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DA 126069

INSTITUTE REPORT NO. 138

FOURTEEN-DAY FEEDING STUDY OF 2,4-DINITROTOLUENE
IN MALE AND FEMALE RATS

EVELYN L. MCGOWN, PhD
JOHN J. KNUDSEN, BS
GEORGE T. MAKOVEC, DVM, CPT VC
and
GLEN E. MARRS, JR., DVM, MS, MAJ VC

DIVISION OF RESEARCH SUPPORT

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Toxicology Series 43

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FOURTEEN-DAY FEEDING STUDY OF 2,4-DINITROTOLUENE IN MALE AND FEMALE RATS
--McGown et al

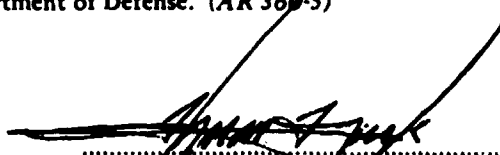
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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER Institute Report No. 138	2. GOVT ACCESSION NO. AD A126069	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Fourteen-Day Feeding Study of 2,4-dinitrotoulene in Male and Female Rats		5. TYPE OF REPORT & PERIOD COVERED Final 18 Feb-25 Mar 81
7. AUTHOR(s) E.L. McGown, PhD; J.J. Knudsen, BS; G.T. Makovec, DVM, CPT VC; and G.E. Marrs, Jr., DVM, MS, MAJ VC		6. PERFORMING ORG. REPORT NUMBER
9. PERFORMING ORGANIZATION NAME AND ADDRESS Division of Research Support, Letterman Army Institute of Research, Presidio of San Francisco, CA 94129		8. CONTRACT OR GRANT NUMBER(s)
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research and Development Command Fort Detrick Frederick, MD 21701		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 611102.56.0.01.350, WU 050
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		12. REPORT DATE February 1983
		13. NUMBER OF PAGES 64
		15. SECURITY CLASS. (of this report) UNCLASSIFIED
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) APPROVED FOR PUBLIC RELEASE; DISTRIPTION IS UNLIMITED		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) 2,4-dinitrotoluene, Rats, Feeding Study		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The purpose of this study was to obtain preliminary data in rats concern- ing effects of 2,4-dinitrotoluene (DNT) on tissue histology, blood chemistry, hematology, and urinary parameters. Male and female rats were fed lab chow diet to which was added 0, 1.0, 1.5, 2.5, or 3.5 g DNT/kg. After 14 days, the animals were farced in metabolic cages, killed, and necropsies were performed. No DNT-related effects were found on blood hematology or urinary parameters. Blood cholesterol was elevated in all groups receiving DNT. A dose-dependent elevation in blood glucose was observed in both sexes, but the effect was		

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statistically significant only in females. Alanine aminotransferase was elevated in all male groups fed the compound. DNT induced the development of hyaline droplets in the epithelium of the proximal convoluted tubules of both males and females. Oligospermatisms with degenerative changes in the testes of male rats was observed in a dose-dependent response.

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ABSTRACT

The purpose of this study was to obtain preliminary data in rats concerning effects of 2,4-dinitrotoluene (DNT) on tissue histology, blood chemistry, hematology, and urinary parameters. Male and female rats were fed a laboratory chow diet to which was added 0, 1.0, 1.5, 2.5, and 3.5 g DNT/kg. After 14 days, the animals were fasted in metabolic cages, killed, and necropsies were performed. No DNT-related effects were found on blood hematology or urinary parameters. Blood cholesterol was elevated in all groups receiving DNT. A dose-dependent elevation in blood glucose was observed in both sexes, but the effect was statistically significant only in the females. Alanine aminotransferase was elevated in all male groups fed the compound. DNT induced the development of hyaline droplets in the epithelium of the proximal convoluted tubules of the kidneys of both males and females. Oligospermatism with degenerative changes in the testes of male rats was observed in a dose-dependent response.

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PREFACE

REPORT: Fourteen-Day Feeding Study (Pilot)

TESTING FACILITY: Division of Research Support
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

SPONSOR: Same as above

PROJECT: Military Toxicology 611102.56.0.OT.350
APC NLO4

WORK UNIT: 050 Toxicology of Explosives and
Explosive By-Products

GLP STUDY NUMBER: 81001

STUDY DIRECTOR: COL John T. Fruin, DVM, PhD, VC,
Diplomate, American College of Veterinary
Preventive Medicine

PRINCIPAL INVESTIGATOR: Evelyn L. McGown, PhD

PATHOLOGY REPORTING: CPT George T. Makovec, DVM, VC
and
MAJ Glen E. Mears, Jr., DVM, MS, VC

STATISTICIAN: Virginia L. Gildengorin, PhD

REPORT AND DATA MANAGEMENT: All raw data, a copy of the final report,
study protocol, and retired SOPs will be
retained in the LAIR Archives.

TEST SUBSTANCE: 2,4-dinitrotoluene

INCLUSIVE STUDY DATES: 18 Feb - 25 Mar 81

PURPOSE OF STUDY: To determine:

- a. Acceptance of test compound in feed.
- b. The best available feeding system.
- c. Preliminary data concerning effects of 2,4-dinitrotoluene on blood chemistry and hematology parameters, urinary parameters and tissue histology.

ACKNOWLEDGMENTS

The authors wish to thank SSG Lance White for preparing the diets, tabulating the daily weight gain and feed consumption data, and general supervision of the operation; SP5 Florence McKinley, BA, and SP5 Marlin McKinley, BA, for their efforts with TOXSYS operation, compilation of clinical signs, and assistance with necropsies and tissue preparation; SP4 Charlotte Speckman, SP4 Thomas Kellner, BS, SP4 Laurence Mullen, BS, and Carolyn Lewis, MS, for performing the daily observations and maintaining the health care of the animals; Mary Lyons for performing the clinical chemistry analyses; Jerry Ann Tillotson, MS, for the diet DNT assays; SP4 Thomas Loughhead for hematologic examinations; SP5 Cecilio Dumlao and Mr. Thomas Hironaga for histologic preparations of tissues; and Ann Wilkinson, R. Gordon Williamson, Jr., and Muriel Paul for typing the manuscript.

SIGNATURES OF PRINCIPAL SCIENTISTS

AND MANAGERS

INVOLVED IN THE STUDY

We, the undersigned, believe the study 81001 described in this report to be scientifically sound and the results in this report and interpretation to be valid. The study was conducted to comply, to the best of our ability, with the Good Laboratory Practice Regulations for Non-Clinical Laboratory Studies outlined by the Food and Drug Administration.

John T. Fruin 4 Oct 82
JOHN T. FRUIN, DVM, PhD / DATE
 COL, VC
 Study Director

Evelyn L. McGown 26 Oct 82
EVELYN L. MCGOWN, PhD / DATE
 DAC, Principal Investigator

Glen E. Marrs Jr. 4 Oct 82
GLEN E. MARRS, DVM / DATE
 CPT, VC

Virginia L. Gildengorin 26 Oct 82
VIRGINIA L. GILDENGORIN, PhD / DATE
 DAC, Statistician

George T. Makovec 7 Oct 82
GEORGE T. MAKOVEC, DVM / DATE
 CPT, VC
 Pathologist

John J. Knudsen 14 Oct 82
JOHN J. KNUDSEN, BS / DATE
 DAC, Data Manager

Paul W. Mellick
PAUL W. MELLICK, DVM, PhD / DATE
 LTC, VC
 Chief, Pathology Services Group



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LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

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ATTENTION OF:

SGRD-ULZ-QA

1 February 1983

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 81001, the following inspections were made:

18 February 1981
2 March 1981, 0800 hours
2 March 1981
18 March 1981, 1045 hours
18 March 1981, 1015 hours
18 March 1981, 1100 hours
18 March 1981, 1430 hours

The report and raw data for this study were audited on 2 Sep 82.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the April 1981 report to management and the Study Director.

JOHN C. JOHNSON
CPT (P), MSC
Quality Assurance Officer

TABLE OF CONTENTS

	Page
Abstract.	i
Preface	iii
Acknowledgments	iv
Signatures of Principal Scientists.	v
Table of Contents	vii
BODY OF REPORT	
INTRODUCTION	1
MATERIALS AND METHODS.	1
Test Substance	1
Historical Listing of Significant Study Events	1
Animal Data.	2
Environmental Conditions	3
Observations	3
Duration of Study.	3
Preparation of Diets	3
Sample Collection and Histopathology	3
Statistical Analyses	4
RESULTS	4
General Observations	4
Diet Consumption and 2,4-DNT Intake.	4
Clinical Blood Chemistry	5
Pathology.	5
DISCUSSION	6

Table of Contents (Cont)

	Page
CONCLUSIONS.	7
RECOMMENDATIONS.	7
REFERENCES	8
APPENDICES	
Appendix A - List of Figures	9
Figures 1-4	
Appendix B - List of Tables.	15
Tables 1-10	
Appendix C	
Gossary of Microscopic Lesions.	49
Appendix D	
Pathology Results	51
DISTRIBUTION LIST	53

FOURTEEN-DAY FEEDING STUDY OF 2,4-DINITROTOLUENE IN MALE AND FEMALE RATS
-McGown et al

"PINK WATERS" are aqueous effluents from plants which manufacture 2,4,6-trinitrotoluene (TNT). One of the toxic constituents of the waste water is 2,4-dinitrotoluene (DNT). The US Army Medical Research and Development Command has sponsored several studies to acquire an adequate data base because of the need to set standards for military-unique product discharge into waters subject to State and Federal regulations.

The major objective of the present 14-day feeding was to establish within LAIR the capability to conduct toxicity assessment feeding studies in compliance with the proposed EPA Good Laboratory Regulations. The purpose was also to collect preliminary data concerning the effects of oral consumption of 2,4-DNT on blood chemistry and hematology parameters, urinary parameters, and tissue histology. The choice of 2,4-DNT as a test substance was logical because of its military relevance and because of the existence of a data base with which the results could be compared.

MATERIALS AND METHODS

Test Substance

The test substance was 2,4-dinitrotoluene purchased from Matheson, Coleman and Bell, Norwood, Ohio 45212. Pertinent chemical data are summarized in Table 1.

Historical Listing of Significant Study Event

18 Feb 81	26 male and 26 female rats (26 days old) arrived at LAIR. They were weighed, ear-tagged, examined for disease and housed individually.
19 Feb 81	One male rat and one female rat were submitted to Pathology for complete necropsy to assure the health of the animals received.

