LEVEL II

MIDWEST RESEARCH INSTITUTE

AD A062016

DDC FILE COPY

REPORT

AD_____

MAMMALIAN TOXICITY OF MUNITIONS COMPOUNDS
PHASE II: EFFECTS OF MULTIPLE DOSES
PART IV: NITROCELLULOSE

PROGRESS REPORT NO. 5
September 1976

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

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

This document has been approved for public release and sale; its distribution is unlimited.

MIDWEST RESEARCH INSTITUTE 425 VOLKER BOULEVARD, KANSAS CITY, MISSOURI 64110 • 816 753 7600

12 04 173

Animal experimentation: Animal experiments were conducted according to the "Guide for Laboratory Animal Facilities and Care" (1972) prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences, National Research Council; the regulations and standards prepared by the Department of Agriculture; and Public Law 91-579, "Laboratory Animal Welfare Act," 1970.

AD_____

MAMMALIAN TOXICITY OF MUNITIONS COMPOUNDS

PHASE II: Effects of Multiple Doses

PART IV: Nitrocellulose

PROGRESS REPORT NO. 5

September 1976

by

Harry V. Ellis, III
John J. Kowalski
John R. Hodgson
Jadgish C. Bhandari
Jaime L. Sanyer
Thomas W. Reddig
Jan L. Minor
Cheng-Chun Lee

1-A047 607

Supported by

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

Contract No. DAMD 17-74-C-4073

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

Midwest Research Institute
Kansas City, Missouri 64110

DDC Distribution Statement

Distribution Unlimited.

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

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: Progress Report No. 5, 1 Mar 431 Aug 75
2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle): MAMMALIAN TOXICITY OF MUNITION COMPOUNDS • PHASE II • Effects of Multiple Doses • PART IV • Nitrocellulose •

5. TYPE OF REPORT & PERIOD COVERED: Progress Report, 1 March 1975 to 31 August 1975

6. PERFORMING ORG. REPORT NUMBER: MRI Project No. 3900-B

7. AUTHOR(s): Harry V. Ellis, III, John J. Kowalski, John R. Hodgson, Jagdish C. Bhandari, Jaime L. Sanyer, Thomas W. Reddig, Jan L. Minor, Cheng-Chun Lee

8. CONTRACT OR GRANT NUMBER(s): DAMD-17-74-C-4073

9. PERFORMING ORGANIZATION NAME AND ADDRESS: Midwest Research Institute, 425 Volker Boulevard, Kansas City, MO 64110

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: 62758A 3A762758A835 00.038; 62720A 3A762720A835 00.038

11. CONTROLLING OFFICE NAME AND ADDRESS: U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD 21701

12. REPORT DATE: Sep 1976

13. NUMBER OF PAGES: 103

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office): 102p.

15. SECURITY CLASS. (of this report): Unclassified

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report): "Distribution Unlimited"

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

18. SUPPLEMENTARY NOTES

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

Toxicity
Oral Toxicity
Subchronic Toxicity
Absorption
Distribution
Nitrocellulose

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

The effects of feeding nitrocellulose (NC) for up to 13 weeks to dogs, rats and mice were studied. Feeding up to 3% NC had no adverse effects. Feeding 10% NC increased feed consumption in all species, decreased weight gain in rats and mice and killed some mice due to impaction of the fibers in the lower intestines. Feeding 10% cotton linters produced the same effects as 10% NC, so these effects are due to the fibers, not the chemical nature of NC.

Rats given oral doses of ^{14}C-labeled NC absorbed none of the dose. The label was recovered from the feces and gastrointestinal tract.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

230350

PREFACE

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

This work was conducted in the Biological Sciences Division, under the direction of Dr. William B. House, between 1 March 1975 and 31 August 1976. The experimental work was directed by Dr. Cheng-Chun Lee, Assistant Director, Biological Sciences, 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, supervised the studies on metabolism, cytogenesis and mutagenesis. Dr. J. C. Bhandari and Dr. Jaime L. Sanyer, Associate Veterinary Pathologists, supervised the necropsy and the histology preparation and performed the microscopic examination. Mr. Thomas W. Reddig (ASCP certified M.T.), Laboratory Supervisor, supervised the hematology and clinical laboratory tests. Mr. Jan L. Minor, Assistant Toxicologist, supervised the computer program and analysis of experimental data. Dr. William P. Duncan, Senior Radiochemist, nitrated radiolabeled cotton furnished by Dr. C. R. Benedict of Texas A and M University. Technical personnel include Robert C. Byrne, Bruce S. Andersen, Mary A. Kowalski, Francis H. Brown, Ellen R. Ellis, Ernesto A. Castillo, Judith D. Girvin, Patricia L. Wilkerson, Bhanu S. Gosalia, Laurel M. Halfpap and William M. Bracken.

Approved for:

MIDWEST RESEARCH INSTITUTE

C. C. Lee

C. C. Lee, Deputy Director
Biological Sciences Division

ACCESSION for
NTIS White Section
DDC Buff Section
UNANNOUNCED
JUSTIFICATION
BY
DISTRIBUTION/AVAILABILITY CODES
DIAL
A

TABLE OF CONTENTS

	Page
Abstract	xi
Introduction	1
I. Dogs	5
A. Subchronic Toxicity and Reversibility	5
1. Introduction	5
2. Material and Methods	5
a. Number of Dogs, Sex and Treatment	5
b. Experimental Procedures	6
c. Experimental Design	6
3. Results	6
a. General Observations, Body Weight and Feed Consumption	6
b. Blood Analysis	7
c. BSP Retention	7
d. Organ Weights	7
e. Gross and Microscopic Examination of Tissues	7
4. Discussion and Conclusions	8
B Immunologic Response to NC	8
1. Introduction	8
2. Material and Methods	8
3. Results and Conclusion	9
C. Summary	9
II. Rats	23
A. Subchronic Toxicity and Reversibility	23
1. Introduction	23
2. Material and Methods	23
a. Number of Rats, Sex and Treatment	23
b. Animal Husbandry	23
c. Feed Preparation	23
d. Experimental Procedure	24
e. Experimental Design	24

TABLE OF CONTENTS (Continued)

Page

3. Results 24

a. General Observations and Weight Gain 24
b. Feed Consumption 25
c. Blood Analyses 25
d. Organ Weights 26
e. Gross and Microscopic Examination of Tissues . 26

4. Discussion and Conclusions 27

B. Cytogenetic Effects of NC 27

1. Introduction 27
2. Material and Methods 27

a. Animals 27
b. Lymphocyte and Kidney Cultures 27
c. Chromosome Analysis 28

3. Results and Conclusion 28

C. Immunologic Response to NC 28

1. Introduction 28
2. Material and Method 28
3. Results and Conclusion 29

D. Summary 29

III. Mice 51

A. Subchronic Toxicity and Reversibility 51

1. Introduction 51
2. Material and Methods 51
3. Results 51

a. General Observations and Weight Gain 51
b. Feed Consumption 52
c. Blood Analysis 53
d. Organ Weights 53
e. Gross and Microscopic Examination of Tissues . 53

4. Discussion 54

TABLE OF CONTENTS (Concluded)

		Page
	B. Other Studies	54
	C. Summary	54
IV.	Absorption	67
	A. Introduction	67
	B. Methodology	67
	C. Results and Conclusions	68
V.	General Summary and Conclusions	71
References		75
Appendix I - Manual for Hematology, Clinical Blood Chemistry, Urinalysis, Histopathology, Statistical Analysis, and Normal Values		77

12 04 173

MAMMALIAN TOXICITY OF MUNITION COMPOUNDS

Phase II: Effects of Multiple Doses

Part IV: Nitrocellulose

(Report Number 5)

EXECUTIVE SUMMARY

The effects of NC after feeding for 13 weeks were investigated in dogs, rats and mice. A study of the absorption and distribution of ^{14}-C-labeled NC was performed in rats.

In dogs, feeding of 1, 3 or 10% of NC or 10% of cotton linters for 13 weeks did not cause any adverse effects. Dogs fed 10% of NC or linters ate somewhat more than the others, due to the non-nutritive bulk effect of these materials. All dogs had some variations in body weight, hematologic and clinical chemistry tests, and commonly seen spontaneous tissue lesions. Feeding NC did not change serum concentrations of IgE.

Rats fed 1 or 3% of NC in the feed for 13 weeks consumed slightly more feed without any adverse effects. Rats fed 10% of NC or cotton linters consumed large amounts of feed, but scattered much of it around their cages. They failed to gain as much weight as the controls, due to not getting enough nutritive intake. These rats did not show any changes in peripheral blood elements or clinical blood chemistry, any lesions, any cytogenetic damage, or any effect on serum IgE.

Mice fed 1 or 3% of NC in the feed for 13 weeks consumed slightly more feed without any adverse effects. High level (10%) of NC or cotton linters did not cause any changes in peripheral blood elements or any lesions. However, a number of mice died during the study due to impaction of fibers in their lower intestinal tract. The survivors fed these doses lost body weight due to insufficient nutritional intake.

Rats given oral suspension of NC-UL-^{14}C absorbed no radioactivity. The ^{14}C was recovered from the feces and gastrointestinal tract.

Program Director

Cheng-Chun Lee

Cheng-Chun Lee, Ph.D.
Deputy Director
Biological Sciences
For Pharmacology and Toxicology
MIDWEST RESEARCH INSTITUTE
425 Volker Boulevard
Kansas City, Missouri 64110

INTRODUCTION

Under Contract No. DAMD-17-74-C-4073, entitled "Munition Compounds Mammalian Toxicity Studies," 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 NC. 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. The absorption of the radiolabeled compound was studied in rats.

