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MAMMALIAN TOXICITY OF MUNITIONS COMPOUNDS
PHASE II: Effects of Multiple Doses
PART II: 2,4-Dinitrotoluene

PROGRESS REPORT NO. 3
November 1978

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For

Project Officer: Dr. Jack C. Dacre
Environmental Protection Research Division
U.S. Army Medical Bioengineering
Research and Development Laboratory
Fort Detrick
Frederick, Maryland 21701

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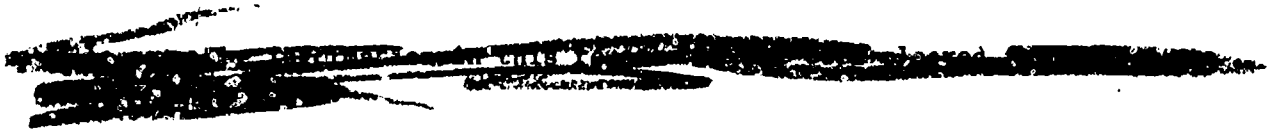

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Mammalian Toxicity of Munitions Compounds

Phase II: Effects of Multiple Doses

Part II: 2,4-Dinitrotoluene

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by

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Supported by

U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

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The effects of 2,4-DNT after oral administration for up to 13 weeks were investigated in dogs, rats, and mice. A detailed study of the disposition and metabolism was performed in rats and the metabolic pathways compared in dogs, rats, mice, rabbits, and monkeys.

The main target organs for the toxic effects of 2,4-DNT were the erythrocytes (methemoglobinemia leading to Heinz bodies and to anemia and its sequelae), the

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20. Abstract (concluded)

testes (depressed spermatogenesis), and the neuromuscular system (a rigid paralysis associated with mild central nervous system lesions). The highest no effect levels were 5 mg/kg/day in male and female dogs, less than 34 mg/kg/day in male rats and less than 38 mg/kg/day in female rats, and 137 mg/kg/day in male mice and 147 mg/kg/day in female mice.

^{14}C-2,4-DNT was well absorbed after oral dosing by all species tested except mice. The radiolabel was concentrated in the liver and kidney. Primary biotransformation reactions were reduction of one or both nitros to amines and oxidation of the methyl to a benzyl alcohol or benzoic acid. Secondary conjugation reactions occurred before excretion in the urine.

PREFACE

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

This work was conducted in the Biological Sciences Division, under the direction of Dr. William B. House, between 1 August 1974 and 28 February 1978. The experimental work was directed by Dr. Cheng-Chun Lee, Deputy Director, Biological Sciences Division, for Pharmacology and Toxicology with the assistance of Dr. Harry V. Ellis, III, Associate Pharmacologist, and Mr. John J. Kowalski, Assistant Biologist. Dr. John R. Hodgson, Head, Biochemical and Developmental Pharmacology Section, supervised the studies on metabolism, cytogenic and mutagenic studies. Dr. Shang W. Hwang, Associate Pharmacologist, assisted the studies on metabolism. Dr. Robert D. Short, Associate Pharmacologist, supervised the in vitro study on metabolism and in vivo study on drug metabolizing enzymes. Dr. J. C. Bhandari and Dr. Jaime L. Sanyer, Associate Veterinary Pathologists, supervised the necropsy and the histology preparation and performed the microscopic examination. Mr. Thomas W. Feddig (ASCP certified M.T.), Laboratory Supervisor, supervised the hematology and clinical laboratory tests. Mr. Jan L. Minor, Assistant Toxicologist, supervised the computer program and analysis of experimental data. Technical personnel included Robert C. Byrne, Bruce S. Andersen, Mary A. Kowalski, Francis H. Brown, Ellen R. Ellis, Ernesto A. Castillo, Judith D. Girvin, Patricia L. Wilkerson, Bhanu S. Gosalia, Laurel M. Halfpap, William M. Bracken, and Rita D. Freeman.

Approved for:

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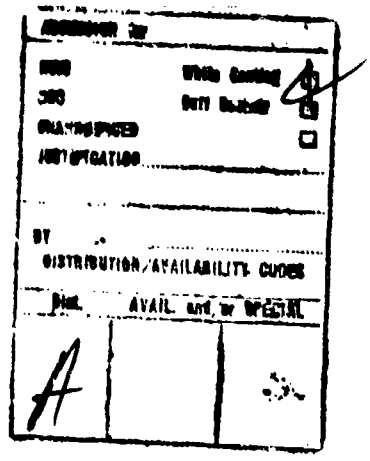
November 1978



MAMMALIAN TOXICITY OF MUNITIONS COMPOUNDS
PHASE II: Effects of Multiple Doses
PART II: 2,4-Dinitrotoluene
(Report No. 3)

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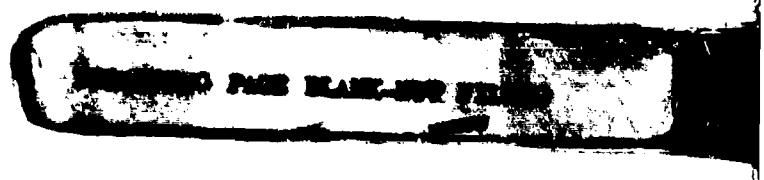

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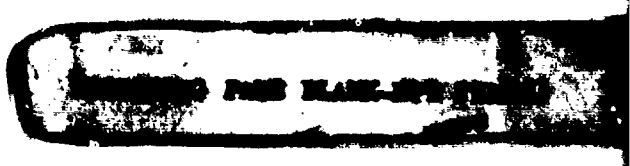
EXECUTIVE SUMMARY

The effects of 2,4-DNT after oral administration for up to 13 weeks were investigated in dogs, rats, and mice. A detailed study of the disposition and metabolism was performed in rats and the metabolic pathways were compared in various species including the liver of human cadavers.

In dogs, daily administration of 1 or 5 mg/kg/day of 2,4-DNT for 13 weeks did not cause any adverse effects. Daily treatment of 25 mg/kg/day was toxic after 12 to 22 days and lethal after 22 or more days. There was great variation in individual susceptibility. Three primary target organs were seen: the neuromuscular system, the erythrocytes and the testes. The neuromuscular effects observed grossly included incoordination and rigid paralysis resulting in anorexia and weight loss. These were associated with mild demyelination, gliosis and edema in the central nervous system. A causal relationship is not certain, but it is significant that demyelination of the optic nerve was seen only in one of the two dogs that suffered from transient blindness. The basic erythrocyte effect was methemoglobinemia. This led to Heinz bodies, anemia, reticulocytosis, hemosiderosis, and extramedullary hematopoiesis. The testicular effect was a decrease in spermatogenesis. This effect was noticeable after treatment for 4 weeks with 25 mg/kg/day of 2,4-DNT and severe after 13 weeks. Severely affected dogs recovered partially in 4 weeks after cessation of treatment, and completely in 8 months. Serum levels of immunoglobin E (IgE) were not affected by 2,4-DNT treatment.

In rats, the 2,4-DNT intake of males fed the low, middle, or high levels of 2,4-DNT in the feed averaged 34, 93, or 266 mg/kg/day, respectively. The female rats consumed an average of 38, 108, or 145 mg/kg/day, respectively. The low level caused a slight depression of weight gain. The middle level caused a more severe depression of weight gain, and hemosiderosis and reticulocytosis, presumably due to methemoglobin, and a depression of spermatogenesis. These effects were more severe and occurred earlier in rats fed the high level. In addition, there were anemia, an unusual gait with widespread and stiff hind legs, and gliosis and/or demyelination in the central nervous system. Ten of the 16 high dosage females died before the end of the third week; whereas, 8 of the 16 high dosage males died before their scheduled necropsy. There was partial recovery 4 weeks after cessation of 2,4-DNT. Chromocomes from lymphocyte and kidney cultures from rats fed the middle



level had increased numbers of chromatid breaks and gaps, but no significant numerical aberrations. Males fed the middle level for 13 weeks and then mated produced low fertility indexes and no viable fetuses, indicating sterility. A cytotoxic level of 2,4-DNT had no mutagenic effect on Chinese hamster ovary cells in vitro. In rats, as in dogs, 2,4-DNT did not affect serum IgE levels.

In mice, the 2,4-DNT intake of males fed the low, middle or high levels of 2,4-DNT in the feed averaged 47, 137, or 413 mg/kg/day, respectively. The female mice consumed an average of 52, 147, or 468 mg/kg/day, respectively. The low and middle levels were nontoxic. The high level caused weight loss, mild anemia, and a few deaths. Two of the four high dosage males terminated after 4 weeks had mild depression of spermatogenesis, but testicular lesions were not seen in those terminated after 13 weeks' feeding. The mice had recovered completely 4 weeks after cessation of treatment. In a dominant lethality test, male mice fed the 0.2% of 2,4-DNT for 13 weeks were normal, while those fed the 0.7% level for 4 weeks had a low fertility index and a normal implant viability index.

After oral administration (Ring-UL-^{14}C) 2,4-DNT was poorly absorbed (8 to 12% of the dose within 24 hours) by two strains of mice, but well absorbed (75% to 85%) by rats, rabbits, dogs and monkeys. The radioactivity was concentrated in the liver and kidney in all species. Metabolism was similar in all species studied. One or both nitros were reduced to aminos. The methyl was oxidized to a benzyl alcohol or further to a reactive benzaldehyde or a benzoic acid. All these products were conjugated to form a glucuronate or sulfate. The final products were excreted in the urine. Little or no 2,4-DNT itself was excreted. No radioactivity from the ring carbons was excreted in the air.

In female rats, after oral administration of radiolabeled TNT and various dinitrotoluenes, radioactivity appeared in the bile within 15 minutes. The time to peak height ranged from 15 minutes to over 6 hours, in the order: 3,4-DNT < 2,3-DNT < TNT = 2,4-DNT < 2,5-DNT = 3,5-DNT < 2,6-DNT < 4-amino-2,6-DNT. The amount of biliary excretion of radioactivity within 24 hours of dosing ranged from 10.3% to 27.3% of the dose in the order: TNT < 2,4-DNT < 2,5-DNT = 3,4-DNT = 3,5-DNT < 4-amino-2,6-DNT < 2,6-DNT < 2,3-DNT. The amount of radioactivity remaining in the GI tract plus contents and feces ranged from 3.1% to 46.8% of the dose, in the order: 2,3-DNT < 2,6-DNT < 2,4-DNT = 3,5-DNT < TNT = 2,5-DNT < 3,4-DNT < 4-amino-2,6-DNT.

2,4-DNT was metabolized in vitro by liver homogenates of mice, rats, rabbits, dogs and monkeys. In these species, between 8 and 27% of the parent compound was metabolized at the end of 1 hour of incubation. Under aerobic conditions, the primary product was 2,4-dinitrobenzyl

alcohol and the second product aminonitrotoluenes. The liver of rabbits formed more metabolites that the other species. Under anaerobic conditions, the amount of 2,4-dinitrobenzyl alcohol was reduced while more aminonitrotoluenes were produced. More aminonitrotoluenes were formed by males than females; and male rats produced the most. When male rats were fed 0.7% of 2,4-DNT, as used in the toxicity studies above, for 2 weeks, neither zoxazolamine paralysis time nor hepatic nitroanisole O-demethylase activity were affected.

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MAMMALIAN TOXICITY OF MUNITION COMPOUNDS
PHASE II: Effects of Multiple Doses
PART II: 2,4-Dinitrotoluene
(Report No. 3)

INTRODUCTION

Under Contract No. DAMD-17-74-C-4073, entitled, "Munition Compounds Mammalian Toxicity Study," we conducted Phase I studies on the effects of acute exposure of various munition compounds.[1/] During Phase II, we studied the effects of multiple exposure to selected compounds including trinitroglycerin (TNG), 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT) and nitrocellulose (NC). This report summarizes the results of Phase II studies on 2,4-DNT. Subacute and subchronic toxicities were performed in dogs, rats, and mice to determine the maximum tolerated dose and to define the biological nature and target organ(s) of the toxic effects. Reversibility of any adverse effects was determined. Mutagenicity of the compound was assessed. Immunologic response was studied by the detection of the serum IgE antibodies. A detailed study on the disposition and metabolism of the radiolabeled compound was performed in rats; the metabolites were isolated and identified. Comparison of the pathways of biotransformation was also studied in vivo in mice, rabbits, dogs and monkeys and in an in vitro system using tissues of the various species including the liver of human cadavers. For comparison, biliary excretion of various nitrotoluenes, including TNT, 2,3-DNT, 2,5-DNT, 2,6-DNT, 3,4-DNT, 3,5-DNT and 4-amino-2,6-DNT in rats were studied. Effects of the test compound on drug metabolizing enzymes were also investigated in in vivo and/or in vitro studies.

I. DOGS

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I. DOGS

A. Subacute and Subchronic Toxicities and Reversibility

1. Introduction

These studies were performed to define the nature and extent of effects of 2,4-DNT on the biological system at the biochemical and cellular levels and to elucidate the dose-response relationship in dogs after administration for 4 weeks and 13 weeks. The reversibility of adverse effects were studied after the treatment of 2,4-DNT was discontinued for 4 weeks.

2. Material and Methods

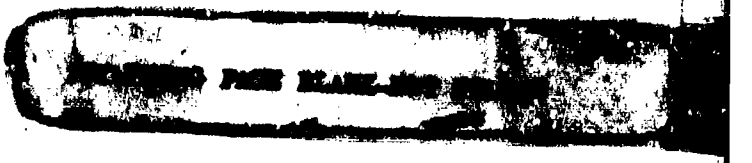
a. Number of Dogs, Sex and Treatment

A total of 32 young healthy beagle dogs (Hazelton Research Animals, Cumberland, Virginia) weighing between 7.5 and 12.5 kg were used for these experiments. The dogs were conditioned and observed carefully in our animal quarters for 3 weeks after their arrival from the supplier. They were divided into four groups, each consisting of four males and four females. The average weights of all groups were kept close.

Three groups of dogs were given 1, 5, or 25 mg/kg/day of 2,4-DNT in capsules. 2,4-DNT was purchased from K and K Laboratories (Cleveland, Ohio), and mixed with lactose, USP, in a ball mill to give mixtures containing 1%, 5%, and 25% of 2,4-DNT, respectively. After the weekly weighing, appropriate amounts of the proper mixture were weighed into capsules, so each dog received the intended dose. The fourth group served as the controls and was given empty capsules daily throughout the experiments. Purina dog chow and water were available ad libitum, except wherever specified.

b. Experimental Procedures

All dogs were observed daily for behavioral changes and toxic signs. Body weights of all dogs were recorded weekly. Blood samples were collected for laboratory tests before treatment and at 4, 8, 13, and/or 17 weeks during experiment. The tests included hematology, clinical blood chemistry tests and serum electrolytes. For fasting blood glucose, the dogs were fasted overnight for 16 hours. At termination or when moribund, the dogs were euthanized with an overdose of sodium pentobarbital and examined for gross lesions. Weights of heart, liver, spleen, kidneys, adrenals, and gonads were recorded, and organ weight to body weight ratios were calculated.



Various tissues were removed, fixed, processed, sectioned and stained for microscopic examination of lesions. The procedures for hematology, clinical blood chemistry tests, and histopathology, and the normal values are given in Appendix I.

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

Bromosulfophthalein (BSP) retention test was performed at termination. A single dose of 5 mg/kg of the sterile test dye (Dade, Miami, Florida) was injected intravenously following fasting for 16 hours. Serum level of the dye at 15 minutes was determined and the percent of retention in the plasma was calculated.[3]

The results of the various parameters were compared with the respective baseline levels and/or with those of the control groups at the respective time intervals according to the Dunnett's multiple comparison procedure.[4]

c. Experimental Design

At the end of 4 and again at 13 weeks of continuous treatment, one male and one female dog from each dosage group were euthanized for necropsy. The treatment for one other male and female dog from each group was discontinued at the end of 4 and 13 weeks and they were then euthanized at the end of 8 and 17 weeks, respectively, to study the reversibility of adverse effects. Several dogs from the high dosage group were moribund and euthanized before the scheduled time. The two dogs in the high dosage group (male No. 59 and female No. 62) who were removed from treatment after 4 weeks were not euthanized until 8 months later to test reversibility after recovery for a longer time.

3. Results

a. General Observations and Weight Gain

The control dogs and dogs receiving 1 or 5 mg/kg/day of 2,4-DNT were healthy throughout the treatment period of 4 or 13 weeks. Their body weights before, during and after treatment are summarized in Table 1. Some dogs consistently gained weight, and others gained or lost small amounts of weight.

In contrast, all of the dogs treated with 25 mg/kg/day began showing toxic signs after 12 to 22 days of treatment. Female No. 62 first had a loss of control of her hind legs on day 12 of the study. After 4 weeks' treatment, she was removed from treatment for recovery studies. Males Nos. 59, 63, and 65 first showed similar symptoms on day 14. No. 65 was killed when moribund after 22 days of treatment, and No. 63 after 24 days. No. 59 was placed in the recovery study with female No. 62 after treatment for 4 weeks. Two females first showed toxic signs on day 20. No. 64 was killed when moribund on day 36; No. 66 survived 13 weeks' treatment and was allowed to recover thereafter for 4 weeks. The last two high dosage dogs first showed symptoms on day 22. Female No. 60 was killed when moribund on day 48; male No. 61 was killed after 13 weeks.

The intensity of toxic signs varied between dogs and also in the same dog from time to time. As noted above, on day 22, male No. 65 was moribund, while male No. 61 was just beginning to show symptoms. Similarly, No. 65 was euthanized 8 days after he first showed symptoms, while male No. 59 survived 77 more days of continued treatment. Frequently, a dog showed severe symptoms for days or weeks, apparently recovered for a while, and then got worse again. When dogs were removed from treatment, the symptoms always lessened. After 4 weeks of recovery, they appeared normal except for occasional poor balance.

Toxic signs seen in virtually all affected dogs included decreased feed consumption, weight loss, yellow stain on and near hind legs, pale gums (sometimes blue-tinged), and a neuromuscular incoordination and paralysis. The weight loss is shown in Table 1, with some dogs losing half their initial body weight. The characteristic effects of 2,4-DNT were those on the neuromuscular system. The first manifestation was a stiffness and incoordination of the hind legs. As this progressed, the animals had difficulty maintaining their balance. Eventually, they were unable to stand, and collapsed, lying on one side. Their muscles stiffened with the legs extended and back arched. The stiffness progressed upward from hind legs to trunk, forelegs, neck, and then head. One dog with moderately severe signs was examined by Dr. Brazil, a veterinary neurologist at the University of

Missouri, Columbia. He found hyperreflexia of the extremities, intact spinal cord and nerves, opisthotonus, and a rolling motion to the right. The dog showed signs similar to those of a middle ear inflammation, but no such lesion was found.

Some toxic signs were seen in only a few of the dogs. Females Nos. 62 and 66 had transient blindness. When examined by Dr. Harlan Jansen, a veterinary ophthalmologist of the University of Missouri, Columbia, Missouri, No. 66 was functionally blind with widely dilated pupils unresponsive to light. Vertical or oval nystagmus was present when the dog's position was changed or when she was stimulated by a loud noise. The fundus appeared normal. Several dogs had occasional tremors; male No. 65 had a grand mal type convulsion shortly before he was euthanized. At times, some dogs salivated excessively. A few vomited occasionally, but the time was not observed.

b. Blood Analysis

Since the hematology and clinical blood chemistry results of the male dogs and the female dogs were not significantly different, the data from both sexes from the control group and the treated groups were combined and are summarized in Tables 2 through 5, respectively. In the low and middle dosage groups, the peripheral blood elements and various clinical chemistry parameters were not apparently altered by 2,4-DNT. However, when compared to baseline levels within the group, or when compared to the control dogs at the same time interval, there were a number of changes. The increase in the erythrocyte count and blood hemoglobin levels with a corresponding decrease in cell volume and cell hemoglobin is characteristic of maturing beagles of the age used. The other changes were slight and inconsistent and usually occurred in both control and treated dogs.

The high dose of 2,4-DNT (25 mg/kg/day) caused some changes in the blood. The erythrocyte count did not increase as did the controls and other treated groups. Hemoglobin, hematocrit and cell volume decreased. The reticulocyte count was high, except for two female dogs (Nos. 60 and 64) tested in week 6. These two dogs were very severely affected and had almost no reticulocytes and elevated BUN.

The treatment of one male and one female from each group was discontinued after 4 weeks to study reversibility of adverse effects. As seen in Table 6, all treatment groups are similar. A reversibility study was also performed after 13 weeks of treatment. Only one female treated with 25 mg/kg/day survived at the end of 13 weeks. The changes of her peripheral blood elements caused by 2,4-DNT were reversible after the treatment was discontinued for 4 weeks, although the erythrocyte count, hematocrit and hemoglobin remained lower than those of the control dogs (Table 7).

Methemoglobin and Heinz bodies were determined after 4, 8 and, 13 weeks of treatment. Results are shown in Table 8. Small amounts of methemoglobin were seen in the surviving high dosage dogs tested at both 8 and 13 weeks and in two of the four middle dosage dogs tested at 13 weeks. All levels found were minimal. Since the assay is the difference of two absorbance readings, some of the values may be artifact. Heinz bodies were seen in all high dosage group dogs at all times tested. When dogs were tested after 4 weeks' recovery, none had any methemoglobin. Only one high dosage group female (No. 62) had 0.4% Heinz bodies at 4 weeks after 4 weeks of 2,4-DNT treatment.

Serum electrolytes were assayed before treatment and after 4, 8, and 13 weeks' treatment. Results are shown in Table 9. No changes occurred during treatment.

c. BSP Retention

BSP retention was determined on the dogs before treatment and the dogs euthanized after 4 or 13 weeks' treatment. The results are shown in Table 9. 2,4-DNT did not cause any retention of BSP in these dogs.

d. Organ Weights

The absolute and relative organ weights of all dogs are shown in Tables 11 through 14. Changes were seen only in the high dosage dogs. Their livers were relatively large, due to decrease in body weight.

e. Gross and Microscopic Examination of the Tissues

The dogs in the control, low dosage and middle dosage groups terminated at various times were in good nutritional condition without any apparent gross changes. After 4 weeks, one control dog (No. 58) had mild inflammation in the lung and liver (Table 15). After 13 weeks, one control dog (No. 53) and both low dosage dogs (Nos. 77 and 78) had similar inflammation in their livers (Table 16). These are spontaneous lesions, not related to 2,4-DNT treatment.

The high dosage dogs were in fair to poor nutritional condition with little or no body fat. Two dogs that were moribund during the 4th week had cloudy swelling in the heart and kidney, gliosis, edema, demyelination in the cerebellum, brainstem and/or spinal cord (Table 15). In addition, one dog (No. 63) had subacute inflammation in the liver, tubular degeneration in the kidney, mucoid degeneration in the gastrointestinal tract, inactive germinal centers in the lymph node and aspermatogenesis in the testes; the other dog (No. 65) had emphysema in the lung and hemosiderosis in the liver. One dog (No. 64) was moribund during the 6th week, one dog (No. 60)

was moribund during the 7th week and one dog (No. 61) was terminated at the end of the 13 weeks. These three dogs had mild cloudy swelling in the heart or kidney, severe hemosiderosis in the liver, spleen or lymph node, mild to moderate gliosis, edema, demyelination in the cerebellum, brainstem or spinal cord and/or severe aspermatogenesis in the testes (Table 16). Other occasional lesions included mild pneumonia and emphysema in the lung, subacute inflammation in the liver, tubular degeneration in the kidney and lymphoid depletion in the spleen. The bone marrows and the myeloid/erythroid (M/E) ratios of these dogs were normal.

The dogs treated for 4 or 13 weeks and allowed to recover for 4 weeks or longer were in good nutritional condition. There were a few mild to moderate lesions in the lung, liver, and lymph nodes (Tables 17 and 18). Two high dosage dogs treated for 4 weeks and allowed to recover for 8 months had only a moderate pneumonia or a mild tonsilitis. The high dosage female dog treated for 13 weeks and allowed to recover for 4 weeks had mild demyelination of the cerebrum and optic nerve.

4. Discussion and Conclusions

Daily treatment of 1 or 5 mg/kg/day of 2,4-DNT for 13 weeks did not cause any adverse effects to dogs. Daily treatment of 25 mg/kg/day was toxic after 12 to 22 days and lethal after 22 or more days. There was great variation in individual susceptibility. Three primary target organs were seen: the neuromuscular system, the erythrocytes and the testes.

The neuromuscular effects observed grossly included incoordination and rigid paralysis resulting in anorexia and weight loss. These were associated with mild demyelination, gliosis and edema in the central nervous system. A casual relationship is not certain, but it is significant that demyelination of the optic nerve was seen only in one of the two dogs that suffered from transient blindness.

The erythrocytic effects are readily explained by the well-known methemoglobin-producing effects of nitro and amino compounds.[5/] 2,4-DNT or some of its metabolites oxidize hemoglobin to methemoglobin. The quantity of methemoglobin is more than the usual hemostatic systems can reduce, so it is destroyed. This process leads to the destruction of erythrocytes, deposits of hemosiderin in liver, spleen, and/or lymph nodes, Heinz bodies in the surviving erythrocytes, and a stimulation of erythropoiesis. The erythropoietic effect leads to an increase in circulating reticulocytes. Most of these effects were observed. Blood samples were normally taken 18 to 20 hours after dosing, and most methemoglobin had been removed from circulation.

The testicular effect was a decrease in spermatogenesis. This effect was noticeable after treatment for 4 weeks with 25 mg/kg/day of 2,4-DNT

and was severe after 13 weeks. However, a subsequent recovery period of 4 weeks or longer was sufficient to restore to normal histologic appearance.

The anorexia and subsequent malnutrition complicated the body's normal repair mechanisms by reducing the supply of exogenous protein and calories. Therefore, the liver was unusually active in catabolizing various tissue constituents. Because of this increased activity, the livers of the affected dogs did not reduce in weight, although these dogs had severe weight loss.

Morton, et al.[6] have recently reported significant increases in SGOT in workers exposed to TNT dust. We found no similar effects. This may be due to different properties of the two compounds or due to species difference.

There was a wide variation in individual susceptibility to the toxic effects of 2,4-DNT: one dog was moribund when two others began to show symptoms. Similar variability has been noted in workers exposed to 2,4-DNT fumes.[7] Furthermore, some dogs apparently adapted to continued ingestion of the compound and partially recovered from the toxic effects.

When treatment with 2,4-DNT ceased, affected dogs began to recover from the various effects. After 4 weeks, one dog had a minor balance problem and some demyelination in the cerebrum and optic nerve. Since this dog was female, we have no data on testicular recovery. Two other dogs kept for 8 months were completely recovered from all effects.

B. Immunologic Response to 2,4-DNT

1. Introduction

In humans, anaphylactic reactions were associated with high titers of immunoglobin E (IgE).[8] IgE, the allergic or hypersensitive antibody, was determined in dogs treated with 2,4-DNT.

2. Material and Methods

The immunodiffusion technique of Mancini et al.[9] was used for determination of serum IgE titer. Replicate samples of serum from the control dogs and dogs treated with various doses of 2,4-DNT at various intervals were placed in wells in an immunodiffusion chamber along with suitable standards. These dogs were used for subacute and subchronic toxicity studies as described in Section I.A. The diffusion chamber was incubated at 37°C for 48 hours and the diameter of the precipitin ring was measured. Since the square root of the diameter is directly proportional to the concentration of the antibody, the IgE concentration was quantitated with the standard antibody reagent.

3. Results and Conclusions

The results of IgE concentration of control dogs and dogs treated with 2,4-DNT are summarized in Table 19. Treatment of various doses of 2,4-DNT for 4, 8, or 13 weeks or treatment for 4 or 13 weeks followed by recovery for 4 weeks did not cause any apparent changes on serum concentration of IgE.

C. Summary

Doses of 1 or 5 mg/kg/day of 2,4-DNT for 13 weeks caused no adverse effects in dogs.

Doses of 25 mg/kg/day were toxic after 12 to 22 days and lethal after 22 or more days, with great variation in individual susceptibility. The target organs were the neuromuscular system (incoordination and rigid paralysis), the erythrocytes (methemoglobinemia and sequelae, including Heinz bodies and anemia) and the testes (decrease in spermatogenesis). Serum IgE levels were not affected by treatment. Severely affected dogs recovered partially in 4 weeks after cessation of treatment and completely in 8 months.

TABLE 1

BODY WEIGHTS OF DOGS TREATED WITH 2,4-DNT IN CAPSULES

Dose (mg/kg/day)	Dog No.	Sex	Body Weights (kg) Initial	4 Weeks	8 Weeks	13 Weeks	17 Weeks
0	57	M	11.0	10.8			
0	58	F	8.6	9.0			
1	81	M	10.6	10.4			
1	82	F	8.0	8.2			
5	73	M	12.2	13.0			
5	74	F	8.2	8.0			
25	63	M	11.8	8.8[a]			
25	65	M	13.2	10.8[a]			
0	55	M	12.4	12.2[b]	12.8		
0	56	F	8.8	9.2[b]	9.2		
1	79	M	12.8	[illegible] 8[b]	13.7		
1	80	F	7.7	.6[b]	7.8		
5	71	M	11.2	12.4[b]	11.9		
5	72	F	8.4	7.8[b]	8.6		
25	64	F	8.0	6.2	4.4[c]		
25	60	F	8.2	6.8	3.8[d]		
0	53	M	12.2	12.8	14.0	15.2	
0	54	F	8.4	9.0	9.0	10.0	
1	77	M	10.8	11.8	12.0	13.4	
1	78	F	8.2	7.8	8.0	9.6	
5	69	M	11.0	10.6	11.8	13.3	
5	70	F	8.0	9.8	7.8	9.0	
25	61	M	13.2	13.2	10.0	9.4	
0	51	M	10.0	11.0	11.5	12.0[b]	12.2
0	52	F	9.0	9.2	9.2	9.2[b]	10.2
1	75	M	10.9	11.0	11.9	12.8[b]	13.4
1	76	F	9.0	8.6	8.9	10.0[b]	10.4
5	67	M	10.6	10.6	10.9	11.2[b]	11.4
5	68	F	9.4	9.8	10.5	11.2[b]	12.0
25	66	F	9.4	8.2	8.7	9.3[b]	10.0
25	59	M	12.2	9.0[b]	9.8	11.5	12.8
25	62	F	8.6	6.2[b]	7.0	8.3	8.8

a/ Terminal weight before necropsy during week 4.
b/ Dosing discontinued thereafter.
c/ Terminal weight before necropsy during week 6.
d/ Terminal weight before necropsy during week 7.

TABLE 2

LABORATORY DATA OF CONTROL DOGS FOR 2,4-DNT

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

WEEKS	0(B. 8)	WEEK 4 (C. 8)	WEEK 8 (C. 4)	WEEK 13 (C. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	5.22 ± .17	6.48 ± .09[a/]	6.72 ± .12[a/]	6.96 ± .21[a/]
RETICULOCYTES, %	.68 ± .07	.86 ± .08	.50 ± .09	.50 ± .08
HEMATOCRIT, VOL. %	41.4 ± 1.4	45.5 ± .7[a/]	47.0 ± .9[a/]	48.5 ± 1.0[a/]
HEMOGLOBIN, GM. %	13.8 ± .5	15.8 ± .3[a/]	15.9 ± .4[a/]	16.7 ± .3[a/]
MCV, CUBIC MICRONS	79.4 ± 1.6	70.2 ± .6[a/]	70.0 ± .6[a/]	69.7 ± 1.1[a/]
MCHB, MICRO MICROGMS.	26.5 ± .5	24.3 ± .3[a/]	23.7 ± .3[a/]	24.0 ± .3[a/]
MCHBC, GM %	33.4 ± .1	34.7 ± .3[a/]	33.8 ± .3	34.5 ± .1[a/]
PLATELETS ($X10^5$ /MM^3)	2.6 ± .2	2.1 ± .2	2.1 ± .2	2.6 ± .1
LEUKOCYTES ($X10^3$ /MM^3)	12.9 ± .8	12.2 ± .5	10.7 ± .6	9.2 ± .5[a/]
NEUTROPHILS, %	62.1 ± 1.4	61.6 ± 3.2	65.3 ± 2.7	59.3 ± 2.3
LYMPHOCYTES, %	34.0 ± 1.7	35.0 ± 3.2	29.8 ± 3.8	29.8 ± 3.5
BANDS, %	.1 ± .1	0.0 ± 0.0	0.0 ± 0.0	1.5 ± .9[a/]
MONOCYTES, %	1.3 ± .3	.3 ± .2	.8 ± .3	3.5 ± .6[a/]
EOSINOPHILS, %	2.1 ± .6	3.1 ± 1.0	4.3 ± 1.4	6.0 ± 2.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
GLUCOSE (FASTING), MG %	86.3 ± 2.0	98.5 ± 2.3[a/]	88.8 ± 4.1	100.5 ± 2.6[a/]
SGOT, IU/L	27.8 ± 1.2	27.3 ± 1.5	30.0 ± 2.7	23.3 ± .8
SGPT, IU/L	27.3 ± 2.1	34.3 ± 3.2	33.3 ± 3.3	35.5 ± 3.6
ALK. PHOS., IU/L	69 ± 7	56 ± 6	40 ± 6[a/]	32 ± 5[a/]
BUN, MG %	12.3 ± .8	12.5 ± .6	13.0 ± 1.1	14.5 ± 1.4

ENTRIES ARE MEAN ± STANDARD ERROR

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

TABLE 3

LABORATORY DATA OF DOGS BEFORE AND DURING ADMINISTRATION OF 2,4-DNT

DOSE 1 MG/KG/DAY

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

WEEKS	0 (B, 8)	WEEK 4 (T, 8)	WEEK 8 (T, 4)	WEEK 13 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	5.16 ± .22	6.48 ± .07[a]	6.85 ± .21[a]	6.90 ± .36[a]
RETICULOCYTES, %	.76 ± .08	1.26 ± .08[a,b]	1.04 ± .08	.64 ± .09
HEMATOCRIT, VOL. %	45.3 ± .9[b]	47.4 ± .3	50.3 ± .9[a]	51.3 ± 1.5[a]
HEMOGLOBIN, GM. %	15.2 ± .3[b]	16.1 ± .1	16.8 ± .5[a]	17.6 ± .7[a]
MCV, CUBIC MICRONS	88.8 ± 4.4	73.1 ± .5[a]	73.5 ± 1.1[a]	74.5 ± 2.1[a]
MCHH, MICRO MICROGMS.	29.7 ± 1.4	24.8 ± .2[a]	24.6 ± .1[a]	25.5 ± .4[a]
MCHHC, GM %	33.5 ± .2	33.9 ± .2	33.5 ± .4	34.3 ± .6
PLATELETS ($X10^5$ /MM^3)	1.9 ± .2[b]	2.0 ± .2	2.1 ± .2	2.2 ± .4
LEUKOCYTES ($X10^3$ /MM^3)	13.1 ± 1.0	15.3 ± 1.5	13.9 ± 2.1	10.4 ± .8
NEUTROPHILS, %	59.8 ± 2.2	60.8 ± 2.4	61.3 ± 4.9	62.8 ± 5.6
LYMPHOCYTES, %	37.1 ± 2.2	36.4 ± 2.2	37.0 ± 5.0	33.0 ± 4.5
BANDS, %	.1 ± .1	0.0 ± 0.0	0.0 ± 0.0	.5 ± .3[a]
MONOCYTES, %	1.1 ± .3	.4 ± .2	.3 ± .3	2.5 ± 1.0
EOSINOPHILS, %	2.4 ± .8	2.5 ± .9	1.8 ± .5	1.3 ± .5
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	.1 ± .1	0.0 ± 0.0	0.0 ± 0.0
GLUCOSE (FASTING), MG %	96.3 ± 2.8[b]	96.3 ± 4.3	94.5 ± 4.3	101.3 ± 1.7
SGOT, IU/L	25.5 ± 1.4	29.1 ± .8[a]	23.3 ± .8	21.0 ± 0.0[a]
SGPT, IU/L	26.8 ± 2.8	26.8 ± 2.[illegible]	34.0 ± 2.4	34.8 ± 3.1
ALK. PHOS., IU/L	76 ± 6	60 ± 5	52 ± 4[a]	45 ± 5[a]
BUN, MG %	13.5 ± .6	12.6 ± .6	12.8 ± 1.7	13.5 ± 1.7

ENTRIES ARE MEAN ± STANDARD ERROR

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

b/ Significantly different from the control dogs at the respective time interval (Dunnett's multiple comparison procedure[4]).

