min

Results: This work indicated 2,6-dinitrotoluene as an impurity in a concentration of 2.2%.

III. Conclusions

Lot 2,4-DNT-4 contains 97.8% 2,4-dinitrotoluene and 2.2% 2,6 dinitrotoluene.

MIDWEST RESEARCH INSTITUTE

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