I. DOGS

TABLE OF CONTENTS

	Page
A. Subchronic Toxicity and Reversibility.	5
1. Introduction	5
2. Material and Methods	5
a. Number of Dogs, Sex and Treatment.	5
b. Experimental Procedures.	6
c. Experimental Design.	6
3. Results.	6
a. General Observations, Body Weight and Feed Consumption.	6
b. Blood Analysis	7
c. BSP Retention.	7
d. Organ Weights.	7
e. Gross and Microscopic Examination of Tissues	7
4. Discussion and Conclusions	8
B. Immunologic Response to NC	8
1. Introduction	8
2. Material and Methods	8
3. Results and Conclusion	9
C. Summary.	9
Tables 1 - 11.	10

I. DOGS

A. Subchronic Toxicity and Reversibility

1. Introduction

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

2. Material and Methods

a. Number of Dogs, Sex and Treatment

A total of 20 young healthy beagle dogs (Hazelton Research Animals, Cumberland, VA) weighing between 7.2 and 13.6 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. After acclimation, the dogs were trained to eat the feed at the same time each day. They were placed individually in metabolism cages with a measured amount of feed for at least 30 minutes. At the end of the period, the dogs were returned to their pen and the feed remaining estimated. Water was available ad libitum in the cage and pen. They were then divided into five groups, each consisting of two males and two females. The average weights of all groups were kept close.

Three groups of dogs were given 1, 3, or 10% of NC in their feed. NC was dipped from the poacher pits at Radford Army Ammunition Plant (Radford, VA) and shipped to MRI in 55 gallon drums. As needed, NC was removed from the drums and dewatered in a Buchner funnel attached to a water aspirator filter pump. The damp NC was mixed with appropriate amounts of Champion Dog Food (kennel formula) and water to produce 10% NC in feed as dry weight in a Univex Model 1222 food mixer with wire whip beaters. Aliquots of this diet were mixed with additional feed to yield 3% and 1%, respectively. These diets were given to the dogs in the metabolism cages as described above. The fourth group received a mixture of 10% of cotton linters (cellulose linters, Military Specification MIL-C-20330, Hercules, Inc., Wilmington, DE) prepared by mixing with feed and water as described above, and served as a cotton control to determine if any effects observed were due to the passage of a non-nutritive bulk through the gastrointestinal tract. The fifth group received moistened feed and served as a normal control.

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 and clinical blood chemistry tests. For fasting blood glucose, the dogs were bled before their daily feeding. At termination, the dogs were euthanized with an overdose of pentobarbital sodium, and examined for gross lesions. Weights of heart, liver, spleen, kidneys, adrenals, pituitary, thyroid, and gonads were recorded; organ weight to body weight or brain 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.

Bromosulfophthalein (BSP) retention test was performed at termination. A single dose of 5 mg/kg of the sterile test dye (Dade, Miami, FL) was injected intravenously following fasting for 16 hours. Serum level of the dye at 15 minutes was determined and the percent of retention in the plasma was calculated.[2/]

The results of the various parameters were compared with the respective baseline levels and/or with those of the control groups at the respective time interval according to Dunnett's Multiple Comparison Procedure.[3/]

c. Experimental Design

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

Since adverse effects were not observed in any dogs and NC-related lesions were not found in the dogs that were euthanized for necropsy at the end of 13 weeks, 17-week necropsy and blood analysis were not performed at 17 weeks.

3. Results

a. General Observations, Body Weight and Feed Consumption

The control dogs and dogs fed various levels of NC or linters were healthy and exhibited no toxic signs throughout the study. Their

body weights are summarized in Table 1. All dogs lost weight during the first 4 weeks of the study and most dogs regained weight thereafter. There were no apparent changes due to treatment with NC.

Feed consumption during the first and last 4 weeks of the study are shown in Table 2. Individual dogs varied slightly in feed consumption from day to day. On the average the normal control dogs ate the least, and all treated dogs ate more. The dogs fed 10% of NC or cotton linters ate about 15% more feed than did the normal control dogs. If one assumes that the NC and linters were non-nutritive bulk, the net consumption by all dogs were very similar. During the recovery period, when all the dogs were fed plain feed, all groups ate less.

b. Blood Analysis

The laboratory data for dogs in the normal control group, the cotton control group and the groups fed the low, middle and high levels of NC are summarized in Tables 3 through 7, respectively. Results for males and females varied little and have been combined for statistical analysis. Feeding nitrocellulose did not produce any toxicologically significant changes in any of the laboratory tests. There were a number of statistically significant differences when compared with the baseline levels or when compared with normal controls at the respective time intervals. The changes were small and inconsistent and all data were within normal limits (Appendix I).

c. BSP Retention

BSP retention was determined in the dogs terminated at the end of 13 weeks. Results are summarized in Table 8. NC did not cause any apparent retention of BSP.

d. Organ Weights

The absolute and relative organ weights of the dogs killed after 13 weeks of NC feeding are listed in Table 9. NC did not cause any apparent change in various organ weights.

e. Gross and Microscopic Examination of Tissues

At necropsy after 13 weeks of feeding, all dogs were in good health with normal body fat. Male No. 1 had a hypertrophic nictitating membrane of the left eye due to hypertrophy of the glands. Female dogs Nos. 2, 6 and 10 had discolored spots on their lungs. Female No. 18 had an enlarged, inflamed right tonsil. Dogs Nos. 2 and 17 had small discolored spots on their livers. Male No. 9 had a slight thickening of the cusps of his aortic valve, but it did not appear pathological.

The results of the microscopic examination are shown in Table 10. The normal control and cotton control dogs and dogs fed the high level of NC had mild or moderate vacuolation in the hepatocytes; special staining showed that these were glycogen deposits. In addition, mild inflammatory changes were seen in the liver of most dogs, in the tonsil of one normal control dog (No. 1), and in the uterus and tonsil of one dog fed the high level of NC. The normal control dog (No. 1) also had focal degeneration of germinal cells and retarded spermatogenesis. These lesions were spontaneous, naturally seen in dogs, and were not caused by feeding of NC. The bone marrow and myeloid/erythroid (M/E) ratios of these dogs were normal. Because no NC-induced lesions were seen in dogs fed the high level of NC, complete examination of tissues from dogs fed the low or middle levels was omitted.

4. Discussion and Conclusions

Feeding 1, 3 or 10% of NC or 10% of cotton linters to dogs for 13 weeks did not cause any adverse effects. Dogs fed 10% of NC or linters ate somewhat more than the others, indicating the test materials were merely non-nutritive bulk. All dogs, including the normal and cotton controls, showed some variations in body weight, peripheral blood elements and various clinical chemistry tests. NC did not cause any gross or microscopic changes in tissues.

B. Immunologic Response to NC

1. Introduction

In humans, anaphylactic reactions were associated with a higher immunoglobin E (IgE) titer.[4] The IgE, the allergic or hypersensitive antibody, of dogs treated with NC was determined.

2. Material and Methods

The immunodiffusion technique of Mancini et al.[5] was used for determination of serum IgE titer. Replicate 1 ml samples of serum from the normal control dogs and dogs treated with various levels of NC at various intervals were placed in wells in an immunodiffusion chamber along with suitable standards. These dogs were used for subchronic toxicity study as described in Section I.A. The diffusion chamber was incubated at 37°C for 48 hours and the diameter of the precipitin ring was measured. Since the square root of the diameter is directly proportional to the concentration of the antibody, the IgE concentration was quantitated with the standard antibody reagent.

3. Results and Conclusion

The results of IgE concentrations of normal control dogs and dogs fed NC or linters are summarized in Table 11. Feeding of NC for up to 13 weeks did not cause any apparent changes in serum concentration of IgE.

C. Summary

Feeding of 1, 3 or 10% of NC or 10% of cotton linters for 13 weeks did not cause any adverse effects. Dogs fed 10% of NC or linters ate somewhat more than the others, due to the non-nutritive bulk effect of these materials. All dogs had some variations in body weight, hematologic and clinical chemistry tests, and commonly seen spontaneous tissue lesions. Feeding NC did not change serum concentrations of IgE.

TABLE 1

BODY WEIGHTS OF DOGS FED NC

Dose (% in Feed)	Dog No.	Sex	Body Weights (kg)				
			Initial	4 Weeks	8 Weeks	13 Weeks	17 Weeks
0	1	M	9.4	8.4	9.4	11.4	
0	2	F	8.4	7.4	7.4	8.6	
10C[a]	5	M	13.6	13.1	13.0	12.8	
10C	6	F	8.0	7.3	7.4	8.5	
1	9	M	11.8	11.5	12.0	12.8	
1	10	F	11.0	10.2	10.3	11.4	
3	13	M	10.8	10.0	10.3	11.8	
3	14	F	8.0	5.9	6.3	7.4	
10	17	M	11.8	10.3	11.3	12.0	
10	18	F	9.8	8.8	9.0	9.5	
0	3	M	12.6	12.0	12.2	12.4	13.2
0	4	F	8.0	7.6	7.3	8.4	6.6
10C	7	M	11.0	10.6	11.1	11.8[b]	12.2
10C	8	F	8.2	7.7	8.8	9.3[b]	7.0
1	11	M	12.8	12.5	13.1	14.2[b]	13.5
1	12	F	12.0	11.9	12.0	12.2[b]	10.0
3	15	M	12.6	12.0	12.3	12.8[b]	13.0
3	16	F	7.2	6.9	8.0	8.6[b]	7.0
10	19	M	9.8	8.9	9.0	9.5[b]	10.0
10	20	F	9.4	8.1	8.2	9.6[b]	8.5

a/ Cotton control, fed 10% of cotton linters.
b/ Feeding of NC or linters discontinued thereafter.

TABLE 2

FEED CONSUMPTION OF DOGS FED NC

Dose (% in Feed)	Dog No.	Sex	Feed Consumption (gm/day) 1-4 Weeks	14-17 Weeks
0	1	M	720	
0	2	F	554	
10C a/	5	M	677	
10C	6	F	641	
1	9	M	637	
1	10	F	652	
3	13	M	709	
3	14	F	639	
10	17	M	701	
10	18	F	730	
0	3	M	570	719
0	4	F	574	424
10C	7	M	788	628
10C	8	F	653	461
1	11	M	606	611
1	12	F	691	523
3	15	M	685	472
3	16	F	631	529
10	19	M	622	407
10	20	F	704	516
0	1-4		605	572
10C	5-8		690	545
1	9-12		647	567
3	13-16		666	501
10	17-20		689	462

a/ Cotton control, fed 10% of cotton linters.