TABLE 4

LABORATORY DATA OF DOGS BEFORE AND DURING ADMINISTRATION OF 2,4-DNT

DOSE 5 MG/KG/DAY

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

WEEKS	0 (B, 8)	WEEK 4 (T, 8)	WEEK 8 (T, 4)	WEEK 13 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	4.98 ± .23	6.18 ± .13[a]	6.32 ± .16[a]	6.59 ± .21[a]
RETICULOCYTES, %	.73 ± .14	1.40 ± .10[a,b]	1.28 ± .22	.90 ± .20
HEMATOCRIT, VOL, %	41.9 ± 1.2	46.1 ± 1.0[a]	45.8 ± 1.5	48.3 ± .9[a]
HEMOGLOBIN, GM, %	14.1 ± .4	15.1 ± .3	15.0 ± .5	16.5 ± .4[a]
MCV, CUBIC MICRONS	85.2 ± 3.8	74.6 ± .6[a,b]	72.3 ± 1.0[a]	73.4 ± 1.3[a]
MCHA, MICRO MICROGMS.	28.7 ± 1.2	24.4 ± .2[a]	23.7 ± .3[a]	25.0 ± .4[a]
MCHBC, GM %	33.7 ± .2	32.7 ± .2[a]	32.8 ± .2[a]	34.1 ± .3
PLATELETS ($X10^5$ /MM^3)	2.3 ± .2	2.4 ± .2	2.9 ± .1	3.2 ± .2
LEUKOCYTES ($X10^3$ /MM^3)	12.6 ± .7	13.4 ± .6	13.0 ± 1.9	10.0 ± 1.3
NEUTROPHILS, %	59.1 ± 2.4	65.1 ± 1.9	57.5 ± 3.8	60.8 ± 5.9
LYMPHOCYTES, %	37.0 ± 2.3	31.8 ± 1.5	40.3 ± 3.1	32.0 ± 5.4
BANDS, %	.3 ± .2	0.0 ± 0.0	0.0 ± 0.0	1.3 ± .6[a]
MONOCYTES, %	.7 ± .3	.1 ± .1	.5 ± .3	3.5 ± 1.0[a]
EOSINOPHILS, %	2.0 ± .5	3.0 ± .7	1.8 ± .8	2.5 ± 1.2
BASOPHILS, %	.4 ± .4	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	.1 ± .1	0.0 ± 0.0	.3 ± .3
GLUCOSE (FASTING), MG %	89.6 ± 2.7	93.6 ± 1.4	97.5 ± 1.8	102.3 ± 2.5[a]
SGOT, IU/L	23.9 ± 1.0	30.1 ± 1.5[a]	26.8 ± 3.4	24.3 ± 1.4
SGPT, IU/L	24.1 ± 1.6	26.5 ± 1.1	27.8 ± 2.4	26.8 ± 2.8
ALK, PHOS., IU/L	69 ± 3	56 ± 2[a]	44 ± 2[a]	39 ± 4[a]
BUN, MG %	13.0 ± 1.1	12.1 ± .9	12.0 ± 1.2	11.3 ± 1.3

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[4]).
b/ Significantly different from the control dogs at the respective time interval (Dunnett's multiple comparison procedure[4]).

TABLE 5

LABORATORY DATA OF DOGS BEFORE AND DURING ADMINISTRATION OF 2,4-DNT

DOSE 25 MG/KG/DAY

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

WEEKS	0 (B. 8)	WEEK 4 (T. 8)	WEEK 6 (T. 7)	WEEK 8 (T. 7)	WEEK 13 (T. 2)
ERYTHROCYTES ($X10^6$ /MM^3)	4.84 ± .17	5.07 ± .21[b]	5.13	4.53	4.91[b]
RETICULOCYTES, %	.61 ± .07	1.53 ± .12[a,b]	.04	2.88[a,b]	.94
HEMATOCRIT, VOL. %	41.8 ± .7	38.3 ± 2.2[b]	38.0	37.0[b]	37.5[b]
HEMOGLOBIN, GM. %	14.0 ± .2	12.7 ± .5[b]	12.6	11.4[b]	7.4[a,b]
MCV, CUBIC MICRONS	86.8 ± 2.0	75.1 ± 1.8[a,b]	73.9[a]	81.3[b]	76.4
MCHB, MICRO MICROGMS.	29.1 ± .7	25.1 ± .4	24.7	25.1[b]	15.0[a]
MCHBC, GM %	33.6 ± .1	33.6 ± 1.0	33.4	30.9[b]	18.9[a]
PLATELETS ($X10^5$ /MM^3)	2.0 ± .2	2.5 ± .2	3.1	3.0	3.4[a]
LEUKOCYTES ($X10^3$ /MM^3)	12.2 ± .8	15.6 ± 3.4	16.2	15.4	10.6
NEUTROPHILS, %	58.8 ± 1.8	72.5 ± 4.5[a]	70.5	62.5	63.0
LYMPHOCYTES, %	38.3 ± 1.5	27.1 ± 4.5	28.0	33.0	31.0
BANDS, %	.1 ± .1	0.0 ± 0.0	.5	0.0	3.0[a]
MONOCYTES, %	.7 ± .4	.3 ± .2	1.0	2.0	1.0
EOSINOPHILS, %	2.1 ± .5	.1 ± .1[a,b]	0.0	2.5	2.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0	0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0	0.0
NUCLEATED RBC, %	.1 ± .1	.9 ± .6	0.0	4.0[a,b]	0.0
GLUCOSE (FASTING), MG %	89.6 ± 2.6	101.5 ± 3.6	132.0[a]	91.0	101.5
SGOT, IU/L	26.6 ± 1.0	28.4 ± 2.4	26.0	24.0	21.0
SGPT, IU/L	21.9 ± 1.6	43.3 ± 7.8[a]	27.5	33.5	29.0
ALK. PHOS., IU/L	68 ± 4	54 ± 4	40	42	43
BUN, MG %	12.8 ± .6	14.9 ± 1.7	33.0[a]	18.0	16.0

ENTRIES ARE MEAN ± STANDARD ERROR

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

b/ Significantly different from the control dogs at the respective time interval (Dunnett's multiple comparison procedure[4]).

TABLE 6

LABORATORY DATA OF DOGS TREATED WITH 2,4-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

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

DOSE: MG/KG/DAY	0 (C. 2)	1 (T. 2)	5 (T. 2)	25 (T. 2)
ERYTHROCYTES ($X10^6$ /MM^3)	6.67	6.47	6.81	6.25
RETICULOCYTES. %	.63	1.06	.95	.53
HEMATOCRIT. VOL. %	47.5	48.0	50.0	47.0
HEMOGLOBIN. GM. %	15.8	15.8	16.1	15.1
MCV. CUBIC MICRONS	71.2	74.1	73.4	75.3
MCHB. MICRO MICROGMS.	23.7	24.3	23.7	24.3
MCHBC. GM %	33.3	32.8	32.2	32.2
PLATELETS ($X10^5$ /MM^3)	2.2	2.6	2.3	2.4
LEUKOCYTES ($X10^3$ /MM^3)	10.3	13.9	13.9	9.9
NEUTROPHILS. %	57.5	65.5	70.5	60.5
LYMPHOCYTES. %	39.0	28.5	29.5	35.0
BANDS. %	0.0	0.0	0.0	0.0
MONOCYTES. %	.5	.5	0.0	1.5
EOSINOPHILS. %	3.0	5.5	0.0	3.0
BASOPHILS. %	0.0	0.0	0.0	0.0
ATYPICAL. %	0.0	0.0	0.0	0.0
NUCLEATED RBC. %	0.0	.5	.5	0.0
GLUCOSE (FASTING). MG %	96.5	95.5	98.5	99.5
SGOT. IU/L	24.5	24.5	21.0	21.0
SGPT. IU/L	31.0	27.5	21.0	26.0
ALK. PHOS.. IU/L	37	45	30	42
BUN. MG %	14.0	10.0	13.0	10.0

ENTRIES ARE MEAN

TABLE 7

LABORATORY DATA OF DOGS TREATED WITH 2,4-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

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

DOSE: MG/KG/DAY	0 (C. 2)	1 (T. 2)	5 (T. 2)	25 (T. 1)
ERYTHROCYTES ($X10^6$ /MM^3)	6.15	5.86	5.90	5.53
RETICULOCYTES, %	.53	.39	.29	.41
HEMATOCRIT, VOL. %	48.5	47.5	47.5	44.0
HEMOGLOBIN, GM. %	16.6	15.9	15.8	14.7
MCV, CUBIC MICRONS	78.9	81.1	80.6	79.6
MCHB, MICRO MICROGMS.	27.1	27.1	26.8	26.6
MCHBC, GM %	34.3	33.4	33.3	33.4
PLATELETS ($X10^5$ /MM^3)	2.0	2.0	2.6	2.0
LEUKOCYTES ($X10^3$ /MM^3)	9.4	9.6	9.4	8.6
NEUTROPHILS, %	64.5	59.0	62.5	67.0
LYMPHOCYTES, %	29.5	36.5	33.0	29.0
BANDS, %	0.0	0.0	0.0	0.0
MONOCYTES, %	1.5	1.5	.5	0.0
EOSINOPHILS, %	4.5	3.0	4.0	4.0
BASOPHILS, %	0.0	0.0	0.0	0.0
ATYPICAL, %	0.0	0.0	0.0	0.0
NUCLEATED RBC, %	0.0	0.0	0.0	0.0
GLUCOSE (FASTING), MG %	89.0	85.5	99.0	86.0
SGOT, IU/L	40.0	26.0	22.5	28.0
SGPT, IU/L	46.0	38.5	34.0	37.0
ALK. PHOS., IU/L	36	47	32	42
BUN, MG %	14.0	15.5	14.0	11.0

ENTRIES ARE MEAN

TABLE 8

METHEMOGLOBIN AND HEINZ BODIES IN CONTROL DOGS AND DOGS TREATED WITH 2,4-DNT

Dosage (mg/kg/day)	Methemoglobin (%)			Heinz Bodies (%)		
	4 Weeks[a/]	8 Weeks	13 Weeks	4 Weeks	8 Weeks	13 Weeks
0	0 (4)[b/]	0 (4)	0 (4)	0 (8)	0 (4)	0 (4)
1	0 (8)	0 (4)	0 (4)	0 (8)	0 (4)	0 (4)
5	0 (8)	0 (4)	1.9 (4)	0 (8)	0 (4)	0 (4)
25	0 (6)	3.5 (2)	3.8 (2)	4.1 (6)	5.5 (2)	4.9 (2)

a/ Weeks of treatment.

b/ Mean (number of dogs tested).

TABLE 9

SERUM ELECTROLYTES OF DOGS TREATED WITH 2,4-DNT

Dose (mg/kg/day)	Serum Electrolytes (meq/ℓ)				
	Na	K	Ca	Mg	Cl
	Before Treatment (8 dogs/group)				
Control	150 ± 2[a]	5.5 ± 0.4	5.5 ± 0.2	1.6 ± 0.1	115 ± 4
1	151 ± 2	5.3 ± 0.2	5.6 ± 0.1	1.5 ± 0.1	110 ± 4
5	148 ± 3	5.2 ± 0.2	5.6 ± 0.2	1.5 ± 0.1	112 ± 2
25	149 ± 2	5.3 ± 0.1	5.4 ± 0.1	1.5 ± 0.1	113 ± 3
	Treatment for 4 Weeks (2 dogs/group)				
Control	151[b]	4.8	5.2	1.6	109
1	153	4.7	5.3	1.4	105
5	154	5.1	5.6	1.5	104
25[c]	157	5.7	5.0	1.8	110
	Treatment for 8 Weeks (2 dogs/group)				
Control	154	5.1	5.5	1.6	108
1	152	4.9	5.3	1.6	106
5	150	4.7	5.1	1.6	104
25[d]	153	5.0	5.2	1.8	107
	Treatment for 13 Weeks (4 dogs/group)				
Control	148 ± 2	4.4 ± 0.2	5.2 ± 0.2	1.5 ± 0.1	104 ± 1
1	146 ± 1	4.4 ± 0.2	5.1 ± 0.1	1.5 ± 0.1	101 ± 2
5	146 ± 3	4.6 ± 0.3	5.0 ± 0.3	1.5 ± 0.1	102 ± 2
25[e]	145	4.7	4.9	1.6	101

a/ Mean ± S.E.
b/ Mean.
c/ One dog.
d/ Three dogs.
e/ Two dogs.

TABLE 10

BSP RETENTION OF DOGS TREATED WITH 2,4-DNT

Dose (mg/kg/day)	Before Treatment		After 4 Weeks		After 13 Weeks	
	Dog No.	% Retention	Dog No.	% Retention	Dog No.	% Retention
Control	51	5	57	4	53	7
Control	52	9	58	3	54	5
Control	53	7				
Control	54	9				
1	75	7	81	5	77	6
1	76	5	82	6	78	7
1	77	5				
1	78	14				
5	67	2	73	4	64	4
5	68	10	74	3	70	5
5	69	8				
5	70	10				
25	59	12			61	4
25	60	5				
25	61	3				
25	62	5				

TABLE 11

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF DOGS AFTER ADMINISTRATION OF 2,4-DNT FOR 4 WEEKS

DNT Dose (mg/kg)	Dog No.	Terminal Body Weight kg	Absolute Organ Weights (gm)					
			Liver	Spleen	Kidneys	Adrenals	Heart	Gonads
0	57 Male	10.8	254	78	54	1.0	70	18
0	58 Female	9.0	210	54	50	1.2	70	0.87
5	73 Male	13.0	306	94	59	1.3	78	22
5	74 Female	8.0	196	104	40	1.0	62	0.78
1	81 Male	10.4	244	50	56	1.1	87	14
1	82 Female	8.2	178	62	42	1.0	88	0.87
25	63 Male[a/]	8.8	305	22	56	1.3		6.9
25	65 Male[b/]	10.8	333	24	70	1.3		16.2

DNT Dose (mg/kg)	Dog No.	Relative Organ Weights (gm/kg Body Weight)						
		Liver	Spleen	Kidneys	Adrenals	Heart	Testes	Ovaries
0	57 Male	23.5	7.2	5.0	0.09	6.5	1.667	
0	58 Female	23.3	6.0	5.6	0.13	7.8		0.097
5	73 Male	23.5	7.2	4.5	0.10	6.0	1.693	
5	74 Female	24.5	13.0	3.1	0.08	7.8		0.097
1	81 Male	23.5	4.8	5.4	0.11	8.4	1.346	
1	82 Female	21.7	7.6	5.1	0.12	10.7		0.106
25	63 Male[a/]	34.7	2.5	6.4	0.14		0.784	
25	65 Male[b/]	30.8	2.2	6.5	0.12		1.500	

a/ Moribund and terminated after 24 days.
b/ Moribund and terminated after 22 days.

TABLE 12

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF DOGS AFTER ADMINISTRATION OF 2,4-DNT FOR 13 WEEKS

Dose (mg/kg)	Dog No.	Terminal Body Weight (kg)	Absolute Organ Weight (gm) Liver	Spleen	Kidneys	Adrenals	Heart	Brain	Gonads
0	53 Male	15.2	304	32	50	1.04	106	86	30.0
0	54 Female	10.0	92	52	46	0.99	71	74	0.79
1	77 Male	13.4	263	65	57	1.42	90	75	24.0
1	78 Female	9.6	219	43	40	1.20	74	78	1.75
5	69 Male	13.3	318	102	56	1.16	99	82	26.0
5	70 Female	9.0	226	92	48	1.05	60	72	0.83
25	64 Female[a/]	4.4	191	12	32	0.70			0.30
25	60 Female[b/]	3.8	178	18	34	1.20	56	70	0.71
25	61 Male	9.4	334	58	50	1.11	88	81	12.0

Dose (mg/kg)	Dog No.	Relative Organ Weights (gm/kg Body Weight) Liver	Spleen	Kidneys	Adrenals	Heart	Brain	Testes	Ovaries
0	53 Male	20.0	2.1	3.3	0.07	7.0	5.7	1.97	
0	54 Female	9.2	5.2	4.6	0.10	7.1	7.4		0.079
1	77 Male	19.6	4.9	4.3	0.11	6.7	5.6	1.72	
1	78 Female	22.8	4.5	4.2	0.13	7.7	8.1		0.182
5	69 Male	23.9	7.7	4.2	0.09	7.4	6.2	1.96	
5	70 Female	25.1	10.2	5.3	0.17	6.7	8.0		0.092
25	64 Female[a/]	43.4	2.7	7.3	0.16				0.068
25	60 Female[b/]	46.8	4.7	8.9	0.37	14.7	18.4		0.186
25	61 Male	35.5	6.2	5.3	0.12	9.4	8.6	1.28	

Dose (mg/kg)	Dog No.	Relative Organ Weights (gm/gm Brain Weight) Liver	Spleen	Kidneys	Adrenals	Heart	Testes	Ovaries
0	53 Male	3.53	0.37	0.58	0.01	1.23	0.34	
0	54 Female	1.24	0.70	0.62	0.01	1.19		0.011
1	77 Male	3.51	0.87	0.76	0.02	1.20	0.31	
1	78 Female	2.81	0.55	0.53	0.02	0.95		0.022
5	69 Male	3.88	1.24	0.68	0.01	1.21	0.32	
5	70 Female	3.14	1.28	0.69	0.02	0.83		0.012
25	60 Female[b/]	2.54	0.25	0.49	0.02	0.80		0.010
25	61 Male	4.12	0.72	0.62	0.01	1.09	0.15	

a/ Moribund and terminated after 36 days.
b/ Moribund and terminated after 48 days.

TABLE 13

ORGAN WEIGHTS AND RELATIVE ORGAN WEIGHTS OF DOGS AFTER ADMINISTRATION OF 2,4-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS OR LONGER

Dose (mg/kg)	Dog No.	Terminal Body Weight (kg)	Absolute Organ Weights (gm) Liver	Spleen	Kidneys	Adrenals	Heart	Brain	Gonads
0	55 Male	12.8	290	64	58	1.28	96	92	16
0	56 Female	9.2	248	62	38	1.05	66	80	1.08
1	79 Male	13.7	310	65	61	0.91	98	84	22
1	80 Female	7.8	228	46	30	1.13	60	68	1.79
5	71 Male	11.9	370	74	70	1.38	100	80	24
5	72 Female	8.6	206	84	38	0.76	90	78	0.87
25	59 Male[a]	ND[b]	364	86	53	2.34	64	65	12
25	62 Female[a]	ND	241	46	41	1.37	60	56	1.46

Dose (mg/kg)	Dog No.	Relative Organ Weights (gm/kg Body Weight) Liver	Spleen	Kidneys	Adrenals	Heart	Brain	Testes	Ovaries
0	55 Male	22.7	5.0	4.5	0.10	7.5	7.2	1.250	
0	56 Female	27.0	6.7	4.1	0.11	7.2	8.7		0.117
1	79 Male	22.6	4.7	4.5	0.07	7.1	6.1	1.606	
1	80 Female	29.2	5.9	3.9	0.15	7.7	8.7		0.229
5	71 Male	31.1	6.2	5.9	0.12	8.4	6.7	2.017	
5	72 Female	24.0	9.8	4.4	0.09	10.5	9.1		0.101

Dose (mg/kg)	Dog No.	Relative Organ Weights (gm/gm Brain Weight) Liver	Spleen	Kidneys	Adrenals	Heart	Testes	Ovaries
0	55 Males	3.15	0.696	0.630	0.014	1.04	0.174	
0	56 Female	3.10	0.775	0.475	0.013	0.83		0.014
1	79 Male	3.69	0.774	0.726	0.011	1.17	0.262	
1	80 Female	3.35	0.676	0.441	0.017	0.88		0.026
5	71 Male	4.63	0.925	0.875	0.017	1.25	0.300	
5	72 Female	2.65	1.077	0.487	0.010	1.15		0.011
25	59 Male[a]	5.60	1.323	0.815	0.036	0.98	0.184	
25	62 Female[a]	4.30	0.821	0.732	0.024	1.07		0.026

a/ Allowed to recover for 8 months.

b/ Not determined.

TABLE 14

ABSOLUTE AND RELATIVE ORGAN WIEGHTS OF DOGS AFTER ADMINISTRATION OF 2,4-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Dose (mg/kg)	Dog No.	Terminal Body Weight (kg)	Absolute Organ Weights (gm)					
			Liver	Spleen	Kidneys	Adrenals	Heart	Gonads
0	51 Male	12.2	243	66	58	1.15	92	17.0
0	52 Female	10.2	250	111	47	1.28	72	1.2
1	75 Male	13.4	320	85	70	1.70	81	20.0
1	76 Female	10.5	281	72	44	1.14	78	1.86
5	67 Male	11.4	230	100	59	1.10	71	15.0
5	68 Female	12.0	250	44	47	1.25	70	2.04
25	66 Female	10.0	313	55	50	1.46	84	0.80

Dose (mg/kg)	Dog No.	Relative Organ Weights (gm/kg Body Weight)						
		Liver	Spleen	Kidneys	Adrenals	Heart	Testes	Ovaries
0	51 Male	19.9	5.4	4.8	0.09	7.5	1.39	
0	52 Female	24.5	10.9	4.6	0.13	7.1		0.118
1	75 Male	23.9	6.3	5.2	0.13	6.0	1.49	
1	76 Female	27.0	6.9	4.2	0.11	7.5		0.177
5	67 Male	20.2	8.8	5.2	0.10	6.2	1.32	
5	68 Female	20.8	3.6	3.9	0.10	5.8		0.170
25	66 Female	31.3	5.5	5.0	0.15	8.0		0.080

TABLE 15

SUMMARY OF TISSUE LESIONS OF DOGS TREATED WITH 2,4-DNT FOR 4 WEEKS

Lesions[a/]	Dose (mg/kg/day) 0		1		5		25	
Dog No.:	57	58	81	82	73	74	63	65
Heart								
Cloudy swelling							+	±
Lung								
Subacute inflammation		+						
Emphysema								±
Liver								
Subacute inflammation		+					+	
Hemosiderosis								++
Kidney								
Cloudy swelling							+	+
Tubular degeneration							+	
Brain								
Gliosis								+
Edema								+
Cerebellar demyelination							+	+
Brainstem demyelination							+	
Spinal cord demyelination							+	
Gastrointestinal Tract								
Mucoid degeneration							+	
Lymph Node								
Inactive centers							+	
Testes								
Aspermatogenesis							+	
Bone Marrow								
M/E	1.5	1.4	1.4	1.3	1.4	1.3	c/	c/

Tissues not listed were normal.

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

b/ Moribund and terminated during the 4th week.

c/ Marrow smear was not prepared.

TABLE 16

SUMMARY OF TISSUE LESIONS OF BEAGLE DOGS
TREATED WITH 2,4-DNT FOR 13 WEEKS

Lesions[a] / Dose (mg/kg/day)	0		1		5		25		
Dog No.:	53	54	77	78	69	70	60[c]	61	64[b]
Heart									
Cloudy swelling							+		
Lung									
Pneumonia									+
Emphysema									±
Liver									
Subacute inflammation	+		+	+				+	
Hemosiderosis							++	+++	
Kidney									
Cloudy swelling							+		+
Tubular degeneration									±
Spleen									
Hemosiderosis							+++		+
Lymphoid depletion							++		
Brain									
Gliosis							++	+	+
Edema							++	+	+
Cerebellar demyelination							+	+	+
Brainstem demyelination							+		
Spinal cord demyelination							+		
Lymph Node									
Hemosiderosis								+++	
Testes									
Aspermatogenesis								+++	
Bone Marrow									
M/E ratio	1.3	1.3	1.1	1.3	1.4	1.2	1.4	1.0	0.9

Tissues not listed were normal.

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

b/ Moribund and terminated during the 6th week.

c/ Moribund and terminated during the 7th week.

TABLE 17

SUMMARY OF TISSUE LESIONS OF DOGS TREATED WITH 2,4-DNT
FOR 4 WEEKS AND ALLOWED TO RECOVER
FOR 4 WEEKS OR LONGER

		Dose (mg/kg/day)							
		0		1		5		25	
Lesions[a]	Dog No.:	55	56	79	80	71	72	59	62
Lungs									
Pneumonia						++	++	++	
Edema						+			
Lymph Node									
Hyperplasia				+			+		
Necrosis				+					
Tonsil									
Inflammation									+
Bone Marrow									
M/E ratio		1.4	1.3	1.6	1.3	1.2	1.5	c/	c/

Tissues not listed were normal.

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

b/ Allowed to recover for 8 months, the others are all for 4 weeks.

c/ Marrow smear was not prepared.

TABLE 18

SUMMARY OF TISSUE LESIONS OF DOGS TREATED WITH 2,4-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

	Dose (mg/kg/day)						
	0		1		5		25
Lesions[a/] Dog No.:	51	52	75	76	67	68	66
Lungs							
Subacute inflammation			+		+		
Liver							
Subacute inflammation	+						++
Hepatic cell necrosis		+					
Brain							
Cerebral demyelination							+
Optic nerve demyelination							±
Lymph Node							
Pigment	++						
Bone Marrow							
N/E ratio	1.3	1.4	1.4	1.3	1.5	1.5	1.4

Tissues not listed were normal.

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

TABLE 19

SERUM IgE (IU/ml) OF DOGS TREATED WITH 2,4-DNT

Dose (mg/kg/day)	Treatment Weeks				
	Baseline[a]	4 Week[b]	8 Week	13 Week	17 Week[d]
Controls	1,696 ± 1,80	800-1,400	1,475-1,875	1,975-2,225	1,500-1,575
1	1,495 ± 308	1,075-1,850	1,425-1,675 1,585-1,675[c]	1,850-2,600	800 (2)
5		1,050-1,750	1,475-1,675 1,675-1,725[c]	1,775-2,475	1,350-1,500
25		700-1,850	1,425-1,625 1,580-1,775[c]	1,150-2,325	1,575 (2)

a/ Mean ± S.E. of eight dogs per group.
b/ Two dogs per group and thereafter.
c/ Treated for 4 weeks and allowed to recover for 4 weeks.
d/ Treated for 13 weeks and allowed to recover for 4 weeks.

II RATS

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II. RATS

A. Subacute and Subchronic Toxicities and Reversibility

1. Introduction

As for the dogs, these studies were performed to define the nature and extent of effects of 2,4-DNT on the biological system at the biochemical and cellular levels and to elucidate the dose-response relationship in the rats fed 2,4-DNT for 4 weeks and 13 weeks. The reversibility of any adverse effects was also studied after the feeding of 2,4-DNT was discontinued for 4 weeks.

2. Material and Methods

a. Number of Rats, Sex and Treatment

A total of 64 male and 64 female young healthy CD® rats (Charles River Breeding Lab.) were used for this study. They were divided into four groups, each consisting of 16 males and 16 females. The average weights of all groups were kept close. Three groups of rats were fed 0.07, 0.2, or 0.7% 2,4-DNT in their feed. The fourth group, serving as control, was given the powdered standard rodent chow (Wayne Laboratory Meal) without 2,4-DNT.

b. Animal Husbandry

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

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

c. Feed Preparation

2,4-DNT was purchased from K and K Laboratories (Cleveland, Ohio) and mixed with rat feed to produce a diet containing the desired 0.7% of 2,4-DNT. The diet was placed in a wooden box (16 x 16 x 20 in.) until

half full. The box was rotated about its long axis for 1 hour in a modified cement mixer at a speed of 20 rpm. Subsequently, portions of this diet were mixed with the standard chow in proper amounts to yield 0.2% and again 0.07% of 2,4-DNT, respectively.

d. Experimental Procedure

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

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

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

(3) BSP retention test was not performed.

e. Experimental Design

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

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

(2) Treatment was discontinued for four male and four female rats from each group at the end of 4 weeks and 13 weeks. These rats were observed for 4 more weeks and then euthanized for necropsy to study the reversibility of the adverse effects.

3. Results

a. General Observations and Weight Gain

The control rats and rats fed 0.07% or 0.2% 2,4-DNT in the feed were healthy throughout the experimental periods of 4 or 13 weeks. Rats fed 0.7% 2,4-DNT soon ate less, were inactive, lost weight, and died. A few animals occasionally had an unusual gait with wide spread and stiff hind legs. This resembled the gait seen in affected dogs, but the ensuing rigid paralysis was not seen.

The female rats in the high dosage group died soon after treatment began. Four died in the first week, four more in the second, and two in the third week. After 4 weeks of treatment, four survivors were

killed for autopsy and two survivors were given control feed without 2,4-DNT to test reversibility of the effects. Deaths in the males occurred later: the first two in week 4, after more than half of the females had died. In addition, there was one death in week 6, one in week 7, two in week 8, one in week 12, and one in week 13. One middle dosage group rat died in week 14, just after the start of the reversibility experiment. All of the rats dying at unscheduled times had lost weight and had little or no body fat remaining.

The body weights of the male and female rats before, during, and after treatment are summarized in Tables 20 and 21, respectively. The weight changes are better illustrated in Figure 1. The control rats consistently gained weight throughout the experiment. The low and middle dosage group rats did not gain as much weight and occasionally lost weight. The high dosage group rats consistently lost weight. During the reversal study all rats gained weight; the lightest ones (high dosage group) gaining the most.

b. Feed Consumption and 2,4-DNT Intake

Feed consumptions of rats fed various doses of 2,4-DNT are summarized in Table 22. These results reflect the weight changes. Low and middle dosage group rats ate somewhat less than control rats; high dosage group rats ate one-third to one-half as much as controls. During the recovery period, all rats ate similar amounts of feed.

The 2,4-DNT intakes of the treated rats are summarized in Table 23. Because of their increase in body weight, intake by the low dosage male rats decreased from 37.6 mg/kg/day in the first 4 weeks to 31.8 mg/kg/day in the last 5 weeks, averaging 34.3 mg/kg/day. Since the weight varied little, intake by the low dosage females fluctuated only slightly between 37.6 and 39.7 mg/kg/day, averaging 38.3 mg/kg/day. In the middle dosage group rats, the males received an average of 92.8 mg/kg/day, ranging from 84.3 to 101.7 mg/kg/day; the females received an average of 108.3 mg/kg/day, ranging from 97.4 to 117.7 mg/kg/day. Because of relatively constant feed consumption at decreasing body weight, 2,4-DNT intake by the high dosage male rats increased from 191.4 mg/kg/day to 292.1 mg/kg/day, averaging 265.6 mg/kg/day. The intake by the high dosage female rats was 145.2 mg/kg/day.

c. Blood Analyses

The hematology results of male control rats and male rats fed the various amounts of 2,4-DNT are summarized in Tables 24 through 27. There were a number of statistically significant fluctuations in the control male rats during the experimental period of 13 weeks (Table 24). These are within

normal limits. At 17 weeks, the erythrocyte, platelet and leukocyte counts of these control males decreased with increases in various blood indices. Similar effects were also apparent in the treated groups. The results from male rats receiving 0.07% or 0.2% 2,4-DNT in the feed were substantially similar to those of the control males (Tables 25 and 26). In addition, there was occasional and slight reticulocytosis. At fourth week, male rats receiving 0.7% 2,4-DNT had decreased erythrocyte count with a compensatory reticulocytosis, increased cell volume, and increased cell hemoglobin concentration (Table 27). These effects and a later decrease in hematocrit and hemoglobin concentration continued throughout the treatment as the anemia worsened. After 13 weeks of treatment and 4 weeks of recovery, the surviving males had returned to the same level as the controls. There were fluctuations in platelet and leucocyte counts; but these changes were not consistent.