20-24 Feb 81 Animals were weighed.

24 Feb-3 Mar 81 Food consumption data were collected.

4 Mar 81 Animal observations daily at 0600 and 1200 hours. Food consumption and animals' weight data were collected every other day.

17 Mar 81 Animals transferred to metabolic cages for urine collection, fasted.

18 Mar 81 Urine samples were taken to clinical lab. All animals were necropsied. Blood and tissue samples were taken for specific measurements.

Animal Data

Species: Rat (*Rattus norvegicus*)

Strain: Sprague-Dawley

Sex: Male and female

Source: Charles River

Pre-Test Conditioning:

1. Quarantine observation: 18 Feb through 24 Feb 81.
2. Diet: Certified Purina Rodent Chow #5002.

Method of Randomization: Manual, Random Number Table, applied to weight-ranked animals.

Number of Animals in Each Group: 5 males and 5 females (except group 4 females had only 4; one removed because of structural defect).

Age of Animals at Start of Study: one month old.

Condition of Animals at Start of Study: Apparently healthy.

Body Weight Range at Beginning of Test Feeding: females 107-139 g;
males 131-164 g.

Animal Identification: eartag (SOP OP-ARG-1).

Environmental Conditions

Number of Animals Per Cage: One.

Type of Cage Used During Study: Stainless steel cage battery,
wire bottom, no bedding.

Water: Ionized water provided by automatic watering devices.

Food: Purina Rodent Chow Certified Diet #5002, ad libitum.

Temperature: 69-72 degrees F (20.6-22.2 C).

Humidity: Generally 49-51%. Occasional peaks as high as 66%.

Photo Period: Lights on at 0600-2000 hours (14 hours).

Observations

Animals were observed daily during the quarantine period. During the feeding study, animals were observed at 0600 and 1800 hours for clinical signs of toxicity. Findings are reported later in this report.

Duration of Study

The feeding study lasted 14 days after a 7-day period of quarantine and acclimatization.

Preparation of Diets

Test compound was incorporated into Certified Purina Rodent Chow #5002 as specified in OP-STX-16 and OP-STX-38. The intended levels were 1.0, 1.5, 2.0, and 3.5 g 2,4-DNT/kg diet. After extraction of the DNT, assay by HPLC (OP-ACH-2) indicated that the actual levels were at 0.9, 1.2, 1.9, and 3.0 g/kg diet.

Sample Collection and Histopathology

Urine samples were collected from fasting rats in metabolic cages on the day before scheduled necropsy. Urines were evaluated for the following: color, specific gravity, urobilinogen, occult blood, bile, ketones, glucose, protein, nitrite, and pH. They were also examined microscopically for the presence of white blood cells, red blood cells, epithelial cells, red blood cell casts, white blood cell casts, hyaline casts, granular casts, bacteria, crystals, and mucous. Following anesthesia with sodium pentobarbital administered intraperitoneally, blood was collected from the abdominal aorta of each rat and submitted for examination (red blood cell count, hemoglobin, packed cell volume, white blood cell count, and white

blood cell differential). Additional blood was submitted to Analytical Chemistry Services Group, Division of Research Support, for chemical analyses. All rats were killed by exsanguination and complete gross necropsy examinations were performed. Tissue specimens from all major organs and systems were fixed in 10% neutral buffered formalin for subsequent histologic examination. Tissues were embedded in paraffin, sectioned at approximately 6 microns thickness and stained with hematoxylin and eosin. All tissues itemized in SOP OP-PSG-13 were examined microscopically in the highest two dosage groups (groups 3 and 1) and the control group (group 4). In the lower two dosage groups (groups 2 and 5), only kidneys and testes were examined microscopically.

Statistical Analyses

A computer package, Minital, on the Eclipse C330 computer was utilized in analyzing the 14-day 2,4-DNT clinical chemistry, hematology, and urine analysis data. Assuming no differences between males and females, the two groups were combined and a one-way analysis of variance was carried out on the blood and urine analysis measurements. Descriptive statistics were calculated for each variable. In addition, individual 95% confidence intervals based on a pooled standard deviation were plotted to examine the group means.

RESULTS

General Observations and Weight Gain

No gross evidence of toxicity was observed during the 14-day period of this study. Summaries of clinical observations for male and female rats are presented in Tables 2 and 3.

The average body weights of the male and female rats during the two-week treatment are summarized in Figures 1 and 2. All treatment groups gained weight consistently after the first four days of the treatment period. Average weight gain was greatest in the control groups and smallest in the high dosage groups (0.35% 2,4-DNT). The low and middle dosage groups (0.1%, 0.15%, and 0.2% 2,4-DNT) averaged weight gains lower than the control groups but greater than the high dosage groups. Statistical analysis was not performed on rat weight gain data.

Diet Consumption and 2,4-DNT Intake

Diet consumption of male and female rats fed various doses of 2,4-DNT are summarized in Figures 3 and 4. All dosage groups appeared to exhibit an initial aversion to the diet, possibly due to an effect of the test compound on diet palatability. Except for this anomaly, the diet results generally reflected weight gain changes. The high dose groups (0.35% 2,4-DNT) ate one-third to one-half as much diet as did the control groups; the low and middle dosage groups ate

intermediate amounts of diet. Statistical analysis was not performed on diet consumption data.

Average daily 2,4-DNT intake as a function of animal weight was not compiled for animals in this study.

Clinical Blood Chemistry

Clinical blood chemistry results for both male and female rats are summarized in Table 4. In some groups, the number of samples/group was less than 5 because plasma sample volumes were too small for all analyses to be performed. (Blood chemistry analyses were conducted in order of priority.) The data were analyzed statistically by analysis of variance followed by Dunnett's multiple comparison procedures. The chosen level of significance was 0.05. Other than as noted below, no significant differences between the control groups and the various dosage groups for the clinical substances measured were observed.

The following significant differences between control groups and the various dosage groups were observed in blood collected at the end of the 14-day study period.

Alanine aminotransferase: All dosage groups were significantly higher than the control group at the 0.05 level for male rats. No significant difference between dosage groups and the control group was observed in the female rats.

Cholesterol: All dosage groups were significantly higher than the control groups at the $p < 0.05$ level for both male and female rats.

Glucose: The 0.35% 2,4-DNT dosage group was significantly higher than the control group for female rats. The males showed the same trend, but the differences were not statistically significant.

Albumin/Globulin Ratio: The 0.15% and 0.35% 2,4-DNT dosage groups were significantly ($p < .05$) higher than the control group for the female rats. No significant difference was observed between any test groups and the control group for male rats.

Pathology

The hematology data are summarized in Table 5. No DNT-related effects were detected on hematologic parameters.

Because of contamination in urine specimens collected in metabolic cages, only pH and specific gravity measurements were considered valid or meaningful. These results are summarized in Table 6. No differences were detected in mean specific gravity values. Mean pH values decreased with increasing DNT dose, but the trend was not statistically significant.

A glossary of the microscopic lesions encountered is in Appendix C. Table 7 contains a key to the microscopic findings listed in Tables 8-10. The incidence and severity of lesions observed in rat tissue obtained at necropsy are tabulated in Tables 8 and 9. Table 10 contains a summary of the incidence of lesions in each tissue according to treatment group. DNT induced the development of hyaline droplets in the epithelium of the proximal convoluted tubules of the kidneys of both male and female rats. The response did not appear to be dose-dependent. The male rats were more sensitive than the females with respect to hyaline droplet formation. DNT induced oligospermia, syncytial cell formation, and decreased thickness of spermatogenic cell layers in the seminiferous tubules in the testes of males. The severity of the pathological lesions in the testes was related to the dose.

Appendix D contains a more detailed summary of pathological observations.

DISCUSSION

Dietary DNT caused a dose-dependent decrease in rate of weight gain. This was presumably due to the dose-dependent depression in food consumption.

Serum cholesterol was the only blood parameter which was significantly different from the controls in all treatment groups, and in both males and females. High serum cholesterol levels have been reported previously (1) in rats and dogs treated with a related compound, 2,4,6-trinitrotoluene (TNT). These compounds possibly impair cholesterol metabolism in the liver, but the biochemical nature of this impairment is unknown.

Serum glucose levels trended upward with increasing dosage of 2,4-DNT, but the elevation was significantly different relative to the control group only in females in the 0.35% 2,4-DNT dosage group. This effect is not likely to be related to differences in food consumption because all animals were fasted for 18 hours before sacrifice and blood collection. Elevated serum glucose levels have been reported previously in 2,4-DNT treated rats (2).

DNT induced the development of hyalin droplets in the epithelium of the proximal convoluted tubules of the kidneys of both male and female rats. The response is not dose-dependent but male rats were more severely affected.

DNT induced oligospermia, syncytial cell formation, and decreased thickness of spermatogenic cell layers in the seminiferous tubules in testes of male rats. The degree of testicular change was dose related. Testicular atrophy, aspermatogenesis, and/or oligospermia have been reported in male chemical workers (3), dogs, rats, and mice (2) after exposure to 2,4-DNT.

CONCLUSIONS

DNT, when fed to rats at 1.0 to 3.5 g/kg diet, causes decreased food consumption, decreased weight gain, elevated blood cholesterol, elevated blood glucose (statistically significant only in the females), and oligospermism with degenerative changes in testes of male rats, all in dose-dependent responses. Hyaline droplet formation (though not a dose-dependent response) was observed in renal tubular epithelial cells. The males were more susceptible than the females to the latter change. The biochemical changes responsible for the above observations are unknown.

RECOMMENDATIONS

None.