TABLE 3

LABORATORY DATA OF NORMAL CONTROL DOGS FOR NC

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

	WK 0 (B. 4)	WK 4 (C. 4)	WK 8 (C. 4)	WK 13 (C. 4)
ERYTHROCYTES ($X10^6/MM^3$)	5.69 ± .19	5.85 ± .23	5.81 ± .07	5.52 ± .16
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.94 ± .07	.39 ± .10 a/	.82 ± .16	.78 ± .11
HEMATOCRIT, VOL. %	41.2 ± 1.2	40.8 ± 1.1	41.8 ± 1.2	43.0 ± 1.6
HEMOGLOBIN, GM. %	14.4 ± .5	14.3 ± .7	14.8 ± .5	14.7 ± .4
METHEMOGLOBIN, %	.6 ± .4	2.1 ± 1.4	1.3 ± .8	2.6 ± 1.5
MCV, CUBIC MICRONS	72.6 ± .	69.8 ± 1.2	71.9 ± 1.4	77.8 ± .9 a/
MCHB, MICRO MICROGMS.	25.4 ± .3	24.5 ± .3	25.5 ± .6	26.7 ± .4
MCHBC, PM %	35.0 ± .4	35.2 ± .9	35.4 ± .2	34.3 ± .4
PLATELETS ($X10^5/MM^3$)	2.3 ± .2	2.5 ± .3	2.6 ± .1	2.6 ± .1
LEUKOCYTES ($X10^3/MM^3$)	12.1 ± .9	11.5 ± 1.7	10.6 ± .7	12.4 ± .9
NEUTROPHILS, %	50.7 ± 4.0	55.3 ± 1.8	52.5 ± 3.5	56.0 ± 1.6
LYMPHOCYTES, %	44.4 ± 4.0	31.0 ± 3.7	37.5 ± 4.0	35.3 ± 2.0
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EUSINOPHILS, %	4.1 ± .6	10.5 ± 4.7	7.5 ± .9	8.0 ± .9
BASOPHILS, %	0.0 ± 0.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.7 ± .3	2.8 ± .6	2.5 ± .6	.8 ± .5
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN.	6.4 ± .2	4.6 ± .6	7.8 ± .7	7.5 ± .2
GLUCOSE (FASTING), MG %	101.7 ± 2.3	87.0 ± 5.0 a/	102.3 ± 1.8	98.3 ± 3.9
SGOT, IU/L	25.7 ± 2.1	23.0 ± 2.9	22.8 ± 2.1	18.0 ± 1.2
SGPT, IU/L	34.9 ± 2.9	34.0 ± 1.7	35.3 ± 4.2	32.3 ± 4.1
ALK. PHOS., IU/L	77 ± 9	38 ± 4 a/	40 ± 5 a/	32 ± 5 a/
CHOLESTEROL, MG %	164 ± 14	161 ± 10	156 ± 6	168 ± 17
BUN, MG %	13.9 ± 1.4	10.0 ± .4 a/	11.5 ± .6	10.3 ± 1.1

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[3]).

TABLE 4

LABORATORY DATA OF DOGS FED COTTON LINTERS

DOSE 10% LINTERS

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

	WK 0 (B, 4)	WK 4 (C, 4)	WK 8 (C, 4)	WK 13 (C, 4)
ERYTHROCYTES ($\times 10^6$ /MM^3)	5.92 ± .10	5.63 ± .43	5.69 ± .09	5.51 ± .11
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.74 ± .08	.27 ± .06 [a]	.72 ± .13	.52 ± .06
HEMATOCRIT, VOL, %	43.5 ± .6	38.0 ± 4.3	40.5 ± .6	42.3 ± 1.7
HEMOGLOBIN, GM, %	15.0 ± .3	13.9 ± .6	14.2 ± .4	14.4 ± .5
METHEMOGLOBIN, %	.2 ± .2	0.0 ± 0.0	.7 ± .7	0.0 ± 0.0
MCV, CUBIC MICRONS	73.5 ± 1.2	66.8 ± 3.5	71.2 ± 1.5	76.8 ± 1.6
MCH, MICRO MICROGMS,	25.4 ± .5	24.9 ± .9	25.0 ± .7	26.1 ± .4
MCHC, GM %	34.5 ± .2	37.8 ± 3.5	35.0 ± .5	34.1 ± .3
PLATELETS ($\times 10^5$ /MM^3)	2.7 ± .3	2.4 ± .3	2.6 ± .1	2.8 ± .3
LEUKOCYTES ($\times 10^3$ /MM^3)	11.4 ± .3	11.0 ± .8	10.8 ± .7	14.5 ± 1.3
NEUTROPHILS, %	56.3 ± 4.3	56.3 ± 4.4	66.0 ± 2.0 [b]	63.5 ± 1.8
LYMPHOCYTES, %	41.2 ± 3.8	38.8 ± 6.2	27.0 ± .6	30.0 ± 2.7
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0
EOSINOPHILS, %	1.6 ± .8	4.5 ± 1.9	5.3 ± 1.5	5.5 ± 2.1
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.8 ± .2	.5 ± .5	1.5 ± .9	1.0 ± 1.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN,	6.3 ± .3	4.4 ± .1 [a]	7.0 ± .4	7.3 ± .5
GLUCOSE (FASTING), MG %	98.0 ± 2.5	84.3 ± 4.2 [a]	97.5 ± 3.8	85.5 ± 2.7 [b]
SGOT, IU/L	29.2 ± 1.7	23.5 ± 2.5	26.8 ± 3.6	23.3 ± .8
SGPT, IU/L	43.8 ± 7.8	59.3 ± 18.5	39.0 ± 5.6	32.5 ± 3.6
ALK, PHOS., IU/L	67 ± 9	38 ± 6 [a]	36 ± 7 [a]	34 ± 7 [a]
CHOLESTEROL, MG %	165 ± 11	158 ± 7	143 ± 10	140 ± 9
BUN, MG %	13.2 ± 1.1	8.5 ± 1.0 [a]	9.3 ± .9 [a]	7.5 ± 1.0 [a]

ENTRIES ARE MEAN ± STANDARD ERROR

[a] Significantly different from the baseline level (Dunnett's multiple comparison procedure[3]).
[b] Significantly different from the controls at the respective time interval (Dunnett's multiple comparison procedure[3]).

TABLE 5

LABORATORY DATA OF DOGS FED NC

DOSE 1% NC

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

	WK 0 (B. 4)	WK 4 (T. 4)	WK 8 (T. 4)	WK 13 (T. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	5.77 ± .17	5.70 ± .09	5.74 ± .14	5.16 ± .56
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.82 ± .03	.24 ± .04 a/	.46 ± .10	.88 ± .17
HEMATOCRIT, VOL. %	41.6 ± 1.2	40.5 ± .5	39.8 ± 1.6	37.8 ± 3.7
HEMOGLOBIN, GM. %	14.3 ± .5	13.7 ± .2	14.0 ± .4	13.1 ± 1.4
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	72.1 ± .6	71.0 ± .8	69.2 ± 1.6	73.4 ± 1.3
MCHB, MICRO MICROGMS.	24.8 ± .3	24.1 ± .2	24.4 ± .3	25.4 ± .5
MCHBC, GM %	34.4 ± .3	33.9 ± .2	35.2 ± .4	34.6 ± .6
PLATELETS ($X10^5$ /MM^3)	2.4 ± .3	2.3 ± .2	2.5 ± .2	2.6 ± .3
LEUKOCYTES ($X10^3$ /MM^3)	11.6 ± 1.1	12.7 ± 1.6	11.8 ± .9	15.5 ± 2.1
NEUTROPHILS, %	54.5 ± 1.2	55.8 ± 4.5	56.4 ± 2.5	61.0 ± 1.8
LYMPHOCYTES, %	42.4 ± 1.0	38.3 ± 3.8	35.5 ± 3.7	31.8 ± 3.2
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	2.8 ± .6	5.5 ± .9	6.3 ± 1.4	6.8 ± 2.7
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.2 ± .2	.5 ± .3	1.5 ± .9	.5 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN.	6.3 ± .4	4.5 ± .4 a/	8.3 ± .6 a/	8.0 ± .4 a/
GLUCOSE (FASTING), MG %	100.1 ± 1.9	89.3 ± 2.7	93.5 ± 4.9	95.0 ± 3.9
SGOT, IU/L	28.0 ± 3.5	22.8 ± 1.8	26.0 ± 2.2	22.0 ± 2.7
SGPT, IU/L	34.2 ± 2.1	30.0 ± 2.7	26.8 ± 5.6	27.8 ± 4.5
ALK. PHOS., IU/L	80 ± 5	44 ± 6	44 ± 4	50 ± 17
CHOLESTEROL, MG %	147 ± 6	145 ± 9	149 ± 12	139 ± 8
BUN, MG %	12.8 ± 1.3	8.0 ± .7	10.8 ± 1.1	11.3 ± 1.8

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[3]).