The clinical blood chemistry results from male rats terminated after treatment or after treatment plus recovery for 4 weeks are shown in Tables 28 and 29, respectively. There were no apparent and consistent changes. High values of fasting blood glucose of the control and treated male rats suggest that these rats were not fasted.

The hematology results of the female control rats and rats fed various levels of 2,4-DNT are summarized in Tables 30 through 33, respectively. As seen in the male control rats, the control females had a number of statistically significant fluctuations during the first 13 weeks and a number of changes at week 17 (Table 30). Similarly, the results from female rats receiving 0.07% or 0.2% 2,4-DNT in the feed were essentially the same as those of the control females (Tables 31 and 32). Occasional and slight reticulocytosis also occurred. The two rats receiving 0.7% of 2,4-DNT which survived for 4 weeks of treatment had decreased erythrocyte count, hematocrit and hemoglobin concentration with compensatory reticulocytosis (Table 33).

The clinical blood chemistry results from female rats terminated after treatment or after treatment plus recovery for 4 weeks are shown in Tables 34 and 35, respectively. As for the male rats, there were no consistent changes. High values of fasting blood glucose of the control and treated female rats suggest that these rats were not fasted.

The results of serum electrolytes of rats terminated at various times are shown in Table 36. Occasionally, there was some statistical significance. However, the values fluctuated within normal limits.

d. Organ Weights

The organ weights of the rats fed 2,4-DNT for 4 or 13 weeks and for 4 or 13 weeks plus 4 weeks recovery are summarized in Tables 37 through 40, respectively. After 4 weeks, the livers of males fed the middle level and females fed the low and high levels were enlarged. The kidneys and hearts of high level males were small, but this was proportional to their smaller body weight. After 13 weeks, based on the body weight, the relative liver, kidney and brain weights of males fed the middle dose and the relative kidney and brain weights of females fed the middle dose were enlarged. The relative weights of these organs of one male fed the high level were also enlarged. All females did not survive after 8 weeks. Based on brain weight, the relative liver, spleen and heart of one male fed the high level and the relative heart of females fed the middle level were smaller than those of their respective controls. Results for rats allowed to recover for 4 weeks showed partial recovery of body weight and similar variations in various organ weights. The liver weights of treated rats after allowed to recover for 4 weeks were elevated. This presumably reflects high metabolic activity during the recovery process.

e. Gross and Microscopic Examination of Tissues

At autopsy, control rats and those receiving 0.07% or 0.2% 2,4-DNT in the feed were in good nutritional condition with no gross lesions. Rats fed 0.7% of 2,4-DNT had little body fat, reflecting their body weight loss, but no gross lesions. Microscopic examination revealed a number of lesions in rats from all groups. Because of the similarities between the middle and low dosage groups, slides from the latter were scanned, but not reported.

After 4 weeks of 2,4-DNT feeding, control rats had a few mild lesions in the lung or liver, as shown in Tables 41 and 42. The rats fed 2,4-DNT had similar lesions. There was hemosiderosis in the spleen, presumably due to removal of methemoglobin from the circulation. In addition, all males fed the high level of 2,4-DNT had moderate atrophy and aspermatogenesis in the testes. The bone marrows and the M/E ratios of these rats were normal.

When 2,4-DNT feeding is continued for 13 weeks, there are more spontaneous lesions in the heart, lung, liver, spleen and kidney of control rats, as seen in Tables 43 and 44. The treated rats had similar lesions. However, the hemosiderosis was more severe. The males fed 0.7% of 2,4-DNT had very severe atrophy and aspermatogenesis in the testes. In these rats, the testes were composed of tubules of epithelium with practically no germ cells in any stage of spermatogenesis. Rats fed the middle level had similar

lesions, ranging in degree from moderate to very severe. One male rat fed the high level also had gliosis in the cerebrum. The bone marrows and the M/E ratios of these rats were normal. Hemosiderosis and the lesions in the testes and brain were similar to those seen in the dogs treated with 2,4-DNT.

Tissue lesions in rats fed 2,4-DNT for 4 or 13 weeks and allowed to recover for 4 weeks are shown in Tables 45 through 48. Hemosiderosis and testicular lesions caused by middle or high levels of 2,4-DNT were apparently not reversible after treatment was discontinued for 4 weeks. Gliosis and demyelination were also observed in the cerebellum of one male rat fed the middle level and one male rat fed the high level of 2,4-DNT. The M/E ratios of these rats were normal.

4. Discussion and Conclusions

The 2,4-DNT intake of the male rats fed the low, middle or high level of 2,4-DNT averaged 34.3, 92.8, or 265.6 mg/kg/day, respectively. The female rats consumed an average of 38.3, 108.3 or at least 145.2 mg/kg/day, respectively. Since many female rats fed the high level of 2,4-DNT stopped eating a few days before they died, the actual 2,4-DNT intake of these female rats would be much higher.

The low level of 2,4-DNT was relatively nontoxic, since the only effect seen was a slight depression of weight gain. This depressed effect of weight gain was accentuated in rats fed the middle level. In addition, hemosiderosis appeared in the spleen of these rats after 4 weeks with a mild reticulocytosis. The 2,4-DNT presumably produced methemoglobin by the well-known mechanism of nitro and amino compounds.[5/] These cells break down and the hemosiderin formed was deposited in the spleen. This resulted in a slight anemia, which was compensated by increased erythrocyte production, reflected in the reticulocytosis. At 13 weeks, there was a major decrease or even complete cessation of spermatogenesis in the rats fed the middle level of 2,4-DNT.

The rats fed the high level of 2,4-DNT had more severe effects than the rats fed the middle level. There were severe weight losses in most rats. Similar lesions in middle level rats appeared. The testicular lesions were apparent by week 4; the rats were anemic by week 13, despite reticulocytosis. In addition, there were some signs of a neuromuscular effect like that seen in the dogs: wide spread and stiff hind legs, with gliosis and/or demyelination in two rats. Finally, deaths occurred. Eight of the 16 males on the high level and one on the middle level died before their scheduled necropsy. The high level female rats were devastated with 10 of 16 dying in the first 3 weeks of study. These deaths occurred so rapidly that tissue lesions may not have developed enough to be visible on microscopic examination. Many of these deaths occurred at night and we were unable to obtain useful blood and tissue samples for examination.

During the recovery studies, the rats rapidly gained weight, particularly the high level rats which were most affected. However, 4 weeks was not sufficient for all the rats to catch up their weight with the controls. The erythropoietic system did return the erythrocyte levels to those seen in controls. The tissue lesions remained. The testicular degeneration apparently continued to worsen during the recovery period. This was presumably the continuing development of a process initiated by 2,4-DNT. It is not apparent from these studies whether the rats can recover from this destruction of the germ cells.

To summarize, rats fed 34.3 to 38.3 mg/kg/day of 2,4-DNT had a decreased weight gain. Those fed 92.8 to 108.3 mg/kg/day had a greater decrease in weight gain, reticulocytosis, hemosiderosis in the spleen, and decreased spermatogenesis. Rats fed 145.2 to 265.6 mg/kg/day had severe weight loss, anemia with reticulocytosis, and a greater degree and more rapid onset of splenic hemosiderosis and aspermatogenesis. Many of these rats died, the females after treatment for 1 to 3 weeks, the males after 4 to 13 weeks. Four weeks' recovery sufficed for large weight gains and correction of the anemia, but did not eliminate the hemosiderosis and did not lessen the aspermatogenesis. A few rats had episodes of wide spread stiff-legged gait in the hind legs; some had mild to moderate gliosis and/or demyelination.

B. Cytogenetic and Mutagenic Effects of 2,4-DNT

1. Introduction

The cytogenetic effect of 2,4-DNT on somatic cell chromosomes was determined by examination of lymphocyte and kidney cell cultures. The capability of 2,4-DNT to induce single gene mutations was studied in Chinese hamster ovary cells in vitro. The mutagenic effect was studied using the dominant lethal mutation test to examine the effect of the testicular lesions described above.

2. Material and Methods

a. Animals

The rats used in these studies were those in the toxicity studies described above or extra animals treated similarly. Some rats were maintained on the diet containing 2,4-DNT for 19 weeks. Extra control females were used in the dominant lethal mutation study.

b. Cytogenetic Tests

(1) Lymphocyte and Kidney Cultures

At the end of 5 and 13 weeks, blood samples were aseptically drawn from the tail vein of both the control and treated rats. The lymphocytes were cultured by the method of Moorhead et al.[10] Kidney tissue samples were removed at necropsy and cultured by the trypsinization method of Fernandes.[11] All cultures were maintained in Eagle's medium as modified by Vogt and Dulbecco.[12]

(2) Chromosome Analysis

Actively dividing kidney cultures and phytohemagglutin-stimulated lymphocytes were arrested in metaphase by short-term colchicine treatment. The cells were trypsinized, swollen in hypotonic solution, and processed for spreading on glass slides by the method of Moorhead and Nowell.[13] Slides were stained with giemsa and scanned under low power optics. Cell polyploidy was estimated by examination of 200 cells. Slides showing minimum scattering of cells in metaphase were selected for analysis under oil immersion optics. Chromosomes were counted and morphological aberrations were examined from photographic negatives of up to 50 metaphase cells.

c. Mutagenic Effects on CHO-K1 Cells In Vitro

Wild type Chinese hamster ovary cells (CHO-K1)[14] capable of growth in both a minimal and an enriched medium were exposed to selected concentrations of 2,4-DNT to test its ability to induce single gene mutations in mammalian somatic cells. The concentrations used were selected from a single cell survival curve obtained according to the method of Puck and Kao.[15] Potential mutants were isolated by the BUdR-visible light technique and confirmed by plating the cells in both media. A mutant was defined as having the capability of growth only in the enriched medium and not in the minimal medium. Mutagenesis was measured relative to the known mutagen ethyl methanesulfonate.[16]

d. Dominant Lethal Mutation Test

Three groups of four or five male rats were fed standard rodent feed or feed containing either 0.02% (low level) or 0.2% (high level) of 2,4-DNT. The detailed procedures were the same as those described in Sections II.A.2.b. and II.A.2.c. After 13 weeks of feeding, each male was mated with three females. The male was left overnight in a cage with two females. Copulation was established by morning vaginal inspections for sperm. Sperm-positive females were removed; that day is considered day 0

of gestation. An additional female was added to the cage with the male and the procedure continued until each male had copulated with three females. On day 13 of gestation, each female was killed and the uterus examined for implantation sites, dead and viable fetuses, and resorptions. Data are expressed as fertility index (confirmed pregnancies/sperm-positive females x 100) and implant viability index (viable fetuses/total implants x 100).

3. Results

a. Cytogenetic Tests

The results on numerical distribution of chromosomes in cultures from rats fed 0.2% of 2,4-DNT are shown in Table 49. Treatment with 2,4-DNT did not cause any significant changes in the chromosome frequency distribution or number of tetraploids in the peripheral lymphocyte cultures. Rats fed 0.2% of 2,4-DNT in the diet for 5 or 13 weeks had increased numbers of tetraploids in the kidney cultures. However, the increases were not statistically significant when compared with those before treatment.

The results on morphological aberrations of chromosomes in these cultures are shown in Table 50. The peripheral lymphocytes sampled at week 13 did not culture properly, additional rats were sampled after 19 weeks' treatment. The number of chromatid breaks and gaps in the lymphocyte cultures were increased after treatment for 5 weeks, but this effect was not statistically significant until after 19 weeks. There were significant increases in the number of chromatid breaks and gaps in the kidney cultures after treatment for 5 or 13 weeks. The number of breaks and gaps increased with the duration of treatment.

b. Mutagenic Effect In Vitro

The preliminary experiment found the 1% and 5% cell survival concentrations to be 193 and 155 µg/ml, respectively. Neither of these concentrations caused mutations, although comparable concentrations of the known mutagen ethyl methanesulfonate did cause mutations.

c. Dominant Lethal Mutation Test

The fertility and implant viability indexes are summarized in Table 51. Rats fed 0.02% of 2,4-DNT for 13 weeks had reduced fertility and implant viability indexes, but the results were not statistically significant. Rats fed 0.2% of 2,4-DNT had significantly reduced fertility indexes. There were no viable fetuses and the implant viability index was zero.

4. Discussion and Conclusions

Feeding 0.2% of 2,4-DNT was toxic to chromosomes in vivo, as indicated by the increased chromatid breaks and gaps in the peripheral lymphocyte or kidney cultures. It is not known if this is the effect of 2,4-DNT itself, its metabolite(s) or combinations of both. Rats converted part of the ingested compound into 2,4-diaminotoluene as discussed below. This compound is a suspected carcinogen[17] and likely will affect the reproductive material of the cell.

The in vitro addition of 2,4-DNT at cytotoxic levels to cultures of Chinese hamster ovary cells did not induce mutations.

Rats fed 0.2% of 2,4-DNT in the diet showed an apparent dominant lethal effect. As found in Section A, toxic levels of 2,4-DNT caused severe testicular lesions and aspermatogenesis. Thus, 2,4-DNT may have reduced the number of sperm and rendered the rats sterile. It may also have produced mutations which failed to implant or caused the resorption of implants. Or 2,4-DNT may have produced both of these effects resulting in a few non-viable fertilized ova. Further testing is required to separate the toxic effect on spermatogenesis from a possible dominant lethal mutation effect.

C. Immunologic Response to 2,4-DNT

1. Introduction

Immunoglobin E (IgE), the allergic or hypersensitive antibody was associated with anaphylactic reactions in humans.[8] Serum concentrations of IgE of rats treated with 2,4-DNT were determined.

2. Material and Methods

As described for dogs in Section I.B.2., the immunodiffusion technique of Mancini[9] was used to determine the serum IgE levels of rats fed various amounts of 2,4-DNT. The terminal blood samples from the toxicity test, Section II.A., were used.

3. Result and Conclusion

Serum concentrations of IgE on control rats and rats fed 0.2% or 0.7% of 2,4-DNT are summarized in Table 52. There were wide variations in the serum concentrations of IgE. 2,4-DNT even at lethal level of 0.7% in the feed did not alter the serum concentration of IgE.

D. Summary

The low dose of 2,4-DNT (intake of 34 mg/kg/day in males and 38 mg/kg/day in females) was slightly toxic, causing only a slight depression in weight gain. The middle dose (93 and 108 mg/kg/day, respectively) was more toxic and the high dose (266 and 145 mg/kg/day) was lethal to over half the rats. Target organs included the neuromuscular system (abnormal gait, gliosis and/or demyelination in the central nervous system), erythrocytes (methemoglobin and sequelae, including anemia and hemosiderosis) and testes (depressed spermatogenesis). Partial recovery occurred 4 weeks after cessation of treatement.

Rats fed the middle dose for 13 weeks had an increased number of chromatid breaks and gaps, but no serious aberrations. No mutagenic effect was seen in Chinese hamters ovary cell cultures. In a dominant lethal mutation study, the males fed the middle dose for 13 weeks were sterile. There was no effect of 2,4-DNT on serum IgE.

TABLE 20

BODY WEIGHTS OF MALE RATS FED 2,4-DNT

% 2,4-DNT in Feed	Body Weights (gm)[a]				
	Initial	4 Weeks	8 Weeks	13 Weeks	17 Weeks
0	413 ± 18	446 ± 24			
0.07	418 ± 13	413 ± 15			
0.2	413 ± 9	414 ± 12			
0.7	470 ± 9	226[b]			
0	395 ± 29	465 ± 34[c]	528 ± 40		
0.07	413 ± 13	455 ± 16[c]	525 ± 19		
0.2	406 ± 30	435 ± 33[c]	496 ± 50		
0.7	439 ± 11	289 ± 4[c]	396 ± 6		
0	411 ± 16	475 ± 25	522 ± 30	531 ± 31	
0.07	394 ± 4	430 ± 5	448 ± 11	401 ± 8	
0.2	419 ± 15	437 ± 16	429 ± 14	396 ± 26	
0.7	414 ± 8	269 ± 12	250[d]	238[d]	
0	404 ± 21	468 ± 21	503 ± 24	560 ± 19[c]	583 ± 31
0.07	418 ± 3	452 ± 3	458 ± 6	481 ± 6[c]	506 ± 10
0.2	419 ± 14	442 ± 16	418 ± 14	418 ± 17[c]	422 ± 20[e]
0.7	444 ± 18	334 ± 7	292 ± 5[f]	305[c,g]	368[g]

a/ Mean ± S.E. of four rats, unless otherwise noted.
b/ Average of two rats; two other rats died in week 4.
c/ 2,4-DNT in feed discontinued thereafter.
d/ One rat; three other rats died in weeks 6, 7 and 8, respectively.
e/ Three rats; one other rat died in week 14.
f/ Three rats; one other rat died in week 8.
g/ One rat; two other rats died in weeks 12 and 13.

TABLE 21

BODY WEIGHTS OF FEMALE RATS FED 2,4-DNT

% 2,4-DNT in Feed	Body Weights (gm)[a]				
	Initial	4 Weeks	8 Weeks	13 Weeks	17 Weeks
0	270 ± 8	258 ± 9			
0.07	249 ± 2	244 ± 6			
0.2	258 ± 8	256 ± 3			
0.7	277 ± 9	197 ± 12			
0	291 ± 15	292 ± 9[b]	323 ± 18		
0.07	268 ± 5	271 ± 4[b]	291 ± 6		
0.2	268 ± 5	255 ± 12[b]	282 ± 9		
0.7	284 ± 4	205[b,c]	270[c]		
0	281 ± 10	278 ± 13	310 ± 12	302 ± 16	
0.07	272 ± 13	286 ± 12	285 ± 12	265 ± 15	
0.2	264 ± 4	267 ± 4	266 ± 4	243 ± 5	
0.7	281 ± 6	[d]	[d]	[d]	
0	255 ± 5	270 ± 8	280 ± 8	303 ± 11[b]	307 ± 10
0.07	261 ± 5	262 ± 9	265 ± 3	272 ± 4[b]	282 ± 7
0.2	275 ± 8	273 ± 3	269 ± 4	274 ± 4[b]	275 ± 7
0.7	271 ± 8	[e]	[e]	[e]	[e]

[a] Mean ± S.E. of four rats, unless otherwise noted.
[b] 2,4-DNT in feed discontinued thereafter.
[c] Two rats; two other rats died in week 1 and week 2.
[d] Two rats died in week 1, one in week 2, one in week 3.
[e] One rat died in week 1, two in week 2, one in week 3.

TABLE 22

AVERAGE FEED CONSUMPTION (gm/rat/day)
OF RATS FED 2,4-DNT

% 2,4-DNT in Feed	Males			
	1-4[a]	5-8	9-13	14-17[b]
0	24.2	24.4	24.4	23.3
0.07	22.8	21.7	20.3	23.2
0.2	21.5	18.2	19.2	25.4
0.7	10.2	9.9	11.6	24.7

% 2,4-DNT in Feed	Females			
	1-4	5-8	9-13	14-17
0	15.6	19.4	16.1	14.7
0.07	14.2	15.6	14.6	15.0
0.2	15.6	13.1	14.4	15.4
0.7	5.0	--	--	--

a/ Weeks.
b/ Recovery period; all rats fed control feed.

TABLE 23

AVERAGE 2,4-DNT INTAKE (mg/kg/day)
OF RATS DURING TREATMENT

% 2,4-DNT in Feed	Males			
	1-4[a]	5-8	9-13	1-13
0.07	37.6	34.0	31.8	34.3
0.2	101.7	84.3	92.5	92.8
0.7	191.4	226.5	292.1	265.6

% 2,4-DNT in Feed	Females			
	1-4	5-8	9-13	1-13
0.07	37.7	39.7	37.6	38.3
0.2	117.7	97.4	109.5	108.3
0.7	145.2	-	-	145.2

a/ Weeks.

TABLE 24

LABORATORY DATA OF CONTROL MALE RATS FOR 2,4-DNT

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

	WKS 0 (B, 4)	WKS 4 (C, 4)	WKS 8 (C, 4)	WKS 13 (C, 4)	WKS 17 (C, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	7.37 ± .22	7.10 ± .04[a/]	8.05 ± .09	7.75 ± .10	4.91 ± .14[a/]
RETICULOCYTES, %	1.33 ± .14	1.23 ± .13	1.21 ± .12	.73 ± .07	3.48 ± 1.20
HEMATOCRIT, VOL. %	53.3 ± 2.5	46.0 ± .4[a/]	51.8 ± .3	55.0 ± 1.1[a/]	45.8 ± .6[a/]
HEMOGLOBIN, GM. %	16.2 ± .3	15.5 ± .1	16.7 ± .1	17.6 ± .4	15.0 ± .3[a/]
MCV, CUBIC MICRONS	72.3 ± 1.7	64.8 ± .4[a/]	64.3 ± .5[a/]	70.9 ± 1.1	93.4 ± 2.4[a/]
MCHS, MICRO MICROGMS.	22.9 ± .2	21.9 ± .2	20.7 ± .2	22.7 ± .3	30.6 ± .9[a/]
MCHBC, GM %	30.5 ± .9	33.8 ± .4[a/]	32.2 ± .2	32.0 ± .5	32.8 ± .2[a/]
PLATELETS ($X10^5$ /MM^3)	7.2 ± .4	6.1 ± .1	6.5 ± .2	7.3 ± .1	5.1 ± .5[a/]
LEUKOCYTES ($X10^3$ /MM^3)	21.0 ± 2.6	15.2 ± 1.4	17.9 ± .6	22.0 ± 1.1	8.4 ± .4[a/]
NEUTROPHILS, %	10.5 ± 1.3	8.0 ± .4	14.0 ± 2.9	16.8 ± 6.5	18.0 ± 3.3
LYMPHOCYTES, %	89.3 ± 1.5	91.0 ± 1.1	83.5 ± 3.4	82.8 ± 6.3	78.0 ± 3.7
BANDS, %	0.0 ± 0.0	0.9 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3
EOSINOPHILS, %	.3 ± .3	1.0 ± 1.0	1.3 ± .3	.5 ± .3	2.5 ± .6[a/]
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3
MONOCYTES, %	0.0 ± 0.0	0.0 ± 0.0	1.3 ± .6	0.0 ± 0.0	1.5 ± .5[a/]
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3

ENTRIES ARE MEAN ± STANDARD ERROR

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

TABLE 25

HEMATOLOGY DATA OF MALE RATS BEFORE, DURING AND AFTER 2,4-DNT FEEDING

DOSE 0.07%

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

	WKS 0 (B, 4)	WKS 4 (T, 4)	WKS 8 (T, 4)	WKS 13 (T, 4)[c]	WKS 17 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	7.14 ± .20	6.49 ± .16[a,b]	7.27 ± .10	6.69 ± .17[b]	4.51 ± .17[a]
RETICULOCYTES, %	1.23 ± .16	2.39 ± .48[a]	.90 ± .11	.70 ± .13	1.92 ± .20
HEMATOCRIT, VOL. %	52.3 ± 2.6	44.3 ± .5[a]	51.3 ± 1.8	50.8 ± 1.9	47.0 ± 1.1
HEMOGLOBIN, GM. %	16.2 ± .4	14.9 ± .3	16.5 ± .2	16.5 ± .6	15.4 ± .5
MCV, CUBIC MICRONS	73.1 ± 2.7	68.2 ± 1.2	70.6 ± 3.3	75.8 ± .9	104.4 ± 1.5[a,b]
MCHB, MICRO MICROGMS.	22.7 ± .2	23.0 ± .3	22.7 ± .5	24.6 ± .4[a]	34.1 ± .3[a,b]
MCHBC, GM %	31.1 ± 1.0	33.8 ± .3[a]	32.3 ± .9	32.5 ± .2	32.7 ± .2
PLATELETS ($X10^5$ /MM^3)	6.6 ± .2	6.7 ± .4	7.3 ± .7	6.7 ± 1.3	6.1 ± .9
LEUKOCYTES ($X10^3$ /MM^3)	20.1 ± 2.7	16.8 ± 1.6	17.8 ± 1.2	28.9 ± 3.7	7.7 ± 1.7[b]
NEUTROPHILS, %	9.3 ± 1.5	10.8 ± 3.2	12.5 ± 1.7	7.5 ± 2.3	10.3 ± 2.9
LYMPHOCYTES, %	89.0 ± .9	86.5 ± 2.4	85.3 ± 1.9	89.8 ± 2.3	88.5 ± 3.2
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.3 ± 1.3	1.3 ± .5	.5 ± .3	2.3 ± .9	.8 ± .5
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.5 ± .3	0.0 ± 0.0	1.8 ± .8	.5 ± .3	.5 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[4]).
b/ Significantly different from the control rats at the respective time interval (Dunnett's multiple comparison procedure[4]).
c/ 2,4-DNT in feed discontinued thereafter.

TABLE 26

HEMATOLOGY DATA OF MALE RATS BEFORE, DURING AND AFTER 2,4-DNT FEEDING

DOSE 0.2%

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

	WKS 0 (B, 4)	WKS 4 (T, 4)	WKS 8 (T, 4)	WKS 13 (T, 4)[c]	WKS 17 (T, 3)
ERYTHROCYTES ($X10^6$ /MM^3)	7.18 ± .15	7.66 ± .12[b]	6.94 ± .13[b]	7.23 ± .21	4.70 ± .27[a]
RETICULOCYTES, %	1.54 ± .24	1.54 ± .23	2.92 ± .34[a]	2.09 ± .2[b]	1.66 ± .48
HEMATOCRIT, VOL. %	50.8 ± .9	51.3 ± 1.[b]	49.0 ± 1.4	49.5 ± 1.2	45.0 ± 2.5
HEMOGLOBIN, GM. %	16.3 ± .2	16.8 ± .4[b]	15.6 ± .1	16.3 ± .5	14.8 ± .8
MCV, CUBIC MICRONS	70.8 ± 1.6	66.9 ± 1.7	70.7 ± 2.0	68.5 ± 1.4	95.9 ± 3.6[a]
MCHB, MICRO MICROGMS.	22.8 ± .5	21.9 ± .4	22.6 ± .6	22.5 ± .5	31.6 ± 1.2[a]
MCNBC, GM %	32.2 ± .2	32.8 ± 1.0	32.0 ± 1.0	32.9 ± .4	33.0 ± .1
PLATELETS ($X10^5$ /MM^3)	6.9 ± .3	6.4 ± .4	9.1 ± .8[a]	9.0 ± .2[a]	4.0 ± .8[a]
LEUKOCYTES ($X10^3$ /MM^3)	18.4 ± 2.4	20.0 ± .6	22.6 ± 1.6	26.5 ± 1.4[a]	9.3 ± 2.2[a]
NEUTROPHILS, %	12.5 ± 1.7	14.5 ± 3.4	11.0 ± 2.9	9.0 ± 2.5	20.7 ± 7.9
LYMPHOCYTES, %	86.5 ± 1.8	84.5 ± 3.3	86.3 ± 3.5	89.0 ± 3.3	78.3 ± 7.3
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.3 ± .3	.8 ± .3	1.0 ± .7	1.0 ± .4	.7 ± .7
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.8 ± .3	.3 ± .3	1.8 ± .8	1.0 ± .7	.3 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

[a] Significantly different from the baseline level (Dunnett's multiple comparison procedure[4]).
[b] Significantly different from the control rats at the respective time interval (Dunnett's multiple comparison procedure[4]).
[c] 2,4-DNT in feed discontinued thereafter.

TABLE 27

HEMATOLOGY DATA OF MALE RATS BEFORE, DURING AND AFTER 2,4-DNT FEEDING

DOSE 0.7%

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

	WKS 0 (B, 4)	WKS 4 (T, 3)	WKS 8 (T, 4)	WKS 13 (T, 2)	WKS 17 (T, 1)
ERYTHROCYTES ($X10^6$ /MM^3)	7.28 ± .07	6.48 ± .16[b]	6.23 ± .43[b]	3.86[a,b]	5.32
RETICULOCYTES, %	1.96 ± .16	4.17 ± .81[b]	5.48 ± .53[a]	2.96[b]	1.70
HEMATOCRIT, VOL. %	51.3 ± .9	51.0 ± -.0[b]	52.3 ± 2.4	33.5[a,b]	47.0
HEMOGLOBIN, GM. %	16.6 ± .1	16.4 ± .2	16.0 ± 1.3	12.3[a,b]	15.5
MCV, CUBIC MICRONS	70.4 ± 1.7	80.6 ± -.0[b]	84.7 ± 5.0[a,b]	85.9[b]	88.3
MCHB, MICRO MICROGMS.	22.8 ± .3	25.3 ± .7[b]	25.7 ± 1.0[b]	32.7[a]	29.1
MCHBC, GM %	32.4 ± .4	31.9 ± -.0	30.6 ± 1.5	38.4	33.0
PLATELETS ($X10^5$ /MM^3)	6.7 ± 1.0	8.3 ± .7[b]	10.7 ± .4[b]	3.0[a,b]	4.8
LEUKOCYTES ($X10^3$ /MM^3)	20.8 ± 1.1	24.2 ± 2.7[b]	18.4 ± 2.0	8.2[a,b]	6.1
NEUTROPHILS, %	8.0 ± 1.1	9.0 ± .6	14.5 ± 2.6	15.5	13.0
LYMPHOCYTES, %	90.3 ± 1.6	89.7 ± .7	84.8 ± 2.3	81.0	84.0
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0
EOSINOPHILS, %	1.3 ± .5	1.3 ± .3	.3 ± .3	2.0	0.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0
MONOCYTES, %	.5 ± .3	0.0 ± 0.0	.5 ± .3	1.5	2.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0

ENTRIES ARE MEAN ± STANDARD ERROR OR MEAN.

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[4/]).
b/ Significantly different from the control rats at the respective time interval (Dunnett's multiple comparison procedure[4/]).
c/ 2,4-DNT in feed discontinued thereafter.

TABLE 28

CLINICAL BLOOD CHEMISTRY DATA OF MALE CONTROL RATS AND RATS FED 2,4-DNT FOR 4 OR 13 WEEKS

	Fed for 4 Weeks	
	Control[a]	0.7% DNT[a]
Glucose (fasting), mg %	96.0 ± 12.5	84.0 ± 5.3
SGOT, IU/L	146.0 ± 16.5	141.5 ± 12.0
SGPT, IU/L	28.8 ± 0.8	51.8 ± 13.1
Alk. Phos., IU/L	56.0 ± 5.5	60.0 ± 6.5
BUN, mg %	15.5 ± 0.6	18.0 ± 1.3

	Fed for 13 Weeks		
	Control[a]	0.2% DNT[a]	0.7% DNT[b]
Glucose (fasting), mg %	120.5 ± 6.7	136.0 ± 5.0	145.0
SGOT, IU/L	72.5 ± 5.1	74.0 ± 3.2	68.0
SGPT, IU/L	25.8 ± 2.8	33.3 ± 2.3	21.0
Alk. Phos., IU/L	46.8 ± 4.5	68.3 ± 9.0	52.0
BUN, mg %	17.3 ± 0.6	18.5 ± 2.4	30.0

a/ Mean ± standard error of four rats.
b/ Mean of two rats.

TABLE 29

CLINICAL BLOOD CHEMISTRY DATA OF MALE CONTROL RATS AND RATS FED 2,4-DNT FOR 4 OR 13 WEEKS AND ALLOWED TO RECOVER 4 WEEKS

	Fed for 4 Weeks and Allowed to Recover 4 Weeks	
	Control[a]	0.7% DNT[b]
Glucose (fasting), mg %	129.7 ± 5.2	153.8 ± 11.3
SGOT, IU/L	97.7 ± 10.7	69.5 ± 3.8[d]
SGPT, IU/L	26.3 ± 2.3	30.0 ± 2.4
Alk. Phos., UL/L	70.7 ± 1.9	72.5 ± 2.6

	Fed for 13 Weeks and Allowed to Recover 4 Weeks		
	Control[b]	0.2% DNT[a]	0.7% DNT[c]
Glucose (fasting), mg %	164.5 ± 8.2	231.7 ± 11.5[d]	210.0
SGOT, IU/L	70.3 ± 9.2	60.7 ± 3.8	99.0
SGFT, IU/L	34.0 ± 2.1	42.0 ± 2.6	65.0
Alk. Phos., IU/L	39.3 ± 1.5	69.0 ± 8.0[d]	63.0
BUN, mg %	17.5 ± 1.0	21.7 ± 1.7	25.0

a/ Mean ± standard error of three rats.
b/ Mean ± standard error of four rats.
c/ One rat.
d/ Significantly different from the control group (Student's t test).