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2. LEE, CHENG-CHUN, H.V. ELLIS, J.J. KOWALSKI, J.R. HODGSON, S.W. HWANG, R.D. SHORT, J.C. BHANDARI, J.L. SANYER, T.W. REDDIG, and J.L. MINOR. Mammalian Toxicity of Munitions Compounds, Phase II: Effects of multiple doses; Part II: 2,4-dinitrotoluene. Progress Report No. 3 for Contract No. DAMD 17-74-C-4073, sponsored by US Army Research and Development Command. November, 1978. pp 9-86
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LIST OF FIGURES

	<u>PAGE</u>
Figure 1 Male rats - weight gain vs. days of study	10
Figure 2 Female rats - weight gain vs. days of study	11
Figure 3 Male rats - diet consumption vs. days of study	12
Figure 4 Female rats - diet consumption vs. days of study	13

APPENDIX A

FIGURE 1 MALE RATS - WEIGHT GAIN VS. DAYS OF STUDY

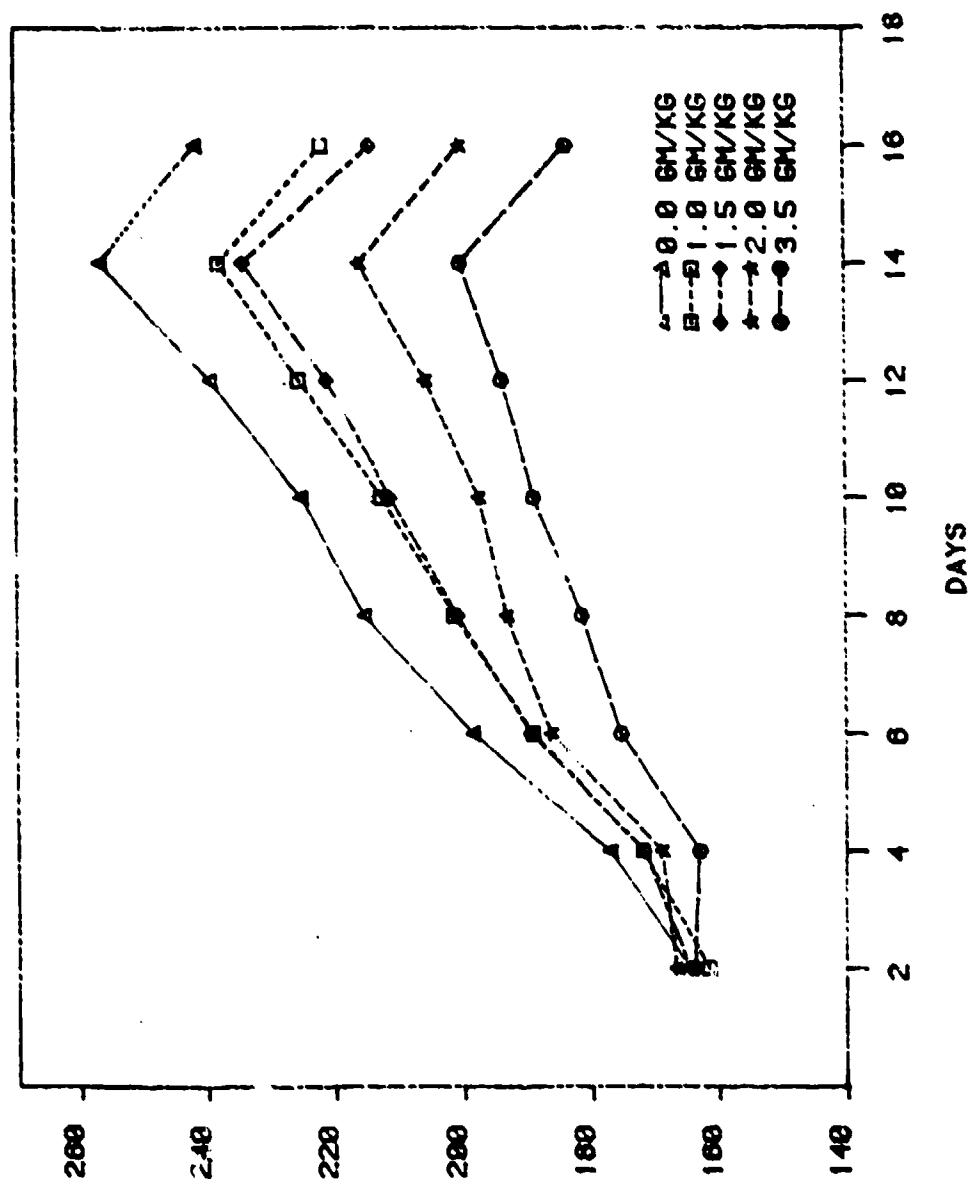


FIGURE 2 FEMALE RATS - WEIGHT GAIN VS. DAYS OF STUDY

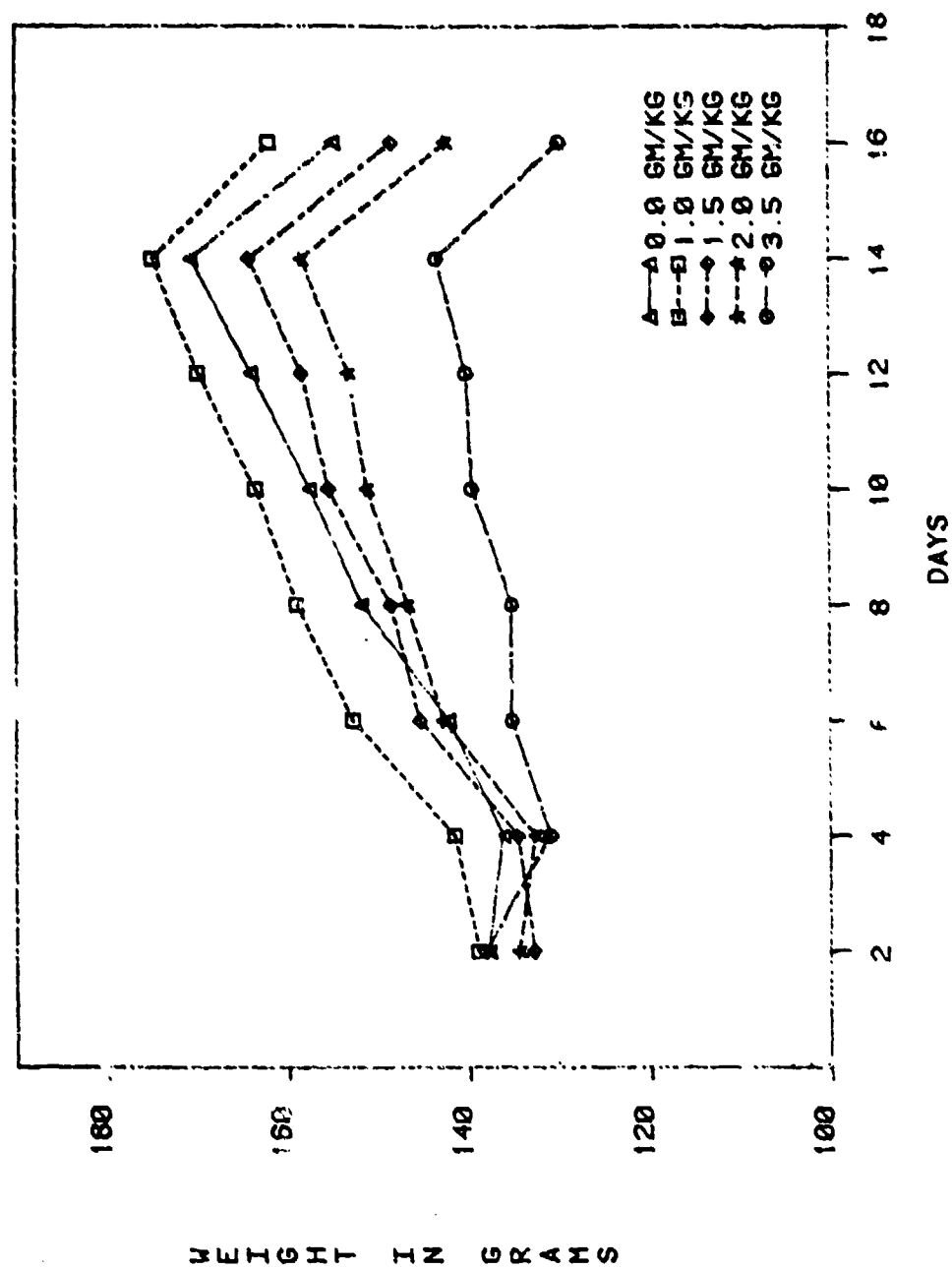


FIGURE 3 MALE RATS - DIET CONSUMPTION VS. DAYS OF STUDY

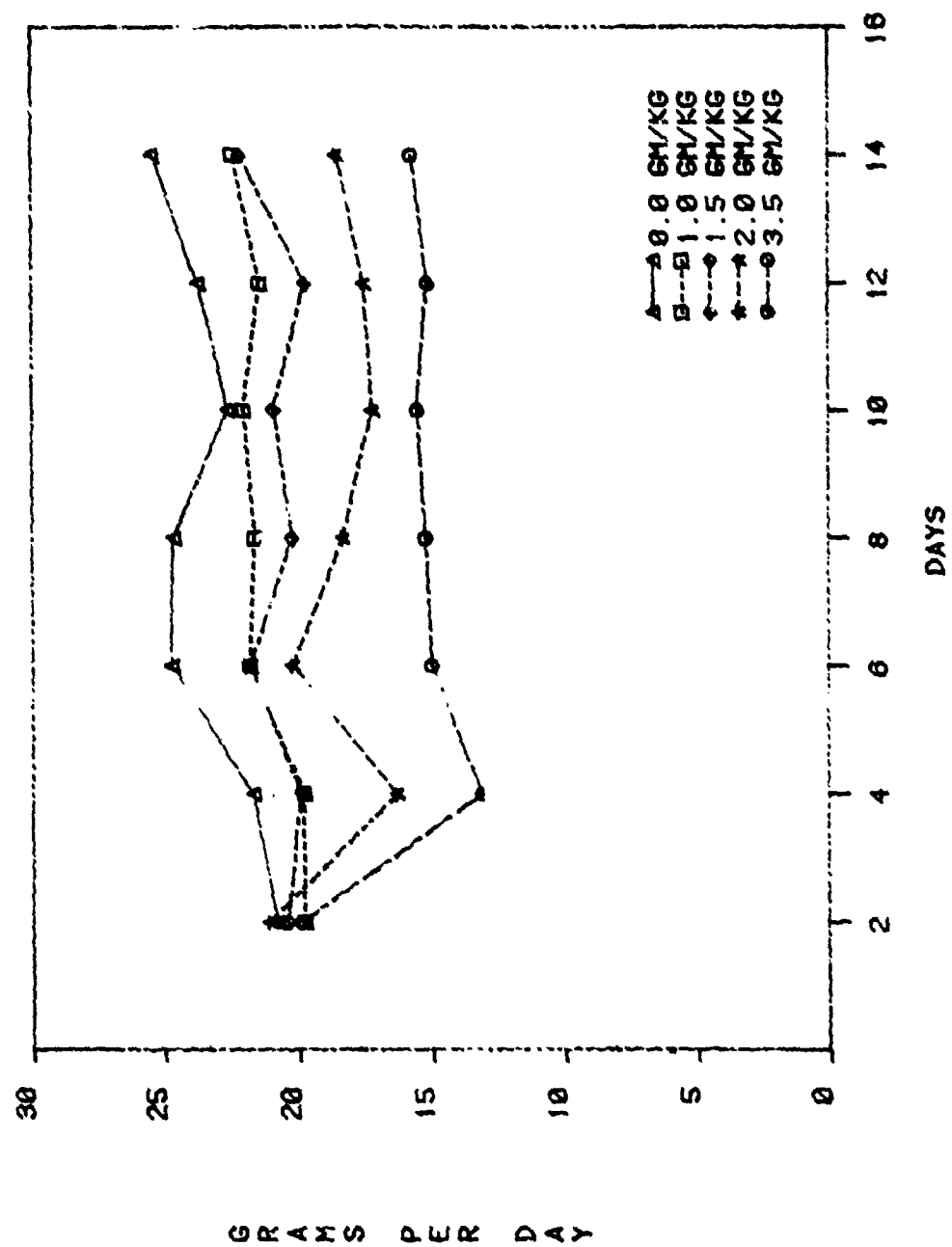
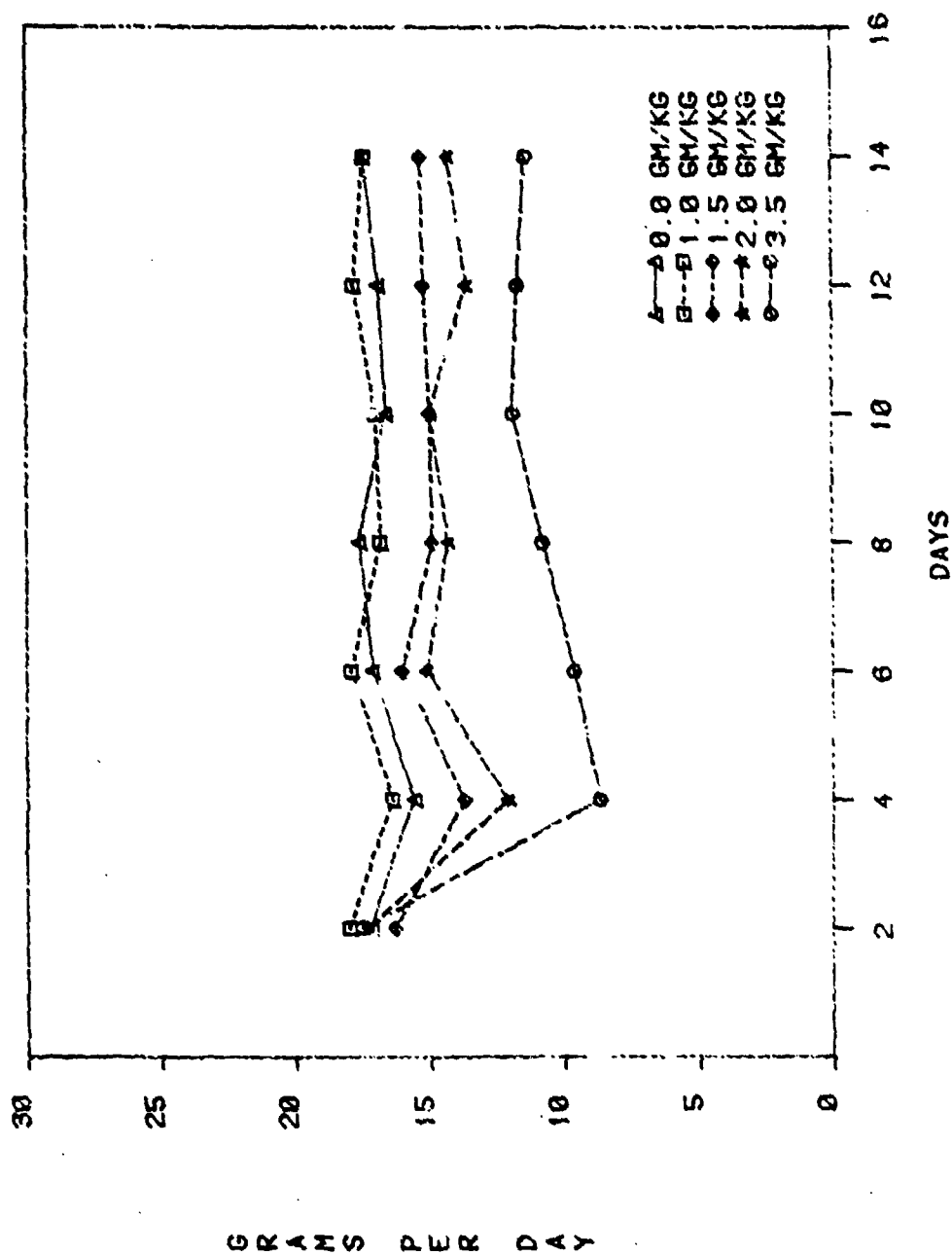


FIGURE 4 FEMALE RATS - D ET CONSUMPTION VS. DAYS OF STUDY



LIST OF TABLES

		<u>PAGE</u>
Table 1	Test Substance Chemical Data	16
Table 2	Summary of Clinical Signs - Females	17
Table 3	Summary of Clinical Signs - Males	18
Table 4	Summary of Blood Chemistry Data	19
Table 5	Summary of Blood Hematology Data	30
Table 6	Summary of Urinalysis Data	37
Table 7	Key to Microscopic Findings	38
Table 8	Gross/Microscopic Incidence, Males	39
Table 9	Gross/Microscopic Incidence, Females	41
Table 10	Summary of Gross/Microscopic Pathology Indicence	43

APPENDIX B

TABLE 1
TEST SUBSTANCE CHEMICAL DATA

Chemical Name (& CAS): 2,4-dinitrotoluene

Molecular Structure:

Molecular Weight: 182.13

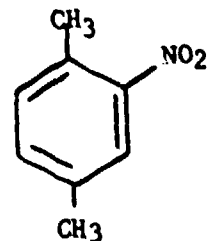
Physical State: Solid, yellow crystals

pH: N/A (dry material)

Melting Point: 69.5 C

Boiling Point: 300 C

Compound Density: 1.521 g/cm³



Compound Refractory Index: 15

Compound Stability: Stable indefinitely when stored
dry in brown bottles.

Contaminants: (Manufacturer's Specifications)

2,6 DNT, 2%

Unspecified, 1%

Source: Matheson, Coleman and Bell, Norwood, Ohio 45212

Manufacturer's Lot No. G-6H09

TABLE 2
SUMMARY OF CLINICAL SIGNS - FEMALES

DNT LEVEL	Signs													
	Slight Sluggish	Light Yellow Urine	Moderate Yellow Urine	Light Yellow Stain - Tail	Moderate Yellow Stain - Tail	Light Brown Stain - Tail	Red Stain - Dorsal	Yellow Material - Nose	Brown Material - Nose	Red Material - Front Leg	Red Material - Nose	Scab - Right Ear	Slight Bleeding - Right Ear	Hair Missing - Right Neck