TABLE 6

LABORATORY DATA OF DOGS FED NC

DOSE 3% NC

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

	WK 0 (B, 4)	WK 4 (C, 4)	WK 8 (C, 4)	WK 13 (C, 4)
ERYTHROCYTES ($X10^6/MM^3$)	5.82 ± .17	6.05 ± .30	5.96 ± .25	5.38 ± .15
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.95 ± .08	.24 ± .08 a/	.76 ± .18	.45 ± .05
HEMATOCRIT, VOL, %	43.3 ± 1.3	44.0 ± 1.3	41.3 ± 1.5	41.5 ± .9
HEMOGLOBIN, GM, %	15.1 ± .6	15.1 ± .7	14.6 ± .6	14.0 ± .2
METHEMOGLOBIN, %	.4 ± .4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	74.5 ± 1.1	72.9 ± 1.5	69.2 ± .5 a/	77.3 ± 1.0
MCHB, MICRO MICROGMS,	25.9 ± .6	24.9 ± .1	24.5 ± .1 a/	26.0 ± .3
MCHBC, GM %	34.8 ± .4	34.3 ± .7	35.4 ± .3	33.7 ± .4
PLATELETS ($X10^5/MM^3$)	2.5 ± .1	2.5 ± .4	2.7 ± .3	2.6 ± .3
LEUKOCYTES ($X10^3/MM^3$)	13.9 ± 1.1	10.9 ± 1.3	11.9 ± 1.7	17.7 ± 1.9
NEUTROPHILS, %	58.6 ± 4.6	61.8 ± 3.3	59.5 ± 3.2	66.4 ± 2.1
LYMPHOCYTES, %	36.6 ± 3.6	30.5 ± 2.9	32.0 ± 3.3	26.8 ± 2.5
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0
EOSINOPHILS, %	3.5 ± 1.4	7.0 ± 2.3	6.8 ± .6	6.3 ± .6
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.3 ± .5	.8 ± .6	1.5 ± .3	.3 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0
CLOTTING TIME, MIN.	6.2 ± .1	4.5 ± .3 a/	6.9 ± .6	7.9 ± .5 a/
GLUCOSE (FASTING), MG %	103.4 ± 2.5	90.8 ± 1.8 a/	98.3 ± 2.3	97.8 ± 2.2
SGOT, IU/L	30.0 ± 2.2	23.4 ± 2.5	24.3 ± 1.4	19.5 ± 1.5 a/
SGPT, IU/L	38.3 ± 4.0	32.3 ± 2.8	28.8 ± .8	30.0 ± 2.7
ALK. PHOS., IU/L	65 ± 7	38 ± 3 a/	37 ± 4 a/	32 ± 2 a/
CHOLESTEROL, MG %	165 ± 9	148 ± 10	139 ± 7	173 ± 11
BUN, MG %	15.4 ± 1.0	9.8 ± .9 a/	10.3 ± 1.4 a/	8.8 ± 1.3 a/

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[3]).

TABLE 7

LABORATORY DATA OF DOGS FED NC

DOSE 10% NC

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

	WK 0 (B, 4)	WK 4 (T, 4)	WK 8 (T, 4)	WK 13 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	5.68 ± .25	5.94 ± .19	5.97 ± .27	5.40 ± .19
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.83 ± .11	.31 ± .04	.85 ± .14	.94 ± .12
HEMATOCRIT, VOL, %	41.7 ± 1.1	41.8 ± 1.1	40.8 ± 1.4	39.5 ± 1.6
HEMOGLOBIN, GM, %	14.4 ± .5	14.6 ± .4	14.2 ± .5	13.7 ± .5
METHEMOGLOBIN, %	.6 ± .2	1.4 ± .8	1.4 ± .8	1.4 ± .8
MCV, CUBIC MICRONS	73.5 ± 1.3	70.3 ± .7	68.4 ± 1.0 a/	73.1 ± 1.2
MCHH, MICRO MICROGMS,	25.4 ± .4	24.5 ± .4	24.0 ± .4	25.3 ± .4
MCHHC, GM %	34.6 ± .3	35.0 ± .5	35.1 ± .2	34.6 ± .2
PLATELETS ($X10^5$ /MM^3)	3.0 ± .3	2.8 ± .4	2.9 ± .4	1.8 ± .6
LEUKOCYTES ($X10^3$ /MM^3)	11.9 ± .7	12.1 1.3	10.1 ± .4	14.9 ± .6
NEUTROPHILS, %	57.6 ± 2.2	62.0 ± 4.0	53.0 ± 4.5	62.5 ± 5.4
LYMPHOCYTES, %	38.3 ± 2.3	29.3 ± 5.9	38.3 ± 5.3	28.6 ± 3.5
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	3.5 ± 1.1	7.3 ± .9	7.0 ± 1.5	8.5 ± 3.7
BASOPHILS, %	0.0 ± 0.0	0.3 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.6 ± .2	1.5 ± .4	1.8 ± .9	.3 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN.	6.5 ± .2	4.9 ± .6	7.8 ± .4	9.5 ± .4 a,b/
GLUCOSE (FASTING), MG %	96.5 ± 1.5	96.0 ± 8.0	98.3 ± 4.2	99.0 ± 1.7
SGOT, IU/L	28.7 ± 1.4	20.3 ± 1.9 a/	24.3 ± 1.4	19.5 ± 2.6 a/
SGPT, IU/L	33.8 ± 1.2	32.5 ± 1.9	30.8 ± 2.9	27.5 ± 3.5
ALK, PHOS., IU/L	76 ± 5	44 ± 6 a/	48 ± 5 a/	44 ± 3 a/
CHOLESTEROL, MG %	159 ± 4	133 ± 4 a/	118 ± 7 a,b/	130 ± 4 a/
BUN, MG %	12.8 ± .4	9.5 ± .6 a/	10.3 ± .6	9.5 ± 1.0 a/

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[3]).
b/ Significantly different from the controls at the respective time interval (Dunnett's multiple comparison procedure[3]).

TABLE 8

BSP RETENTION OF DOGS FED NC FOR 13 WEEKS

Dose (% in Feed)	Dog No.	% Retention
0	1	5
C	2	2
10C[a/]	5	4
10C	6	2
1	9	2
1	10	4
3	13	5
3	14	5
10	17	4
10	18	3

a/ Cotton control, fed 10% of cotton linters.

TABLE 9

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF DOGS FED NC FOR 13 WEEKS

Dose (% in Feed)	Dog No.	Terminal Body Weight (kg)	Absolute Organ Weights (gm)									
			Heart	Liver	Kidneys	Spleen	Adrenals	Pituitary	Thyroid	Testes	Ovaries	Brain
0	1	11.4	74.9	258.8	50.5	39.9	1.28	0.06	0.68	10.8	--	88.3
0	2	8.6	58.7	320.0	39.2	60.7	1.16	0.04	0.55	--	1.80	68.7
10C[a/]	5	12.8	114.5	406.1	70.1	89.0	1.56	0.08	0.89	12.7	--	71.5
10C	6	8.5	54.1	275.0	42.8	32.3	1.27	0.06	0.55	--	0.51	82.9
1	9	12.8	106.2	371.1	73.4	37.8	1.51	0.07	0.84	16.4	--	78.2
1	10	11.4	82.2	319.3	51.7	63.4	1.26	0.07	0.75	--	0.94	72.8
3	13	11.8	85.6	361.9	53.9	100.2	1.22	0.08	0.65	11.7	--	79.6
3	14	7.4	72.6	284.4	34.1	28.0	1.58	0.07	0.63	--	1.11	66.2
10	17	12.0	91.9	424.2	69.6	33.4	0.94	0.05	0.75	17.4	--	78.9
10	18	9.5	71.4	284.4	42.9	25.6	1.41	0.07	0.68	--	0.94	77.2

Dose (% in Feed)	Dog No.	Relative Organ Weights (gm/kg body weight)									
		Heart	Liver	Kidneys	Spleen	Adrenals	Pituitary	Thyroid	Testes	Ovaries	Brain
0	1	6.57	22.7	4.43	3.50	0.112	0.005	0.060	0.95	--	7.75
0	2	6.83	37.2	4.56	7.06	0.135	0.005	0.064	--	0.209	7.99
10C	5	8.95	31.7	5.48	6.95	0.122	0.006	0.070	0.99	--	5.59
10C	6	6.36	32.4	5.04	3.80	0.149	0.007	0.065	--	0.107	9.75
1	9	8.30	29.0	5.73	2.95	0.118	0.005	0.066	1.13	--	6.11
1	10	7.21	28.0	4.54	5.56	0.111	0.006	0.066	--	0.082	6.39
3	13	7.25	24.1	4.56	8.49	0.103	0.007	0.055	0.99	--	6.75
3	14	9.81	38.4	4.61	3.78	0.214	0.009	0.085	--	0.150	8.95
10	17	7.66	35.4	5.80	2.78	0.078	0.004	0.063	1.45	--	6.58
10	18	7.52	30.0	4.52	2.69	0.148	0.007	0.072	--	0.099	8.13

Dose (% in Feed)	Dog No.	Relative Organ Weights (gm/gm Brain Weight)								
		Heart	Liver	Kidneys	Spleen	Adrenals	Pituitary	Thyroid	Testes	Ovaries
0	1	0.848	2.93	0.572	0.452	0.0145	0.0007	0.0077	0.122	--
0	2	0.854	4.66	0.571	0.884	0.0169	0.0006	0.0080	--	0.0262
10C	5	1.601	5.09	0.980	1.244	0.0218	0.0011	0.0124	0.178	--
10C	6	0.653	3.32	0.516	0.390	0.0153	0.0007	0.0066	--	0.0110
1	9	1.358	4.75	0.939	0.483	0.0193	0.0009	0.0107	0.184	--
1	10	1.129	4.39	0.710	0.871	0.0173	0.0010	0.0103	--	0.0129
3	13	1.075	4.55	0.677	1.259	0.0153	0.0010	0.0081	0.147	--
3	14	1.097	4.30	0.515	0.423	0.0239	0.0011	0.0095	--	0.0168
10	17	1.165	5.38	0.882	0.423	0.0119	0.0006	0.0095	0.221	--
10	18	0.925	3.69	0.556	0.331	0.0183	0.0009	0.0088	--	0.0121

a/ Cotton control, fed 10% of cotton linters.

TABLE 10

SUMMARY OF TISSUE LESIONS IN DOGS FED NITROCELLULOSE FOR 13 WEEKS

Lesions[a]	Dose (% in feed)					
	0		10C[b]		10	
Dog No.:	1	2	5	6	17	18
Eye						
Hypertrophy of glans nictitans	2					
Liver						
Pigment deposits	1					
Glycogenic infiltration in hepatocytes	1	2	1	2	2	2
Chronic inflammation and focal fibrosis			1			
Mononuclear cell microfoci				1	1	
Microgranuloma						1
Leucocytic infiltration	1					
Eosinophilic infiltration						1
Testis						
Focal degeneration of germinal cells and retarded spermatogenesis	1					
Uterus						
Mononuclear cell microfoci						1
Tonsil						
Inflammation	1					1
Bone marrow						
M/E ratio	1.3	1.2	1.1	1.3	1.2	1.1

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = markedly severe.

b/ Cotton control, fed 10% of cotton linters.