TABLE 30

HEMATOLOGY DATA OF FEMALE CONTROL RATS FOR 2,4-DNT

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

	WKS 0 (B, 4)	WKS 4 (C, 4)	WKS 8 (C, 4)	WKS 13 (C, 4)	WKS 17 (C, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.81 ± .16	7.82 ± .07 a/	6.79 ± .05	6.48 ± .03	4.66 ± .21 a/
RETICULOCYTES, %	1.47 ± .02	1.58 ± .25	1.10 ± .18	.75 ± .24	1.94 ± .28
HEMATOCRIT, VOL. %	50.8 ± 1.4	48.3 ± .3 a/	47.0 ± .8	48.8 ± .9	40.7 ± .9 a/
HEMOGLOBIN, GM. %	16.0 ± .2	16.3 ± .1	15.4 ± .1	15.5 ± .4	13.9 ± .4 a/
MCV, CUBIC MICRONS	74.6 ± 1.2	61.7 ± .7 a/	69.2 ± .8	75.2 ± 1.7	87.5 ± 3.6 b/
MCHB, MICRO MICROGMS.	23.6 ± .5	20.8 ± .2 a/	22.7 ± .3	24.0 ± .6	29.8 ± .6 a/
MCHBC, GM %	31.7 ± .6	33.7 ± .1	32.9 ± .4	32.0 ± 1.3	34.1 ± .7
PLATELETS ($X10^5$ /MM^3)	7.7 ± .3	5.5 ± .1	7.0 ± .5	5.8 ± .4	5.8 ± 1.7
LEUKOCYTES ($X10^3$ /MM^3)	14.3 ± 1.1	20.7 ± 1.4 a/	18.0 ± .2 a/	13.4 ± .4	4.9 ± .7 a/
NEUTROPHILS, %	8.5 ± 2.4	15.8 ± 1.1	9.3 ± 3.1	10.0 ± 1.1	23.5 ± 5.0 a/
LYMPHOCYTES, %	89.0 ± 1.9	83.3 ± 1.1	86.8 ± 3.0	87.8 ± 1.1	71.5 ± 4.2 a/
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.0 ± .7	1.0 ± 0.0	2.8 ± 1.1	.8 ± .3	2.5 ± 1.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.5 ± 1.0	0.0 ± 0.0	1.3 ± .8	1.5 ± .3	2.5 ± .5
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

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

TABLE 31

HEMATOLOGY DATA OF FEMALE RATS BEFORE, DURING AND AFTER, 2,4-DNT FEEDING

DOSE 0.07%

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

	WKS 0 (B, 4)	WKS 4 (T, 4)	WKS 8 (T, 4)	WKS 13 (T, 4)[c]	WKS 17 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.87 ± .24	6.79 ± .19	6.77 ± .28	6.67 ± .36	5.29 ± .18 [a,b]
RETICULOCYTES, %	1.29 ± .21	2.93 ± .29 [a]	1.43 ± .16	1.01 ± .23	1.24 ± .11
HEMATOCRIT, VOL, %	50.0 ± 1.1	45.0 ± .7 [a]	48.3 ± .9	49.8 ± 1.1	46.0 ± 1.2 [b]
HEMOGLOBIN, GM, %	15.9 ± .2	14.7 ± .3 [a]	16.1 ± .2	16.6 ± .3	15.3 ± .3 [b]
MCV, CUBIC MICRONS	73.7 ± 2.1	66.3 ± 1.1	72.2 ± 3.7	75.0 ± 3.4	87.1 ± 1.2 [a]
MCMB, MICRO MICROGMS.	23.5 ± .8	21.7 ± .4	24.0 ± .9	25.0 ± 1.3	28.9 ± .5 [a]
MCMBC, GM %	31.9 ± .7	32.7 ± .1	33.3 ± .4	33.4 ± 1.3	33.2 ± .1
PLATELETS ($X10^5$ /MM^3)	6.7 ± .5	6.5 ± .5	5.8 ± .4	5.8 ± .4	4.8 ± .8
LEUKOCYTES ($X10^3$ /MM^3)	15.1 ± 1.0	23.0 ± 2.3 [a]	14.8 ± 2.1	18.9 ± 2.2 [b]	5.5 ± .4 [a]
NEUTROPHILS, %	6.5 ± 2.4	14.8 ± 1.8	7.3 ± .9	6.5 ± 1.9	21.0 ± 7.8
LYMPHOCYTES, %	91.3 ± 3.2	84.0 ± 1.8	91.0 ± 1.2	91.0 ± 1.7	77.3 ± 7.7
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.0 ± .4	.8 ± .3	1.3 ± .6	2.3 ± .3	1.0 ± .4
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.3 ± .6	.5 ± .3	.5 ± .3	.3 ± .3 [b]	.8 ± .5
ATYPICAL, %	0.9 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[4]).
b/ Significantly different from the control rats at the respective time interval (Dunnett's multiple comparison procedure[4]).
c/ 2,4-DNT in feed discontinued thereafter.

TABLE 32

HEMATOLOGY DATA OF FEMALE RATS BEFORE, DURING AND AFTER 2,4-DNT FEEDING

DOSE 0.2%

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

	WKS 0 (B. 4)	WKS 4 (T. 4)	WKS 8 (T. 4)	WKS 13 (T. 4)[c]	WKS 17 (T. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	7.31 ± .15	7.51 ± .30	6.73 ± .21	6.93 ± .14	5.31 ± .8[a,b]
RETICULOCYTES, %	1.23 ± .11	1.50 ± .37	2.48 ± .59[b]	1.20 ± .16	1.40 ± .35
HEMATOCRIT, VOL. %	52.8 ± 1.3	49.3 ± 1.7	48.5 ± 2.2	49.5 ± 1.2	40.5 ± .3[a]
HEMOGLOBIN, GM. %	16.4 ± .5	16.3 ± .4	15.7 ± .6	16.1 ± .4	14.1 ± .3[a]
MCV, CUBIC MICRONS	72.2 ± .3	65.7 ± 1.1[a]	72.0 ± 1.2	71.4 ± 1.0	76.2 ± .7[a,b]
MCHB, MICRO MICROGMS.	22.5 ± .3	21.8 ± .6	23.3 ± .3	23.2 ± .3	26.5 ± .5[a,b]
MCHBC, GM %	31.2 ± .4	33.2 ± .4[a]	32.4 ± .7	32.5 ± .3	34.8 ± .5[a]
PLATELETS ($X10^5$ /MM^3)	7.0 ± .4	8.1 ± .6[b]	6.7 ± .1	5.3 ± .5	4.2 ± .6[a]
LEUKOCYTES ($X10^3$ /MM^3)	23.7 ± 2.0[b]	26.3 ± 1.5	20.2 ± 2.8	19.5 ± .5[b]	5.7 ± .6[a]
NEUTROPHILS, %	10.5 ± 1.8	15.8 ± 1.9	11.0 ± 1.2	5.8 ± 1.0	11.5 ± 3.2
LYMPHOCYTES, %	87.0 ± 1.8	82.3 ± 2.3	88.5 ± 1.4	92.5 ± .3[b]	85.0 ± 3.7
BANDS, %	6.0 ± 0.0	1.8 ± .6[a]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.8 ± .3	0.0 ± 0.0[b]	.5 ± .3	1.8 ± .9	.8 ± .8
BASOPHILS, %	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.8 ± .8	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a,b]	2.8 ± .5
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

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[a] Significantly different from the baseline level (Dunnett's multiple comparison procedure[4]).
[b] Significantly different from the control rats at the respective time interval (Dunnett's multiple comparison procedure[4]).
[c] 2,4-DNT in feed discontinued thereafter.

TABLE 33

HEMATOLOGY DATA OF FEMALE RATS BEFORE AND DURING 2,4-DNT FEEDING

DOSE: 0.7%

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

	WKS 0 (B, 4)	WKS 4 (T, 2)
ERYTHROCYTES ($X10^6$ /MM^3)	6.78 ± .16	5.56[a]
RETICULOCYTES, %	1.61 ± .13	6.13[a]
HEMATOCRIT, VOL. %	47.8 ± 1.1	41.0[a]
HEMOGLOBIN, GM. %	16.0 ± .3	13.7[a]
MCV, CUBIC MICRONS	70.6 ± 2.6	74.3[a]
MCHB, MICRO MICROGMS.	23.7 ± .7	24.7[a]
MCHBC, GM %	33.6 ± .3	33.3
PLATELETS ($X10^5$ /MM^3)	5.9 ± .3	5.5
LEUKOCYTES ($X10^3$ /MM^3)	18.5 ± 2.5	16.5
NEUTROPHILS, %	8.5 ± 1.7	8.0[a]
LYMPHOCYTES, %	89.5 ± 2.7	91.5[a]
BANDS, %	0.0 ± 0.0	0.0[a]
EOSINOPHILS, %	1.5 ± .9	0.0[a]
BASOPHILS, %	0.0 ± 0.0	0.0
MONOCYTES, %	.5 ± .3	.5
ATYPICAL [illegible]	0.0 ± 0.0	0.0
NUCLEATED RBC'S	0.0 ± 0.0	0.0

ENTRIES ARE MEAN ± STANDARD ERROR OR MEAN.

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

TABLE 34

CLINICAL BLOOD CHEMISTRY DATA OF FEMALE CONTROL RATS AND RATS FED 2,4-DNT FOR 4 OR 13 WEEKS

	Fed for 4 Weeks	
	Control[a]	0.7% in Feed[b]
Glucose (fasting), mg %	96.0 ± 5.6	101.0
SGOT, IU/L	93.3 ± 6.2	79.5
SGPT, IU/L	24.3 ± 1.4	37.0
Alk. Phos., IU/L	33.0 ± 2.0	37.0
BUN, mg %	17.5 ± 0.6	17.5

	Fed for 13 Weeks	
	Control[a]	0.2% in Feed[a]
Glucose (fasting), mg %	129.0 ± 9.0	138.3 ± 6.2
SGOT, IU/L	103.5 ± 14.7	81.0 ± 12.1
SGPT, IU/L	35.5 ± 2.9	29.3 ± 2.1
Alk. Phos., IU/L	23.8 ± 1.8	43.5 ± 6.7[c]
BUN, mg %	17.3 ± 1.3	19.0 ± 0.6

a/ Mean ± standard error of four rats.
b/ Mean of two rats.
c/ Significantly different from the control group (Student's t test).

TABLE 35

CLINICAL BLOOD CHEMISTRY DATA OF FEMALE CONTROL RATS AND RATS FED 2,4-DNT FOR 4 OR 13 WEEKS AND ALLOWED TO RECOVER 4 WEEKS

	Fed for 4 Weeks and Allowed to Recover 4 Weeks	
	Control[a]	0.7% in Feed[b]
Glucose (fasting), mg %	165.0 ± 8.1	181.0
SGOT, IU/L	110.5 ± 10.3	74.0
SGPT, IU/L	42.5 ± 7.0	24.0
Slk. Phos., IU/L	59.0 ± 2.1	49.0
BUN, mg %	19.5 ± 0.6	19.5

	Fed for 13 Weeks and Allowed to Recover 4 Weeks	
	Control[a]	0.2% in Feed[a]
Glucose (fasting), mg %	166.0 ± 5.9	181.0 ± 13.6
SGOT, IU/L	57.0 ± 6.1	65.5 ± 3.6
SGPT, IU/L	29.3 ± 2.1	25.3 ± 1.7
Alk. Phos., IU/L	30.0 ± 5.5	26.5 ± 6.0
BUN, mg %	20.5 ± 2.0	21.0 ± 1.9

a/ Mean ± standard error of four rats.
b/ Mean of two rats.

TABLE 36

SERUM ELECTROLYTES OF RATS FED 2,4-DNT

2,4-DNT in Feed (%)	Sex	Serum Electrolytes (meq/ℓ)[a] Na	K	Ca	Mg	Cl
		Fed for 4 Weeks				
0	Male	152 ± 2	6.3 ± 0.2	5.2 ± 0.1	2.3 ± 0.2	100 ± 0
0	Female	148 ± 2	5.2 ± 0.2	4.9 ± 0.1	2.1 ± 0.1	103 ± 1
0.7	Male	147 ± 0[b]	6.5 ± 0.4	5.2 ± 0.2	2.7 ± 0.2	100 ± 1
0.7	Female[d]	149	5.1	5.0	2.2	103
		Fed for 13 Weeks				
0	Male	145 ± 1	5.0 ± 0.2	5.4 ± 0.1	1.6 ± 0.1	98 ± 1
0	Female	146 ± 1	5.3 ± 0.3	5.6 ± 0.1	1.8 ± 0.1	101 ± 1
0.2	Male	158 ± 1[b]	5.3 ± 0.1	5.2 ± 0.1[b]	1.8 ± 0.2	101 ± 2
0.2	Female	152 ± 2[b]	4.8 ± 0.1	4.9 ± 0.1[b]	1.9 ± 0.1	96 ± 2
0.7	Male[e]	162	5.5	4.9	2.4	105
		Fed for 4 Weeks and Allowed to Recover for 4 Weeks				
0	Male[c]	151 ± 2	6.1 ± 0.3	5.5 ± 0.1	2.2 ± 0.2	102 ± 2
0	Female	150 ± 2	5.1 ± 0.3	5.5 ± 0.1	2.5 ± 0.2	103 ± 1
0.7	Male	148 ± 1	5.9 ± 0.2	5.5 ± 0.1	2.0 ± 0.2	102 ± 1
0.7	Female[d]	150	4.9	5.6	2.1	102
		Fed for 13 Weeks and Allowed to Recover for 4 Weeks				
0	Male	147 ± 0	4.9 ± 0.2	5.2 ± 0.1	1.9 ± 0.1	102 ± 2
0	Female	144 ± 1	4.6 ± 0.3	5.2 ± 0.0	2.0 ± 0.0	102 ± 2
0.2	Male[c]	150 ± 1[b]	4.7 ± 0.2	5.8 ± 0.1[b]	2.0 ± 0.1	97 ± 1
0.2	Female	145 ± 2	4.2 ± 0.2	5.5 ± 0.2	1.9 ± 0.2	100 ± 3
0.7	Male[e]	145	5.3	5.6	2.2	99

a/ Mean ± standard error of four rats, unless stated otherwise.
b/ Significantly different from control (Student's t test).
c/ Mean ± standerd error of three rats.
d/ Mean of two rats.
e/ One rat.

TABLE 37

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

Sex	% 2,4-DNT in Feed	Terminal Weight (gm)	Absolute Organ Weights (gm) Liver	Spleen	Kidneys	Heart
Male	0	446±23[a]	13.5±1.1	0.72±0.08	3.20±0.14	1.35±0.10
	0.07	413±15	14.4±0.9	0.99±0.08	3.06±0.17	1.17±0.08
	0.2	414±12	19.1±0.8[b]	0.76±0.06	2.91±0.18	1.30±0.05
	0.7	329±2[b]	12.1±0.9	0.74±0.09	2.49±0.14[b]	0.99±0.03[b]
Female	0	258±9	7.6±0.4	0.54±0.04	1.65±0.05	0.80±0.09
	0.07	244±6	10.7±0.5[b]	0.54±0.05	1.75±0.05	0.85±0.17
	0.2	256±3	9.2±0.6	0.42±0.08	1.62±0.14	0.79±0.04
	0.7[c]	212±13[b]	10.4±0.8[b]	0.38±0.13	1.52±0.13	0.63±0.07
			Relative Organ Weights (gm/100 gm Body Weight) Liver	Spleen	Kidneys	Heart
Male	0		3.0±0.2	0.16±0.02	0.72±0.04	0.30±0.01
	0.07		3.5±0.1	0.24±0.01[b]	0.74±0.02	0.28±0.01
	0.2		4.6±0.2[b]	0.18±0.01	0.70±0.03	0.31±0.01
	0.7		3.7±0.3	0.22±0.03	0.76±0.05	0.30±0.01
Female	0		3.0±0.1	0.21±0.01	0.64±0.04	0.31±0.03
	0.07		4.4±0.2[b]	0.22±0.02	0.72±0.03	0.35±0.06
	0.2		3.6±0.2	0.17±0.03	0.64±0.05	0.31±0.01
	0.7[c]		4.9±0.1[b]	0.17±0.05	0.73±0.10	0.29±0.02

a/ Mean ± standard error of four rats.

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

c/ Two rats.

TABLE 38

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF RATS FED 2,4-DNT FOR 13 WEEKS

Sex	% 2,4-DNT in Feed	Terminal Weight (gm)	Absolute Organ Weight (gm)				
			Liver	Spleen	Kidneys	Heart	Brain
Male	0	531±31[a]	14.4±1.2	0.85±0.07	3.16±0.11	1.40 0.10	2.03±0.01
	0.07	401±8[b]	16.0±0.6	0.64±0.03[b]	3.76±0.16	1.26±0.06	1.93±0.03
	0.2	396±26[b]	17.9±1.5	0.73±0.03	3.49±0.13	1.32±0.08	2.01±0.02
	0.7[c]	238	9.6	0.35	3.64	1.01	2.03
Female	0	302±16	9.4 1.2	0.56±0.04	2.03±0.12	0.98±0.04	2.02±0.08
	0.07	265±15	7.8±0.4	0.48±0.04	1.91±0.09	0.86±0.02[b]	1.97±0.04
	0.2	243±5	9.2±1.0	0.52±0.03	1.80±0.08	0.84±0.02[b]	2.01±0.04
			Relative Organ Weights (gm/100 gm Body Weight)				
			Liver	Spleen	Kidneys	Heart	Brain
Male	0		2.7±0.1	0.16±0.02	0.60±0.03	0.26±0.02	0.39±0.03
	0.07		4.0±0.2[b]	0.16±0.01	0.81±0.04	0.31±0.02	0.49±0.01
	0.2		4.5±0.3[b]	0.19±0.01	0.89±0.06[b]	0.34±0.02	0.51±0.04[b]
	0.7[c]		4.0	0.15	1.54	0.42	0.85
Female	0		3.1±0.3	0.19±0.01	0.67±0.01	0.33±0.01	0.68±0.05
	0.07		3.0±0.1	0.18±0.01	0.72±0.01	0.33±0.01	0.75±0.03
	0.2		3.8±0.4	0.21±0.01	0.74±0.02[b]	0.35±0.00	0.83±0.02[b]
			Relative Organ Weights (gm/gm Brain Weight)				
			Liver	Spleen	Kidneys	Heart	
Male	0		7.1±0.6	0.42±0.04	1.56±0.06	0.69±0.05	
	0.07		8.2±0.4	0.33±0.01	1.67±0.10	0.64±0.03	
	0.2		8.9±0.8	0.36±0.02	1.74±0.08	0.66±0.04	
	0.7[c]		4.7	0.17	1.80	0.49	
Female	0		4.7±0.8	0.28±0.03	1.01±0.08	0.49±0.02	
	0.07		4.0±0.2	0.24±0.02	0.97±0.04	0.44±0.01	
	0.2		4.6±0.5	0.26±0.02	0.90±0.04	0.42±0.01[b]	

a/ Mean ± standard error of four rats.

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

c/ One rat.

TABLE 39

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF RATS FED 2,4-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Sex	% 2,4-DNT in Feed	Terminal Weight (gm)	Absolute Organ Weights (gm) Liver	Spleen	Kidneys	Heart
Male	0	528±40[a]	16.5±3.7	0.68±0.15	3.53±0.26	1.00±0.11
	0.07	525±19	21.1±1.7	0.92±0.05	3.00±0.04	1.05±0.03
	0.2	496±50	21.7±3.1	0.55±0.16	3.10±0.20	1.27±0.21
	0.7	396±6[b]	20.0±0.3	0.92±0.11	3.00±0.27	1.17±0.06
Female	0	323±18	10.9±0.7	0.52±0.08	2.43±0.20	0.85±0.05
	0.07	288±6	10.6±0.5	0.55±0.18	2.03±0.06	0.82±0.03
	0.2	282±9	9.7±0.7	0.37±0.11	1.33±0.17[b]	0.85±0.05
	0.7[c]	267±4	15.4±3.9	0.35±0.05	1.95±0.05	0.90±0.10
			Relative Organ Weights (gm/100 gm Body Weight) Liver	Spleen	Kidneys	Heart
Male	0		3.1±0.6	0.14±0.04	0.67±0.05	0.19±0.01
	0.07		4.0±0.2	0.18±0.01	0.57±0.02	0.20±0.00
	0.2		4.3±0.4	0.11±0.02	0.63±0.03	0.27±0.06
	0.7		5.1±0.0[b]	0.23±0.03	0.76±0.06	0.30±0.02
Female	0		3.4±0.2	0.16±0.02	0.77±0.09	0.27±0.02
	0.07		3.7±0.1	0.19±0.07	0.70±0.03	0.29±0.01
	0.2		3.4±0.2	0.13±0.04	0.47±0.05[b]	0.30±0.01
	0.7[c]		5.8±1.5[b]	0.13±0.02	0.73±0.03	0.34±0.03

[a] Mean ± standard error of four rats.

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

[c] Two rats.

TABLE 40

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF RATS FED 2,4-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Sex	% 2,4-DNT in Feed	Terminal Weight (gm)	Absolute Organ Weights (gm) Liver	Spleen	Kidneys	Heart	Brain
Male	0	569±21[a]	16.1±1.5	0.79±0.04	3.57±0.28	1.51±0.05	2.15±0.05
	0.07	482±6[b]	18.7±0.4	0.75±0.08	3.77±0.21	1.52±0.04	2.06±0.06
	0.2[c]	391±21[b]	28.0±4.3[b]	0.61±0.03	4.06±0.34	1.45±0.13	2.06±0.08
	0.7[d]	352	25.2	0.53	2.43	1.42	1.80
Female	0	302±10	7.3±0.2	0.47±0.01	1.77±0.09	0.95±0.04	2.06±0.04
	0.07	259±6[b]	8.0±0.5	0.47±0.04	1.88±0.07	0.92±0.04	2.01±0.05
	0.2	256±1[b]	9.6±0.4[b]	0.41±0.02	1.98±0.12	1.03±0.02	2.00±0.07

Sex	% 2,4-DNT in Feed	Relative Organ Weights (gm/100 gm Body Weight) Liver	Spleen	Kidneys	Heart	Brain
Male	0	2.8±0.2	0.14±0.01	0.62±0.03	0.27±0.01	0.38±0.01
	0.07	3.9±0.1	0.16±0.01	0.78±0.04[b]	0.32±0.01[b]	0.43±0.01[b]
	0.2[c]	7.1±0.7[b]	0.16±0.00	1.04±0.04[b]	0.37±0.01[b]	0.53±0.01[b]
	0.7[d]	7.2	0.15	0.69	0.40	0.51
Female	0	2.4±0.1	0.15±0.01	0.59±0.03	0.32±0.02	0.68±0.03
	0.07	3.1±0.2[b]	0.18±0.01	0.73±0.02[b]	0.36±0.02	0.78±0.02
	0.2	3.8±0.2[b]	0.16±0.01	0.77±0.05[b]	0.40±0.01	0.78±0.03

Sex	% 2,4-DNT in Feed	Relative Organ Weights (gm/gm Brain Weight) Liver	Spleen	Kidneys	Heart
Male	0	7.5±0.6	0.37±0.02	1.65±0.10	0.70±0.03
	0.07	9.1±0.3	0.37±0.04	1.84±0.14	0.74±0.01
	0.2[c]	13.5±1.6[b]	0.29±0.00	1.97±0.10	0.70±0.04
	0.7[d]	14.0	0.30	1.35	0.79
Female	0	3.4±0.1	0.23±0.00	0.86±0.02	0.76±0.01
	0.07	4.0±0.2	0.23±0.02	0.94±0.03	0.46±0.03
	0.2	4.8±0.2[b]	0.20±0.01	0.99±0.05	0.52±0.01

[a] Mean ± standard error of four rats.
[b] Significantly different from the control rats (Dunnett's multiple comparison procedure[4]).
[c] Three rats.
[d] One rat.

TABLE 41

SUMMARY OF TISSUE LESIONS IN MALE RATS FED 2,4-DNT FOR 4 WEEKS

Lesions[a]	Dose (% in Feed)											
	0				0.2				0.7			
Rat No.:	113	114	115	116	163	164	165	166	188	189	190	191
Heart												
Myocarditis								+				
Lungs												
Lymphoid hyperplasia		+					+		+	+	+	
Pneumonitis			±	+								
Liver												
Hepatic cell necrosis												++
Spleen												
Hemosiderosis					±	+	++			++	±	+
Adrenal												
Fatty degeneration							±					
Testes												
Atrophy									++	++	++	++
Aspermatogenesis									++	++	++	++
Bone Marrow												
M/E Ratio	1.5	1.4	1.3	1.4	1.7	1.9	1.6	1.7	1.6	1.3	1.4	1.5

Tissue not listed were normal.

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

TABLE

SUMMARY OF TISSUE LESIONS IN REMALE RATS FED 2,4-DNT FOR 4 WEEKS

Lesions[a/]	Dose (% in Feed)									
	0				0.2				0.7	
Rat No.:	213	214	215	216	263	264	265	266	289	294
Lung										
Lymphoid hyperplasia				+	+	+	+	+	+	+
Pneumonitis		±							+	++
Liver										
Hepatic cell necrosis	+									
Spleen										
Hemosiderosis					+			+++		++
Bone Marrow										
M/E Ratio	1.2	1.5	1.3	1.4	1.8	1.8	1.6	b/	1.2	1.6

Tissue not listed were normal.

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

b/ Marrow smear was not prepared.

TABLE 43

SUMMARY OF TISSUE LESIONS IN MALE RATS FED 2,4-DNT FOR 13 WEEKS

Lesions[a/]	Dose (% in Feed)									
	0				0.2				0.7	
Rat No.:	105	106	107	108	155	156	157	158	179	181
Heart										
Myocarditis	±			+	±	+	±	+	++	+
Lung										
Lymphoid hyperplasia	+					+		+		
Pneumonitis									++	++
Edema					++					
Liver										
Subacute inflammation		+		+				++		
Hepatic cell necrosis			+	+					+	++
Hemosiderosis		+								+
Fatty changes					++		+		±	
Spleen										
Hemosiderosis	++			++	+++	+++	++	+++	+++	++
Kidney										
Albuminoid		++								
Cerebrum										
Gliosis										±
Adrenals										
Fatty necrosis						+++				
Testes										
Atrophy					++	++	++++	++	++++	++++
Aspermatogenesis					++	++	+++	++++	++++	++++
Bone Marrow										
M/E Ratio	1.4	1.6	1.7	1.4	1.6	1.7	1.6	1.5	1.4	1.5

Tissue not listed were normal.

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

TABLE 44

SUMMARY OF TISSUE LESIONS OF FEMALE RATS FED 2,4-DNT FOR 13 WEEKS

Lesions[a/]	Dose (% in feed)							
	0				0.2			
Rat No.:	205	206	207	208	255	256	257	258
Heart								
Myocarditis		±				±	+	±
Lung								
Lymphoid hyperplasia	±							±
Liver								
Subacute inflammation	±							±
Kidney								
Albuminoid							+	
Spleen								
Hemosiderosis	+++	+	++	+	++	+	+++	+++
Extramedullary hematopoiesis						±		
Bone Marrow								
M/E ratio	1.5	1.4	1.7	1.6	1.6	1.7	1.5	1.6

Tissues not listed were normal.

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

TABLE 45

SUMMARY OF TISSUE LESIONS IN MALE RATS FED 2,4-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Lesions[a/]	Dose (% in Feed) 0				0.2				0.7			
Rat No.:	109	110	111	112	159	160	161	162	184	185	186	187
Heart												
Myocarditis											±	
Lung												
Lymphoid hyperplasia	+				+		+	+		+	+	+
Pneumonitis		++	±			++			±			
Liver												
Subacute inflammation												
Hepatic cell necrosis		+									±	
Spleen												
Hemosiderosis								++	+++	++	+++	++
Kidney												
Casts										+		
Adrenals												
Fatty necrosis								+				
Pancreas												
Acinar necrosis	±								++			
Testes												
Atrophy								++	++++	+++	+++	+++
Aspermatogenesis								+++	++++	+++	++++	+++
Bone Marrow												
M/E Ratio	1.6	1.5	1.7	1.5	1.7	1.6	1.5	1.8	1.4	1.6	1.5	1.6

Tissue not listed were normal.

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

TABLE 46

SUMMARY OF TISSUE LESIONS IN FEMALE RATS FED 2,4-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

	Dose (% in feed)									
	0				0.2				0.7	
Lesions[a/] Rat No.:	209	210	211	212	259	260	261	262	297	298
Heart										
Myocarditis			+							
Lung										
Lymphoid hyperplasia	++		+					+	+	
Liver										
Subacute inflammation										+
Hepatic cell necrosis					±		±			
Kidney										
Interstitial nephritis					+					
Spleen										
Hemosiderosis	+				+++	+++	++	+	+	±
Bone Marrow										
M/E ratio	1.4	1.6	1.5	1.3	1.7	1.6	1.5	1.7	1.4	1.5

Tissues not listed were normal.

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

TABLE 47

SUMMARY OF TISSUE LESIONS IN MALE RATS FED 2,4-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

	Dose (% in feed)							
	0				0.2			0.7
Lesions[a/] Rat No.:	101	102	103	104	151	152	153	177
Heart								
Myocarditis			±		+	++	++	++
Lungs								
Lymphoid hyperplasia	+	+	+		+	+		
Pneumonitis				+			+++	+++
Liver								
Subacute inflammation		+		+				+
Vacuolated hepatocytes (degeneration)					+++	++	+++	++
Cerebellum								
Gliosis							+	+
Demyelination							+	±
Spleen								
Hemosiderosis					+	+++	++++	++
Kidney								
Albuminoid	+				++	+	+	+
Glomerulonephritis							++	
Casts							+	
Pancreas								
Hemosiderotic cell inclusions					+	±		
Testes								
Atrophy					++++	++++	++++	++++
Aspermatogenesis					++++	++++	++++	++++
Bone Marrow								
M/E ratio	1.6	1.5	1.4	1.7	1.6	1.8	1.5	1.4

Tissues not listed were normal.

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

TABLE 48

SUMMARY OF TISSUE LESIONS IN FEMALE RATS FED 2,4-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

	Dose (% in feed)							
	0				0.2			
Lesions[a/] Rat No.:	201	202	203	204	251	252	253	254
Heart								
Myocarditis				+	++	++	++	±
Lung								
Lymphoid hyperplasia	±	+		+	+		+	±
Liver								
Subacute inflammation					+			
Hepatic cell necrosis					+	±	+	
Spleen								
Hemosiderosis	±	+	±	+	++++		+	
Kidney								
Tubular coagulation necrosis						+		
Glomerulonephritis								±
Pancreas								
Necrosis						±	+	
Lymph Node								
Hemosiderosis					+			
Bone Marrow								
M/E ratio	1.6	1.4	1.7	1.5	1.7	1.7	1.8	1.7

Tissues not listed were normal.

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

TABLE 49

NUMERICAL DISTRIBUTION OF CHROMOSOMES
FROM RATS FED 0.2% OF 2,4-DNT

Treatment[a]	No. of Rats	Chromosome Frequency <40	41	42	43	≥44	Tetraploids per 100 Cells
Control							
Lymphocyte	4	3[b]	5	40	2	0	0.26 ± 0.15[c]
Kidney	4	4	5	39	2	0	0.48 ± 0.18
2,4-DNT for 5 weeks							
Lymphocyte	4	2	4	42	2	0	0.25 ± 0.15
Kidney	3	4	4	41	1	0	0.83 ± 0.60
2,4-DNT for 13 weeks							
Kidney	3	4	4	40	2	0	2.17 ± 0.72
2,4-DNT for 19 weeks							
Lymphocyte	4	6	5	37	2	0	0.50 ± 0.35

a/ No lymphocyte cultures were obtained from rats fed the diet for 13 weeks; however, rats were bled again at 19 weeks and the lymphocyte cultures were examined.

b/ Mean.

c/ Mean ± S.E.

TABLE 50

MORPHOLOGICAL ABERRATIONS OF CHROMOSOMES FROM RATS FED 0.2% OF 2,4-DNT

Treatment[a]	No. of Rats	Chromatid Breaks and Gaps per 50 Cells	Translocations per 50 Cells	Total Aberrations per 50 Cells
Control				
Lymphocytes	4	0.75 ± 0.25[b]	0.25 ± 0.25	1.00 ± 0.40
Kidneys	4	1.50 ± 0.29	0.50 ± 0.29	1.25 ± 0.48
2,4-DNT for 5 weeks				
Lymphocytes	5	2.23 ± 0.85	0.43 ± 0.26	2.05 ± 0.67
Kidneys	3	2.80 ± 0.17[c]	0.67 ± 0.33	3.50 ± 0.50[c]
2,4-DNT for 13 weeks				
Kidneys	4	5.25 ± 1.03[c]	0.75 ± 0.47	6.00 ± 1.35[c]
2,4-DNT for 19 weeks[a]				
Lymphocytes	4	4.28 ± 0.77[c]	0.37 ± 0.37	4.66 ± 1.06[c]

a/ No lymphocyte cultures were obtained from rats fed the diet for 13 weeks; however, rats were bled at 19 weeks and the lymphocyte cultures were examined.

b/ Mean ± S.E.

c/ Significantly different from the control (Student's t test).

TABLE 51

DOMINANT LETHAL EFFECTS IN RATS FED 2,4-DNT FOR 13 WEEKS

Treatment	No. of Males	Fertility Index[a]	Implant Viability Index[b]
Control	4	92 ± 8[c]	92 ± 1
2,4-DNT (0.02%)	4	67 ± 14	62 ± 24
2,4-DNT (0.20%)	5	20 ± 13[d]	0[d]

a/ Confirmed pregnancies/sperm positive females x 100.
b/ Viable fetuses/implants x 100.
c/ Mean ± S.E.
d/ Significantly different from the control (Student's t test).

TABLE 52

SERUM IgE OF RATS FED 2,4-DNT

Sex	% 2,4-DNT in Feed	IgE (IU/ml) 4 Weeks[a]	4 + 4 Weeks[b]	13 Weeks	13 + 4 Weeks[b]
Male	0	<500	1,350[e]	850 ± 100	1,325 ± 50
	0.2			1,350 ± 75	1,330 ± 150[c]
	0.7	<500	1,450 ± 75	1,400[d]	1,225[e]
Female	0	<500	1,150 ± 100	1,400 ± 200	1,350 ± 75
	0.2			1,700 ± 175	1,375 ± 50
	0.7	675[d]	1,300[d]		

a/ Weeks of feeding. Entries are mean ± S.E. of four rats.
b/ Treatment for 4 or 13 weeks, then allowed to recovery for 4 weeks.
c/ Three rats.
d/ Two rats.
e/ One rat.