0 g/kg				1								1	1	
1.0 g/kg		1		2	1	2					2	1	1	
1.5 g/kg		1		2	2						1			
2.0 g/kg		2		1				1						
3.5 g/kg	1	2	1	3		1	2		1	1	2	2		1

TABLE 3

SUMMARY OF CLINICAL SIGNS - MALES

DNT LEVEL	Signs												
	Slight Inactive	Slight Sluggish	Moderate Sluggish	Light Yellow Urine	Light Yellow Stain - Tail	Moderate Yellow Stain - Tail	Red Material - Eye	Red Material - Nose	Brown Material - Nose	Scab - Right Front Leg	Scab - Right Ear	Slight Bleeding - Right Ear	Pus - Right Ear
0 g/kg					1								
1.0 g/kg				1	1	1	1	1			2		
1.5 g/kg				1	3	3						1	1
2.0 g/kg				3	2	1		3		1		1	
3.5 g/kg	1	1	1	3	4	1		1			1		

TABLE 4
SUMMARY OF BLOOD CHEMISTRY DATA
CALCIUM (MG/DL)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	10.81	10.80	10.91	11.11	11.07
Std. Deviation =	.759	.855	.649	.976	1.016
No. of Animals =	9	10	10	10	9
Males					
Mean Value =	10.32	10.06	10.42	10.42	10.46
Std. Deviation =	.249	.329	.268	.698	.737
No. of Animals =	5	5	5	5	5
Females					
Mean Value =	11.43	11.54	11.40	11.80	11.83
Std. Deviation =	.741	.410	.525	.682	.806
No. of Animals =	4	5	5	5	4

CHLORIDE (MEQ/LITER)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	107.14	105.13	111.13	118.29	111.25
Std. Deviation =	8.071	3.314	6.854	13.250	9.394
No. of Animals =	7	8	8	7	4
Males					
Mean Value =	104.40	103.60	108.61	113.00	106.67
Std. Deviation =	4.930	3.209	2.191	12.832	2.517
No. of Animals =	5	5	5	4	3
Females					
Mean Value =	114.00	107.67	115.33	125.33	125.00
Std. Deviation =	12.728	1.528	10.599	12.220	.000
No. of Animals =	2	3	3	3	1

TABLE 4 (Cont)
SUMMARY OF BLOOD CHEMISTRY DATA
PHOSPHORUS (MG/DL)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	11.08	10.89	10.85	11.32	10.68
Std. Deviation =	.620	1.249	1.076	1.386	1.288
No. of Animals =	9	10	10	10	9
Males					
Mean Value =	11.08	11.36	11.08	11.94	10.94
Std. Deviation =	.507	1.374	1.248	1.021	1.224
No. of Animals =	5	5	5	5	5
Females					
Mean Value =	11.07	10.12	10.62	10.70	10.35
Std. Deviation =	.826	1.033	.958	1.523	1.473
No. of Animals =	4	5	5	5	4

SODIUM (MEQ/LITER)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	160.71	160.33	159.29	157.20	162.00
Std. Deviation =	3.451	6.285	1.604	3.272	2.915
No. of Animals =	7	9	7	5	5
Males					
Mean Value =	159.80	158.40	159.20	157.00	162.00
Std. Deviation =	3.115	3.288	1.924	3.742	3.367
No. of Animals =	5	5	5	4	4
Females					
Mean Value =	163.00	162.75	159.50	158.00	162.00
Std. Deviation =	4.243	8.770	.707	.000	.000
No. of Animals =	2	4	2	1	1