TABLE 11

SERUM IgE (IU/ml) OF DOGS FED NC

Dose (% in Feed)	Treatment Weeks			
	Week 0	Week 4	Week 8	Week 13
0	925±98 [b/]	400, 1500	1075±229	1531±142
10 [a/]	975, 1175 [c/]			1488±128
1	400, 400			
3	925		1500, 1525	
10	923±41	656±93	1444±41	1431±16

a/ Cotton control, fed 10% of cotton linters.
b/ Mean ± standard error of four dogs.
c/ Individual values.

II. RATS

TABLE OF CONTENTS

	Page
A. Subchronic Toxicity and Reversibility	23
1. Introduction	23
2. Material and Methods	23
a. Number of Rats, Sex and Treatment	23
b. Animal Husbandry	23
c. Feed Preparation	23
d. Experimental Procedure	24
e. Experimental Design	24
3. Results	24
a. General Observations and Weight Gain	24
b. Feed Consumption	25
c. Blood Analyses	25
d. Organ Weights	26
e. Gross and Microscopic Examination of Tissues	26
4. Discussion and Conclusions	27
B. Cytogenetic Effects of NC	27
1. Introduction	27
2. Material and Methods	27
a. Animals	27
b. Lymphocyte and Kidney Cultures	27
c. Chromosome Analysis	28
3. Results and Conclusion	28
C. Immunologic Response to NC	28
1. Introduction	28
2. Material and Method	28
3. Results and Conclusion	29
D. Summary	29
Tables 12 - 29	30

II. RATS

A. Subchronic Toxicity and Reversibility

1. Introduction

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

2. Material and Methods

a. Number of Rats, Sex and Treatment

A total of 40 male and 40 female young healthy CD® rats (Charles River Breeding Lab., Wilmington, Mass.) were used for this study. They were divided into five groups, each consisting of eight males and eight females. The average weights of all groups were kept close. Three groups of rats were fed 1, 3 or 10% of NC in the feed. The 4th group, referred to as the cotton controls, received 10% of cotton linters in the feed. The 5th group, referred to as the normal controls, was given the powdered standard rodent chow (Wayne Laboratory Meal) without NC.

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 $\pm$ 5°F and the relative humidity at 50 $\pm$ 10%. The light cycle in all animal rooms is kept at 12-hour on and 12-hour off with a timer.

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

c. Feed Preparation

Wet NC was dewatered, as described for the dogs in Section I.A.2.a., and an appropriate amount added to the rodent chow to yield

a diet containing 10% NC on a dry basis. 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 hr in a modified cement mixer at a speed of 20 rpm. Subsequently, appropriate amounts of this diet were mixed with the standard chow to produce 3% and again 1% of NC, respectively. The diet for the cotton control group consisted of 10% of cellulose linters mixed as for the NC diets.

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) 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.

(2) 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 13 weeks, four male and four female rats from each group were euthanized for necropsy.

(2) The treatment for four males and four female rats from each group were discontinued at the end of 13 weeks. They were kept for observation. Since adverse effects were not observed in any rats and NC did not cause any lesions in the rats that were euthanized at the end of 13 weeks, the rats for the reversibility study were not necropsied for examination at 17 weeks as scheduled.

3. Results

a. General Observations and Weight Gain

The rats fed linters or various levels of NC were generally healthy throughout the experiment. However, during week 4, one low dosage male (No. 155) became inactive, occasionally twitching, and had a large lump on his forehead. During the next weeks he gained little weight, his fur became rough, and his back arched. In week 6, he lost 41 gm in 2 days, so he was killed. Necropsy showed severe hydrocephalus, apparently congenital. One night in week 8, one low dosage female (No. 256)

caught her left front leg in the wire mesh cage top. By morning the leg was black due to a tourniquet effect, so she was killed. There were no other gross adverse effects.

The body weights of male and female rats before, during and after NC feeding are shown in Table 12. The weight gains of the males and females fed the low (1%) and middle (3%) levels of NC were comparable to those of the control group. Males fed the high level (10%) of NC or cotton linters had reduced weight gain, with the cotton control rats being somewhat more affected. When returned to plain feed for the recovery study, the body weights of the high level males approached the weights of the normal control males, whereas those of the cotton control males were still less than those of the normal control males. The weight gains of the female rats fed 10% of cotton linters were also depressed as compared with the normal control females.

b. Feed Consumption

Feed consumption of rats fed the cotton linters or various levels of NC are shown in Table 13. Rats fed the cotton linters or the high level (10%) of NC were readily identified by the enormous mounds of white fluffy material scattered all around the cages. Apparently, the rats tried to discard the fiber while trying to get at the feed. Therefore, these rats had high apparent feed consumptions. Rats fed the low level of NC ate somewhat more feed than the control rats. Rats fed the middle level ate considerably more. Since there was no apparent increase in feed scattering, these rats ate more feed to compensate for the non-nutritive fiber in their diet. During the recovery period, most rats consumed less feed than the controls.

c. Blood Analyses

The laboratory results from control male rats and male rats fed linters or various levels of NC are summarized in Tables 14 through 18, respectively. The control males had increases in erythrocytes and hemoglobin, a decrease in reticulocytes, and various changes in the cell indices. These changes are normal in maturing rats like those used in these studies. The terminal blood samples have statistically lower hematocrits and leucocyte and platelet counts. The leucocyte count is below normal (see Appendix I, Table L). One control male (No. 136) had very high SGOT (595 IU/liter), SGPT (803 IU/liter) and alkaline phosphatase (111 IU/liter) values. The other peripheral blood elements fluctuated within normal limits. Similar changes and fluctuations in peripheral blood elements occurred in rats fed various levels of NC or cotton linters. The changes and fluctuations were small, inconsistent, and were not due to NC.

The laboratory results from control female rats and female rats fed linters or various levels of NC are summarized in Tables 19 through 23, respectively. The results were similar to those seen in the males. There were increases in erythrocyte count, hematocrit, and/or hemoglobin concentration, and/or decrease in reticulocytes during the experiment in the normal control and cotton control rats, and rats fed various levels of NC. Other changes and fluctuations were small and of no clinical significance.

d. Organ Weights

The organ weights of rats fed 10% linters or various levels of NC for 13 weeks are summarized in Table 24. When compared with the normal control rats, the males fed 10% of cotton linters or NC had significantly smaller weights of liver, kidney, and/or spleen with similar weights of testes and brain. These males also had considerably smaller body weights. Based on body weights, their relative weights of testes and brain were larger, and their liver, kidney and spleen weights were similar to those of the normal control males. Based on brain weight, the relative weights of the various organs of the treated males were not significantly different from those of the normal controls. For the females, there were not any consistent differences in various organ weights.

e. Gross and Microscopic Examination of Tissues

The rats fed linters or various levels of NC were in good nutritional condition with no gross lesions when necropsied after 13 weeks of feeding. Results of the microscopic examintion are shown in Tables 25 and 26. The control rats and the rats fed the cotton linters or the high level of NC had inflammatory lesions in the myocardium, lung, salivary gland, liver, kidneys and/or adrenal. Other occasional lesions occurred in both the control rats and rats fed linters or NC. The lesions included retinal rosettes, epithelial hyperplasia, corneal thickening and/or chorioretinopathy of the eye, pinworms in the large intestine, pelvic dilation and/or microcalculi in the kidney, or a cystic and hypoplastic thyroid. These lesions were spontaneous and were not caused by cotton linters or NC. The bone marrows and their M/E ratios were normal.

Since 10% of cotton linters or NC in the feed did not cause any lesions, the tissue slides prepared from rats fed the middle (3%) or low (1%) of NC were not examined.

4. Discussion and Conclusions

Male rats fed the low, middle or high levels of NC consumed 26.9, 31.7 or 58.1 gm/rat/day of feed, respectively. Female rats consumed 20.1, 22.9 or 46.5 gm/rat/day, respectively. Much of the feed, particularly the cotton fibers for the rats fed the high level (10%) of NC, was spread about the cages. Rats fed control feed ate less averaging 25.9 and 17.5 gm/rat/day for the males and females, respectively. Rats fed 10% of cotton linters ate and removed considerably more feed averaging 67.3 and 52.2 gm/rat/day for the males and females, respectively. The NC and linters apparently acted as non-nutritive bulk ingredient in the feed, and the rats attempted to remove it. These rats did not get enough nutritive portion of the feed, and they gained less weight than did the normal control rats. The male and the female rats fed the low or middle levels of NC apparently received enough nutritional intake. They gained weight comparable to the controls.

NC did not cause any significant changes in peripheral blood elements or clinical blood chemistry or any lesions. The changes in organ weights were due to depressed body weight gain. One control rat (No. 136) had high serum transaminases and alkaline phosphatase due to marked hepatic necrosis. Other noted effects were mild and inconsistent.

B. Cytogenetic Effects of NC

1. Introduction

The cytogenetic effect of NC on somatic cell chromosomes was studied. The lymphocyte and kidney cultures from rats fed NC were obtained and examined for any damages.

2. Material and Methods

a. Animals

Rats fed the high level of NC from the subchronic toxicity study were used. These rats were fed 10% of NC in the feed for 13 weeks.

b. Lymphocyte and Kidney Cultures

At the end of 13 weeks, blood samples were aspetically drawn from the tail vein of both the cotton control and treated rats. The cotton control rats fed 10% of cotton linters were used to eliminate any effects

due to the non-nutritive bulk fibers. The lymphocytes were cultured by the method of Moorhead et al.[6] Kidney tissue samples were removed at necropsy and cultured by the trypsinization method of Fernandes.[7] All cultures were maintained in Eagle's medium as modified by Vogt and Dulbecco.[8]

c. Chromosome Analysis

Actively dividing kidney cultures and phytohemagglutin-stimulated lymphocytes were arrested in metaphase by short-term colchicine treatment. The cells were trypsinized, swollen in hypotonic solution, and processed for spreading on glass slides by the method of Moorhead and Nowell.[9] Slides were stained with giemsa and scanned under low power optics. Cell polyploidy was estimated by examination of 200 cells. Slides showing minimum scattering of cells in metaphase were selected for analysis under oil immersion optics. Chromosomes were counted and morphological aberrations were examined from photographic negatives of up to 50 metaphase cells.