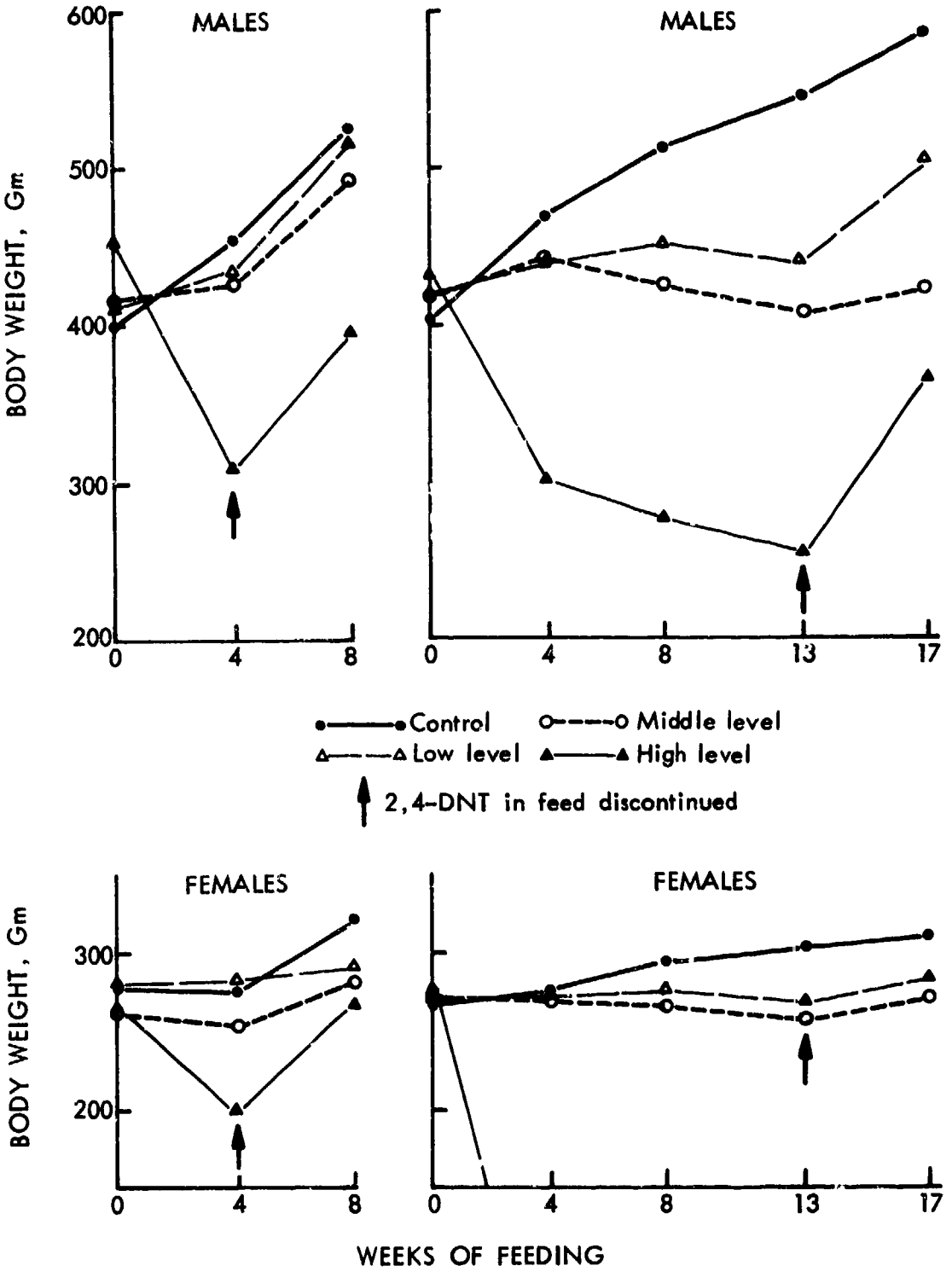

Figure 1 - Body Weights of Rats Fed Various Levels of 2,4-DNT

III. MICE

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III. MICE

A. Subacute and Subchronic Toxicities and Reversibility

1. Introduction

As for the dogs and mice, these studies were performed to define the nature and extent of effects of 2,4-DNT on the biological system at the biochemical and cellular levels and to elucidate the dose-response relationship in the mice fed 2,4-DNT for 4 and 13 weeks. The reversibility of adverse effects was also studied in mice after the feeding of 2,4-DNT was discontinued for 4 weeks.

2. Material and Methods

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

(a) A total of 64 male and 64 female young healthy albino Swiss mice (National Laboratory Animals, O'Fallon, Missouri) were used for this study. They were divided into four groups, each consisting of 16 males and 16 females. The average weights of all groups were kept close. Three groups of mice were fed 0.07, 0.2, or 0.7% of 2,4-DNT, in powdered standard rodent chow (Wayne Laboratory Meal). The fourth group served as controls and were given the powdered standard rodent chow.

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

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

3. Results

a. General Observations and Weight Gain

The control mice and mice fed low (0.07%) and middle (0.2%) levels of 2,4-DNT were healthy. They maintained their weight or gained slightly. These data are listed in Tables 53 and 54 and shown graphically in Figure 2. Mice fed the high level (0.7%) of 2,4-DNT lost weight. During the recovery period, most of these mice regained the weight they had lost. Five deaths occurred: one low dosage male died during week 11, two high

dosage males died in week 5; one had just entered the recovery period, the other was continuing on study; one high dosage female died in week 7; and one high dosage male died in week 10. None of the mice had any obvious signs of morbidity.

b. Feed Consumption and 2,4-DNT Intake

Feed consumption of the mice fed 2,4-DNT is summarized in Table 55. Mice fed the high level ate less, as reflected in their weight loss. The other groups consumed comparable amounts throughout the experiment.

Intake of 2,4-DNT is summarized in Table 56. The 2,4-DNT intake of the male mice fed 0.07, 0.02 or 0.7% fluctuated slightly and averaged 47, 137, and 413 mg/kg/day, respectively. Similarly, intake of the females averaged 52, 147, and 468 mg/kg/day, respectively.

c. Blood Analysis

The hematology results of the control males and males fed various levels of 2,4-DNT for 4 or 13 weeks, with and without recovery, are shown in Tables 57 through 60. Results for females are shown in Tables 61 through 64.

The various peripheral blood elements of mice fed the low or middle level of 2,4-DNT were not apparently altered. When compared with the respective controls, there were occasional differences at the various time intervals. However, these differences were slight and were inconsistent. Mice fed the high level of 2,4-DNT for 13 weeks had significant decreases in hematocrit and/or hemoglobin concentrations when compared with those of the controls (Tables 58 and 62). In addition, there was a compensatory increase in reticulocyte count, although the increase was not statistically significant. The middle dosage males (Table 58) had lesser effects and the changes were not statistically significant. After allowed to recover for 4 weeks, these blood elements recovered and the differences disappeared (Tables 60 and 64).

d. Organ Weights

The various organ weights of the mice fed various levels of 2,4-DNT for 13 weeks, or for 4 or 13 weeks and allowed to recover for 4 weeks, are shown in Tables 65, 66, and 67. There were occasional increases in kidney, liver, or brain weight, or occasional decrease or increase in spleen weight when compared with those of the respective controls. The differences were slight and the changes were inconsistent.

e. Gross and Microscopic Examination of Tissues

At necropsy, the control mice and mice fed 0.07 or 0.2% of 2,4-DNT were in good nutritional condition. Most mice fed 0.7% of 2,4-DNT for 4 or 13 weeks had little, if any, body fat. When 2,4-DNT in feed was discontinued for 4 weeks, the condition of most mice recovered.

After 4 weeks of feeding, there were a number of mild tissue lesions in males and females, as shown in Tables 68 and 69, respectively. Spontaneous lesions included interstitial nephritis, hematopoiesis in the spleen, myocarditis and focal necrosis in the liver. Two of the males fed the high level of 2,4-DNT had mild depression on spermatogenesis.

Control mice and mice fed 2,4-DNT for 13 weeks had a few additional spontaneous lesions, including lymphoid hyperplasia and pneumonia in the lung and subacute inflammation in the liver and kidney as shown in Tables 70 and 71. There were no apparent 2,4-DNT-induced lesions in the testes or other organs.

Control mice and mice fed 2,4-DNT for 4 or 13 weeks and allowed to recover for 4 weeks also had a variety of spontaneous lesions, as shown in Tables 72 through 75. In addition, male and female mice in the latter group fed the high level of 2,4-DNT had a number of liver lesions, including inclusion bodies, cytomegaly and pigmented Kupffer cells (Tables 74 and 75). These lesions were mild and were not seen in mice terminated before the recovery period.

Bone marrows and M/E ratios of all mice in all groups were normal.

4. Discussion and Conclusions

The 2,4-DNT intake for males and females, respectively, averaged 47 or 52 mg/kg/day in the low level group, 137 or 147 mg/kg/day in the middle level group, and 413 or 468 mg/kg/day in the high level group.

The low and middle levels of 2,4-DNT were nontoxic to mice. Mice fed the high level had weight loss and a few deaths. There was mild anemia as evidenced by decreases in hematocrit and hemoglobin concentration and an increase in reticulocyte count. Two high dosage males terminated after 4 weeks had mild depression on spermatogenesis, but testicular lesions were not seen in those terminated after 13 weeks. This may reflect tolerance or may be incidental. After allowed to recover for 4 weeks, the mice recovered.

The mice were relatively resistant to the toxic effects of 2,4-DNT. They ingested more 2,4-DNT related to their body weight than the rats did. Unlike rats, mice had no apparent behavioral effects. The anemia was mild and occurred later. There were only few deaths. Mild aspermatogenesis was seen in two of four mice fed the high level of 2,4-DNT for 4 weeks, but not in mice for 13 weeks. This species difference was also observed in acute toxicity.[1/] The LD_{50}'s of 2,4-DNT for mice were two to four times larger than those for rats. One possible explanation is the difference in the absorption and metabolism of 2,4-DNT between mice and rats as discussed below in Section IV.

B. Mutagenic Effects of 2,4-DNT

1. Introduction

The mutagenic effect of 2,4-DNT was studied in male mice using the dominant lethal mutation test.

2. Material and Methods

The methods used were the same as in the rat study, Section II.B.2.d., except that mating was determined by the presence of a vaginal plug and females were allowed to deliver.

3. Results and Discussion

Male mice were fed 0.2% of 2,4-DNT for 13 weeks, or 0.7% for 4 weeks. The results are shown in Table 76. The low level of 2,4-DNT produced no effects. The high level greatly reduced the fertility index but had no effect on the implant viability index. The reduced fertility index was due to a majority of the females having no implants. Females bearing young had normal numbers of implants and pups. These results suggest preimplant losses, possibly related to the testicular lesions discussed above.

4. Conclusions

DNT at 0.7% in the diet for 4 weeks greatly reduced the fertility index without effect on the implant viability index. A majority of the females mated to the treated males had no implants, whereas females bearing young had normal numbers of implants and pups.

C. Summary

Male mice fed up to 137 mg/kg/day of 2,4-DNT and female mice fed up to 147 mg/kg/day for 13 weeks were unaffected. The high dose, giving 413 and 468 mg/kg/day to males and females, respectively, caused weight loss, mild anemia, some decreases in spermatogenesis (decreasing fertility) and a few deaths. Surviving mice recovered completely after cessation of treatment.

TABLE 53

BODY WEIGHTS OF MALE MICE FED 2,4-DNT

% 2,4-DNT in Feed	Body Weights (gm)[a]				
	Initial	4 Weeks	8 Weeks	13 Weeks	17 Weeks
0	31.8±1.8	29.8±2.2			
0.07	30.5±0.9	30.5±2.0			
0.2	35.5±1.0	34.5±1.0			
0.7	32.8±2.3	26.5±2.0			
0	28.5±1.7	33.5±1.7[b]	32.0±1.5		
0.07	29.0±0.4	30.8±0.5[b]	31.5±0.9		
0.2	29.5±2.1	31.5±1.5[b]	33.0±2.3		
0.7	31.0±1.9	24.3±1.4[b]	33.3±0.7[c]		
0	31.3±0.5	33.8±0.6	38.5±0.9	35.0±2.2	
0.07	30.3±0.5	31.5±1.2	33.5±1.0	33.7±0.3[d]	
0.2	32.3±1.8	34.0±0.8	34.3±1.8	33.5±1.3	
0.7	31.3±1.4	27.8±0.6	26.8±1.2	25.7±0.9[e]	
0	31.0±0.7	34.3±1.3	36.3±1.1	37.3±1.3[b]	35.0±0.9
0.07	32.8±1.8	34.8±1.5	35.3±2.0	36.0±2.1[b]	33.0±1.7
0.2	30.3±1.9	31.0±1.0	31.0±1.4	33.0±1.5[b]	30.3±2.1
0.7	29.3±1.7	28.0±0.3	28.7±1.7[c]	29.0±1.8[b,c]	32.3±0.6[c]

a/ Mean ± S.E. of four mice, unless otherwise noted.
b/ 2,4-DNT in feed discontinued thereafter.
c/ Three mice; one other mouse died in week 5.
d/ Three mice; one other mouse died in week 11.
e/ Three mice; one other mouse died in week 10.

TABLE 54

BODY WEIGHTS OF FEMALE MICE FED 2,4-DNT

% 2,4-DNT in Feed	Body Weights (gm)[a]				
	Initial	4 Weeks	8 Weeks	13 Weeks	17 Weeks
0	26.0±0.4	26.0±1.4			
0.07	26.3±0.6	23.3±1.1			
0.2	27.0±1.2	25.3±1.7			
0.7	25.0±1.1	22.5±0.3			
0	27.5±0.5	28.7±0.8[b]	28.0±0.7		
0.07	25.5±0.7	27.3±1.1[b]	26.3±1.1		
0.2	25.8±0.9	28.3±1.0[b]	26.5±0.9		
0.7	26.3±1.0	24.0±1.1[b]	28.0±0.9		
0	26.5±0.7	29.0±1.3	28.6±1.1	29.3±0.8	
0.07	26.8±0.9	27.8±0.9	30.5±1.3	29.5±1.2	
0.2	26.3±1.1	27.8±0.9	27.5±1.2	27.8±0.8	
0.7	26.3±0.9	23.3±1.3	25.7±0.9[c]	25.0±1.0[c]	
0	26.5±0.7	27.0±0.6	27.3±0.5	29.5±1.3[b]	26.8±0.6
0.07	25.8±0.8	28.8±0.8	29.5±1.0	31.3±1.3[b]	27.5±1.0
0.2	26.3±0.6	27.8±0.9	29.8±0.9	31.8±1.3[b]	28.5±1.0
0.7	26.0±0.8	24.0±0.4	24.8±0.6	26.7±1.0[b]	24.3±1.7

a/ Mean ± S.E. of four mice, unless otherwise noted.

b/ 2,4-DNT in feed discontinued thereafter.

c/ Three mice; one other mouse died in week 7.

TABLE 55

AVERAGE FEED CONSUMPTION (GM/DAY/MOUSE) OF MICE FED 2,4-DNT

% 2,4-DNT In Feed	Males			
	1 - 4[a]	5 - 8	9 - 13	14 - 17[b]
0	2.2	2.3	2.6	1.9
0.07	2.0	2.5	2.2	2.0
0.2	2.1	2.4	2.3	2.1
0.7	1.4	2.0	2.0	2.3
	Females			
	1 - 4	5 - 8	9 - 13	14 - 17
0	2.2	2.3	2.2	1.9
0.07	2.0	2.3	2.0	1.6
0.2	1.9	2.3	2.0	1.8
0.7	1.5	1.9	1.7	1.7

a/ Weeks.
b/ Recovery period; all mice fed control feed.

TABLE 56

AVERAGE 2,4-DNT INTAKE (MG/KG/DAY) OF MICE DURING TREATMENT

% 2,4-DNT In Feed	Males			
	1 - 4[a/]	5 - 8	9 - 13	1 - 13
0.07	46.0	51.0	44.0	47.0
0.2	132.0	149.0	138.0	137.0
0.7	332.0	490.0	498.0	413.0
	Females			
	1 - 4	5 - 8	9 - 13	1 - 13
0.07	53.0	54.0	47.0	52.0
0.2	142.0	165.0	140.0	147.0
0.7	434.0	547.0	458.0	468.0

TABLE 57

HEMATOLOGY DATA OF MALE MICE FED 2,4-DNT FOR 4 WEEKS

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

DOSE: MG/KG/DAY	0.00 (C, 4)	0.07 (T, 4)	0.2 (T, 4)	0.7 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	5.91 ± .46	6.06 ± .63	5.50 ± .08	5.75 ± .35
RETICULOCYTES, %	2.91 ± .34	1.22 ± .18	1.96 ± .31	2.29 ± .35
HEMATOCRIT, VOL. %	43.3 ± 1.5	42.3 ± 1.7	42.5 ± .3	42.0 ± 2.3
HEMOGLOBIN, GM. %	13.5 ± .8	13.6 ± .7	13.9 ± .4	13.4 ± .9
MCV, CUBIC MICRONS	74.1 ± 4.8	71.3 ± 5.3	77.3 ± .7	73.3 ± 3.1
MCMB, MICRO MICROGMS.	23.1 ± 1.3	22.9 ± 1.5	25.3 ± .4	23.3 ± 1.3
MCHRC, GM %	31.2 ± .6	32.2 ± .3	32.7 ± .8	31.8 ± .4
PLATELETS ($X10^5$ /MM^3)	5.8 ± .7	7.5 ± .7	7.1 ± .4	10.4 ± .9[a/]
LEUKOCYTES ($X10^3$ /MM^3)	10.4 ± 2.0	5.6 ± .6	16.8 ± 4.2	7.4 ± .4
NEUTROPHILS, %	46.3 ± 13.9	13.5 ± 2.3[a/]	19.5 ± .6	30.3 ± 5.9
LYMPHOCYTES, %	50.8 ± 13.9	86.0 ± 2.5[a/]	79.5 ± 1.0	69.5 ± 6.1
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.0 ± .3	.5 ± .3[a/]	1.0 ± .4	.3 ± .3[a/]
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

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

TABLE 58

HEMATOLOGY DATA OF MALE MICE FED 2,4-DNT FOR 13 WEEKS

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

DOSE: MG/KG/DAY	0.00 (C, 4)	0.07 (T, 3)	[illegible] (T, 4)	0.7 (T, 3)
ERYTHROCYTES (X10^6 /MM^3)	6.83 ± .30	6.20 ± .14	4.43 ± .71	5.27 ± .62
RETICULOCYTES, %	1.25 ± .30	1.55 ± .33	4.84 ± 1.44	5.32 ± 1.77
HEMATOCRIT, VOL, %	41.0 ± 1.8	41.0 ± 1.2	38.5 ± 1.6	30.7 ± 2.8[a/]
HEMOGLOBIN, GM, %	14.0 ± .9	14.0 ± .3	12.6 ± .6	10.6 ± 1.0[a/]
MCV, CUBIC MICRONS	60.0 ± .2	66.2 ± 2.2	94.4 ± 16.9	58.6 ± 1.7
MCHM, MICRO MICROGRAMS,	20.5 ± .5	22.6 ± .5	30.9 ± 5.5	20.2 ± .5
MCHC, GM %	34.2 ± .9	34.2 ± .4	32.6 ± .5	34.4 ± .4
PLATELETS (X10^5 /MM^3)	5.9 ± .7	6.8 ± .2	7.5 ± .9	7.5 ± 4.0
LEUKOCYTES (X10^3 /MM^3)	6.7 ± .3	7.0 ± 2.0	7.8 ± .5	4.5 ± .8
NEUTROPHILS, %	16.3 ± 2.6	32.3 ± 11.0	9.8 ± 2.9	10.7 ± 4.2
LYMPHOCYTES, %	82.5 ± 3.3	65.3 ± 11.2	89.8 ± 2.8	88.7 ± 3.8
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3
EOSINOPHILS, %	1.3 ± 1.3	2.0 ± 1.2	.5 ± .3	.3 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3

ENTRIES ARE MEAN ± STANDARD ERROR

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

TABLE 59

HEMATOLOGY DATA OF MALE MICE FED 2,4-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

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

DOSE: MG/KG/DAY	0.00 (C, 3)	0.07 (T, 4)	0.02 (T, 3)	0.7 (T, 3)
ERYTHROCYTES ($X10^6$ /MM^3)	7.41 ± .40	6.47 ± .30	6.66 ± .44	6.71 ± .10
RETICULOCYTES, %	1.76 ± .22	2.09 ± .50	1.66 ± .07	1.30 ± .08
HEMATOCRIT, VOL. %	42.7 ± 2.0	41.5 ± 1.5	44.5 ± .5	40.0 ± 1.0
HEMOGLOBIN, GM. %	14.1 ± .8	12.6 ± .8	14.6 ± .0	12.9 ± .3
MCV, CUBIC MICRONS	57.6 ± .4	64.1 ± .7	63.2 ± 2.3[a/]	59.6 ± 1.1
MCHB, MICRO MICROGMS.	19.0 ± .1	19.4 ± .3	20.7 ± 1.1	19.2 ± .3
MCHBC, GM %	32.9 ± .3	30.3 ± .8	32.7 ± .5	32.3 ± .1[a/]
PLATELETS ($X10^5$ /MM^3)	6.7 ± .2	ND[b/]	6.4 ± .6	8.1[c/]
LEUKOCYTES ($X10^3$ /MM^3)	7.0 ± .7	7.9 ± .5	7.6 ± .8	7.6 ± .7
NEUTROPHILS, %	22.0 ± 5.9	28.3 ± 12.5	19.3 ± 3.8	25.0 ± 5.6
LYMPHOCYTES, %	76.0 ± 6.8	70.0 ± 11.7	80.3 ± 3.9	73.0 ± 6.0
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.3 ± .3	.8 ± .5	.3 ± .3	.7 ± .7
EOSINOPHILS, %	1.7 ± .9	1.0 ± .7	0.0 ± 0.0	1.3 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the controls (Dunnett's multiple comparison procedure[4/]).
b/ ND-No Data.
c/ One mouse.

TABLE 60

HEMATOLOGY DATA OF MALE MICE FED 2,4-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

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

DOSE: MG/KG/DAY	0.00 (C, 4)	0.07 (T, 4)	0.20 (T, 4)	0.70 (T, 3)
ERYTHROCYTES ($X10^6$ /MM^3)	4.54 ± .24	3.62 ± .20[a/]	3.71 ± .17[a/]	3.76 ± .28
RETICULOCYTES, %	1.59 ± .35	3.33 ± .78	6.00 ± 3.03	2.09 ± .03
HEMATOCRIT, VOL. %	42.5 ± 1.8	39.5 ± 2.3	41.8 ± 3.3	40.7 ± .9
HEMOGLOBIN, GM. %	13.9 ± .7	13.2 ± .8	13.9 ± 1.2	13.6 ± .4
MCV, CUBIC MICRONS	93.7 ± 1.8	109.3 ± 3.9[a/]	111.9 ± 4.2[a/]	109.1 ± 6.5
MCHR, MICRO MICROGMS.	30.7 ± .4	36.6 ± 1.3[a/]	37.2 ± .6[a/]	36.5 ± 2.1[a/]
MCHRC, GM %	32.7 ± .4	33.5 ± .2	33.2 ± .3	33.4 ± .2
PLATELETS ($X10^5$ /MM^3)	4.4 ± 1.2	9.9 ± 2.0[a/]	10.4 ± 1.1[a/]	4.6 ± .7
LEUKOCYTES ($X10^3$ /MM^3)	6.0 ± .7	5.2 ± .4	7.8 ± 1.1	6.8 ± .6
NEUTROPHILS, %	21.5 ± 5.4	23.5 ± 4.5	20.5 ± 3.9	9.3 ± 3.0
LYMPHOCYTES, %	77.8 ± 5.6	75.3 ± 4.1	79.3 ± 4.1	89.7 ± 2.8
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.5 ± .3	.3 ± .3	0.0 ± 0.0	.3 ± .3
EOSINOPHILS, %	0.0 ± 0.0	1.0 ± .6	.3 ± .3	.7 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

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

TABLE 61

HEMATOLOGY DATA OF FEMALE MICE FED 2,4-DNT FOR 4 WEEKS

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

DOSE: MG/KG/DAY	0.00	(C, 4)	0.07	(T, 4)	0.2	(T, 4)	0.7	(T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.05	± .13	6.25	± .35	5.78	± .37	5.96	± .31
RETICULOCYTES, %	1.55	± .22	1.78	± .22	1.62	± .11	2.59	± 1.06
HEMATOCRIT, VOL. %	45.8	± .8	45.3	± .6	44.0	± 1.5	44.5	± 2.3
HEMOGLOBIN, GM. %	15.0	± .2	14.8	± .2	14.1	± .2	13.9	± .8
MCV, CUBIC MICRONS	75.6	± 1.2	73.0	± 3.7	76.9	± 5.0	74.8	± 3.0
MCHB, MICRO MICROGMS.	24.8	± .4	23.9	± 1.5	24.7	± 1.8	23.3	± .6
MCHBC, GM %	32.7	± .3	32.7	± .4	32.1	± .8	31.4	± 1.8
PLATELETS ($X10^5$ /MM^3)	6.5	± .7	7.3	± .7	6.1	± 1.0	7.6	± .2
LEUKOCYTES ($X10^3$ /MM^3)	10.7	± 1.5	10.5	± 1.0	10.7	± 1.4	14.4	± 1.3
NEUTROPHILS, %	21.5	± 3.3	17.8	± 7.1	10.8	± 2.1	19.3	± 7.6
LYMPHOCYTES, %	78.5	± 3.3	82.3	± 7.1	89.3	± 2.1	79.5	± 7.9
BANDS, %	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0
MONOCYTES, %	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0
EOSINOPHILS, %	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	1.3	± .5[a/]
BASOPHILS, %	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0
ATYPICAL, %	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0
NUCLEATED RBC, %	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

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

TABLE 62

HEMATOLOGY DATA OF FEMALE MICE FED 2,4-DNT FOR 13 WEEKS

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

DOSE: MG/KG/DAY	0.00 (C, 4)	0.02 (T, 4)	0.20 (T, 4)	0.7 (T, 3)
ERYTHROCYTES ($X10^6$ /MM^3)	7.37 ± .13	7.33 ± .40	7.20 ± .28	6.46 ± .43
RETICULOCYTES, %	1.99 ± .53	1.85 ± .31	1.57 ± .46	2.19 ± .48
HEMATOCRIT, VOL. %	44.5 ± .9	43.8 ± 1.1	44.3 ± .5	42.0 ± .6
HEMOGLOBIN, GM. %	15.5 ± .3	15.3 ± .4	15.0 ± .2	14.2 ± .4[a/]
MCV, CUBIC MICRONS	60.4 ± .4	60.0 ± 2.4	61.8 ± 2.6	55.5 ± 3.8
MCHB, MICRO MICROGMS.	21.1 ± .3	21.1 ± 1.2	20.9 ± 1.0	22.2 ± 1.0
MCHBC, GM %	35.0 ± .4	35.1 ± .7	33.9 ± .5	33.9 ± .6
PLATELETS ($X10^5$ /MM^3)	5.5 ± .4	3.6 ± 1.1	6.5 ± 1.1	3.5[b/]
LEUKOCYTES ($X10^3$ /MM^3)	6.7 ± .8	6.1 ± .9	7.0 ± 1.2	6.9 ± 2.0
NEUTROPHILS, %	8.0 ± 2.5	9.0 ± 1.4	8.0 ± 2.4	19.7 ± 6.0
LYMPHOCYTES, %	91.5 ± 2.5	89.8 ± 1.4	91.5 ± 2.2	80.0 ± 6.2
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.5 ± .5	1.3 ± .3	.3 ± .3	.3 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the controls (Dunnett's multiple comparison procedure[4/]).
b/ One mouse.

TABLE 63

HEMATOLOGY DATA OF FEMALE MICE FED 2,4-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

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

DOSE: MG/KG/DAY	0.00 (C, 3)	0.07 (T, 2)	0.2 (T, 4)	0.7 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.23 ± .49	5.91 ± .38	6.47 ± .04	5.85 ± .31
RETICULOCYTES, %	2.18 ± .69	2.13 ± .03	2.27 ± .58	1.12 ± .16
HEMATOCRIT, VOL. %	41.3 ± 1.2	43.0 ± 1.0	44.3 ± .5	43.8 ± 2.0
HEMOGLOBIN, GM. %	13.2 ± .9	13.3 ± .3	14.0 ± .2	13.8 ± .5
MCV, CUBIC MICRONS	66.8 ± 3.4	73.0 ± 2.9	68.4 ± 1.0	75.0 ± 2.9
MCHR, MICRO MICROGMS.	21.3 ± .4	22.6 ± .9	21.6 ± .3	23.7 ± .9
MCHBC, GM %	31.9 ± 1.2	30.9 ± .0	31.6 ± .2	31.6 ± .3
PLATELETS ($X10^5$ /MM^3)	4.5[b]	6.5 ± .7	6.1 ± .5	6.0[b]
LEUKOCYTES ($X10^3$ /MM^3)	7.0 ± .7	8.9 ± .3	7.8 ± .5	7.5±0.4
NEUTROPHILS, %	10.3 ± 3.3	18.5 ± 10.5	13.3 ± 2.8	18.3 ± 4.0
LYMPHOCYTES, %	88.0 ± 3.1	80.0 ± 9.0	85.3 ± 2.7	80.5 ± 4.1
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.3 ± .3	.5 ± .5	.8 ± .5	0.0 ± 0.0
EOSINOPHILS, %	1.3 ± .3	1.0 ± 1.0	.8 ± .5	1.3 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	4.3 ± 1.5[a]
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the controls (Dunnett's multiple comparison procedure[4]).
b/ One mouse.

TABLE 64

HEMATOLOGY DATA OF FEMALE MICE FED 2,4-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

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

DOSE: MG/KG/DAY	0.00 (C, 4)	0.07 (T, 4)	0.2 (T, 4)	0.7 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	4.05 ± .22	3.89 ± .23	4.34 ± .34	4.68 ± .18
RETICULOCYTES, %	1.23 ± .32	1.17 ± .21	1.27 ± .29	1.14 ± .11
HEMATOCRIT, VOL. %	44.8 ± 1.3	44.5 ± 1.7	45.8 ± 1.9	44.3 ± 1.3
HEMOGLOBIN, GM. %	14.6 ± .6	14.8 ± .6	15.1 ± .6	14.7 ± .7
MCV, CUBIC MICRONS	111.0 ± 3.5	114.7 ± 3.1	106.4 ± 5.2	95.1 ± 5.6
MCHB, MICRO MICROGMS.	36.3 ± .8	38.2 ± 1.4	35.2 ± 1.6	31.7 ± 2.4
MCHBC, GM %	32.7 ± .3	33.3 ± .4	33.1 ± .2	33.3 ± .6
PLATELETS ($X10^5$ /MM^3)	8.2 ± .8	5.9 ± 1.2	5.0 ± 1.3	4.6 ± 1.4
LEUKOCYTES ($X10^3$ /MM^3)	5.2 ± .4	7.0 ± .5	6.7 ± .7	6.5 ± .8
NEUTROPHILS, %	15.0 ± 2.7	12.5 ± 2.6	12.0 ± 3.5	17.8 ± 3.9
LYMPHOCYTES, %	84.0 ± 3.4	86.8 ± 2.5	87.3 ± 3.7	82.0 ± 4.0
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.3 ± .3	.8 ± .3	1.0 ± 0.0[a/]	1.0 ± 0.0 [a/]
EOSINOPHILS, %	3.0 ± 0.0	2.8 ± .3	.8 ± .5[a/]	.3 ± .3 [a/]
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

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

TABLE 65

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MICE FED 2,4-DNT FOR 13 WEEKS

Sex	% 2,4-DNT in Feed	Terminal Body Weight (gm)	Absolute Weight (gm)				
			Liver	Spleen	Kidneys	Heart	Brain
Male	0	35.0±2.2[a]	1.78±0.16	0.12±0.03	0.62±0.05	0.21±0.06	0.43±0.03
	0.07[b]	33.7±0.3	1.89±0.26	0.09±0.00	0.43±0.05[c]	0.17±0.02	0.45±0.01
	0.2	33.5±1.3	2.03±0.19	0.18±0.06	0.52±0.04	0.15±0.00	0.46±0.02
	0.7[b]	25.7±0.9[c]	1.67±0.24	0.15±0.05	0.42±0.02[c]	0.13±0.01	0.43±0.02
Female	0	29.3±0.8	1.43±0.17	0.13±0.02	0.41±0.03	0.17±0.04	0.50±0.06
	0.07	29.5±1.2	1.49±0.15	0.09±0.01	0.40±0.01	0.12±0.00	0.48±0.01
	0.2	27.8±0.8	1.51±0.02	0.12±0.01	0.44±0.01	0.13±0.00	0.49±0.04
	0.7[b]	25.0±1.0[c]	1.50±0.20	0.12±0.01	0.35±0.01	0.11±0.00	0.45±0.01

Sex	% 2,4-DNT in Feed	Relative Organ Weight (gm/100 gm body weight)				
		Liver	Spleen	Kidneys	Heart	Brain
Male	0	5.06±0.16	0.34±0.06	1.76±0.04	0.59±0.14	1.24±0.10
	0.07[b]	5.64±0.82	0.27±0.01	1.27±0.15[c]	0.51±0.05	1.33±0.03
	0.2	6.03±0.40	0.54±0.17	1.56±0.07	0.48±0.03	1.38±0.06
	0.7[b]	6.52±0.86	0.60±0.19	1.63±0.09	0.52±0.01	1.68±0.12[c]
Female	0	4.86±0.49	0.44±0.05	1.39±0.03	0.57±0.13	1.74±0.22
	0.07	5.01±0.31	0.30±0.02	1.37±0.03	0.40±0.01	1.64±0.08
	0.2	5.46±0.17	0.45±0.03	1.60±0.03[c]	0.46±0.01	1.76±0.13
	0.7[b]	5.95±0.55	0.47±0.06	1.40±0.03	0.45±0.01	1.81±0.10

Sex	% 2,4-DNT in Feed	Relative Organ Weight (gm/gm brain weight)			
		Liver	Spleen	Kidneys	Heart
Male	0	4.20±0.47	0.28±0.06	1.45±0.14	0.48±0.12
	0.07[b]	4.24±0.57	0.21±0.00	0.96±0.11[c]	0.38±0.04
	0.2	4.38±0.31	0.39±0.13	1.14±0.10	0.35±0.02
	0.7[b]	3.97±0.78	0.37±0.14	0.98±0.09[c]	0.31±0.03
Female	0	2.97±0.56	0.27±0.06	0.86±0.16	0.34±0.08
	0.07	3.10±0.34	0.19±0.02	0.84±0.03	0.24±0.01
	0.2	3.17±0.29	0.26±0.03	0.93±0.08	0.27±0.02
	0.7[b]	3.35±0.50	0.26±0.03	0.78±0.03	0.25±0.01

a/ Mean ± standard error of four mice.
b/ Data from three surviving mice.
c/ Significantly different from control mice (Dunnett's multiple comparison procedure[4]).