TABLE 4 (Cont)
SUMMARY OF BLOOD CHEMISTRY DATA
ASP. AMINOTRANSFERASE (I.U./LITER)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	99.56	97.10	108.50	106.00	150.60
Std. Deviation =	39.960	41.752	49.554	42.867	110.090
No. of Animals =	9	10	10	10	10
Males					
Mean Value =	81.40	113.80	109.20	102.40	110.20
Std. Deviation =	19.308	22.428	29.978	32.631	39.347
No. of Animals =	5	5	5	5	5
Females					
Mean Value =	122.25	80.40	107.80	109.60	151.00
Std. Deviation =	50.249	21.824	68.009	55.112	157.102
No. of Animals =	4	5	5	5	5

LACTATE DEHYDROGENASE (I.U./LITER)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	525.22	456.60	548.70	421.10	485.00
Std. Deviation =	167.663	283.408	164.944	199.554	165.836
No. of Animals =	9	10	10	10	10
Males					
Mean Value =	544.00	613.80	654.80	408.20	503.20
Std. Deviation =	219.999	318.195	96.344	212.013	175.988
No. of Animals =	5	5	5	5	5
Females					
Mean Value =	501.75	299.40	442.60	434.00	466.80
Std. Deviation =	95.430	133.011	154.238	210.316	173.431
No. of Animals =	4	5	5	5	5

TABLE 4 (Cont)
SUMMARY OF BLOOD CHEMISTRY DATA
TRIGLYCERIDES (MG/DL)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	55.96	61.24	47.61	45.36	42.17
Std. Deviation =	19.423	31.809	21.024	21.737	12.538
No. of Animals =	9	10	10	10	10
Males					
Mean Value =	64.12	62.34	43.26	60.62	49.94
Std. Deviation =	18.723	12.678	22.384	21.354	5.219
No. of Animals =	5	5	5	5	5
Females					
Mean Value =	48.00	60.14	51.96	50.10	34.40
Std. Deviation =	18.604	45.965	21.123	5.001	12.519
No. of Animals =	4	5	5	5	5

CREATININE (MG/DL)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	.37	.35	.37	.33	.39
Std. Deviation =	.080	.058	.056	.109	.140
No. of Animals =	9	10	10	10	7
Males					
Mean Value =	.34	.31	.33	.34	.33
Std. Deviation =	.083	.027	.046	.098	.120
No. of Animals =	5	5	5	5	5
Females					
Mean Value =	.40	.39	.41	.32	.52
Std. Deviation =	.065	.058	.031	.130	.078
No. of Animals =	4	5	5	5	2

TABLE 4 (Cont)
SUMMARY OF BLOOD CHEMISTRY DATA
BLOOD UREA NITROGEN (MG/DL)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	12.50	12.24	13.00	11.82	12.08
Std. Deviation =	1.826	1.514	2.970	4.272	7.292
No. of Animals =	9	10	10	10	10
Males					
Mean Value =	11.74	11.34	11.50	11.76	13.90
Std. Deviation =	1.973	1.553	.660	1.498	2.198
No. of Animals =	5	5	5	5	5
Females					
Mean Value =	13.45	13.14	15.70	11.68	11.80
Std. Deviation =	1.240	.847	2.896	6.230	10.579
No. of Animals =	4	5	5	5	5

TOTAL BILIRUBIN (MG/DL)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	.19	.18	.16	.14	.13
Std. Deviation =	.127	.103	.097	.107	.134
No. of Animals =	9	10	10	10	10
Males					
Mean Value =	.18	.16	.14	.12	.14
Std. Deviation =	.110	.089	.134	.130	.167
No. of Animals =	5	5	5	5	5
Females					
Mean Value =	.20	.20	.18	.16	.12
Std. Deviation =	.163	.122	.045	.039	.110
No. of Animals =	4	5	5	5	5

TABLE 4 (Cont)
SUMMARY OF BLOOD CHEMISTRY DATA
ALKALINE PHOSPHATASE (I.U./LITER)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	124.89	124.60	118.50	127.50	118.60
Std. Deviation =	43.705	51.026	40.258	54.372	33.965
No. of Animals =	9	10	10	10	10
Males					
Mean Value =	154.40	16.00	152.40	170.40	138.40
Std. Deviation =	30.088	31.456	22.996	44.534	34.019
No. of Animals =	5	5	5	5	5
Females					
Mean Value =	88.00	82.20	84.60	84.60	98.00
Std. Deviation =	24.913	19.344	15.646	8.204	21.406
No. of Animals =	4	5	5	5	5
ALBUMIN (GM/DL)					
DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	4.69	4.57	4.59	4.46	4.74
Std. Deviation =	.289	.254	.828	.504	.538
No. of Animals =	9	10	10	10	10
Males					
Mean Value =	4.50	4.42	4.02	4.26	4.34
Std. Deviation =	.173	.228	.698	.456	.207
No. of Animals =	5	5	5	5	5
Females					
Mean Value =	4.92	4.72	5.16	4.66	5.14
Std. Deviation =	.222	.192	.493	.513	.456
No. of Animals =	4	5	5	5	5

TABLE 4 (Cont)
SUMMARY OF BLOOD CHEMISTRY DATA
TOTAL PROTEIN (G/DL)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	5.84	5.65	5.70	5.57	5.65
Std. Deviation =	.301	.254	.397	.350	.514
No. of Animals =	10	9	10	10	10
Males					
Mean Value =	5.65	5.52	5.40	5.38	5.34
Std. Deviation =	.251	.110	.187	.312	.270
No. of Animals =	5	5	5	5	5
Females					
Mean Value =	6.07	5.74	6.00	5.76	6.02
Std. Deviation =	.171	.321	.308	.241	.462
No. of Animals =	4	5	5	5	5

URIC ACID (MG/DL)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	1.21	1.12	1.29	2.15	1.91
Std. Deviation =	.669	.299	.711	1.398	1.193
No. of Animals =	8	9	10	10	9
Males					
Mean Value =	1.17	1.05	.98	2.00	1.46
Std. Deviation =	.299	.238	.226	2.342	.950
No. of Animals =	4	4	5	5	5
Females					
Mean Value =	1.25	1.18	1.60	2.30	2.47
Std. Deviation =	.975	.356	.919	1.600	1.300
No. of Animals =	4	5	5	5	4

TABLE 4 (Cont)
SUMMARY OF BLOOD CHEMISTRY DATA
METHEMOGLOBIN (% OF HGB)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	.43	.38	.37	.17	.04
Std. Deviation =	.407	.444	.771	.287	.091
No. of Animals =	9	10	10	10	10
Males					
Mean Value =	.49	.58	.17	.35	.09
Std. Deviation =	.373	.531	1.047	.332	.118
No. of Animals =	5	5	5	5	5
Females					
Mean Value =	.37	.18	.17	.00	.00
Std. Deviation =	.496	.244	.376	.000	.000
No. of Animals =	4	5	5	5	5

CREAT. PHOSPHOKINASE (I.U./LITER)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	595.33	639.10	703.75	451.70	506.90
Std. Deviation =	585.703	543.776	769.574	383.706	455.685
No. of Animals =	9	10	8	10	10
Males					
Mean Value =	398.40	625.00	396.60	409.60	541.40
Std. Deviation =	224.415	506.998	86.584	188.455	379.656
No. of Animals =	5	5	5	5	5
Females					
Mean Value =	841.50	653.20	1215.67	493.80	472.40
Std. Deviation =	837.980	638.565	1195.374	539.742	567.582
No. of Animals =	4	5	3	5	5

TABLE 4 (Cont)
SUMMARY OF BLOOD CHEMISTRY DATA
POTASSIUM (MEQ/LITER)

DMT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	7.34	6.66	6.66	7.60	7.58
Std. Deviation =	2.169	1.293	.506	2.178	.719
No. of Animals =	7	9	7	5	5
Males					
Mean Value =	6.12	6.72	6.68	7.90	7.80
Std. Deviation =	.701	1.642	.614	2.393	.606
No. of Animals =	5	5	5	4	4
Females					
Mean Value =	10.40	6.57	6.60	6.40	6.70
Std. Deviation =	.283	.922	.141	.000	.000
No. of Animals =	2	4	2	1	1

GLUCOSE (MG/DL)

DMT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	95.22	103.00	121.50	132.10	151.30
Std. Deviation =	10.575	19.944	43.028	18.406	31.598
No. of Animals =	9	10	10	10	10
Males					
Mean Value =	91.00	103.20	117.80	131.20	135.40
Std. Deviation =	11.554	29.047	34.142	21.324	24.265
No. of Animals =	5	5	5	5	5
Females					
Mean Value =	100.50	102.80	125.20	133.00	167.20*
Std. Deviation =	8.737	7.155	54.458	17.479	32.027
No. of Animals =	4	5	5	5	5

TABLE 4 (Cont)
SUMMARY OF BLOOD CHEMISTRY DATA
GLOBULIN (GM/DL)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	1.16	1.06	1.11	1.11	.94
Std. Deviation =	.235	.151	.479	.363	.259
No. of Animals =	9	10	10	10	10
Males					
Mean Value =	1.16	1.10	1.38	1.12	1.00
Std. Deviation =	.297	.141	.540	.217	.235
N of Animals =	5	5	5	5	5
Females					
Mean Value =	1.15	1.02	.84	1.10	.88
Std. Deviation =	.173	.164	.207	.500	.295
No. of Animals =	4	5	5	5	5

ALBUMIN/GLOBULIN RATIO

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	4.23	4.40	4.95	4.78	5.49
Std. Deviation =	.992	.712	2.449	2.966	2.014
No. of Animals =	9	10	10	10	10
Males					
Mean Value =	4.13	4.09	3.33	3.96	4.54
Std. Deviation =	1.209	.764	1.388	1.037	1.088
No. of Animals =	5	5	5	5	5
Females					
Mean Value =	4.37	4.70	6.57*	5.61	6.45*
Std. Deviation =	.795	.573	2.238	4.126	2.376
No. of Animals =	4	5	5	5	5