3. Results and Conclusion

The results on numerical and morphological aberrations of chromosomes are shown in Tables 27 and 28, respectively. Rats fed 10% of NC for 13 weeks did not show any changes in chromosome frequency distribution, number of tetraploids, or frequency of chromatid breaks, gaps or translocation in the peripheral lymphocyte and kidney cultures. NC was not absorbed through the gastrointestinal tract as discussed below in Part IV, so these results are expected.

C. Immunologic Response to NC

1. Introduction

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

2. Material and Method

As described for the dogs in Section I.B.2., the immunodiffusion technique of Mancini[5] was used to determine the serum IgE of rats fed 10% of NC for 13 weeks. These rats were used in the subchronic toxicity study described in Section II.A.

3. Results and Conclusion

Serum concentration of IgE of cotton control rats and rats fed 10% NC for 13 weeks are summarized in Table 29. NC did not apparently alter the serum concentration of IgE.

D. Summary

Rats fed 1 or 3% of NC in the feed for 13 weeks consumed slightly more feed without any adverse effects. Rats fed 10% of NC or of cotton linters consumed large amounts of feed, but scattered much of it around their cages. They failed to gain as much weight as the controls, due to not getting enough nutritive intake. These rats did not show any changes in peripheral blood elements or clinical blood chemistry, any lesions, any cytogenetic damage, or any effect on serum IgE.

TABLE 12

BODY WEIGHTS OF RATS FED NC

Sex	% NC in Feed	Body Weights (gm) Initial	4 Weeks	8 Weeks	13 Weeks	17 Weeks
Male	0	250 ± 5[b]	394 ± 9	477 ± 15	540 ± 16	
	10C[a]	240 ± 5	326 ± 4	361 ± 14	420 ± 11	
	1	254 ± 6	389 ± 15	472 ± 23	545 ± 38	
	3	239 ± 12	385 ± 26	456 ± 24	520 ± 27	
	10	232 ± 5	329 ± 5	391 ± 4	446 ± 5	
Female	0	179 ± 5	250 ± 5	278 ± 7	306 ± 6	
	10C	188 ± 3	231 ± 6	270 ± 3	298 ± 6	
	1	171 ± 7	236 ± 14	266 ± 9	283 ± 11	
	3	181 ± 3	252 ± 9	293 ± 13	330 ± 14	
	10	182 ± 2	242 ± 5	274 ± 7	304 ± 15	
Male	0	246 ± 3	399 ± 9	487 ± 21	555 ± 22	600 ± 22
	10C	251 ± 3	322 ± 9	376 ± 11	438 ± 15[c]	557 ± 25
	1	246 ± 3	368 ± 9	442 ± 9	507 ± 17[c]	550 ± 13
	3	246 ± 11	393 ± 22	456 ± 25	525 ± 30[c]	576 ± 38
	10	253 ± 6	350 ± 13	408 ± 14	476 ± 12[c]	596 ± 17
Female	0	186 ± 5	247 ± 7	274 ± 7	308 ± 10	328 ± 9
	10C	192 ± 11	231 ± 6	272 ± 4	287 ± 5[c]	315 ± 2
	1	175 ± 5	239 ± 7	282 ± 12	308 ± 12[c]	321 ± 15
	3	172 ± 4	243 ± 6	277 ± 7	310 ± 9[c]	317 ± 10
	10	187 ± 4	250 ± 9	283 ± 9	311 ± 13[c]	339 ± 2

a/ Cotton control; fed 10% of cotton linters.
b/ Mean ± standard error of four rats.
c/ NC or linters in feed discontinued thereafter.

TABLE 13

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

% NC in Feed	Males				
	1 - 4[b]	5 - 8	9 - 13	1 - 13	14 - 17[c]
0	24.7	27.1	26.0	26.0	25.1
10C[a]	70.6	65.8	65.5	67.3	23.7
1	23.0	28.1	29.7	26.9	19.4
3	29.7	35.2	30.3	31.7	23.2
10	55.6	59.7	59.0	58.1	26.0
	Female				
	1 - 4	5 - 8	9 - 13	1 - 13	14 - 17[c]
0	16.7	17.9	17.9	17.5	16.2
10C	51.0	55.6	50.1	52.2	14.0
1	17.7	19.6	23.1	20.1	11.9
3	21.0	24.5	23.3	22.9	14.0
10	45.6	48.0	46.0	46.5	13.1

a/ Cotton control; fed 10% of cotton linters.
b/ Weeks.
c/ Recovery period; all rats fed control feed.

TABLE 14

LABORATORY DATA OF NORMAL CONTROL MALE RATS FOR NC

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

	WK 0 (B. 4)	WK 4 (C. 4)	WK 8 (C. 4)	WK 13 (C. 4)
ERYTHROCYTES ($X10^5$ /MM^3)	5.81 ± .09	6.79 ± .19	6.56 ± .29	7.15 ± .30[a/]
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	1.90 ± .53	1.35 ± .27	1.55 ± .22	1.11 ± .10
HEMATOCRIT, VOL. %	51.3 ± 3.2	52.0 ± 1.6	50.5 ± .9	42.0 ± 2.4[a/]
HEMOGLOBIN, GM. %	14.2 ± .2	16.2 ± .4[a/]	16.4 ± .2[a/]	14.3 ± .8
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3
MCV, CUBIC MICRONS	88.0 ± 4.4	76.6 ± 1.7[a/]	77.2 ± 2.3[a/]	58.7 ± .4[a/]
MCHB, MICRO MICROGMS.	24.4 ± .0	23.8 ± .2	25.1 ± .7	20.1 ± .1[a/]
MCHBC, GM %	27.9 ± 1.4	31.1 ± .5[a/]	32.5 ± .3[a/]	34.2 ± .3[a/]
PLATELETS ($X10^5$ /MM^3)	8.0 ± .7	6.5 ± .5	7.1 ± .3	4.6 ± .3[a/]
LEUKOCYTES ($X10^3$ /MM^3)	18.3 ± 2.9	21.0 ± 1.5	20.2 ± 1.4	7.0 ± 1.9[a/]
NEUTROPHILS, %	12.3 ± 2.1	5.0 ± .1	6.3 ± 2.4	23.8 ± 8.1
LYMPHOCYTES, %	86.3 ± 2.0	92.0 ± .9	91.5 ± 2.8	73.8 ± 8.0
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.5 ± .5	2.3 ± 1.0	1.5 ± 1.0	.5 ± .5
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.0 ± .4	.8 ± .5	.8 ± .5	2.0 ± .4
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
GLUCOSE (FASTING), MG %				148.5 ± 6.9
SGOT, IU/L				239 ± 120
SGPT, IU/L				228 ± 192
ALK. PHOS., IU/L				63 ± 17
CHOLESTEROL, MG %				78 ± 18
BUN, MG %				15.3 ± 3.2

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[3/]).

TABLE 15

LABORATORY DATA OF MALE RATS FED COTTON LINTERS

DOSE 10 % IN FEED

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

	WKS 0 (B. 4)	WKS 4 (T. 4)	WKS 8 (T. 4)	WKS 13 (C. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	5.78 ± .07	7.11 ± .34[a/]	6.46 ± .41	7.39 ± .23[a/]
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	1.65 ± .20	1.45 ± .03	1.11 ± .26	1.18 ± .09
HEMATOCRIT, VOL. %	49.3 ± 2.4	54.5 ± .9	51.0 ± 1.4	44.8 ± .9
HEMOGLOBIN, GM. %	14.0 ± .2	16.7 ± .3 [a/]	16.1 ± .3[a/]	15.0 ± .2
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	.6 ± .6	0.0 ± 0.0
MCV, CUBIC MICRONS	85.4 ± 5.1	77.1 ± 2.9	79.5 ± 3.5	60.7 ± 1.7[a/]
MCH, MICRO MICROGMS.	24.3 ± .4	23.7 ± 1.3	25.1 ± 1.1	20.3 ± .6[a/]
MCHC, GM %	28.6 ± 1.0	30.7 ± .7	31.5 ± .2[a/]	33.5 ± .2[a/]
PLATELETS ($X10^5$ /MM^3)	7.2 ± .4	6.0 ± 1.0	6.6 ± .5	5.6 ± .2
LEUKOCYTES ($X10^3$ /MM^3)	19.2 ± 1.1	23.9 ± 1.2	23.0 ± 1.6	5.5 ± 1.1[a/]
NEUTROPHILS, %	7.0 ± 1.4	9.8 ± 1.0	8.5 ± .9	22.5 ± 3.9[a/]
LYMPHOCYTES, %	92.0 ± 1.1	87.3 ± 1.5	90.5 ± .5	75.3 ± 3.6[a/]
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	0.0 ± 0.0	1.0 ± .6	.5 ± .3	1.0 ± .4
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.0 ± .4	2.0 ± .7	.5 ± .3	1.3 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0
GLUCOSE (FASTING), MG %				110.8 ± 10.8[b/]
SGOT, IU/L				86.3 ± 7.7
SGPT, IU/L				31.8 ± 2.3
ALK. PHOS., IU/L				49 ± 5
CHOLESTEROL, MG %				53 ± 4
BUN, MG %				17.8 ± 2.7

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[3/]).
b/ Significantly different from the controls at the respective time interval (Dunnett's multiple comparison procedure[3/]).