TABLE 66

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MICE FED 2,4-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Sex	% 2,4-DNT in Feed	Terminal Body Weight (gm)	Absolute Weight (gm) Liver	Spleen	Kidneys	Heart
Male	0	32.0±1.5[a]	1.27±0.08	0.17±0.06	0.53±0.04	0.13±0.01
	0.07	31.5±0.9	1.15±0.01	0.09±0.01	0.49±0.02	0.17±0.01
	0.2	33.0±2.3	1.58±0.15	0.14±0.01	0.50±0.02	0.14±0.00
	0.7[b]	33.3±0.7	1.66±0.06[c]	0.15±0.02	0.48±0.03	0.18±0.03
Female	0	28.0±0.7	1.28±0.03	0.16±0.02	0.39±0.01	0.14±0.01
	0.07	26.3±1.1	1.14±0.06	0.09±0.01[c]	0.38±0.01	0.13±0.01
	0.2	26.5±0.9	1.21±0.07	0.11±0.01[c]	0.40±0.02	0.15±0.01
	0.7	28.0±0.9	1.36±0.08	0.10±0.01[c]	0.42±0.01	0.15±0.01
			Relative Organ Weight (gm/100 gm body weight) Liver	Spleen	Kidneys	Heart
Male	0		3.96±0.15	0.52±0.15	1.66±0.12	0.54±0.03
	0.07		3.68±0.12	0.29±0.01	1.57±0.08	0.54±0.01
	0.2		4.95±0.60	0.43±0.06	1.54±0.16	0.44±0.04
	0.7[b]		4.98±0.29	0.45±0.06	1.44±0.10	0.54±0.09
Female	0		4.58±0.19	0.58±0.10	1.41±0.08	0.49±0.03
	0.07		4.34±0.17	0.34±0.04[c]	1.45±0.04	0.48±0.01
	0.2		4.58±0.11	0.42±0.04	1.52±0.04	0.57±0.02
	0.7		4.86±0.24	0.35±0.04[c]	1.49±0.03	0.53±0.03

[a] Mean ± standard error of four mice.
[b] Data from three surviving mice.
[c] Significantly different from control mice (Dunnett's multiple comparison procedure[4]).

TABLE 67

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MICE FED 2,4-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Sex	% 2,4-DNT in Feed	Terminal Body Weight (gm)	Absolute Weight (gm)				
			Liver	Spleen	Kidneys	Heart	Brain
Male	0	35.0±0.9[a]	1.49±0.03	0.08±0.01	0,65±0.03	0.18±0.01	0.42±0.02
	0.07	33.0±1.7	1.43±0.11	0.09±0.01	0.43±0.05[c]	0.17±0.02	0.46±0.02
	0.2	30.3±2.1	1.38±0.10	0.20±0.04[c]	0.46±0.03[c]	0.17±0.01	0.48±0.03
	0.7[b]	32.3±0.7	1.76±0.08	0.11±0.00	0.47±0.04[c]	0.18±0.00	0.46±0.01
Female	0	26.8±0.6	1.19±0.08	0.08±0.01	0.42±0.01	0.14±0.01	0.47±0.02
	0.07	27.5±1.0	1.14±0.05	0.11±0.02	0.41±0.02	0.14±0.01	0.47±0.01
	0.2	28.5±1.0	1.12±0.07	0.09±0.00	0.42±0.02	0.13±0.00	0.43±0.02
	0.7	24.3±1.7	1.14±0.08	0.12±0.00	0.35±0.02[c]	0.13±0.02	0.44±0.02
			Relative Organ Weight (gm/100 gm body weight)				
			Liver	Spleen	Kidneys	Heart	Brain
Male	0		4.27±0.04	0.24±0.02	1.87±0.14	0.53±0.03	1.2G±0.08
	0.07		4.35±0.27	0.28±0.04	1.31±0.12[c]	0.52±0.05	1.38±0.03
	0.2		4.57±0.17	0.66±0.14[c]	1.51±0.02	0.57±0.03	1.60±0.03[c]
	0.7[b]		5.43±0.14[c]	0.34±0.00	1.45±0.15	0.57±0.01	1.42±0.02[c]
Female	0		4.44±0.25	0.31±0.03	1.57±0.08	0.54±0.06	1.76±0.13
	0.07		4.18±0.31	0.41±0.10	1.50±0.03	0.50±0.03	1.70±0.07
	0.2		3.93±0.12	0.33±0.01	1.46±0.06	0.46±0.02	1.50±0.05
	0.7		4.73±0.15	0.48 0.05	1.44±0.08	0.53±0.04	1.85±0.17
			Relative Organ Weight (gm/gm brain weight)				
			Liver	Spleen	Kidneys	Heart	
Male	0		3.61±0.23	0.20±0.62	1.58±0.14	0.45±0.04	
	0.07		3.16±0.26	0.20±0.03	0.95±0.09[c]	0.38±0.04	
	0.2		2.85±0.05[c]	0.42±0.02[c]	0.95±0.03[c]	0.35±0.02	
	0.7[b]		3.83±0.10	0.24±0.00	1.03±0.12[c]	0.40±0.01	
Female	0		2.55±0.13	0.18±0.02	0.90±0.02	0.30±0.02	
	0.07		2.44±0.10	0.24±0.05	0.88±0.04	0.29±0.01	
	0.2		2.63±0.09	0.22±0.01	0.98±0.03	0.31±0.02	
	0.7		2.62±0.28	0.27±0.04	0.79±0.05	0.30±0.04	

a/ Mean ± standard error of four mice.
b/ Data from three surviving mice.
c/ Significantly different from control mice (Dunnett's multiple comparison procedure[4]).

TABLE 68

SUMMARY OF TISSUE LESIONS OF MALE MICE
FED 2,4-DNT FOR 4 WEEKS

Lesions[a/]	Dose (% in Feed)											
	0				0.2				0.7			
Mouse No.:	301	302	303	304	351	352	353	354	376	377	378	379
Kidney												
Interstitial nephritis		+				+					+	
Spleen												
Extramedullary hematopoiesis				±								
Testes												
Aspermatogenesis									±		±	
Bone Marrow												
M/E ratio	1.8	1.6	1.8	1.7	1.7	1.6	1.6	1.8	1.7	1.5	1.8	1.6

Tissues not listed were normal.

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

TABLE 69

SUMMARY OF TISSUE LESIONS OF FEMALE MICE
FED 2,4-DNT FOR 4 WEEKS

Lesions[a]	Dose (% in Feed)											
	0				0.2				0.7			
Mouse No.:	401	402	403	404	451	452	453	454	476	477	478	479
Heart												
Myocarditis						+						
Liver												
Focal necrosis		±	±			+	+			±		
Bone Marrow												
M/E ratio	1.6	1.5	1.8	1.7	1.7	1.6	1.8	1.7	1.7	1.4	1.8	1.5

Tissues not listed were normal.

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

TABLE 70

SUMMARY OF TISSUE LESIONS OF MALE MICE
FED 2,4-DNT FOR 13 WEEKS

Lesions[a/] / Mouse No.:	Dose (% in Feed) 0				0.2				0.7		
	309	310	311	312	359	360	361	362	384	385	386
Lung											
Lymphoid hyerplasia	+++							+			
Pneumonia						+				±	
Liver											
Subacute inflammation		+	+							+	
Focal necrosis											±
Kidney											
Interstitial nephritis			+	±	+		+				±
Spleen											
Extramedullary hematopoiesis	±										
Bone Marrow											
M/E ratio	1.6	1.7	1.7	1.5	1.7	1.6	1.6	1.7	1.6	1.8	1.7

Tissues not listed were normal.

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

TABLE 71

SUMMARY OF TISSUE LESIONS OF FEMALE MICE
FED 2,4-DNT FOR 13 WEEKS

Lesions[a/] / Mouse No.:	Dose (% in Feed) 0				0.2				0.7		
	409	410	411	412	459	460	461	462	484	485	486
Lung											
Lymphoid hyperplasia		+			±						
Liver											
Subacute inflammation					±		±		+		
Kidney											
Subacute inflammation										+	
Interstitial nephritis								±			+
Bone Marrow											
M/E ratio	1.6	1.7	1.6	1.8	b/	1.7	1.6	1.5	1.5	1.6	1.7

Tissues not listed were normal.

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

b/ Marrow smear was not prepared.

TABLE 72

SUMMARY OF TISSUE LESIONS OF MALE MICE FED
2,4-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Lesions[a]	Mouse No.:	Dose (% in Feed) 0			0.2				0.7		
		305	306	307	355	356	357	358	380	381	382
Liver											
Subacute inflammation				+++	++			++		++	+
Kidney											
Interstitial nephritis					±	±		±			
Bone Marrow											
M/E ratio		1.6	[b]	1.5	1.5	[b]	1.6	1.7	1.7	1.5	1.6

Tissues not listed were normal

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

[b] Marrow smear was not prepared.

TABLE 73

SUMMARY OF TISSUE LESIONS OF FEMALE MICE FED
2,4-DNT FOR 4 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Lesions[a/]	Dose (% in Feed)											
	0				0.2				0.7			
Mouse No.:	405	406	407	408	455	456	457	458	480	481	482	483
Heart												
Myocarditis				+								
Lung												
Lymphoid hyperplasia									+			
Liver												
Subacute inflammation	+	++	+	++								
Kidney												
Interstitial nephritis			+		±							
Adrenals												
Hypertrophy									+			
Bone Marrow												
M/E ratio	1.7	1.5	1.8	1.7	1.5	1.7	1.9	1.6	1.7	1.5	1.8	1.7

Tissues not listed were normal.

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

TABLE 74

SUMMARY OF TISSUE LESIONS IN MALE MICE FED
2,4-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

Lesions[a/]	Dose (% in Feed)										
	0				0.2				0.7		
Mouse No.:	313	314	315	316	363	364	365	366	388	389	390
Trachea											
Lymphoid hyerplasia									+		
Liver											
Extranuclear inclusion bodies								+	+	+	+
Cytomegaly										+	
Pigmented Kupffer cells										+	+
Kidney											
Pyelonephritis	+										+
Interstitial nephritis							±				
Bone Marrow											
M/E ratio	1.7	1.6	1.6	1.5	1.6	1.7	1.5	1.6	1.6	1.5	1.8

Tissues not listed were normal.

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

TABLE 75

SUMMARY OF TISSUE LESIONS ON FEMALE MICE FED 2,4-DNT FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

	Dose (% in Feed)											
	0				0.2				0.7			
Lesions[a/] Mouse No.:	413	414	415	416	463	464	465	466	488	489	490	491
Lung												
Lymphoid hyperplasia	+											
Atelectasis	+											
Liver												
Focal necrosis									+	+	+	
Nuclear inclusion bodies									+			
Pigment in Kupffer cells									+			+
Adrenals												
Fatty change (cortex)							±					
Kidney												
Interstitial nephritis	+				±				+		+	
Spleen												
Pigment in R-E cells												+
Bone Marrow												
M/E ratio	1.5	1.6	1.4	1.5	1.6	1.7	1.5	1.5	1.5	1.7	1.8	1.7

Tissues not listed were normal.

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

TABLE 76

DOMINANT LETHAL EFFECTS IN MICE FED 2,4-DNT

% 2,4-DNT in Feed	No. of Males Mated[a/]	Fertility Index[b/]	Litter (Pups/Dam)	Implants (Number/Dam)	Implant Viability Index[c/]
0	4	75 ± 8	8.0 ± 0.5	9.5 ± 0.6	84 ± 3
0.2% (13 weeks)	5	87 ± 8	8.8 ± 1.3	10.0 ± 1.1	86 ± 7
0.7% (4 weeks)	3	17 ± 8[d/]	1.9 ± 1.0[d/]	2.5 ± 1.4[d/]	75 ± 8

a/ Mating confirmed by vaginal plug.
b/ Confirmed pregnancies/females with vaginal plugs x 100.
c/ Viable birth/total implants x 100.
d/ Significantly different from the controls (Student's t test).

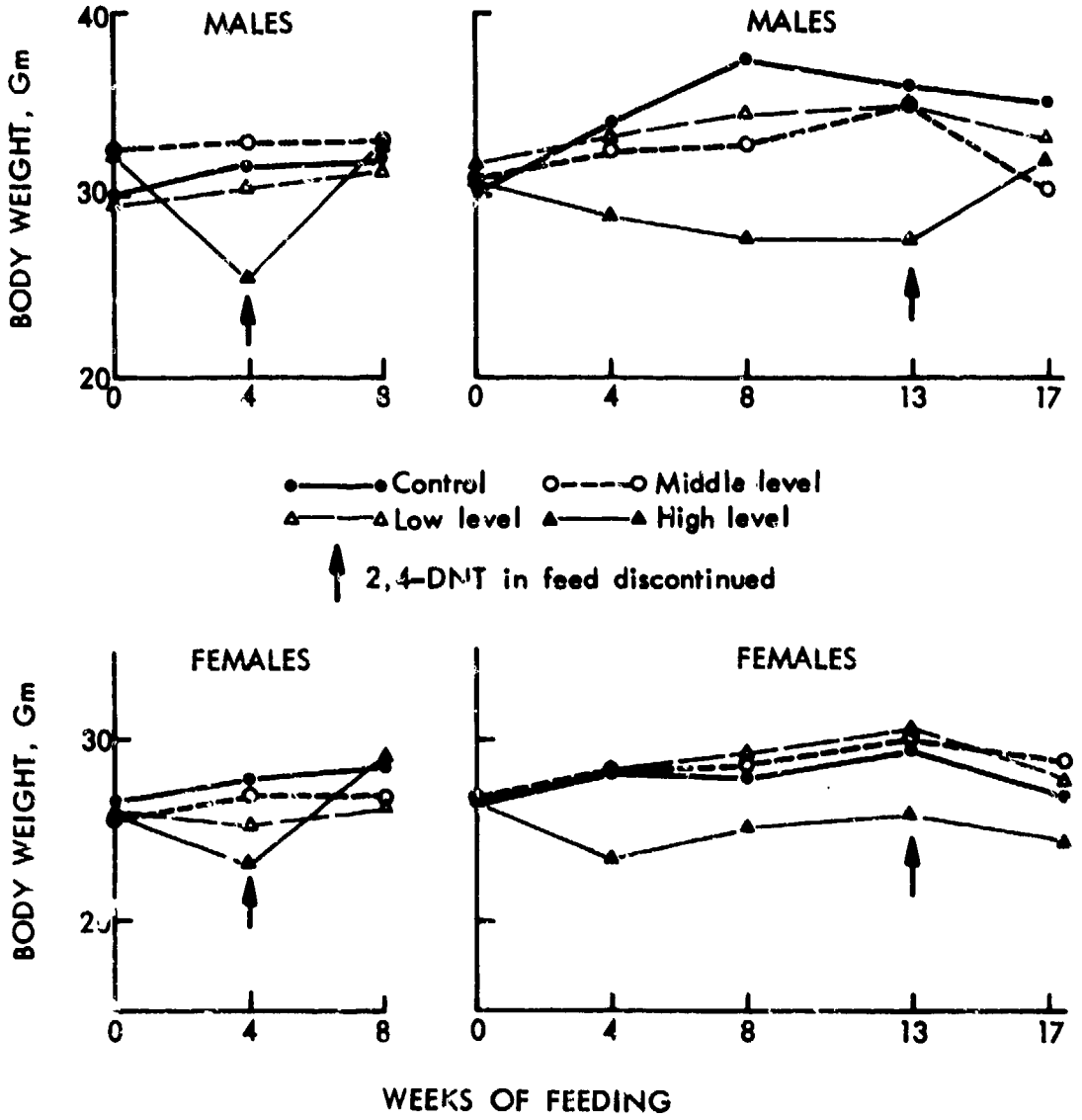

Figure 2 - Body Weights of Mice Fed Various Levels of 2,4-DNT.

IV. DISPOSITION AND METABOLISM

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IV. DISPOSITION AND METABOLISM

A. Disposition and Metabolism of 2,4-DNT in Various Species

1. Introduction

The absorption, distribution, biotransformation, and excretion of 2,4-DNT were studied previously in rats.[1] 2,4-DNT and its metabolites were characterized by the available conventional analytical methods and radioassay. In the experiments reported here, similarities and differences in the disposition and pathways of biotransformation were investigated in mice, rabbits, dogs and monkeys.

2. Material and Methods

The procedure and methods described for rats[1] were generally used to study the detailed disposition and metabolism of 2,4-DNT.

a. Animal Species

Various species used for these studies included female albino CD-1 and B6C3F1 mice (Charles River Breeding Lab.) weighing 28 to 35 gm and 18 to 26 gm, respectively, female New Zealand rabbits (Small Stock Industries, Pea Ridge, Arkansas) weighing 1.69 to 1.87 kg, female beagle dogs (Hazelton Research Animals) weighing 8.2 to 11.8 kg, and female rhesus monkeys (Primate Imports, Port Washington, New York) weighing 3.0 to 3.7 kg.

b. Experimental Procedure

Each animal was fasted overnight. A single oral dose of 2,4-DNT, approximately 10% of the acute LD_{50}, spiked with 10 µCi of 2,4-DNT (Ring-UL-^{14}C, specific activity of 3.55 mCi/mM), was given via an intragastric metal or rubber tube. The 2,4-DNT with ^{14}C-labeled 2,4-DNT was suspended in peanut oil and given at a volume of 1 ml/100 gm to mice and rats, 2 ml/kg to rabbits, or 1 ml/kg to dogs and monkeys. After dosing, the mouse or rat was placed immediately in a "Roth-Delmar" metabolism cage.[17] The rabbit, dog or monkey was placed in a stainless steel animal cage (24 in. x 24 in. x 20 in.) All animals were given feed and water ad libitum. Feces and urine were collected separately in the apparatus. At termination, each animal was anesthetized with ether or pentobarbital sodium. Aortic blood was collected and various tissues and feces were removed, weighed, and digested in 10 volumes of 2 N NaOH. Blood samples were decolorized by the addition of H_2O_2. Aliquots of tissue and fecal digests, blood, plasma and urine were neutralized with Beckman BBS-2, solubilized in Beckman BBS-3 and counted in the scintillation solution using a Packard Tricarb 3375 liquid scintillation spectrophotometer.

c. Methods of Identification

Thin-layer chromatography (TLC): Precoated silica gel plates (without fluorescent indicator, 0.25 mm thickness, Brinkman Instruments, Inc.) were used for all experiments. All samples were spotted 2.0 cm from the bottom of the plate and developed at least 10 cm. The solvents used were (I) benzene:ethyl acetate (4:1 v/v); (II) ethyl acetate:n-heptane (9:1, v/v); and (III) n-butanol:acetic acid:water (10:1:1, v/v/v).

Gas-liquid chromatography (GLC): A Hewlett-Packard model 5736A gas chromatograph equipped with a flame ionization detector set at 250°C was used for GLC. For the separation and identification of nitro- and aminonitrotoluenes, a stainless steel column (0.125 in. i.d. x 3 ft) packed with 10% UCW-982 on WAW-DMCS (80-100 mesh) was used. The oven temperature was maintained at 150°C and nitrogen carrier gas at a flow rate of 60 ml/min. For the nitro- and aminonitrobenzyl alcohols, a stainless steel column (0.125 in. i.d. x 3 ft) packed with 10% OV-1 on Chromosorb P (80-100 mesh) was used. The oven temperature was programmed from 150°C to 180°C at 2°/min, and nitrogen carrier gas was maintained at a flow rate of 60 ml/min. For 2,4-dinitrobenzoic acid silyl ester, a glass column (0.25 in. i.d. x 4 ft) packed with 1.5% DC LSX-3-0295 and 1.5% GE-XE-60 on Gas Chrom Q (60-80 mesh) was used. The oven temperature was programmed from 100 to 150°C at 4°/min. and nitrogen carrier gas was maintained at a flow rate of 30 ml/min. The silyl ester of 2,4-dinitrobenzoic acid was prepared by adding bis(trimethylsilyl)acetamide to an ether solution containing dried urine residue or pure 2,4-dinitrobenzoic acid.

Chemical detection tests: Aryl nitrates were detected using 5% diphenylamine in absolute ethanol.[18] Aryl amines were detected using the Bratton-Marshall reagent.[19]

Enzyme hydrolysis: Samples were prepared for enzyme treatment by eluting the metabolites from TLC scrapings with water. The pH was adjusted to 5.6 by the addition of 13.6 mg/ml of sodium acetate and treated with β-glucuronidase or aryl sulfatase (Sigma Chemical Company, St. Louis, Missouri). β-Glucuronidase was added at a final concentration of 5,000 units/ml; aryl sulfatase was added at a final concentration of 225 units/ml. All incubations were carried out for 18 hours at 37°C. After incubation, enzyme activity was terminated by extraction with 5 volumes of $CHCl_3$:MeOH (2:1). The resulting aqueous and nonaqueous phases were concentrated by evaporation and the metabolites identified by TLC and GLC.

3. Results

a. Distribution and Excretion

The results on the disposition of 2,4-DNT in rats reported previously[1] are included here for comparison. The distribution and excretion of radioactivity after a single oral dose of 2,4-DNT (Ring-UL-^{14}C) in various species are summarized in Table 77. In mice, an average of 78.2% of the administered dose was recovered from the feces and gastrointestinal tract (GI) plus contents in 24 hours, while only 9.5% to 17.3% was recovered from the rats, rabbits, dogs, and monkeys during the same period. Both strains of mice were similar. The total recovery from various tissues and urine was 6.5% in mice and 75.8% to 83.5% in the other species. This suggests that the net absorption of 2,4-DNT in mice approximated 17% of the administered dose and that the other species absorbed about 75% to 85% of the dose.

In all species, the majority of the absorbed radioactivity was excreted in the urine. The urinary excretion ranged 16.3% of the administered dose for mice and 75.2 to 81.3% of the administered dose for rats, rabbits, dogs and monkeys in 24 hours. No radioactivity was found in the expired air.

In the rodents and rabbits, the various tissues including blood contained less than 1% of the dose. The tissues of dogs and monkeys contained an estimated 3.6% and 2.2% of the dose, respectively. The tissue/plasma radioactivity ratios after a single dose of 2,4-DNT are shown in Table 78. Since the second strain of mice (B6C3F1) were intended to check the unusual lack of absorption, they are omitted from the table. Radioactivity was highly concentrated in the liver of all species. The concentration ratio of radioactivity in the liver 24 hours after dosing was 18.1 for rats, 17.8 for monkeys and 6.3 to 8.7 for mice, rabbits and dogs. The concentration ratio was also high in the kidney reflecting the excretion of radioactivity in the urine. The radioactivity was also concentrated in the lung and spleen in which the concentration ratios were greater than one. In addition, the skeletal muscle and brain of rats and monkeys contained more radioactivity than that in the plasma.

b. Metabolites in Urine

As detailed below, 2,4-DNT is metabolized in two phases. The first phase, illustrated in Figure 3, consists of reduction of the nitro groups and/or oxidation of the methyl group. These reactions are well-known.[20] One or both nitros may be reduced to amines by the nitro reductase systems found in liver microsomes and other tissues. The methyl group may be oxidized to a benzyl alcohol by the liver microsomal oxidation system which oxidizes many compounds. The alcohol can then be further oxidized to a benzaldehyde by alcohol dehydrogenase and to a benzoic acid by aldehyde dehydrogenase. These last two enzymes are the relatively non specific soluble enzymes which metabolize ethanol. These oxidative and/or reductive products may then

undergo the second phase of metabolism, conjugation, to form glucuronates, ethereal sulfates and perhaps other compounds, which are then excreted.

These compounds are chemically similar; their separation is difficult. Various methods used for separation and characterization are listed in Table 79. TLC system 1 was the most generally useful one. GLC was necessary to separate some isomers, such as the aminonitrotoluenes (compounds II and III). We were unable to separate the aminonitrobenzyl alcohols (compounds VI and VII), and we could not obtain authentic samples of compounds X, XI, XII, XIV, XV, and XVI for use as standards to identify these compounds.

Various metabolites found in the urine of mice, rats, rabbits, dogs and monkeys are listed in Tables 80 through 84, respectively. In mice (Table 80), 57.0% of the urinary radioactivity was found to be glucuronide conjugates, primarily compounds V (19.6%) and VI + VII (24.5%). Other major metabolites included the sulfate conjugate of compound II (10.3%) and the glucuronate of compound III (7.3%). The parent compound (I) was present in very small amounts (0.3%). A small amount (3.9%) of the oxidized benzoic acid derivative (compound XIII) was present, thus the benzaldehyde derivative (compound IX) was probably produced as an intermediate, even though none was detected in the urine. A total of 19.6% of the urinary radioactivity in mice was not identified.

Despite the different amounts of radioactivity excreted in the urine, the relative amounts of the various metabolites in rats, rabbits, dogs, or monkeys (Tables 81 through 84, respectively) were similar to mice. A majority of the urinary metabolites was present as glucuronide conjugates, ranging from 54.1% for monkeys to 66.4% for rabbits. The most common metabolites were the unreduced and partially reduced benzyl alcohols, compounds V, VI, and VII. Very little of the parent 2,4-DNT (compound I) was present, ranging from none for rats to 2.6% for monkeys. Although 3.2% to 9.5% of the urinary radioactivity in these species was completely oxidized benzoic acid derivative (compound XIII), none of the intermediate benzaldehyde derivative (compound IX) was found. A substantial amount of the urinary radioactivity, ranging from 7.9% for rabbit to 30.7% for monkeys, was not identified.

Total recoveries of various urinary metabolites as a percent of the administered dose in 24 hours regardless of conjugated forms, are shown in Table 85. Noteworthy is the fact that 0.6% to 8.8% of the dose was converted to 2,4-diaminotoluene (compound IV), a suspected carcinogen,[21] and excreted in the urine of the various species studied in 24 hours.

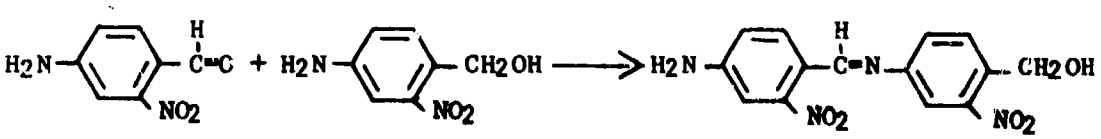
4. Discussion and Conclusions

Mice absorbed about 17% of the administered radioactivity in 24 hours after a single oral dose of 2,4-DNT (Ring-UL-^{14}C). A large amount of radioactivity was recovered in the feces and GI tract plus contents. After noting this anomalous result in our usual albino strain (CD-1), we did a parallel experimnet in the pigmented B6C3F1 strain, but found the same results (Table 77). One B6C3F1 mouse had very aberrant values (16.4% of dose in feces, 91.2% in urine). She was omitted from the table as an outlier; if this datum was included, there would still be no significant differences between strains of mice. This could be the result of poor absorption after ingestion or a rapid absorption and metabolism, excretion in the bile, and non absorption of the metabolite(s). In view of the similar pattern of urinary metabolites in the five species studied including mice, a sufficiently different metabolism and excretion of 2,4-DNT through the biliary system of the mice is unlikely. Furthermore, 2,4-DNT is significantly less toxic to mice than to rats or dogs. This difference in toxicity is apparently due to poor absorption through the GI tract in mice. On the other hand, most of the ingested 2,4-DNT was well absorbed in rats, rabbits, dogs, and monkeys and excreted in the urine within 24 hours.

After oral administration of ^{14}C-labeled 2,4-DNT, the radioactivity was concentrated in the liver and kidney with less in other organs. The liver is apparently the main organ of metabolism and site of biliary excretion, and the kidney is the main site of urinary excretion.

After oral administration, the absorbed 2,4-DNT was extensively metabolized with no or only a small amount of the parent compound excreted in the urine. Metabolism of 2,4-DNT was similar in all five species studied. The most common pathway was oxidation to a benzyl alcohol, perhaps with reduction to an aminonitrobenzyl alcohol, followed by glucuronide conjugation and excretion. Variations included reduction of one or both nitro groups to amines, oxidation to a benzoic acid, and excretion as sulfates or as the free compounds.

A substantial fraction of the administered 2,4-DNT was excreted as unidentified metabolites. We did not have authentic samples of some of the metabolites as standards for identification. The reduced benzoic acids (compounds XIV, XV, and XVI as shown in Figure 3) are likely to be minor components, because their formation requires four or five successive enzymatic reactions and a molecule is likely to be excreted before these reactions take place. Isolation of the authentic benzaldehydes (compound IX through XII) is unlikely because of the facile formation of stable Schiff bases, such as shown in the following reaction:



If any benzaldehyde derivatives are left in aqueous solution, it would readily react with any amine. This reaction would produce a wide variety of products depending on which benzaldehyde reacts with which amine. Other possible metabolites include other routes of conjugation. An amino compound could be acetylated by acetyl-CoA, as is sulfanilamide. A benzoic acid derivative could react with coenzyme A and then with glycine to form a hippuric acid derivative. To investigate these possibilities would be very tedious.

B. Biliary Excretion of Nitrotoluenes in Rats

1. Introduction

As discussed in the preceding Section IV.A., 2,4-DNT was highly concentrated in the liver of various species. Liver serves both as a site for metabolic biotransformation of foreign compounds and as an organ for biliary excretion. In these experiments, biliary excretion of 2,4-DNT was studied. For comparison, TNT and its dinitroisomers (DNT's) including 4-amino-2,6-DNT were also studied.

2. Material and Methods

Female CD® rats (Charles River Breeding Lab.) weighing 280 to 320 gm, were fasted overnight before use. Under ether anesthesia, the common bile duct was cannulated with PE-10 plastic tubing through a midline abdominal incision. After the incision had been closed, a dose of TNT, DNT's, or 4-amino-2,6-DNT approximating 10% of the acute LD_{50}, spiked with about 10 µCi of the respective ^{14}C-labeled compounds (Ring-UL-^{14}C, specific activity of 3.02 to 4.90 mCi/mM), was dissolved in peanut oil and administered orally by intragastric intubation at 1 ml/100 gm body weight. The rats were then confined individually in restrictive animal holders (Stoelting Company, Chicago, Illinois). Purina Rodent Chow and water were freely accessible to the animals.

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

Radioactivities in the bile, blood, plasma and the GI tract were measured using a Packard Tricarb 3375 liquid scintillation spectrophotometer as described in Section IV.A.2.b. Bile was counted directly in the

scintillation solution. Blood, plasma and the GI tract were digested with NaOH, decolored with H_2O_2, solubilized in the scintillation solution and counted.

3. Results

The biliary excretions of radioactivity in female rats after oral administration of TNT and various DNT's (Ring-UL-^{14}C) are summarized in Tables 86 through 93. The DNT's included all isomers (2,3; 2,4; 2,5; 2,6; 3,4; and 3,5) and 4-amino-2,6-DNT, a metabolite of TNT. Some radioactivity appeared in the bile within 15 minutes after oral administration of the various ^{14}C-labeled nitrotoluenes. The rate of biliary excretion increased with time and reached a peak in 15 minutes for 3,4-DNT, in 1 hour for 2,3-DNT, in 2 hours for TNT and 2,4-DNT, in 4 hours for 2,5-DNT and 3,5-DNT, and in 6 hours for 2,6-DNT. Thereafter, the rate of excretion decreased. Blood concentration of radioactivity correlated with the rate of biliary excretion. The blood concentration and rate of biliary excretion of 4-amino-2,6-DNT increased slightly during the first 6 hours and continued to increase throughout the collection period.

The total radioactivity excreted in bile and that remaining in the GI tract plus contents and feces are summarized in Table 94. After 24 hours, the total biliary excretion of radioactivity averaged 10.3% and 10.9% of the dose for TNT and 2,4-DNT; 14.4% to 14.5% for 2,5-DNT, 3,4-DNT and 3,5-DNT; 17.1% for 4-amino-2,6-DNT, and 24.8% and 27.3% for 2,6-DNT and 2,3-DNT. On the other hand, the radioactivity recovered from the GI tract plus contents and feces averaged 3.1% to 8.1% of the dose for 2,3-DNT, 2,4-DNT, 2,6-DNT, and 3,5-DNT; 14.2% to 17.9% for TNT, 2,5-DNT and 3,4-DNT; and 46.8% for 4-amino-2,6-DNT.