TABLE 4 (Cont.)
SUMMARY OF BLOOD CHEMISTRY DATA
CHOLESTEROL (MG/DL)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	45.29	79.04	97.22	88.76	107.65
Std. Deviation =	19.158	23.103	19.880	20.858	23.689
No. of Animals =	9	10	10	10	10
Males					
Mean Value =	41.12	77.28*	86.76*	87.58*	89.98*
Std. Deviation =	9.417	18.118	22.017	25.281	14.647
No. of Animals =	5	5	5	5	5
Females					
Mean Value =	50.50	80.80*	107.68*	89.94*	125.32*
Std. Deviation =	28.202	29.410	11.444	18.339	16.355
No. of Animals =	4	5	5	5	5

AL. AMINOTRANSFERASE (I.U./LITER)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	20.33	25.30	26.00	27.20	27.40
Std. Deviation =	3.905	8.994	9.321	9.976	6.552
No. of Animals =	9	10	10	10	10
Males					
Mean Value =	20.40	32.60*	31.60*	34.20*	30.20*
Std. Deviation =	2.702	6.693	7.925	3.556	5.450
No. of Animals =	5	5	5	5	5
Females					
Mean Value =	20.25	18.00	20.40	20.20	24.60
Std. Deviation =	5.560	2.000	7.369	5.310	6.878
No. of Animals =	4	5	5	5	5

* = Significantly different from the control group at $p < 0.05$

TABLE 5
SUMMARY OF BLOOD HEMATOLOGY DATA
MEAN CORPUSCULAR HEMOGLOBIN (PG)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	23.0	22.6	23.0	22.3	22.1
Std. Deviation =	0.761	0.673	0.901	0.893	0.645
No. of Animals =	7	9	8	8	7
Males					
Mean Value =	23.2	22.3	22.8	22.4	21.8
Std. Deviation =	0.416	0.823	0.555	1.283	0.618
No. of Animals =	1	5	5	4	1
Females					
Mean Value =	22.5	22.9	23.2	22.2	22.5
Std. Deviation =	1.414	0.141	1.457	0.432	0.513
No. of Animals =	2	4	3	4	3

MEAN CORPUSCULAR VOLUME (FL)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	63.3	62.0	61.7	61.6	60.6
Std. Deviation =	2.498	2.000	1.669	2.973	1.988
No. of animals =	7	9	8	8	7
Males					
Mean Value =	62.6	63.4	62.4	60.7	60.0
Std. Deviation =	1.673	1.342	1.817	1.258	2.582
No. of Animals =	5	5	5	4	4
Females					
Mean Value =	65.0	60.2	60.7	62.5	61.3
Std. Deviation =	4.243	0.957	0.577	4.123	0.577
No. of Animals =	2	4	3	4	3

TABLE 5 (Cont)
SUMMARY OF BLOOD HEMATOLOGY DATA
NEUTROBANDS (1000/ μ l)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	0.0	0.0	0.0	0.0	0.0
Std. Deviation =	0.000	0.000	0.000	0.000	0.000
No. of Animals =	7	9	8	8	7
Males					
Mean Value =	0.0	0.0	0.0	0.0	0.0
Std. Deviation =	0.000	0.000	0.000	0.000	0.000
No. of Animals =	5	5	5	4	4
Femal					
Mean Value =	0.0	0.0	0.0	0.0	0.0
Std. Deviation =	0.000	0.000	0.000	0.000	0.000
No. of Animals =	2	4	3	4	3

NEUTROSEGS (1000/ μ l)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	0.5	0.9	0.7	1.2	0.6
Std. Deviation =	0.349	0.780	0.297	0.849	0.843
No. of Animals =	7	9	8	8	7
Males					
Mean Value =	0.6	1.1	0.6	1.2	0.9
Std. Deviation =	0.395	1.002	0.293	0.964	1.061
No. of Animals =	5	5	5	4	4
Females					
Mean Value =	0.4	0.7	0.8	1.1	0.3
Std. Deviation =	0.042	0.374	0.348	0.867	0.327
No. of Animals =	2	4	3	4	3

TABLE 5 (Cont)
SUMMARY OF BLOOD HEMATOLOGY DATA
LYMPHOCYTES (1000/ μ l)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	5.9	8.0	7.3	6.3	5.2
Std. Deviation =	2.124	2.630	2.029	2.744	2.727
No. of Animals =	7	9	8	8	7
Males					
Mean Value =	6.0	7.9	6.5	5.5	5.6
Std. Deviation =	1.747	1.878	1.115	1.791	2.657
No. of Animals =	5	5	5	4	4
Females					
Mean Value =	4.2	8.3	8.6	7.0	4.7
Std. Deviation =	2.548	3.690	2.774	3.575	3.301
No. of Animals =	2	4	3	4	3

EOSINOPHILS (1000/ μ l)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	0.0	0.0	0.0	0.0	0.0
Std. Deviation =	0.030	0.040	0.025	0.000	0.011
No. of Animals =	7	9	8	8	7
Males					
Mean Value =	0.0	0.0	0.0	0.0	0.0
Std. Deviation =	0.035	0.054	0.032	0.000	0.000
No. of Animals =	5	5	5	4	4
Females					
Mean Value =	0.0	0.0	0.0	0.0	0.0
Std. Deviation =	0.019	0.000	0.000	0.000	0.017
No. of Animals =	2	4	3	4	3

TABLE 5 (Cont)
SUMMARY OF BLOOD HEMATOLOGY DATA
BASOPHILS (1000/ μ l)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	0.0	0.0	0.0	0.0	0.0
Std. Deviation =	0.000	0.000	0.000	0.000	0.000
No. of Animals =	7	9	8	8	7
Males					
Mean Value =	0.0	0.0	0.0	0.0	0.0
Std. Deviation =	0.000	0.000	0.000	0.000	0.000
No. of Animals =	5	5	5	4	4
Females					
Mean Value =	0.0	0.0	0.0	0.0	0.0
Std. Deviation =	0.000	0.000	0.000	0.000	0.000
No. of Animals =	2	4	3	4	3

MONOCYTES (1000/ μ l)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	0.1	0.1	0.1	0.1	0.1
Std. Deviation =	0.064	0.072	0.163	0.119	0.076
No. of Animals =	7	9	8	8	7
Males					
Mean Value =	0.1	0.1	0.2	0.2	0.1
Std. Deviation =	0.047	0.086	0.176	0.137	0.092
No. of Animals =	5	5	5	4	4
Females					
Mean Value =	0.0	0.1	0.0	0.0	0.0
Std. Deviation =	0.045	0.039	0.000	0.042	0.017
No. of Animals =	2	4	3	4	3

TABLE 5 (Cont)
SUMMARY OF BLOOD HEMATOLOGY DATA
RETICULOCYTES (%)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	2.7	2.4	1.9	1.7	2.5
Std. Deviation =	1.479	1.698	1.397	1.761	1.771
No. of Animals =	7	9	8	8	7
Males					
Mean Value =	3.4	3.7	2.3	2.8	3.3
Std. Deviation =	0.965	0.942	1.641	1.938	2.005
No. of Animals =	5	5	5	4	4
Females					
Mean Value =	0.9	0.7	1.1	0.6	1.3
Std. Deviation =	0.566	0.387	0.289	0.320	0.252
No. of Animals =	2	4	3	4	3

MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (%)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	35.9	36.6	36.7	36.3	36.4
Std. Deviation =	2.314	1.891	1.286	1.429	0.800
No. of Animals =	7	9	8	8	7
Males					
Mean Value =	36.3	35.4	36.8	36.8	36.3
Std. Deviation =	1.436	1.593	1.313	1.201	0.888
No. of Animals =	5	5	5	4	4
Females					
Mean Value =	34.9	38.2	36.5	35.9	36.6
Std. Deviation =	4.596	0.602	1.501	1.668	0.814
No. of Animals =	2	4	3	4	3

TABLE 5 (Cont)
SUMMARY OF BLOOD HEMATOLOGY DATA
HEMATOCRIT (%)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	42.1	41.2	42.1	42.4	41.5
Std. Deviation =	3.200	1.182	2.160	2.731	2.145
No. of Animals =	7	9	5	8	7
Males					
Mean Value =	41.0	41.7	43.1	41.5	42.0
Std. Deviation =	2.652	1.092	1.836	0.640	2.722
No. of Animals =	5	5	5	4	4
Females					
Mean Value =	44.7	40.4	40.5	43.4	40.8
Std. Deviation =	3.677	0.925	1.756	3.820	1.234
No. of Animals =	2	4	3	4	3

WHITE BLOOD CELL COUNT (1000/ μ l)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	6.5	9.1	6.1	7.5	5.9
Std. Deviation =	2.293	3.000	2.120	3.115	3.354
No. of Animals =	7	9	3	3	7
Males					
Mean Value =	7.3	9.2	7.3	6.8	6.6
Std. Deviation =	1.842	2.617	1.006	2.198	3.552
No. of Animals =	5	5	5	4	4
Females					
Mean Value =	4.5	9.0	9.4	3.2	4.9
Std. Deviation =	2.616	3.654	3.119	4.069	3.612
No. of Animals =	2	4	3	4	3

TABLE 5 (Cont)
SUMMARY OF BLOOD HEMATOLOGY DATA
PLATELETS (1×10^6 /CUBIC μ l)

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	9.042	9.000	14.000	14.575	11.071
Std. Deviation =	4.352	5.584	5.162	3.671	2.921
No. of Animals =	7	9	8	8	7
Males					
Mean Value =	9.200	9.940	16.700	16.250	9.750
Std. Deviation =	3.662	7.551	3.526	2.327	3.201
No. of Animals =	5	5	5	4	4
Females					
Mean Value =	10.750	9.625	9.500	12.500	12.133
Std. Deviation =	6.638	2.657	4.444	4.032	1.443
No. of Animals =	2	4	5	4	3

TABLE 6
SUMMARY OF URINE DATA

URINE pH

GLP STUDY #: 81001

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	7.4	6.5	6.9	6.8	6.4
Std. Deviation =	1.054	.707	.738	.890	.459
No. of Animals =	9	10	10	10	10
Males					
Mean Value =	8.2	7.0	7.2	7.2	6.5
Std. Deviation =	.447	.707	.906	1.036	.500
No. of Animals =	5	5	5	5	5
Females					
Mean Value =	6.4	6.0	6.6	6.3	6.3
Std. Deviation =	.479	.000	.415	.447	.447
No. of Animals =	4	5	5	5	5

URINE SPECIFIC GRAVITY

GLP STUDY #: 81001

DNT LEVEL (g/kg)	0	1.0	1.5	2.0	3.5
Combined "M" and "F"					
Mean Value =	1.029	1.028	1.022	1.027	1.028
Std. Deviation =	.016	.013	.014	.011	.012
No. of animals =	9	9	10	10	10
Males					
Mean Value =	1.022	1.021	1.019	1.029	1.031
Std. Deviation =	.043	.007	.007	.008	.014
No. of Animals =	5	5	5	5	5
Females					
Mean Value =	1.040	1.038	1.025	1.024	1.025
Std. Deviation =	.021	.013	.019	.015	.015
No. of Animals =	4	4	5	5	5

TABLE 7

Key to Microscopic Findings (Tables 8-10)

(+) = Tissue or organ present, no significant lesions were observed unless recorded as present (P) or graded as to severity (1-5).