TABLE 16

LABORATORY DATA OF MALE RATS FED NC

DOSE 1 % IN FEED

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

	WKS 0 (B. 4)	WKS 4 (T. 4)	WKS 8 (T. 4)	WKS 13 (C. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	5.48 ± .14	7.51 ± .26a/	6.96 ± .19a/	8.05 ± .12a/
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	2.77 ± .68	.99 ± .16a/	1.29 ± .22	1.03 ± .09a/
HEMATOCRIT, VOL, %	49.0 ± 3.0	53.0 ± 2.3	50.5 ± 1.2	46.5 ± .5
HEMOGLOBIN, GM, %	14.3 ± .4	16.2 ± .6a/	16.1 ± .3a/	15.6 ± .2
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	83.7 ± 5.1	70.6 ± 1.1a/	72.6 ± 1.0a/	57.8 ± .6a/
MCHA, MICRO MICROGMS,	24.5 ± .3	21.6 ± .1a/	23.2 ± .2a/	19.4 ± .1a/
MCHHC, GM %	29.5 ± 1.4	30.6 ± .5	32.0 ± .3	33.6 ± .3a/
PLATELETS ($X10^5$ /MM^3)	7.7 ± 1.5	7.4 ± .4	6.9 ± .3	5.4 ± .8
LEUKOCYTES ($X10^3$ /MM^3)	22.9 ± 1.9	22.6 ± .1	20.4 ± .7	9.8 ± .8a/
NEUTROPHILS, %	10.3 ± 1.8	8.5 ± .9	7.3 ± 2.2	28.5 ± 4.2a/
LYMPHOCYTES, %	89.0 ± 2.1	90.0 ± .7	91.5 ± 2.3	69.0 ± 4.8a/
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.5 ± .5	1.3 ± .4	.8 ± .5	1.0 ± .4
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.3 ± .3	.3 ± .3	.5 ± .3	1.5 ± .6
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 %				136.3 ± 6.1
SGOT, IU/L				157 ± 68
SGPT, IU/L				104 ± 66
ALK, PHOS., IU/L				58 ± 7
CHOLESTEROL, MG %				75 ± 12
BUN, MG %				16.8 ± 1.3

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure3/).

TABLE 17

LABORATORY DATA OF MALE RATS FED NC

DOSE 3 % IN FEED

(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)
ERYTHROCYTES ($\times 10^6$ /MM^3)	5.70 ± .09	6.93 ± .18[a]	6.99 ± .16[a]	7.75 ± .16[a]
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	2.32 ± .38	1.03 ± .12[a]	.99 ± .14[a]	1.07 ± .08[a]
HEMATOCRIT, VOL. %	46.0 ± 1.0	52.0 ± .4[a]	50.5 ± 1.2[a]	46.4 ± .3[b]
HEMOGLOBIN, GM. %	14.2 ± .1	15.7 ± .1[a]	16.2 ± .4[a]	15.9 ± .1[a,b]
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	80.8 ± 2.3	75.1 ± 1.6	73.3 ± 1.4[a]	60.3 ± 1.1[a]
MCHH, MICRO MICROGMS.	24.9 ± .3	22.7 ± .5[a]	23.5 ± .4	20.6 ± .4[a]
MCHBC, GM %	30.9 ± .5	30.2 ± .4	32.1 ± .1	34.1 ± .2[a]
PLATELETS ($\times 10^5$ /MM^3)	6.7 ± .5	5.6 ± .6	7.9 ± .7	5.8 ± .5
LEUKOCYTES ($\times 10^3$ /MM^3)	21.2 ± 2.5	23.2 ± 1.6	20.9 ± .8	8.3 ± .9[a]
NEUTROPHILS, %	7.0 ± 1.0	9.3 ± 2.1	11.3 ± 1.8	16.0 ± 1.5[a]
LYMPHOCYTES, %	92.3 ± .9	90.0 ± 2.0	87.5 ± 2.1	82.5 ± 2.1[a]
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.3 ± .3	.3 ± .3	1.3 ± .6	1.0 ± .4
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.5 ± .5	.5 ± .3	0.0 ± 0.0	.5 ± .5
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
GLUCOSE (FASTING), MG %				124.5 ± 3.7
SGOT, IU/L				75.5 ± 3.1
SGPT, IU/L				27.5 ± 2.4
ALK. PHOS., IU/L				51 ± 2
CHOLESTEROL, MG %				98 ± 5
BUN, MG %				16.0 ± .7

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[3]).
b/ Significantly different from the controls at the respective time interval (Dunnett's multiple comparison procedure[3]).

TABLE 18

LABORATORY DATA OF MALE RATS FED NC

DOSE 10 % IN FEED

(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)
ERYTHROCYTES ($X10^6$ /MM^3)	5.35 ± .18	6.86 ± .16[a/]	6.48 ± .24[a/]	7.35 ± .10[a/]
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	2.90 ± .95	.98 ± .19	1.27 ± .26	.86 ± .09[a/]
HEMATOCRIT, VOL, %	44.8 ± 1.8	56.7 ± 2.7[a/]	49.0 ± 1.0	45.3 ± .6
HEMOGLOBIN, GM, %	13.7 ± .5	16.4 ± .1[a/]	15.5 ± .1[a/]	15.5 ± .2[a/]
METHEMOGLOBIN, %	0.0 ± 0.0	1.8 ± .6[a/]	0.0 ± 0.0	.6 ± .4
MCV, CUBIC MICRONS	83.7 ± 2.5	81.2 ± 3.8	75.8 ± 1.6	61.6 ± .9[a/]
MCHH, MICRO MICROGMS,	25.6 ± .5	24.0 ± .5	24.1 ± .8	21.1 ± .3[a/]
MCHBC, GM %	30.6 ± .3	29.3 ± 1.4	31.8 ± .6	34.3 ± .2[a/]
PLATELETS ($X10^5$ /MM^3)	5.8 ± 1.3	5.7 ± .3	5.0 ± 1.2	4.2 ± .8
LEUKOCYTES ($X10^3$ /MM^3)	19.8 ± 2.1	23.1 ± 2.2	19.8 ± 1.7	6.8 ± 1.0[a/]
NEUTROPHILS, %	7.3 ± 1.3	8.5 ± 1.0	7.0 ± 2.3	25.0 ± 4.3[a/]
LYMPHOCYTES, %	91.8 ± 2.0	90.5 ± 1.0	92.0 ± 2.5	73.5 ± 4.6[a/]
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.8 ± .5	1.0 ± 0.0	1.0 ± .4	1.3 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3[b/]
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 %				124.3 ± 4.0
SGOT, IU/L				104 ± 21
SGPT, IU/L				28.3 ± 4.4
ALK. PHOS., IU/L				53 ± 5
CHOLESTEROL, MG %				55 ± 5
BUN, MG %				18.0 ± .8

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[3/]).
b/ Significantly different from the controls at the respective time interval (Dunnett's multiple comparison procedure[3/]).

TABLE 19

LABORATORY DATA OF NORMAL CONTROL FEMALE RATS FOR NC

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

	WK 0 (B, 4)	WK 4 (C, 4)	WK 8 (C, 4)	WK 13 (C, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.16 ± .13	6.56 ± .23	6.45 ± .46	6.99 ± .22
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	2.17 ± .43	.99 ± .19	1.54 ± .34	1.47 ± .14
HEMATOCRIT, VOL, %	42.3 ± 1.4	50.5 ± 1.3a/	47.8 ± 1.4a/	43.0 ± .8
HEMOGLOBIN, GM, %	14.3 ± .2	16.3 ± .3a/	15.1 ± .6	15.0 ± .4
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3
MCV, CUBIC MICRONS	68.7 ± 2.8	77.1 ± 1.1	82.7 ± 5.0a/	61.6 ± 1.2
MCHB, MICRO MICROGMS,	23.2 ± .3	24.9 ± .5	26.1 ± 1.3a/	21.5 ± .3
MCHBC, GM %	33.9 ± 1.5	32.3 ± .6	31.6 ± .3	35.0 ± .4
PLATELETS ($X10^5$ /MM^3)	7.2 ± .4	5.8 ± .6	6.4 ± .1	4.9 ± .4a/
LEUKOCYTES ($X10^3$ /MM^3)	16.5 ± 2.6	18.7 ± 3.3	17.8 ± 2.1	5.4 ± .7a/
NEUTROPHILS, %	11.3 ± 3.7	5.3 ± 1.7	9.8 ± 4.4	18.0 ± 2.4
LYMPHOCYTES, %	87.0 ± 3.4	93.8 ± 1.7	88.8 ± 4.8	80.3 ± 2.8
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.5 ± .9	.5 ± .3	1.0 ± .6	.5 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.3 ± .3	.5 ± .3	.5 ± .5	1.3 ± .6
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 %				107.8 ± 8.1
SGOT, IU/L				88.8 ± 16.3
SGPT, IU/L				37.8 ± 3.9
ALK, PHOS., IU/L				39 ± 9
CHOLESTEROL, MG %				77 ± 9
BUN, MG %				15.0 ± 1.7

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[1/]).

TABLE 20

LABORATORY DATA OF FEMALE RATS FED COTTON LINTERS

DOSE 10 % IN FEED

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

	WK 0 (B. 4)	WK 4 (T. 4)	WK 8 (T. 4)	WK 13 (T. 4)
ERYTHROCYTES ($\times 10^6$ /MM^3)	5.88 ± .19	5.66 ± .65	5.78 ± .21	6.72 ± .20
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	3.55 ± .15	.58 ± .18 a/	1.38 ± .45 a/	1.60 ± .13 a/
HEMATOCRIT, VOL. %	45.0 ± 1.1	48.0 ± .6	48.0 ± 1.2	42.0 ± .9
HEMOGLOBIN, GM. %	13.6 ± .4	16.4 ± .4 a/	14.9 ± .3	14.4 ± .3
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	.7 ± .7	0.0 ± 0.0
MCV, CUBIC MICRONS	76.7 ± 2.5	88.5 ± 10.1	83.3 ± 3.3	62.9 ± 1.3
MCH, MICRO MICROGMS.	23.2 ± .7	29.9 ± 2.8 a/	25.8 ± .9	21.5 ± .4
MCHBC, GM %	30.3 ± .4 b/	34.2 ± 1.1 a/	31.0 ± .3	34.4 ± .3 a/
PLATELETS ($\times 10^5$ /MM^3)	7.4 ± .9	6.5 ± .7	6.1 ± .2	4.9 ± .1
LEUKOCYTES ($\times 10^3$ /MM^3)	13.1 ± 2.1	19.9 ± 2.4 a/	22.0 ± 1.1 a/	5.2 ± .6
NEUTROPHILS, %	15.8 ± 2.5	7.. ± .9 b/	7.0 ± .9 a/	11.0 ± 3.1
LYMPHOCYTES, %	84.0 ± 2.3	9 3 ± .5 a/	92.0 ± 1.2 a/	87.5 ± 2.2
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.3 ± .3	.5 ± .3	.8 ± .5	1.3 ± .8
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.3 ± .3	1.0 ± .7	.3 ± .3	.3 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
GLUCOSE (FASTING), MG %				124.5 ± 6.8
SGOT, IU/L				105 ± 18
SGPT, IU/L				27.8 ± 2.4
ALK. PHOS., IU/L				39 ± 3
CHOLESTEROL, MG %				72 ± 6
BUN, MG %				12.8 ± .8

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[3]).
b/ Significantly different from the controls at the respective time interval (Dunnett's multiple comparison procedure[3]).