4. Discussion and Conclusions

After oral administration of TNT and various dinitrotoluenes (Ring-UL-^{14}C) to rats, the radioactivity appeared in the bile within 15 minutes. The rate of biliary excretion reached a peak in 15 minutes for 3,4-DNT, in 6 hours for 2,6-DNT and in 1 to 4 hours for TNT and the other DNT's. The blood concentrations of radioactivity increased and decreased in close relation to the rate of biliary excretion. The blood concentration and rate of biliary excretion of 4-amino-2,6-DNT continued to increase throughout the collection period. The biliary excretion rate increased slowly during the first 6 hours and increased only slightly during the 24th hour. The peak rate was probably reached before the 23rd hour.

The amount of biliary excretion was relatively high for 2,3-DNT and 2,6-DNT and somewhat lower for TNT and the other dinitrotoluenes. The amount of radioactivity remaining in the GI tract and feces 24 hours after dosing was large for 4-amino-2,6-DNT, small for 2,3-DNT, 2,4-DNT, 2,6-DNT and 3,5-DNT and intermediate for TNT and the other DNT's.

C. Metabolism of 2,4-DNT In Vitro

1. Introduction

This part of the study was to describe the in vitro metabolism of 2,4-DNT by homogenates of livers from various species. These data in conjunction with the in vivo observations may be utilized to predict how humans metabolize 2,4-DNT.

2. Material and Methods

Animals were sacrificed by decapitation (rats), cervical dislocation (mice), air embolism (rabbits), or an overdose of magensium sulfate (dogs and monkeys), and the livers removed, weighed, and homogenized in three volumes of 1.15% KCl. The homogenate was centrifuged at 10,000 g for 30 minutes at 4°C. The incubation medium contained 5 mM magnesium chloride, 5 mM glucose-6-phosphate, 0.8 mM nicotinamide adenine dinucleotide phosphate, 1 mM ^{14}C-2,4-DNT (0.1 μg/ml), 1.0 ml of 0.2 M Tris-HCL pH 7.4, and 0.5 ml of the 10,000 g supernatant for a final volume of 2.5 ml. All reactions were conducted in 50 ml Erlenmeyer flasks at 37°C for 1 hour in a shaking incubator. Aerobic reactions were performed in room air while anaerobic reactions were conducted in sealed flasks which had been gassed with nitrogen for 30 seconds before incubation. All reactions were terminated with 2.5 ml of acetone. The supernatants were chromatographed with standards on silica gel TLC plate in a solvent system of benzene and ethyl acetate (4:1). The standards were visualized with ultraviolet light, and the radioactivity associated with the standards was quantified using liquid scintillation counting. Protein determinations[22] were made on the 10,000 g supernatant using bovine serum albumin as the standard. The enzymatic formation of metabolites was corrected for nonenzymatic degradation, and the reults were expressed as nmoles metabolite/mg protein.

A preliminary study was conducted to determine the ability of our in vitro system to detect the difference in 2,4-DNT metabolism. Groups of four male rats were given 80 mg/kg of phenobarbital sodium intraperitoneally daily for 4 days before sacrifice on the 5th day, or 50 mg/kg of SKF-525A intraperitoneally for 1 hour before sacrifice, or no treatment. The livers of these animals were removed, prepared, incubated, and analyzed as above.

3. Results

The results of the preliminary experiment involving pretreatment of phenobarbital sodium or SKF-525A indicated that both compounds modified the ability of livers from these rats to metabolize 2,4-DNT in the in vitro system (Table 95). Consequently, we concluded that this system was suitable to detect species difference in 2,4-DNT metabolism.

A species comparison of 2,4-DNT metabolism under aerobic and anaerobic conditions is presented in Tables 96 and 97, respectively. Between 8 and 27% of the parent compound was metabolized, at the end of 1 hour, in all of the species tested. Under aerobic conditions, 2,4-dinitrobenzyl alcohol was the major metabolite in all the species tested. If incubations were conducted under anaerobic conditions, then the amount of 2,4-dinitrobenzyl alcohol was reduced while the amount of aminonitrotoluenes was increased relative to aerobic conditions. Differences between aerobic and anaerobic incubations, however, were not as apparent when the quantity of unidentified metabolites produced was compared. In addition, this study suggested the sex differences in 2,4-DNT metabolism. Female mice and rabbits and male dogs and rats produced more 2,4-dinitrobenzyl alcohol under aerobic conditions than did the opposite sex of each species. In contrast, more aminonitrotoluenes were produced under anaerobic conditions by males of all the species tested. There were no apparent sex differences in the amount of unidentified metabolites formed under either aerobic or anaerobic conditions.

4. Discussion and Conclusion

Phenobarbital and SKF-525A pretreatment modified the ability of livers from male rats to metabolize 2,4-DNT in an in vitro system. Similar effects of phenobarbital and SKF-525A on drug metabolism have been reported.[20/] If the disappearance of 2,4-DNT was used as a basis of comparison, then the species tested were similar.

Under aerobic conditions, the liver of rabbits formed more metabolic products than the other species. Under anaerobic conditions, more aminonitrotoluenes were formed by males than females; and male rats produced the most. The production of unidentified metabolites was similar for all of the species tested and did not provide information for comparison. We tried to obtain human cadaver liver for parallel experiments, but were not successful.

D. Effect of 2,4-DNT on Drug Metabolizing Enzymes

1. Introduction

Having seen what the liver drug metabolizing enzymes do to 2,4-DNT, we then studied what 2,4-DNT did to the liver drug metabolizing enzymes. Activities of these enzymes were assayed both in vivo by the zoxazolamine paralysis time and in vitro by the hepatic nitroanisole O-demethylase activity.

2. Material and Methods

Male rats were fed diets that contained 0.7% of 2,4-DNT for 2 weeks. Rats in the positive control group received 50 mg/kg of phenobarbital sodium twice daily for 3 days. At the end of treatment, the zoxazolamine paralysis time and nitroanisole O-demethylase activity were determined.

Zoxazolamine was administered i.p. to rats at a dose of 45 mg/kg in a vehicle of 0.2 N HCl. The duration of paralysis was measured in terms of the loss of the righting reflex. The values are reported as the mean ± S.E., and the test of significance was the two-sample rank test.[23] The level of significance was selected as $p < 0.05$.

The metabolism of nitroanisole by livers was measured in an in vitro system. Rats were sacrificed by decapitation. The livers were removed, weighed, and homogenized in four volumes of 1.15% potassium chloride. The homogenate was centrifuged at 9,000 x g for 30 minutes. The incubation medium contained 15 µmoles magnesium chloride, 15 µmoles glucose-6-phosphate, 3 µmoles p-nitroanisole, 0.5 ml of the 9,000 x g liver supernatant, and 0.5 ml of 0.5 M sodium phosphate buffer pH 7.8. Reactions were conducted for 20 minutes in a shaking water bath at 37°C. The reaction was terminated by the addition of 0.5 ml of 40% formalin and the color was developed with 0.5 ml of 0.8 N sodium hydroxide. The product formed was measured spectrophotometrically at 420 mm. The relationship of µmoles p-nitrophenol formed = Absorbance Units/10.22 was used to quantitate product formed. The Lowry protein assay[22] was used to measure protein content. The activity was expressed as nmoles p-nitrophenol/mg protein. The values are reported as the mean ± S.E. The test and level of significance were the same as described above.

3. Results

Phenobarbital pretreatment significantly decreased the duration of zoxazolamine paralysis, as shown in Table 98. Two weeks of 0.7% of 2,4-DNT in the feed did not significantly affect the duration.

This same diet regimen did not change the in vitro ability of the liver to convert nitroanisole to nitrophenol, as shown in Table 99.

4. Conclusion

Feeding 0.7% of 2,4-DNT to male rats for 2 weeks neither affected the liver enzymes involved in the metabolism of zoxazolamine in vivo nor affected the in vitro liver nitroanisole O-demethylase activity.

E. Summary

Oral doses of radiolabelled 2,4-DNT were poorly absorbed (8 to 12% of the dose) by two strains of mice, but well absorbed (75 to 85%) by rats, rabbits, dogs and monkeys. Once absorbed, the compound was handled similarly in all species. Concentrations of radiolabel were found in the liver and kidney. No radiolabel was found in exhaled air, but most of that absorbed was excreted in the urine within 24 hours. Metabolic processes, as determined from urinary metabolites, included reduction of one or both nitros to aminos, oxidation of the methyl to an alcohol or benzoic acid, and conjugation with sulfate or glucuonate.

In female rats, portions of oral doses of radiolabelled TNT or DNT isomers were excreted into the bile within 15 minutes of dosing. There were variations between the compounds with respect to time to peak biliary excretion rats (15 minutes for 3,4-DNT to 6 hours for 4-amino-2,6-DNT), and radioactivity remaining in the GI tract, its contents, and feces (3.1% of dose for 2,3-DNT to 46.8% for 4-amino-2,6-DNT).

In vitro liver homogenates from mice, rats, rabbits, dogs and monkeys metabolized 8 to 27% of added 2,4-DNT within 1 hour. The primary product under aerobic conditions was 2,4-dinitrobenzyl alcohol; under anaerobic, aminonitrotoluenes. These compounds are the first products formed in vivo, also.

Feeding 0.7% 2,4-DNT (high dose in toxicity study) to rats for 2 weeks did not affect liver enzymes, as determined by zoxazolamine paralysis time and hepatic nitroanisole O-demethylase activity.

TABLE 77

DISTRIBUTION AND EXCRETION OF RADIOACTIVITY IN VARIOUS SPECIES OF ANIMALS 24 HR AFTER ORAL ADMINISTRATION OF A SINGLE DOSE OF 2,4-DNT (RING-UL-^{14}C)

	% of Administered Dose					
	Mice					
	CD-1	B6C3F1	Rats	Rabbits	Dogs	Monkeys
Gastrointestinal Tract Plus Contents	2.1 ± 1.3[c]	0.6 ± 0.2[d]	2.8 ± 1.5[e]	10.9[f]	8.6[f]	4.7[f]
Feces	81.0 ± 4.3	84.0 ± 2.9	9.1 ± 3.0	3.1	8.7	4.8
Whole Blood[a]	0.1 ± 0.0	<0.1	0.1 ± 0.0	0.1	0.6	0.3
Urine	11.3 ± 3.7	7.2 ± 1.9	75.9 ± 2.6	75.2	76.4	81.3
Spleen	<0.1	<0.1	--	<0.1	<0.1	<0.1
Liver	0.2 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	<0.3	1.1	0.7
Kidney	<0.1	<0.1	<0.1	<0.1	0.2	<0.1
Brain	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Lung	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Skeletal Muscle[b]	0.1 ± 0.0	<0.1	0.3 ± 0.1	0.2	1.6	1.2
Recovery	94.8 ± 2.3	92.0 ± 3.2	88.5 ± 2.6	89.8	97.3	93.0

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

TABLE 78

TISSUE/PLASMA RADIOACTIVITY RATIOS[a] IN VARIOUS SPECIES OF ANIMALS 24 HR AFTER ORAL ADMINISTRATION OF A SINGLE DOSE OF 2,4-DNT (RING-UL-^{14}C)

	Mice	Rats	Rabbits	Dogs	Monkeys
Spleen	1.7±0.3[b]	--	1.3[d]	1.1[d]	2.6[d]
Liver	6.3±0.8	18.1±0.7[c]	8.7	6.9	17.8
Kidneys	3.4±0.4	7.4±0.5	7.0	4.9	6.4
Brain	0.6±0.1	1.5±0.1	0.6	0.5	1.3
Lungs	1.9±0.3	6.1±3.2	2.2	1.7	1.9
Skeletal Muscle	0.6±0.1	1.8±0.2	0.5	0.5	1.5

a/ Radioactivity in 1 ml or gm of wet tissue per radioactivity in 1 ml of plasma

b/ Mean ± S.E. of four CD-1 mice.

c/ Mean ± S.E. of three rats.

d/ Average of two animals.

TABLE 79

IDENTIFICATION OF 2,4-DNT METABOLITES IN URINES OF VARIOUS SPECIES

Metabolite	Thin-Layer Chromatography Rf Values			Gas-Liquid Chromatography Retention Time	Arylamine Test[d]
	System 1[a]	System 2[b]	System 3[c]		
2,4-Dinitrotoluene (I)	0.71	0.85	0.78	9.4[e]	-
4-Amino-2-nitrotoluene (II)	0.55	0.85	0.78	10.8[e]	+
2-Amino-4-nitrotoluene (III)	0.55	0.85	0.78	12.8[e]	+
2,4-Diaminotoluene (IV)	0.06	0.46	0.36	5.0[e]	+
2,4-Dinitrobenzyl alcohol (V)	0.37	0.85	0.78	6.3[f]	-
4-Amino-2-nitrobenzyl alcohol (VI)	0.13	0.70	0.72	6.9[f]	+
2-Amino-4-nitrobenzyl alcohol (VII)	0.13	0.70	0.72	6.9[f]	+
2,4-Diaminobenzyl alcohol (VIII)	0.13	0.70	0.72	10.8[f]	+
2,4-Dinitrobenzaldehyde (IX)	0.81	0.90	0.80	--	--
2,4-Dinitrobenzoic Acid (XIII)	0	0	0.36	2.5[g]	-

a/ Benzene:ethyl acetate (4:1)
b/ Ethyl acetate:n-heptane (9:1)
c/ Butanol:acetic acid:water (10:1:1)
d/ Bratton-Marshall reagent 19/
e/ 10% UCW-982 on 80-100 mesh WAW-DMCS in a 3 ft steel column.
f/ 10% OV-1 on 80-100 mesh Ghp in a 3 ft steel column.
g/ 1.5% DC-LSX-3-0295 and 1.5% XE-60 on 60-80 mesh GCQ in a 4 ft glass column; samples were silylated before injection.

TABLE 80

METABOLITES OF 2,4-DNT IN MOUSE URINE COLLECTED FOR 24 HR AFTER ORAL ADMINISTRATION OF A SINGLE DOSE OF 2,4-DNT (RING-UL-^{14}C)

Metabolites	Free	Conjugates	
		Glucuronide	Sulfate
2,4-Dinitrotoluene (I)	0.3[a/]	0	0
4-Amino-2-nitrotoluene (II)	1.0	1.2	10.3
2-Amino-4-nitrotoluene (III)	0.1	7.3	2.1
2,4-Diaminotoluene (IV)	0.1	3.1	2.0
2,4-Dinitrobenzyl alcohol (V)	0.3	19.6	2.7
2,4-Aminonitrobenzyl alcohols (VI, VII)	0.1	24.5	0.5
2,4-Diaminobenzyl alcohol (VIII)	0.2	1.4	0
2,4-Dinitrobenzoic acid (XIII)	3.8	0.1	0
All Identified	5.8	57.0	17.5
Unidentified	19.6		

a/ Percent of ^{14}C-radioactivity in urine. Average of four CD-1 mice.

TABLE 81

METABOLITES OF 2,4-DNT IN RAT URINE COLLECTED FOR 24 HR AFTER ORAL ADMINISTRATION OF A SINGLE DOSE OF 2,4-DNT (RING-UL-^{14}C)

Metabolites	Free	Conjugates	
		Glucuronide	Sulfate
2,4-Dinitrotoluene (I)	0	0	0
4-Amino-2-nitrotoluene (II)	0.4[a/]	1.2	0.9
2-Amino-4-nitrotoluene (III)	0	0.1	0.6
2,4-Diaminotoluene (IV)	1.3	5.9	4.3
2,4-Dinitrobenzyl alcohol (V)	3.2	27.1	2.9
2,4-Aminonitrobenzyl alcohols (VI, VII)	2.2	22.5	0.7
2,4-Diaminobenzyl alcohol (VIII)	0.9	1.7	3.4
2,4-Dinitrobenzoic acid (XIII)	8.0	0.2	0
All identified	16.0	58.7	12.9
Unidentified	12.5		

a/ Percent of ^{14}C-radioactivity in urine. Average of three rats.

TABLE 82

METABOLITES OF 2,4-DNT IN RABBIT URINE COLLECTED FOR 24 HR AFTER ORAL ADMINISTRATION OF A SINGLE DOSE OF 2,4-DNT (RING-UL-^{14}C)

Metabolites	Free	Conjugates	
		Glucuronide	Sulfate
2,4-Dinitrotoluene (I)	0.3[a/]	0	0
4-Amino-2-nitrotoluene (II)	0.4	0.5	7.9
2-Amino-4-nitrotoluene (III)	0.6	4.2	1.8
2,4-Diaminotoluene (IV)	0.9	4.0	0.7
2,4-Dinitrobenzyl alcohol (V)	0.5	40.3	3.0
2,4-Aminonitrobenzyl alcohols (VI, VII)	0.3	9.4	0.5
2,4-Diaminobenzyl alcohol (VIII)	1.0	5.9	0.6
2,4-Dinitrobenzoic acid (XIII)	7.5	2.0	0
All identified	11.4	66.4	14.5
Unidentified	7.9		

a/ Percent of ^{14}C-radioactivity in urine. Average of two rabbits.

TABLE 83

METABOLITES OF 2,4-DNT IN DOG URINE COLLECTED FOR 24 HR AFTER ORAL ADMINISTRATION OF A SINGLE DOSE OF 2,4-DNT (RING-UL-^{14}C)

Metabolites	Free	Conjugates	
		Glucuronide	Sulfate
2,4-Dinitrotoluene (I)	0.2[a/]	0.5	0
4-Amino-2-nitrotoluene (II)	0.1	4.6	3.7
2-Amino-4-nitrotoluene (III)	0.2	2.9	0.4
2,4-Diaminotoluene (IV)	0.2	4.8	0.4
2,4-Dinitrobenzyl alcohol (V)	0.1	33.1	1.6
2,4-Aminonitrobenzyl alcohols (VI, VII)	0.4	17.9	0.2
2,4-Diaminobenzyl alcohol (VIII)	0.5	1.4	1.5
2,4-Dinitrobenzoic acid (XIII)	5.7	1.0	0
All identified	7.4	66.2	7.8
Unidentified	18.6		

a/ Percent of ^{14}C-radioactivity in urine. Average of two dogs.

TABLE 84

METABOLITES OF 2,4-DNT IN MONKEY URINE COLLECTED FOR 24 HR AFTER ORAL ADMINISTRATION OF A SINGLE DOSE OF 2,4-DNT (RING-UL-^{14}C)

Metabolites	Free	Conjugates	
		Glucuronide	Sulfate
2,4-Dinitrotoluene (I)	0.4[a/]	2.2	0
4-Amino-2-nitrotoluene (II)	1 0	0.7	0.4
2-Amino-4-nitrotoluene (III)	0.3	6.8	1.6
2,4-Diaminotoluene (IV)	0.4	3.3	1.1
2,4-Dinitrobenzyl alcohol (V)	1.5	21.5	2.3
2,4-Aminonitrobenzyl alcohols (VI, VII)	0	17.9	0.2
2,4-Diaminobenzyl alcohol (VIII)	0.5	1.3	1.4
2,4-Dinitrobenzoic acid (XIII)	4.5	0.4	0
All identified	8.2	54.1	6.9
Unidentified	30.7		

a/ Percent of ^{14}C-radioactivity in urine. Average of two monkeys.

TABLE 85

METABOLITES OF 2,4-DNT IN URINE OF VARIOUS SPECIES COLLECTED FOR 24 HR AFTER ORAL ADMINISTRATION OF A SINGLE DOSE OF 2,4-DNT (RING-UL-14C)

Metabolite	% of Administered Dose[a]				
	Mice	Rats	Rabbits	Dogs	Monkeys
2,4-Dinitrotoluene (I)	0[b]	0[c]	0.2[d]	0.6[d]	2.1[d]
4-Amino-2-nitrotoluene (II)	1.4	1.9	6.6	6.3	1.8
2-Amino-4-nitrotoluene (III)	1.1	0.6	5.0	2.7	7.1
2,4-Diaminotoluene (IV)	0.6	8.8	4.2	4.0	3.8
2,4-Dinitrobenzyl alcohol (V)	2.6	25.2	32.9	26.6	20.2
2,4-Aminonitrobenzyl alcohols (VI, VII)	2.8	19.2	7.6	14.2	14.8
2,4-Diaminobenzyl alcohol (VIII)	0.2	4.6	5.6	2.6	2.5
2,4-Dinitrobenzoic acid (XIII)	0.4	6.2	7.2	5.1	4.0
Unidentified	2.2	9.5	5.9	14.2	25.0
Total	11.3	75.9	75.2	76.4	81.3

a/ Includes free compound and glucuronide and sulfate conjugates.
b/ Mean of four animals.
c/ Mean of three animals.
d/ Mean of two animals.

TABLE 36

BILIARY EXCRETION OF RADIOACTIVITY IN RATS AFTER A SINGLE ORAL DOSE OF TNT (RING-UL-^{14}C)

Time After Dosing (hours)	Bile Volume (ml)	Excretion in Bile (% of Dose)	Excretion in Bile (Cumulative %)	Excretion Rate (% of dose/min)	Blood Concentration (dpm/ml)
1/4	0.13 ± 0.04[a]	0.02 ± 0.01	0.02 ± 0.01	$1.25 \pm 0.10 \times 10^{-3}$	$8.59 \pm 1.42 \times 10^{3}$
1/2	0.16 ± 0.06	0.04 ± 0.01	0.06 ± 0.02	$2.42 \pm 0.09 \times 10^{-3}$	—
1	0.32 ± 0.07	0.34 ± 0.09	0.40 ± 0.10	$4.34 \pm 0.76 \times 10^{-3}$	$32.69 \pm 7.21 \times 10^{3}$
2	0.63 ± 0.02	1.07 ± 0.42	1.47 ± 0.68	$17.[illegible] \pm 2.82 \times 10^{-3}$	$39.45 \pm 6.24 \times 10^{3}$
4	1.24 ± 0.36	1.52 ± 0.73	2.97 ± 0.87	$12.[illegible]4 \pm 3.31 \times 10^{-3}$	$40.23 \pm 5.32 \times 10^{3}$
6	[illegible].17 ± 0.31	0.71 ± 0.12	3.70 ± 0.46	$5.92 \pm 0.97 \times 10^{-3}$	$29.65 \pm 3.21 \times 10^{3}$
23	6.91 ± 2.10	5.89 ± 0.90	9.59 ± 0.96	$5.71 \pm 0.94 \times 10^{-3}$	—
24	0.46 ± 0.14	0.69 ± 0.21	10.28 ± 0.97	$4.34 \pm 1.21 \times 10^{-3}$	$28.05 \pm 4.35 \times 10^{3}$

a/ Mean ± S.E. of three rats.

TABLE 87

BILIARY EXCRETION OF RADIOACTIVITY IN RATS AFTER A SINGLE ORAL DOSE OF 2,3-DNT (RING-UL-^{14}C)

Time After Dosing (hours)	Bile Volume (ml)	Excretion in Bile (% of Dose)	Excretion in Bile (Cumulative %)	Excretion Rate (% of dose/min)	Blood Concentration (dpm/ml)
1/4	0.28 ± 0.04[a]	0.50 ± 0.06	0.50 ± 0.06	$33.7 \pm 4.3 \times 10^{-3}$	$0.85 \pm 0.08 \times 10^{3}$
1/2	0.18 ± 0.05	0.57 ± 0.17	1.07 ± 0.13	$38.3 \pm 8.4 \times 10^{-3}$	--
1	0.51 ± 0.07	1.27 ± 0.42	2.34 ± 0.83	$42.0 \pm 9.3 \times 10^{-3}$	$1.06 \pm 0.09 \times 10^{3}$
2	1.12 ± 0.07	2.23 ± 0.90	4.57 ± 1.93	$37.3 \pm 6.9 \times 10^{-3}$	$0.95 \pm 0.14 \times 10^{3}$
4	2.11 ± 0.09	4.52 ± 0.82	9.09 ± 2.49	$37.6 \pm 6.9 \times 10^{-3}$	$0.90 \pm 0.03 \times 10^{3}$
6	1.84 ± 0.24	6.50 ± 1.44	15.59 ± 4.91	$54.2 \pm 10.3 \times 10^{-3}$	$0.87 \pm 0.07 \times 10^{3}$
23	8.78 ± 2.22	11.55 ± 1.81	27.14 ± 4.04	$11.3 \pm 2.9 \times 10^{-3}$	--
24	0.48 ± 0.19	0.12 ± 0.07	27.26 ± 4.04	$2.7 \pm 1.2 \times 10^{-3}$	$0.74 \pm 0.17 \times 10^{3}$

[a] Mean ± S.E. of three rats.

TABLE 88

BILIARY EXCRETION OF RADIOACTIVITY IN RATS AFTER A SINGLE ORAL DOSE OF 2,4-DNT (RING-UL-^{14}C)

Time After Dosing (hours)	Bile Volume (ml)	Excretion in Bile (% of Dose)	Excretion in Bile (Cumulative %)	Excretion Rate (% of dose/min)	Blood Concentration (dpm/ml)
1/4	0.32 ± 0.03[a]	0.09 ± 0.03	0.09 ± 0.03	5.8 ± 1.8 x 10^{-3}	1.6 ± 0.3 x 10^{3}
1/2	0.31 ± 0.04	0.20 ± 0.04	0.29 ± 0.07	13.3 ± 2.5 x 10^{-3}	2.0 ± 0.3 x 10^{3}
1	0.59 ± 0.10	0.62 ± 0.10	0.01 ± 0.12	20.5 ± 3.4 x 10^{-3}	2.5 ± 0.3 x 10^{3}
1-1/2	0.59 ± 0.10	0.58 ± 0.03	1.40 ± 0.11	19.4 ± 1.1 x 10^{-3}	3.2 ± 0.1 x 10^{3}
2	0.73 ± 0.09	0.96 ± 0.18	2.45 ± 0.17	31.7 ± 6.3 x 10^{-3}	4.1 ± 0.3 x 10^{3}
3	1.24 ± 0.10	0.92 ± 0.16	3.37 ± 0.01	15.2 ± 2.7 x 10^{-3}	5.8 ± 1.0 x 10^{3}
4	1.18 ± 0.07	0.92 ± 0.33	4.19 ± 0.32	15.3 ± 5.4 x 10^{-3}	6.2 ± 1.1 x 10^{3}
5	1.08 ± 0.08	0.78 ± 0.11	5.07 ± 0.34	13.0 ± 1.8 x 10^{-3}	5.0 ± 1.1 x 10^{3}
6	0.89 ± 0.05	0.55 ± 0.13	5.62 ± 0.33	9.2 ± 2.2 x 10^{-3}	5.2 ± 1.2 x 10^{3}
23	10.66 ± 0.52	5.11 ± 0.47	10.73 ± 0.23	5.0 ± 0.5 x 10^{-3}	—
24	0.61 ± 0.03	0.18 ± 0.06	10.91 ± 0.69	2.9 ± 0.9 x 10^{-3}	3.3 ± 0.5 x 10^{3}

a/ Mean ± S.E. of three rats.

TABLE 89

BILIARY EXCRETION OF RADIOACTIVITY IN RATS AFTER A SINGLE ORAL DOSE OF 2,5-DNT (RING-UL-^{14}C)

Time After Dosing (hours)	Bile Volume (ml)	Excretion in Bile (% of Dose)	Excretion in Bile (Cumulative %)	Excretion Rate (% of dose/min)	Blood Concentration (dpm/ml)
1/4	0.19 ± 0.03[a/]	0.21 ± 0.01	0.21 ± 0.01	$14.3 \pm 0.9 \times 10^{-3}$	$3.48 \pm 1.15 \times 10^{3}$
1/2	0.19 ± 0.03	0.20 ± 0.05	0.41 ± 0.05	$13.8 \pm 2.6 \times 10^{-3}$	--
1	0.29 ± 0.04	0.33 ± 0.06	0.74 ± 0.12	$11.0 \pm 1.9 \times 10^{-3}$	$3.90 \pm 0.9 \times 10^{3}$
2	0.61 ± 0.10	0.63 ± 0.17	1.37 ± 0.26	$10.3 \pm 2.3 \times 10^{-3}$	$3.89 \pm 0.9 \times 10^{3}$
4	1.43 ± 0.10	1.82 ± 0.10	3.19 ± 0.27	$15.1 \pm 0.3 \times 10^{-3}$	$4.06 \pm 0.5 \times 10^{3}$
6	1.51 ± 0.10	1.42 ± 0.05	4.61 ± 0.24	$11.8 \pm 3.4 \times 10^{-3}$	$4.13 \pm 1.2 \times 10^{3}$
23	8.98 ± 1.15	9.62 ± 0.55	14.23 ± 0.19	$9.4 \pm 1.8 \times 10^{-3}$	--
24	0.39 ± 0.09	0.17 ± 0.02	14.40 ± 0.20	$3.0 \pm 0.6 \times 10^{-3}$	$2.98 \pm 0.7 \times 10^{3}$

a/ Mean ± S.E. of three rats.

TABLE 90

BILIARY EXCRETION OF RADIOACTIVITY IN RATS AFTER A SINGLE ORAL DOSE OF 2,6-DNT (RING-UL-^{14}C)

Time After Dosing (hours)	Bile Volume (ml)	Excretion in Bile (% of Dose)	Excretion in Bile (Cumulative %)	Excretion Rate (% of dose/min)	Blood Concentration (dpm/ml)
1/4	0.24 ± 0.01[a/]	0.05 ± 0.01	0.05 ± 0.01	$3.3 \pm 0.3 \times 10^{-3}$	$1.20 \pm 0.50 \times 10^{3}$
1/2	0.25 ± 0.03	0.13 ± 0.02	0.18 ± 0.04	$8.6 \pm 1.7 \times 10^{-3}$	--
1	0.56 ± 0.08	0.49 ± 0.03	0.67 ± 0.08	$16.3 \pm 1.2 \times 10^{-3}$	$1.64 \pm 0.42 \times 10^{3}$
2	1.16 ± 0.13	1.13 ± 0.06	1.80 ± 0.08	$18.7 \pm 0.9 \times 10^{-3}$	$2.21 \pm 0.64 \times 10^{3}$
4	2.10 ± 0.16	2.38 ± 0.72	4.18 ± 0.78	$19.8 \pm 1.4 \times 10^{-3}$	$3.83 \pm 0.90 \times 10^{3}$
6	2.05 ± 0.12	2.81 ± 1.12	6.99 ± 1.89	$23.3 \pm 9.2 \times 10^{-3}$	$5.60 \pm 1.67 \times 10^{3}$
23	9.52 ± 1.70	17.52 ± 2.98	24.51 ± 1.80	$17.2 \pm 2.7 \times 10^{-3}$	$4.57 \pm 1.70 \times 10^{3}$
24	0.43 ± 0.16	0.26 ± 0.16	24.77 ± 1.80	$4.3 \pm 0.4 \times 10^{-3}$	--

a/ Mean ± S.E. of three rats.

TABLE 91

BILIARY EXCRETION OF RADIOACTIVITY IN RATS AFTER A SINGLE ORAL DOSE OF 3,4-DNT (RING-UL-^{14}C)

Time After Dosing (hours)	Bile Volume (ml)	Excretion in Bile (% of Dose)	Excretion in Bile (Cumulative %)	Excretion Rate (% of dose/min)	Blood Concentration (dpm/ml)
1/4	0.18 ± 0.04[a]	0.36 ± 0.08	0.36 ± 0.08	24.0 ± 5.2 x 10^{-3}	0.52 ± 0.12 x 10^{3}
1/2	0.17 ± 0.05	0.31 ± 0.03	0.67 ± 0.03	20.7 ± 4.7 x 10^{-3}	--
1	0.28 ± 0.03	0.53 ± 0.03	1.20 ± 0.24	17.6 ± 3.6 x 10^{-3}	0.45 ± 0.14 x 10^{3}
2	0.69 ± 0.08	0.78 ± 0.05	1.98 ± 0.44	13.4 ± 2.8 x 10^{-3}	0.46 ± 0.17 x 10^{3}
4	1.22 ± 0.11	0.71 ± 0.13	2.69 ± 0.72	5.0 ± 1.0 x 10^{-3}	0.23 ± 0.09 x 10^{3}
6	1.08 ± 0.10	0.83 ± 0.12	3.52 ± 0.89	6.9 ± 1.2 x 10^{-3}	0.21 ± 0.08 x 10^{3}
23	8.76 ± 0.67	10.74 ± 2.70	14.26 ± 2.80	10.4 ± 3.2 x 10^{-3}	--
24	0.33 ± 0.01	0.11 ± 0.10	14.37 ± 2.80	1.8 ± 0.3 x 10^{-3}	0.21 ± 0.12 x 10^{3}

a/ Mean ± S.E. of three rats.