(-) = Tissue or organ not present.

(P) = Lesion recorded as present and not graded as to severity.

Grading for severity of lesion is as follows:

- 1 = minimal
- 2 = mild
- 3 = moderate
- 4 = marked
- 5 = severe

() = Gross lesions observed during necropsy.

TABLE 8
GROSS/MICROSCOPIC INCIDENCE, MALES

MALE RATS	Gp 4-control				Ip 3-3500 mg/kg				Ip 1-2000 mg/kg				Ip 5-1500 mg/kg				Ip 2-1000 mg/kg			
	30388	30393	30396	30399	30406	30387	30389	30391	30401	30408	30390	30395	30402	30409	30411	30394	30398	30403	30407	30410
Project Title: 2,4-DHT - 14 Day Feeding																				
GLP Study #81-001																				
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1. Ectasia, ductal, submucosa		2	2	2	3		2	1	3	2	2		3		3					
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SALIVARY GLANDS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1. Myocarditis, histiocytic, focal														2						
LUNG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MESENTERIC LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1. Hepatitis, histiocytic, focal																				
2. Mononuclear aggregates, multifocal					2						2			2						
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
(1. White focal area, subcapsular)																				
1. Hyaline droplet accumulation																				
2. Nephritis, interstitial, focal						3	2	3	2		3	3	2		2	4	3	3	2	1
3. Concretions, intratubular									2											
UTERINE BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
(1. White calculus, 5x3x2 mm)	P																			
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SEMINAL VESICLE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE 8 (Cont)

MALE RATS		GROSS/MICROSCOPIC INCIDENCE, MALES											
Project Title: 2,4-DNT - 14 Day Feeding GLP Study #81-001		Gp 4-control			Dp 3-3500 mg/kg			Dp 1-2000 mg/kg			Dp 5-1500 mg/kg		
		30388	30393	30396	30399	30406	30387	30388	30391	30401	30409	30411	30394
TESTES		+	+	+	+	+	+	+	+	+	+	+	+
1. Oligospermia							4	4	4	4	4	4	+
2. Syncytial giant cells								3	3	3*	4	4	
3. Granuloma, spermatic, focal											4	4	
4. Decreased thickness of spermato-								2			2	3	
4. Decreased thickness of spermato-											3	2	
UTERUS		-	-	-	-	-	-	-	-	-	-	-	-
1. Neutrophilic inflammation,													
2. Fecundity, luminal													
OVARIES		-	-	-	-	-	-	-	-	-	-	-	-
STOMACH		+	+	+	+	+	+	+	+	+	+	+	+
PANCREAS		+	+	+	+	+	+	+	+	+	+	+	+
SMALL INTESTINE		+	+	+	+	+	+	+	+	+	+	+	+
LARGE INTESTINE		+	+	+	+	+	+	+	+	+	+	+	+
CECUM		+	+	+	+	+	+	+	+	+	+	+	+
SKELETAL MUSCLE		+	+	+	+	+	+	+	+	+	+	+	+
SKIN		+	+	+	+	+	+	+	+	+	+	+	+
NASAL CROSS-SECTION		+	+	+	+	+	+	+	+	+	+	+	+
SPINAL CORD		+	+	+	+	+	+	+	+	+	+	+	+
RIBS		+	+	+	+	+	+	+	+	+	+	+	+
FEMUR		+	+	+	+	+	+	+	+	+	+	+	+
ADRENALS		+	+	+	+	+	+	+	+	+	+	+	+
PITUITARY		+	+	+	+	+	+	+	+	+	+	+	+
EYES		+	+	+	+	+	+	+	+	+	+	+	+
1. Fusion of nuclear layers, reti-			P										
2. Diffuse focal distribution													

TABLE 9
GROSS/MICROSCOPIC INCIDENCE, FEMALES

GROSS/ MICROSCOPIC INCIDENCE																							
FEMALE RATS	Gp 4-control				Tp 3-3500 mg/kg				Tp 1-2000 mg/kg				Tp 5-1500 mg/kg				Tp 2-1000 mg/kg						
	30412	30413	30423	30431	30418	30422	30427	30434	30435	30417	30425	30430	30432	30433	30414	30415	30416	30419	30426	30420	30421	30424	
Project Title: 2,4-DNT - 14 Day Feeding																							
I.P. Study #81-001																							
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
1. Ectasia, ductal, submucosa	2	2	2	2	2	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
SALIVARY GLANDS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
1. Myocarditis, histiocytic, focal																							
LUNG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
MESENTERIC LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
1. Hepatitis, histiocytic, focal																							
2. Mononuclear aggregates, multi-focal	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
(1. White focal area, subcapsular)																							
1. Hyaline droplet accumulation	1																						
2. Nephritis, interstitial, focal																							
3. Concretions, intratubular																							
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
(1. White calculus, 5x3x2 mm)																							
PROSTATE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
SEMINAL VESICLE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

TABLE 9 (Cont)
GROSS/MICROSCOPIC INCIDENCE, FEMALES

FEMALE RATS					Gp 4-control				ip -3500 mg/kg				ip 1-2000 mg/kg				ip 5-1500 mg/kg				ip 2-1000 mg/kg															
Project Title: 2,4-DNT - 14 Day Feeding GLP Study #81-001					30412	30413	30423	30431	30418	30422	30427	30434	30435	30417	30425	30430	30432	30433	30414	30415	30416	30419	30426	30420	30421	30424	30428	30429								
					TESTES																															
										1. Oligospermia																										
															2. Syncytial giant cells																					
																				3. Granuloma, spermatic, focal																
4. Decreased thickness of spermatic genital layers																																				
					UTERUS																															
1. Neutrophilic infiltration,																																				
2. Ectasia, luminal																																				
OVARIES																																				
STOMACH																																				
PANCREAS																																				
SMALL INTESTINE																																				
LARGE INTESTINE																																				
CECUM																																				
SKELETAL MUSCLE																																				
SKIN																																				
NASAL CROSS-SECTION																																				
SPINAL CORD																																				
KIDS																																				
THYROID																																				
ADRENALS																																				
PITUITARY																																				
EYES																																				
1. Fusion of nuclear layers, retina					P																															

TABLE 10
SUMMARY OF GROSS/MICROSCOPIC PATHOLOGY INCIDENCE

Tissue/Response	Dose Level mg/kg	Animals on Study	Animals Logged	Incidence of Response									
				Group 4		Group 1		Group 3		Group 5		Group 2	
				Control 0	3500	2000	1500	1000	500	0	0	0	0
Brain.....				5	4	5	5	5	5	0	0	0	0
Not Remarkable.....				5	4	5	5	5	5				
Missing													
Autolysis													
Trachea.....				5	4	5	5	5	5	0	0	0	0
NR.....				1	1	2	2	2	2				
His													
Aut				4	3	4	4	4	3				
Ectasia, ductal, submucosa.....													
Esophagus.....				5	4	5	5	5	5	0	0	0	0
NR.....				5	4	5	5	5	5				
His													
Aut													
Thyroid.....				5	4	5	3	3	4	0	0	0	0
NR.....				5	4	5	3	3	4				
His.....							2	2	1				
Aut													
Adrenals.....				5	4	4	5	5	5	0	0	0	0
NR.....				5	4	4	5	5	5				
His.....							1						
Aut													
Pituitary.....				4	4	3	3	3	4	0	0	0	0
NR.....				4	4	3	3	4	4				
His.....				1	2	1	2	1	1				
Aut													

TABLE 10 (Cont)
SUMMARY OF GROSS/MICROSCOPIC PATHOLOGY INCIDENCE

Tissue/Response	Dose Level mg/kg Animals on Study Animals Logged	Incidence of Response									
		Group 4		Group 3		Group 1		Group 5		Group 2	
		Control	0	3500	5000	2000	5000	1500	1000	500	0
		M	F	M	F	M	F	M	F	M	F
		5	4	5	5	5	5	5	5	5	5
		5	4	5	5	5	5	5	5	5	5
Eyes.....		5	4	5	5	5	5	0	0	0	0
HR.....		4	3	5	5	5	5				
His.....											
Aut.....		1	1								
Fusion of nuclear layers, retina											
Salivary Glands.....		5	4	5	5	4	5	0	0	0	0
NR.....		5	4	5	5	4	5				
His.....						1					
Aut.....											
Heart.....		5	4	5	5	5	5	0	0	0	0
HR.....		5	4	5	5	4	5				
His.....											
Aut.....											
Myocarditis, histiocytic, focal.....						1					
Lung.....		5	4	5	5	5	5	0	0	0	0
HR.....		5	4	5	5	5	5				
His.....											
Aut.....											
Thyroid.....		5	4	4	5	5	5	0	0	0	0
HR.....		5	4	4	5	5	5				
His.....				1							
Aut.....											
Spleen.....		4	4	5	5	5	5	0	0	0	0
NR.....		4	4	5	5	5	5				
His.....		1									
Aut.....											

TABLE 10 (Cont)
SUMMARY OF GROSS/MICROSCOPIC PATHOLOGY INCIDENCE

Tissue/Response	Dose Level mg/kg		Group 3		Group 1		Group 5		Group 2	
	Animals on Study	Animals Logged	Group 4	Group 3	Group 1	Group 5	Group 5	Group 5	Group 2	Group 2
Seminal Vesicle.....			Control	3500	2000	1500	1000			
HR.....			H	M	M	M	N	F	N	F
NIS			5	5	5	5	5	5	5	5
Aut			4	5	5	5	5	5	5	5
Testes.....			5	5	5	5	5	5	5	5
HR.....			5	5	5	5	5	5	5	5
NIS			5	5	5	5	5	5	5	5
Aut			5	5	5	5	5	5	5	5
Oligospermia.....										
Syncytial giant cells.....										
Granuloma, spermatic, focal.....										
Decreased thickness of spermatogenic layers.....										
Uterus.....			0	0	0	0	0	0	0	0
HR.....			4	4	4	4	4	4	4	4
NIS			4	4	4	4	4	4	4	4
Aut			4	4	4	4	4	4	4	4
Neutrophilic infiltration, endometrium.....										
Ectasia, luminal.....										
Ovaries.....										
HR.....										
NIS										
Aut										
Stomach.....										
HR.....										
NIS										
Aut										

TABLE 10 (Cont)
SUMMARY OF GROSS/MICROSCOPIC PATHOLOGY INCIDENCE

Tissue/Response	Dose Level mg/kg	Incidence of Response							
		Group 4 Control	Group 3 3500	Group 1 2000	Group 5 1500	Group 2 1000			
	Animals on Study	N	N	N	N	N			
	Animals Logged	5	5	5	5	5			
Skin.....		5	4	5	0	0			
NR.....		5	4	5					
Mis.....			4						
Aut.....			1						
Nasal Cross-section.....									
NR.....		5	5	5	0	0			
Mis.....		5	5	5					
Aut.....									
Spinal Cord.....									
NR.....		5	5	5	0	0			
Mis.....		5	5	5					
Aut.....									
Ribs.....									
NR.....		5	5	5	0	0			
Mis.....		5	5	5					
Aut.....									
Femur.....									
NR.....		5	5	5	0	0			
Mis.....		5	5	5					
Aut.....									

TABLE 10 (Cont)
SUMMARY OF GROSS/MICROSCOPIC PATHOLOGY INCIDENCE

Tissue/Response	Dose Level mg/kg	Animals on Study Animals Logged	Incidence of Response							
			Group 4		Group 3		Group 1		Group 5	
			Control	3500	2000	1500	UHM	0	0	0
Pancreas.....										
NR.....			4	3	5	5	5	5	0	0
Mis.....			4	3	5	5	5	5		
Aut.....			1	1						
Small Intestine.....										
NR.....			5	4	5	5	5	5	0	0
Mis.....			5	4	5	5	5	5		
Aut.....										
Large Intestine.....										
NR.....			5	4	5	5	5	5	0	0
Mis.....			5	4	5	5	5	5		
Aut.....										
Cecum.....										
NR.....			5	4	5	5	5	5	0	0
Mis.....			5	4	5	5	5	5		
Aut.....										
Skeletal Muscle.....										
NR.....			5	4	5	5	5	5	0	0
Mis.....			5	4	5	5	5	5		
Aut.....										

TABLE 10 (Cont)
SUMMARY OF GROSS/MICROSCOPIC PATHOLOGY INCIDENCE

Tissue/Response	Dose Level mg/kg Animals on Study Animals Logged	Incidence of Response							
		Group 4		Group 3		Group 1		Group 5	
		Certified	N	Certified	N	Certified	N	Certified	N
Hesenteric Lymph Nodes.....		4	4	5	3	4	4	0	0
IR.....		4	4	5	3	4	4		
His.....		1			2	1	1		
Aut.....									
Liver.....		5	4	5	5	5	5	0	0
IR.....		4		5	5	3	3		
His.....									
Aut.....									
Hepatitis, histiocytic, focal.....									
Mononuclear aggregates, multifocal.....		1	4			2	1		
Kidney.....		5	4	5	5	5	5	5	5
IR.....		5	2	1	1	1	2		
His.....									
Aut.....									
(White focal area, subcapsular).....							(1)		
Hyaline, Droplet Accumulation.....		1		4	4	4	3	5	5
Nephritis, interstitial, chronic, focal.....				1				1	1
Concretions, intratubular.....		1				1	1	2	
Urinary Bladder.....		5	4	5	5	5	4	0	0
IR.....		5	4	5	5	5	4		
His.....							1		
Aut.....		(1)							
(White calculus, 5 x 3 x 2 mm)									
Prostate.....		5	0	5	0	5	0	0	0
IR.....		5		5		5			
His.....									
Aut.....									

() - Gross lesions

APPENDIX C

Glossary of Microscopic Lesions Encountered in Fourteen-day Subacute Study of 2,4-Dinitrotoluene (2,4-DNT) Study Number 81-0001

KIDNEY

Concretions, intratubular - Focal, dense, granular, basophilic to amphophilic material is present within the lumen of distal collecting tubules.

Hyaline droplet accumulation - Epithelial cells of the proximal convoluted tubules contain variable numbers of intracytoplasmic, round, 1-4 micron diameter, dense, eosinophilic globules.

Nephritis, interstitial, chronic, focal - A few small interstitial areas, usually limited to the cortex, contain cellular infiltrate that is characterized primarily by macrophages and lymphocytes. Indentation of cortical surface is sometimes present.

TESTES

Decreased thickness of spermatogenic layers - Seminiferous tubules with wider lumens due to a decrease in the numbers of spermatogenic precursors lining the tubules.

Granuloma, spermatic, focal - A localized area containing interstitial spermatozoa and a mixed inflammatory cell infiltrate.

Oligospermia - Seminiferous tubules contain spermatogonium and primary spermatocytes. Developing spermatids and spermatozoa are absent or in very low numbers.

Synctial giant cells - One to several multinucleate (2-8/cell) giant cells, with 2-8 oval or round nuclei, are free in the lumen of seminiferous tubules.

TRACHEA

Ectasia, ductal, submucosa - Most tracheal gland ducts are moderately dilated, are often lined by flattened cuboidal epithelial cells, and contain variable amounts of eosinophilic fibrillar material.

EYES

Fusion of nuclear layers, retina - There is a focal transposition

APPENDIX C

and fusion of inner and outer nuclear layers, extending into the rod and cone layers.

HEART

Myocarditis, histiocytic, focal - An aggregate of macrophages in the right ventricular wall is adjacent to a cardiac myofiber that has a pyknotic nucleus and granular eosinophilic cytoplasm.

LIVER

Hepatitis, histiocytic, focal - A small focus of macrophages and lymphocytes mixed with cellular debris and eosinophilic material is located midzonal in an acinus.

Mononuclear aggregates, multifocal - Aggregates of lymphocytes and macrophages are adjacent to blood vessels, central veins, and within or adjacent to portal triads.

UTERUS

Ectasia, luminal - The uterine lumen is mildly to moderately dilated.

Neutrophilic infiltration, endometrium - A diffuse cellular infiltrate of neutrophils is throughout the lamina propria.

APPENDIX D

Pathology

Clinical pathology: One-way variance of analysis was performed on white cell counts, red cell counts, hemoglobin levels, and hematocrit values of blood and the pH and specific gravities of urine to determine if there were any differences between the 5 groups of control and test rats. The males and females were combined in each group for this purpose. In each case the "P-value" was greater than .05. We conclude that there are no differences between the groups.

Gross necropsy: There were two gross findings: One male group 4 rat had a small urinary calculus and one female group 1 rat had a white focal subcapsular area on the kidney (may have been adipose tissue). Both of these observations are interpreted as incidental findings unrelated to ingestion of the test compound.

Histologic findings: There was a higher incidence of hyaline droplet accumulation in the epithelium of the proximal convoluted tubules in the kidneys of male and female rats in test groups than in the control groups. Hyaline droplets were present in the kidneys of 7/10 rats in group 1 (4/5 males, 3/5 females), 10/10 rats in group 2 (5/5 males, 5/5 females), 8/10 rats in group 3 (4/5 males, 4/5 females), 10/10 rats in group 5 (5/5 males, 5/5 females), and only 1/9 control rats in group 4 (0/5 males, 1/4 females). Staining characteristics of the droplets indicate that they contain proteinic material. This lesion is compound related, but not clearly dose dependent. One male rat in group 3 (1/5 males), one male rat in group 2 (1/5 males) and 3/10 rats in group 5 (1/5 males, 2/5 females) had focal chronic interstitial nephritis. This lesion is a common finding and usually becomes more severe as the rat ages. It is not compound related. One female rat in group 4 (1/4 females) and one female rat in group 1 (1/5 females) has intratubular concretions in their kidneys that were incidental finding and were not test compound related.

Male rats fed the 2,4-DNT containing diet developed testicular lesions that were not observed in the male controls. Five of five rats in group 3 and group 1 and 1/5 rats in group 5 had oligospermia of varying degrees. Syncytial giant cells were present in the seminiferous tubules of 4/5 group 3 rats, 3/5 group 1 rats, and 1/5 group 5 rats. The presence of syncytial cells indicates a degenerative process. A decreased thickness of spermatogenic cell layers lining seminiferous tubules, considered to be an initial change leading to oligospermia, was present in 1/5 group 5 rats and 2/5 group 2 rats. The oligospermia, syncytial giant cell formation, and decreased thickness of spermatogenic cell layers appear to be both compound related and dose dependent (1). Spermatic granulomas were

present in 1/5 group 1 rats and 2/5 group 5 rats. Spermatic granulomas are usually the result of physical trauma that ruptures a seminiferous tubule. The associated inflammation represents a local foreign body response. The lesion is not considered to be test compound related.

Ectasia of the submucosal tracheal ducts was present in 7/9 rats in group 4 (4/5 males, 3/4 females), 8/10 rats in group 3 (4/5 males, 4/5 females), and 6/10 rats in group 1 (3/5 males, 3/5 females). The cause of this duct lesion is not known. It is not test compound related and is not considered to be a significant lesion.

There were 2/9 rats in group 4 (1/5 males, 1/4 females) with focal mixing of retinal layers. The retinal lesion is congenital and is not test compound related.

One male rat from group 1 (1/5 males) had focal histiocytic myocarditis. The focal myocarditis observed in this single male rat is not considered to be test compound related.

Focal histiocytic hepatitis was present in one rat from group 1 (1/5 females). The focal hepatitis observed in this single female rat is not considered to be test compound related.

Mononuclear aggregates were present in the livers of 5/9 rats in group 4 (1/5 males, 4/4 females) and 3/10 rats in group 1 (2/5 males, 1/5 females). This lesion is commonly observed in rats. The significance is not known, but it not compound related.

Endometrial neutrophilic infiltration was present in 2/5 group 1 rats and ectasia of the uterine lumen was present in 1/5 group 3 rats and 2/5 group 1 rats. This observation is probably a physiologic response related to the estrus cycle of the female rats.

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