TABLE 92

BILIARY EXCRETION OF RADIOACTIVITY IN RATS AFTER A SINGLE ORAL DOSE OF 3,5-DNT (RING-UL-^{14}C)

Time After Dosing (hours)	Bile Volume (ml)	Excretion in Bile (% of Dose)	Excretion in Bile (Cumulative %)	Excretion Rate (% of dose/min)	Blood Concentration (dpm/ml)
1/4	0.33 ± 0.4[a/]	0.03 ± 0.01	0.03 ± 0.01	2.1 ± 0.1 x 10^{-3}	3.97 ± 1.69 x 10^{3}
1/2	0.28 ± 0.03	0.08 ± 0.02	0.11 ± 0.03	5.2 ± 1.2 x 10^{-3}	--
1	0.56 ± 0.08	0.34 ± 0.03	0.45 ± 0.05	11 1 ± 0.1 x 10^{-3}	11.24 ± 0.26 x 10^{3}
2	0.97 ± 0.18	1.01 ± 0.23	1.46 ± 0.23	16.8 ± 3.9 x 10^{-3}	11.21 ± 0.26 x 10^{3}
4	2.03 ± 0.20	2.81 ± 0.88	4.27 ± 1.31	20.0 ± 4.6 x 10^{-3}	13.07 ± 0.36 x 10^{3}
6	1.70 ± 0.21	2.12 ± 0.80	6.39 ± 1.21	18.0 ± 7.5 x 10^{-3}	11.22 ± 4.55 x 10^{3}
23	8.14 ± 0.70	8.00 ± 0.40	14.39 ± 2.39	7.8 ± 0.4 x 10^{-3}	--
24	0.61 ± 0.03	0.10 ± 0.02	14.49 ± 2.37	1.7 ± 0.4 x 10^{-3}	1.75 ± 0.08 x 10^{3}

a/ Mean ± S.E. of three rats.

TABLE 93

BILIARY EXCRETION OF RADIOACTIVITY IN RATS AFTER A SINGLE ORAL DOSE OF 4-AMINO-2,6-DNT (RING-UL-^{14}C)

Time After Dosing (hours)	Bile Volume (ml)	Excretion in Bile (% of Dose)	Excretion in Bile (Cumulative %)	Excretion Rate (% of dose/min)	Blood Concentration (dpm/ml)
1/4	0.24 ± 0.3[a/]	0.01 ± 0.001	0.01 ± 0.001	0.7 ± 0.2 x 10^{-3}	0.48 ± 0.08 x 10^3
1/2	0.25 ± 0.02	0.05 ± 0.01	0.06 ± 0.01	3.0 ± 0.6 x 10^{-3}	--
1	0.48 ± 0.05	0.22 ± 0.06	0.28 ± 0.04	7.3 ± 1.3 x 10^{-3}	1.87 ± 0.35 x 10^3
2	0.91 ± 0.16	0.39 ± 0.07	0.67 ± 0.03	6.5 ± 0.3 x 10^{-3}	2.05 ± 0.32 x 10^3
4	1.92 ± 0.10	0.95 ± 0.48	1.62 ± 0.15	7.3 ± 1.5 x 10^{-3}	2.98 ± 0.64 x 10^3
6	1.48 ± 0.16	0.89 ± 0.30	2.51 ± 0.34	7.4 ± 2.7 x 10^{-3}	3.11 ± 0.89 x 10^3
23	10.88 ± 0.92	14.02 ± 3.46	16.53 ± 3.30	13.7 ± 4.2 x 10^{-3}	--
24	0.69 ± 0.03	0.57 ± 0.08	17.10 ± 3.29	9.5 ± 2.9 x 10^{-3}	5.24 ± 1.05 x 10^3

a/ Mean ± S.E. of three rats.

TABLE 94

TOTAL RADIOACTIVITY EXCRETED IN THE BILE AND REMAINING IN THE GASTROINTESTINAL TRACT IN RATS 24 HOURS AFTER A SINGLE ORAL DOSE OF NITROTOLUENES (RING-UL-^{14}C)

Compound	Excretion in Bile (% of Dose)			Radioactivity in GI Tract[b] (% of Dose)
	0 - 4 hr	4 - 24 hr	Total	
TNT	3.0 ± 0.9[a]	7.3 ± 2.0	10.3 ± 1.0	14.0 ± 3.7
2,3-DNT	9.1 ± 2.5	18.2 ± 4.2	27.3 ± 4.0	3.1 ± 0.8
2,4-DNT	4.2 ± 0.3	6.6 ± 1.0	10.9 ± 0.7	7.6 ± 2.2
2,5-DNT	3.2 ± 0.3	9.2 ± 1.1	14.4 ± 0.2	14.2 ± 5.3
2,6-DNT	4.2 ± 0.8	20.5 ± 2.1	24.8 ± 1.8	6.9 ± 0.2
3,4-DNT	2.7 ± 0.7	11.7 ± 0.9	14.4 ± 2.8	17.9 ± 3.6
3,5-DNT	4.3 ± 1.3	10.2 ± 1.1	14.5 ± 2.4	8.1 ± 1.6
4-Amino-2,6-DNT	1.6 ± 0.2	15.5 ± 3.4	17.1 ± 3.3	46.8 ± 2.5

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

TABLE 95

METABOLISM OF 2,4-DNT (RING-UL-^{14}C) BY RAT LIVERS AFTER PRETREATMENT WITH PHENOBARBITAL OR SKF-525A

Pretreatment	Aerobic Incubation (nmoles/mg Protein)			
	2,4-Dinitrobenzyl Alcohol	Aminonitrotoluenes	2,4-Dinitrotoluene	Others [a]
Control	9.7 ± 0.6 [b]	2.7 ± 0.6	112 ± 4	2.4 ± 0.1
Phenobarbital	22.5 ± 2.4	1.4 ± 0.4	94 ± 2	8.3 ± 1.3
SKF-525A	4.8 ± 0.5	1.3 ± 0.5	124 ± 4	3.3 ± 1.3
	Anaerobic Incubation (nmoles/mg Protein)			
Control	6.8 ± 1.0	9.5 ± 3.0	110 ± 4	2.2 ± 0.2
Phenobarbital	8.3 ± 2.1	39.7 ± 13.2	66 ± 11	11.2 ± 0.7
SKF-525A	3.9 ± 1.1	13.4 ± 1.7	112 ± 3	3.4 ± 0.7

a/ Unknown compounds remaining at origin of TLC.

b/ Mean ± standard error.

TABLE 96

AEROBIC METABOLISM OF 2,4-DNT (RING-UL-^{14}C) BY LIVERS OF VARIOUS SPECIES

Species	Sex	Number of Determinations	nmoles/mg Protein			
			2,4-Dinitrobenzyl Alcohol	Aminonitrotoluenes	2,4-Dinitrotoluene	Others [b]
Mouse	Male	4	11.0 ± 0.8 [a]	4.0 ± 0.2	107 ± 4	3.9 ± 0.1
	Female	4	16.6 ± 0.7	1.3 ± 0.1	120 ± 4	3.4 ± 0.5
Rat	Male	4	11.1 ± 0.6	1.8 ± 0.5	129 ± 1	3.8 ± 0.5
	Female	4	8.0 ± 1.0	1.3 ± 0.1	123 ± 8	1.8 ± 0.2
Rabbit	Male	4	21.5 ± 1.3	7.6 ± 0.4	123 ± 8	5.4 ± 0.2
	Female	4	28.8 ± 1.5	8.3 ± 0.4	124 ± 7	6.4 ± 0.4
Dog	Male	4	17.1 ± 1.0	0.7 ± 0.1	116 ± 4	1.5 ± 0.2
	Female	3	14.0 ± 1.5	0.6 ± 0.1	126 ± 3	1.6 ± 0.2
Monkey	Male	3	20.1 ± 7.6	15.8 ± 1.7	128 ± 13	7.2 ± 2.6
	Female	1	25.0	17.3	84	10.6

a/ Mean standard error or mean.

b/ Unknown compounds remaining at origin of TLC.

TABLE 97

ANAEROBIC METABOLISM OF 2,4-DNT (RING-UL-^{14}C) BY LIVERS OF VARIOUS SPECIES

Species	Sex	Number of Determinations	nmoles/mg Protein			
			2,4-Dinitrobenzyl Alcohol	Aminonitrotoluenes	2,4-Dinitrotoluene	Others [b]
Mouse	Male	4	8.4 ± 0.7 [a]	10.3 ± 2.8	103 ± 4	3.9 ± 0.2
	Female	4	13.0 ± 1.8	5.1 ± 2.0	120 ± 1	3.1 ± 0.3
Rat	Male	4	4.6 ± 1.6	30.4 ± 9.4	105 ± 9	4.2 ± 0.3
	Female	4	6.1 ± 0.6	3.1 ± 0.8	123 ± 1	1.8 ± 0.2
Rabbit	Male	4	16.8 ± 0.6	20.3 ± 2.1	116 ± 7	5.1 ± 0.2
	Female	4	24.4 ± 1.1	13.7 ± 0.7	124 ± 8	5.2 ± 0.7
Dog	Male	4	8.6 ± 3.4	8.0 ± 5.7	117 ± 4	1.1 ± 0.3
	Female	3	11.4 ± 1.5	1.1 ± 0.3	128 ± 4	1.2 ± 0.3
Monkey	Male	3	13.4 ± 7.3	55.8 ± 5.4	89.3 ± 7.2	13.9 ± 5.1
	Female	1	26.8	22.1	78	9.8

a/ Mean ± standard error or mean.

b/ Unknown compounds remaining at origin of TLC.

TABLE 98

DURATION OF ZOXAZOLAMINE PARALYSIS IN MALE RATS PRETREATED WITH PHENOBARBITAL OR FED A CONTROL DIET OR A DIET CONTAINING 0.7% OF 2,4-DNT

Treatment	Duration of Paralysis (minutes)
Experiment I	
Control	98 ± 11 (10)[a]
Phenobarbital	34 ± 3 (7)[b]
Experiment II	
Control	134 ± 14 (8)
2,4-DNT	105 ± 6 (6)

a/ Mean ± standard error (number of rats)
b/ Significantly different from control[21]

TABLE 99

NITROANISOLE O-DEMETHYLASE ACTIVITY IN LIVERS OF RATS FED A CONTROL DIET OR A DIET CONTAINING 0.7% OF 2,4-DNT

Treatment	Activity[a/]
Control	1.32 ± 0.17 (3)[b/]
2,4-DNT	1.14 ± 0.01 (3)

a/ nmoles p-nitrophenol/mg prctein in 9,000 x g supernatant.

b/ Mean ± standard error (number of rats).

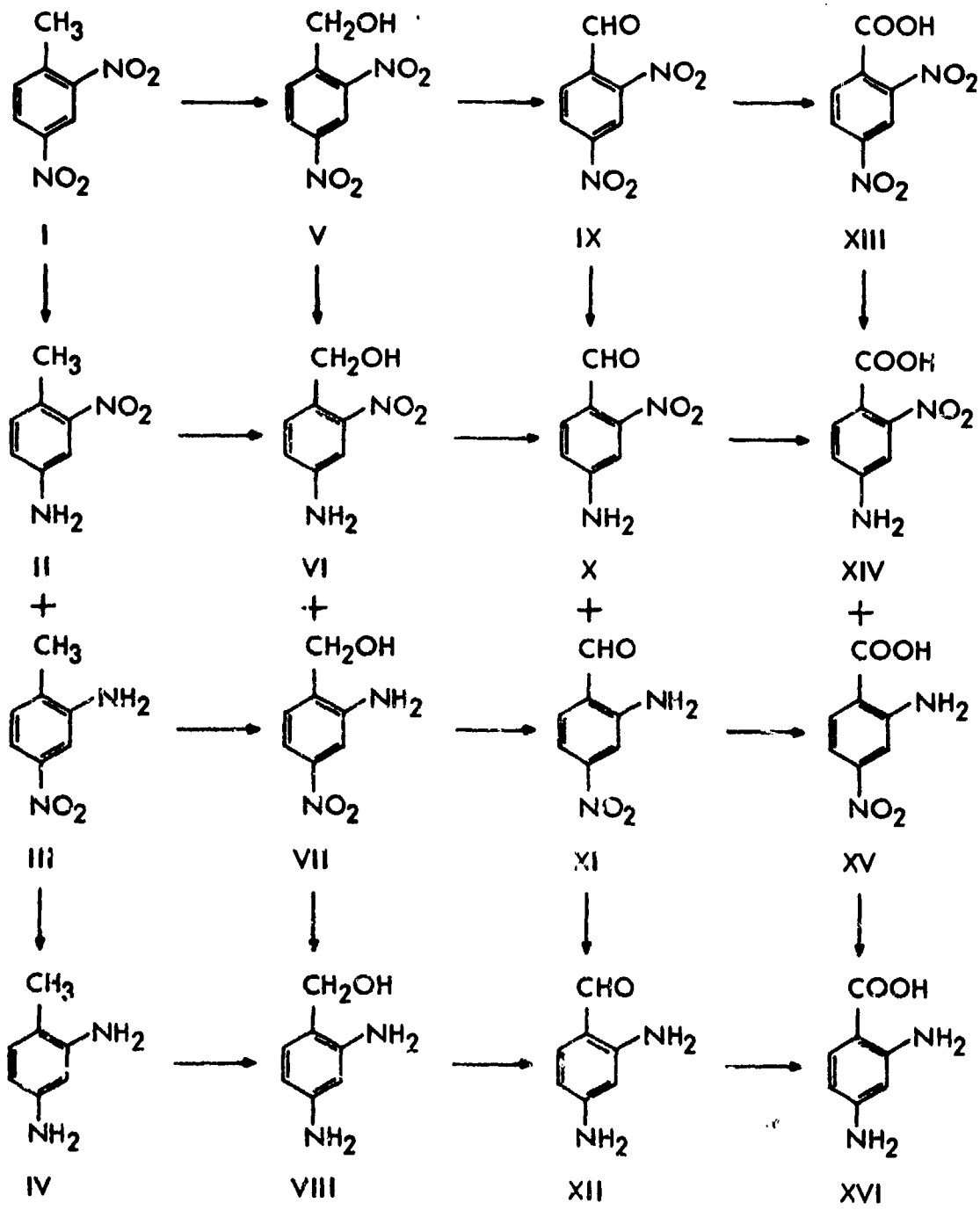


Figure 3 - Possible Early Steps (Phase I) of Metabolic Pathways of 2,4-DNT in Mammals

V. GENERAL SUMMARY AND CONCLUSIONS

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V. GENERAL SUMMARY AND CONCLUSIONS

A. Toxic Effects

1. Toxic and Nontoxic Doses

Dogs were the most sensitive species tested in these subchronic dose studies, with 5 mg/kg/day having no effects and 25 mg/kg/day being toxic to all and lethal to some. In rats, a dose in feed giving 34 mg/kg/day to males and 38 mg/kg/day to females was slightly toxic; no nontoxic dose was found in this study. Mice were much less affected, since intakes of 137 mg/kg/day by males and 147 mg/kg/day be females had no adverse effects, and intakes of the high dose of 415 mg/kg/day by males and 468 mg/kg/day were not as toxic as much lower doses given dogs or rats.

The cause of the relative nontoxicity of 2,4-DNT to mice is apparent in the absorption studies. Mice only absorbed 8 to 12% of an oral dose, while rats, rabbits, dogs and monkeys absorbed 75 to 85% of the dose. When this effect was seen in the albino strain used in the toxicity study (CD-1®), we considered the possibility of a peculiarity in that strain. However, virtually identical results were seen in the pigmented B6C3F1 strain (F_1 hybrids of C57 BL/6 black mice and C3H agouti mice), the standard strain of the NCI Carcinogenesis Bioassay Program. Therefore, the low absorption of 2,4-DNT is a peculiarity of the species Mus musculus.

A noteworthy phenomenon is the extreme individual variation in susceptibility to 2,4-DNT toxicity. A good example is the dogs given 25 mg/kg/day. On day 22, one died, but two others were finally having minimal toxic signs.

2. Target Organs

Typical non-specific toxic effects (decreased weight gain or even weight loss) were seen in the rodents. This was the only effect seen in the low-dose rats.

All species had methemoglobinemia and its sequelae (Heinz bodies, anemia, reticulocytosis, hemosiderosis, etc.) in varying degrees.

Dogs and, to a lesser extent, rats had neuromuscular symptoms of incoordination and rigid paralysis. Some gliosis and demyeliniation was seen, but the connection between lesions and symptoms is not clear.

Males of all species tested had decreased spermatogenesis. In sufficient dose, this could produce functional sterility as seen in the dominant lethal mutation study in rats.

3. Special Toxicity Tests

No increases of immunoglobulin E were seen in dogs and rats.

No unequivocal mutagenic effect was seen in the cytogenetics, dominant lethal mutation, and cell culture studies. The toxic effect of 2,4-DNT on spermatogenesis confounded the dominant lethal mutation study. The chromatid breaks and gaps seen in the cytogenetics study have dubious predictive value.

B. Disposition and Metabolism

1. Absorption, Distribution, Metabolism and Excretion Studies

Oral doses of radiolabelled 2,4-DNT were poorly absorbed (8 to 12% of the dose) by two strains of mice, but well absorbed (75 to 85%) by rats, rabbits, dogs and monkeys. Once absorbed, the compound was handled similarly in all species. Concentrations of radiolabel were found in the liver and kidney. No radiolabel was found in exhaled air, but most of that absorbed was excreted in the urine within 24 hours. Metabolic processes, as determined from urinary metabolites, included reduction of one or both nitros to aminos, oxidation of the methyl to an alcohol or benzoic acid, and conjugation with sulfate or glucuronate.

2. Biliary Excretion Studies

In the female rats, portions of oral doses of radiolabelled TNT or DNT isomers were excreted into the bile within 15 minutes of dosing. There were variations between the compounds with respect to time to peak biliary excretion rate (15 minutes for 3,4-DNT to 6 hours for 4-amino-2,6-DNT), total biliary excretion in 24 hours (10.3% of dose for TNT to 27.3% for 2,3-DNT), and radioactivity remaining in the GI tract, its contents, and feces (3.1% of dose for 2,3-DNT to 46.8% for 4-amino-2,6-DNT).

3. In Vitro Metabolism Studies

In vitro liver homogenates from mice, rats, rabbits, dogs and monkeys metabolized 8 to 27% of added 2,4-DNT within 1 hour. The primary product under aerobic conditions was 2,4-dinitrobenzyl alcohol; under anaerobic, aminonitrotoluenes. These compounds are the first products form in vivo, also.

4. Metabolic Interaction Studies

Feeding 0.7% 2,4-DNT (high dose in toxicity study) to rats for 2 weeks did not affect liver enzymes, as determined by zoxazolamine paralysis time and hepatic nitroanisole O-demethylase activity.

C. Future Research

1. Exposure Standards

2,4-DNT is obviously toxic to mammals. Setting a reliable exposure standard will require a lifetime exposure to evaluate carcinogenicity. Because of their aberrant absorption, mice would be of relatively little use in standard setting.

2. Toxic Effects

Additional work will be necessary to fully understand some of the toxic effects of 2,4-DNT.

Methemoglobinemia is a well known effect.[5] The only remaining question is what exposure level in humans produces no effect, a routine industrial hygiene problem. Heinz bodies seem to be a useful diagnostic tool.

The neuromuscular effect has not been reported previously. Its mechanism is unknown. It may be related to some neurologic complaints of 2,4-DNT worker,[7] but that is a guess. Because of this lack of knowledge, extrapolation to humans is less certain than for better-studied effects, such as methemoglobinemia.

The depressed spermatogenesis is also a new effect for these compounds. The most serious unanswered question is its reversibility--how great a depression can the victim recover from? The accompanying toxicity is so great that it is unlikely that 2,4-DNT would be a useful lead compound for development of a male contraceptive.

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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:

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

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

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

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

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

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

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

B. Clinical Blood Tests

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

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

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

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

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

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

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

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

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

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

C. Urinalysis

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

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

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

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

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

D. Occult Blood in Feces

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

E. Precision of Hematology and Clinical Blood Chemistry Tests

1. Reproducibility

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

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

2. Reproducibility Within a Test Day

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

3. Proficiency Test Service

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

II. HISTOPATHOLOGY

A. Necropsy and Gross Examination

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

B. Organ Weights

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

C. Tissues for Microscopic Examination

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

D. Fixation and Staining of Tissues

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

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

III. STATISTICAL ANALYSIS

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

IV. NORMAL VALUES

A. Hematology, Clinical Laboratory Tests and Bone Marrow

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

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

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

B. Absolute and Relative Organ Weights

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

C. Presence of Various Substances in the Urine

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

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

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

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

D. Occult Blood in the Feces

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

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TABLE A

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

	No. of Determinations	Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)			
Normal level	20	4.51 ± 0.07	4.36 - 4.67
Abnormal level	20	2.32 ± 0.04	2.25 - 2.40
Hematocrit (vol %)			
Normal level	20	44.3 ± 0.40	44 - 45
Abnormal level	20	22.8 ± 0.60	22 - 24
Hemoglobin (gm %)			
Normal level	20	14.2 ± 0.20	13.6 - 14.5
Abnormal level	20	7.4 ± 0.20	6.9 - 7.8
Leukocyte Counts (x 10^3/mm^3)			
Normal level	20	7.3 ± 0.50	6.8 - 8.7
Abnormal level	20	17.6 ± 0.80	16.3 - 18.7
Fasting Blood Glucose (mg %)	20	163.0 ± 7.5	151 - 178
SGOT (IU/ℓ)	23	61.7 ± 3.9	55 - 68
SGPT (IU/ℓ)	23	51.3 ± 2.6	46 - 55
Creatinine (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/]

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

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

TABLE C

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

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

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

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

TABLE D

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

	Male Rhesus Monkeys			
	Number Studied	Body Weight (kg) Mean ± S.D.	Observed Results Mean ± S.D.	Range
Erythrocytes (x $10^6/mm^3$)	108	3.74 ± 0.50	5.51 ± 0.45	3.75 - 6.61
Reticulocytes (%)	108	3.74 ± 0.50	0.97 ± 0.82	0.07 - 2.41
Hematocrit (vol %)	108	3.74 ± 0.50	43.0 ± 2.6	37.0 - 50.0
Hemoglobin (gm %)	108	3.74 ± 0.50	13.4 ± 0.8	10.8 - 15.4
MCV (μ^3)	108	3.74 ± 0.50	77.8 ± 7.0	69.5 - 117.3
MCHb ($\mu\mu g$)	108	3.74 ± 0.50	24.4 ± 1.8	21.0 - 33.6
MCHbC (mg %)	108	3.74 ± 0.50	31.4 ± 1.3	27.2 - 34.1
Platelets (x $10^5/mm^3$)	99	3.74 ± 0.50	3.08 ± 0.45	0.80 - 7.10
Leukocytes (x $10^3/mm^3$)	108	3.74 ± 0.50	10.4 ± 4.9	3.8 - 30.1
Neutrophils I (%)	108	3.74 ± 0.50	0.18 ± 0.45	0 - 2
Neutrophils M (%)	108	3.74 ± 0.50	39.30 ± 17.72	10 - 83
Lymphocytes (%)	108	3.74 ± 0.50	56.83 ± 17.74	13 - 84
Eosinophils (%)	108	3.74 ± 0.50	1.91 ± 2.42	0 - 13
Monophils (%)	108	3.74 ± 0.50	1.37 ± 1.58	0 - 7
Basophils (%)	108	3.74 ± 0.50	0.04 ± 0.20	0 - 2
Atypical cells (%)	108	3.74 ± 0.50	0.00 ± 0.00	0 - 0
Nucleated RBC (%)		3.74 ± 0.50	0.00 ± 0.00	0 - 0
Fasting Glucose (mg %)	100	3.76 ± 0.51	96.9 ± 15.2	59 - 127
SGOT (IU/ℓ)	100	3.76 ± 0.51	33.7 ± 9.2	20 - 60
SGPT (IU/ℓ)	100	3.76 ± 0.51	31.3 ± 7.8	15 - 46
Alkaline Phosphatase (IU/ℓ)	100	3.76 ± 0.51	360.0 ± 116.0	143 - 501
BUN (mg %)	100	3.76 ± 0.51	19.5 ± 7.3	12 - 65
Proth Time (sec)	62	3.91 ± 0.44	10.2 ± 0.7	9.3 - 11.9
Serum Creat. (mg %)	100	3.76 ± 0.51	1.1 ± 0.3	0.6 - 1.8
Bilirubin				
Total (mg %)	62	3.91 ± 0.44	0.1 ± 0.2	0.0 - 0.8
Direct (mg %)	62	3.91 ± 0.44	0.0 ± 0.0	0.0 - 0.0
BSP 15 min (% ret.)	62	3.91 ± 0.44	18.0 ± 7.4	2 - 34
Na (mEq/ℓ)	62	3.91 ± 0.44	154.0 ± 19.1	144 - 179
K (mEq/ℓ)	62	3.91 ± 0.44	4.8 ± 0.6	3.9 - 5.7
Cl (mEq/ℓ)	62	3.91 ± 0.44	109.0 ± 6.4	93 - 118
Ca (mEq/ℓ)	62	3.91 ± 0.44	5.2 ± 0.4	4.2 - 6.3
Mg (mEq/ℓ)	62	3.91 ± 0.44	1.6 ± 0.1	1.2 - 1.8
Bone Marrow				
Myeloid/erythroid ratio	15	3.65 ± 0.41	1.5 ± 0.3	1.5 - 2.1

a/ Data collected between June 1971 and December 1976.

TABLE E

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

	Female Rhesus Monkeys			
	Number Studied	Body Weight (kg) Mean ± S.D.	Observed Results Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)	81	3.51 ± 0.48	5.33 ± 0.49	4.25 - 6.03
Reticulocytes (%)	81	3.51 ± 0.48	1.07 ± 0.54	0.35 - 3.31
Hematocrit (vol %)	81	3.51 ± 0.48	41.5 ± 2.8	30.0 - 46.0
Hemoglobin (gm %)	81	3.51 ± 0.48	13.1 ± 1.0	7.9 - 14.1
MCV (μ^3)	81	3.51 ± 0.48	77.7 ± 5.3	66.5 - 95.2
MCHb ($\mu\mu$g)	81	3.51 ± 0.48	24.6 ± 1.7	17.6 - 29.7
MCHbC (mg %)	81	3.51 ± 0.48	31.6 ± 1.4	26.6 - 34.2
Platelets (x 10^5/mm^3)	81	3.51 ± 0.48	3.11 ± 1.23	1.85 - 7.90
Leukocytes (x 10^3/mm^3)	81	3.51 ± 0.48	9.5 ± 3.9	3.2 - 24.8
Neutrophils I (%)	81	3.51 ± 0.48	0.10 ± 0.43	0 - 3
Neutrophils M (%)	81	3.51 ± 0.48	36.41 ± 13.32	13 - 56
Lymphocytes (%)	81	3.51 ± 0.48	60.38 ± 13.26	41 - 79
Eosinophils (%)	81	3.51 ± 0.48	2.28 ± 3.10	0 - 18
Monophils (%)	81	3.51 ± 0.48	0.75 ± 0.98	0 - 4
Basophils (%)	81	3.51 ± 0.48	0.05 ± 0.22	0 - 1
Atypical cells (%)	81	3.51 ± 0.48	0.00 ± 0.00	0 - 0
Nucleated RBC (%)	74	3.56 ± 0.50	0.00 ± 0.00	0 - 0
Fasting Glucose (m	81	3.51 ± 0.48	92.1 ± 15.3	57 - 116
SGOT (IU/ℓ)	81	3.51 ± 0.48	32.1 ± 7.6	20 - 70
SGPT (IU/ℓ)	81	3.51 ± 0.48	30.1 ± 7.6	12 - 39
Alkaline Phosphatase (IU/ℓ)	81	3.51 ± 0.48	349.9 ± 112.3	148 - 572
BUN (mg %)	81	3.51 ± 0.48	17.3 ± 4.2	13 - 29
Proth. Time (sec)	59	3.56 ± 0.43	10.5 ± 0.9	9.7 - 12.3
Serum Creat. (mg %)	81	3.51 ± 0.48	1.1 ± 0.3	0.6 - 1.7
Bilirubin				
Total (mg %)	81	3.51 ± 0.48	0.1 ± 0.1	0.0 - 0.8
Direct (mg %)	81	3.51 ± 0.48	0.0 ± 0.0	0.0 - 0.0
BSP 15 min (% ret.)	59	3.56 ± 0.43	16.4 ± 8.3	5 - 34
Na (mEq/ℓ)	59	3.56 ± 0.43	158.2 ± 6.5	147 - 174
K (mEq/ℓ)	59	3.56 ± 0.43	4.8 ± 0.7	3.9 - 6.2
Cl (mEq/ℓ)	59	3.56 ± 0.43	109.0 ± 6.1	95 - 113
Ca (mEq/ℓ)	59	3.56 ± 0.43	5.3 ± 0.5	4.3 - 6.3
Mg (mEq/ℓ)	59	3.56 ± 0.43	1.6 ± 0.2	1.3 - 2.0
Bone Marrow				
Myeloid/erythroid ratio	11	3.49 ± 0.62	1.4 ± 0.3	1.0 - 1.8

a/ Data collected between June 1971 and December 1976.

TABLE F

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

Organ Weight	Absolute Mean ± S.D.	Range
Liver (gm)	82 ± 17	64 - 122
Spleen (gm)	4.6 ± 1.8	2.0 - 9.3
Kidneys (gm)	15.1 ± 3.8	8.0 - 22.0
Adrenals (gm)	0.73 ± 0.15	0.45 - 0.86
Thyroids (gm)	0.57 ± 1.30	0.37 - 0.81
Testes (gm)	1.29 ± 0.67	0.53 - 3.30
	Relative (per kg body weight) Mean ± S.D.	Range
Liver (gm)	23.4 ± 2.5	18.8 - 30.4
Spleen (gm)	1.25 ± 0.47	0.57 - 2.38
Kidneys (gm)	4.13 ± 0.92	2.20 - 6.43
Adrenals (mg)	201 ± 44	129 - 254
Thyroids (mg)	154 ± 42	86 - 250
Testes (gm)	0.34 ± 0.11	0.18 - 0.53

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

TABLE G

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

Organ Weight	Absolute Mean ± S.D.	Range
Liver (gm)	83 ± 17	64 - 122
Spleen (gm)	3.8 ± 1.4	2.0 - 6.0
Kidneys (gm)	14.5 ± 2.8	11.0 - 20.0
Adrenals (gm)	0.68 ± 0.16	0.53 - 1.14
Thyroids (gm)	0.60 ± 0.20	0.37 - 1.11
Ovaries (gm)	0.28 ± 0.10	0.14 - 0.45
	Relative (per kg body weight) Mean ± S.D.	Range
Liver (gm)	25.4 ± 5.8	19.2 - 37.4
Spleen (gm)	1.16 ± 0.49	0.60 - 1.89
Kidneys (gm)	4.40 ± 0.86	3.20 - 6.25
Adrenals (mg)	212 ± 80	138 - 438
Thyroids (mg)	173 ± 66	97 - 346
Ovaries (mg)	82 ± 28	43 - 140

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

TABLE H

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

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

a/ Data collected between September 1971 and December 1976.

TABLE I

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

	Female Beagle Dogs			Observed Results	
	Number Studied	Age (months)	Body Weight (kg) Mean ± S.D.	Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)	257	4 - 7	6.9 ± 1.3	5.59 ± 0.73	3.27 - 7.75
Reticulocytes (%)	265	4 - 7	6.9 ± 1.3	0.74 ± 0.52	0.04 - 5.05
Hematocrit (vol %)	257	4 - 7	6.9 ± 1.3	42.3 ± 3.5	32 - 51
Hemoglobin (gm %)	257	4 - 7	6.9 ± 1.3	13.7 ± 1.3	11.0 - 18.6
MCV (μ^3)	257	4 - 7	6.9 ± 1.3	76.7 ± 9.7	55.8 - 128.4
MCHb ($\mu\mu$g)	257	4 - 7	6.9 ± 1.3	24.8 ± 3.3	17.1 - 41.6
MCHbC (mg %)	257	4 - 7	6.9 ± 1.3	32.3 ± 1.6	28.7 - 40.4
Platelets (x 10^5/mm^3)	227	4 - 7	6.9 ± 1.3	3.08 ± 1.15	1.08 - 7.95
Leukocytes (x 10^3/mm^3)	265	4 - 7	6.9 ± 1.3	10.9 ± 3.4	3.8 - 26.9
Neutrophils I (%)	265	4 - 7	6.9 ± 1.3	0.54 ± 1.16	0 - 7
Neutrophils M (%)	265	4 - 7	6.9 ± 1.3	57.08 ± 10.10	31 - 85
Lymphocytes (%)	265	4 - 7	6.9 ± 1.3	37.15 ± 10.46	10 - 61
Eosinophils (%)	265	4 - 7	6.9 ± 1.3	2.37 ± 2.25	0 - 13
Monophils (%)	265	4 - 7	6.9 ± 1.3	1.94 ± 2.01	0 - 9
Basophils (%)	265	4 - 7	6.9 ± 1.3	0.01 ± 0.09	0 - 1
Atypical cells (%)	265	4 - 7	6.9 ± 1.3	0.11 ± 0.43	0 - 4
Nucleated RBC (%)	265	4 - 7	6.9 ± 1.3	0.03 ± 0.17	0 - 2
Fasting Glucose (mg %)	248	4 - 7	6.9 ± 1.3	99.6 ± 14.4	55 - 130
SGOT (IU/ℓ)	257	4 - 7	6.9 ± 1.3	23.5 ± 7.2	6 - 52
SGPT (IU/ℓ)	257	4 - 7	6.9 ± 1.3	25.3 ± 7.0	8 - 49
Alkaline Phosphatase (IU/ℓ)	257	4 - 7	6.9 ± 1.3	73.5 ± 19.2	30 - 146
BUN (mg %)	265	4 - 7	6.9 ± 1.3	12.4 ± 3.3	4 - 26
Bone Marrow					
Myeloid/erythroid ratio	34	5 - 9	7.8 ± 1.4	1.4 ± 0.3	1.1 - 2.4

a/ Data collected between September 1971 and December 1976.

TABLE J

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

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

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

TABLE K

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

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

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

TABLE L

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

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

a/ Data collected between September 1971 and December 1976.

TABLE M

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

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

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

TABLE N

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

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

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

TABLE O

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

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

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

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

c/ Percent of total (number of samples).