TABLE 21

LABORATORY DATA OF FEMALE RATS FED NC

DOSE 1 % IN FEED

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

	WK 0 (B. 4)	WK 4 (T. 4)	WK 8 (T. 4)	WK 13 (T. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.44 ± .05	6.53 ± .30	6.11 ± .34	7.25 ± .15
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	2.11 ± .47	1.42 ± .08	1.83 ± .22	1.33 ± .09
HEMATOCRIT, VOL. %	45.0 ± .6	50.0 ± 1.5a/	48.5 ± .9	45.0 ± .7
HEMOGLOBIN, GM. %	14.5 ± .1	16.0 ± .3a/	15.6 ± .4a/	15.6 ± .1
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	69.9 ± .6	76.8 ± 2.1	79.9 ± 3.2a/	62.1 ± .6a/
MCHS, MICRO MICROGMS.	22.5 ± .2	24.6 ± .9	25.7 ± 1.0 a/	21.5 ± .3
MCHBC, GM %	32.2 ± .2	32.1 ± .5	32.2 ± .4	34.6 ± .3 a/
PLATELETS ($X10^5$ /MM^3)	9.5 ± .4	6.9 ± .4 a/	7.8 ± .4 a/	5.3 ± .5 a/
LEUKOCYTES ($X10^3$ /MM^3)	16.1 ± .8	20.6 ± 1.9 a/	19.1 ± .5	7.6 ± .9 a/
NEUTROPHILS, %	5.3 ± 1.1	6.3 ± 2.9	4.0 ± 1.2	12.5 ± 1.0 a/
LYMPHOCYTES, %	92.5 ± 1.6	92.5 ± 2.8	94.8 ± 1.9	86.5 ± 1.0
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	2.3 ± 1.1	.8 ± .5	0.0 ± 0.0	.5 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	.5 ± .5	1.3 ± .8	.5 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3
GLUCOSE (FASTING), MG %				110.3 ± 2.2
SGOT, IU/L				59.5 ± 4.0
SGPT, IU/L				34.0 ± 5.0
ALK. PHOS., IU/L				31 ± 2
CHOLESTEROL, MG %				69 ± 6
BUN, MG %				14.8 ± 1.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[3]).

TABLE 22

LABORATORY DATA OF FEMALE RATS FED NC

DOSE 1 % IN FEED

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

	WK 0 (B. 4)	WK 4 (T. 4)	WK 8 (T. 4)	WK 13 (T. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.25 ± .16	6.68 ± .24	6.27 ± .23	6.93 ± .12
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	3.26 ± .23	.89 ± .14 a/	1.52 ± .15 a/	1.33 ± .14 a/
HEMATOCRIT, VOL. %	45.3 ± .5	50.8 ± 1.1 a/	48.5 ± .6 a/	43.5 ± .9
HEMOGLOBIN, GM. %	13.8 ± .3	15.9 ± .5 a/	15.3 ± .3 a/	15.2 ± .3 a/
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	72.5 ± 1.7	76.0 ± 1.3	77.5 ± 2.1	62.8 ± .2 b/
MCHB, MICRO MICROGMS.	22.1 ± .2	23.9 ± .4 a/	24.5 ± .7 a/	22.0 ± .2
MCHBC, GM %	30.5 ± .5 b/	31.4 ± .3	31.5 ± .2	35.0 ± .3 a/
PLATELETS ($X10^5$ /MM^3)	8.7 ± .5	6.4 ± .2 a/	6.5 ± .6 a/	5.5 ± .3 a/
LEUKOCYTES ($X10^3$ /MM^3)	16.5 ± 2.5	16.1 ± 1.3	15.7 ± 2.0	7.1 ± .5 a/
NEUTROPHILS, %	13.0 ± 3.4	8.5 ± 2.5	6.5 ± 1.4	10.8 ± 3.5
LYMPHOCYTES, %	85.5 ± 3.8	90.0 ± 2.9	92.3 ± 1.8	87.0 ± 3.9
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.8 ± .5	1.0 ± .4	.5 ± .3	1.3 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.8 ± .4	.5 ± .3	.8 ± .5	1.0 ± .4
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
GLUCOSE (FASTING), MG %				124.8 ± 7.5
SGOT, IU/L				79.5 ± 8.5
SGPT, IU/L				31.0 ± 1.2
ALK. PHOS., IU/L				41 ± 4
CHOLESTEROL, MG %				71 ± 6
BUN, MG %				14.8 ± 1.3

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[3]).
b/ Significantly different from the controls at the respective time interval (Dunnett's multiple comparison procedure[3]).

TABLE 23

LABORATORY DATA OF FEMALE RATS FED NC

DOSE 10 % IN FEED

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

	WK 0 (B. 4)	WK 4 (T. 4)	WK 8 (T. 4)	WK 13 (T. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	5.72 ± .14	6.58 ± .28	5.90 ± .34	7.30 ± .26[a/]
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	3.45 ± .67	.98 ± .12[a/]	1.27 ± .15[a/]	1.54 ± .15[a/]
HEMATOCRIT, VOL. %	45.0 ± .4	54.0 ± 2.1[a/]	47.5 ± 1.0	43.3 ± .8
HEMOGLOBIN, GM. %	13.0 ± .2 [b/]	15.9 ± .4[a/]	15.3 ± .4[a/]	15.4 ± .3[a/]
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	78.8 ± 2.1[b/]	82.4 ± 4.7	81.3 ± 4.9	59.4 ± 2.0[a/]
MCHB, MICRO MICROGMS.	22.8 ± .1	24.2 ± .7	26.1 ± 1.1[a/]	21.1 ± .6
MCHBC, GM %	29.0 ± .7[b/]	29.6 ± 1.2	32.2 ± 1.1	35.5 ± .3[a/]
PLATELETS ($X10^5$ /MM^3)	6.5 ± .6	5.3 ± .2	5.7 ± .5	5.1 ± .5
LEUKOCYTES ($X10^3$ /MM^3)	10.2 ± 1.0	22.1 ± 1.9[a/]	15.9 ± 1.9[a/]	6.0 ± .7
NEUTROPHILS, %	11.5 ± 1.5	4.8 ± 1.4	6.8 ± 2.9	12.8 ± .5
LYMPHOCYTES, %	87.0 ± 1.9	94.5 ± 1.3[a/]	93.0 ± 2.9	84.8 ± 1.3
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.5 ± .5	.3 ± .3	.3 ± .3	2.3 ± 1.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.0 ± .6	.5 ± .3	0.0 ± 0.0	.3 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
GLUCOSE (FASTING), MG %				111.8 ± 3.6
SGOT, IU/L				86.0 ± 8.2
SGPT, IU/L				37.3 ± 8.3
ALK. PHOS., IU/L				39 ± 1
CHOLESTEROL, MG %				71 ± 12
BUN, MG %				13.0 ± 1.5

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from the baseline level (Dunnett's multiple comparison procedure[3/]).
b/ Significantly different from the controls at the respective time interval (Dunnett's multiple comparison procedure[3/]).

TABLE 24

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

Sex	% NC in Feed	Terminal Body Weight (gm)	Absolute Organ Weight (gm) Liver	Kidneys	Spleen	Testes	Ovaries	Brain
Male	0	540±16[b]	15.2±1.1	3.54±0.11	0.78±0.04	2.95±0.16		1.94±0.02
	10C[a]	420±11[c]	11.6±0.4[c]	3.08±0.11	0.61±0.02[c]	3.35±0.06		1.92±0.06
	1	545±30	14.2±0.8	3.36±0.09	0.81±0.05	3.28±0.05		1.89±0.12
	3	520±27	13.5±0.6	3.65±0.21	0.74±0.02	3.34±0.18		1.90±0.07
	10	446±5[c]	10.4±0.3[c]	2.80±0.12[c]	0.64±0.01[c]	3.18±0.07		1.98±0.03
Female	0	306±6	8.4±0.6	2.16±0.09	0.59±0.05		0.153±0.035	1.97±0.08
	10C	298±6	8.1±0.3	1.49±0.24[c]	0.58±0.01		0.132±0.005	2.05±0.03
	1	283±11	6.9±0.3	1.75±0.10	0.51±0.05		0.127±0.018	1.88±0.04
	3	330±14	8.6±0.4	1.93±0.08	0.55±0.02		0.128±0.014	2.00±0.03
	10	304±15	7.9±0.6	1.83±0.25	0.55±0.06		0.143±0.022	2.01±0.06

Sex	% NC in Feed	Relative Organ Weights (gm/100 gm Body Weight) Liver	Kidneys	Spleen	Testes	Ovaries	Brain
Male	0	2.80±0.13	0.66±0.04	0.144±0.003	0.55±0.02		0.36±0.01
	10C	2.76±0.05	0.73±0.02	0.145±0.006	0.80±0.04[c]		0.46±0.02[c]
	1	2.63±0.17	0.62±0.04	0.151±0.013	0.61±0.03		0.35±0.04
	3	2.62±0.18	0.71±0.04	0.144±0.005	0.65±0.07		0.37±0.02
	10	2.33±0.06	0.63±0.03	0.143±0.004	0.71±0.02[c]		0.44±0.01[c]
Female	0	2.73±0.20	0.71±0.03	0.194±0.016		0.051±0.012	0.64±0.03
	10C	2.72±0.06	0.50±0.08[c]	0.194±0.003		0.044±0.001	0.69±0.02
	1	2.43±0.08	0.62±0.01	0.180±0.015		0.045±0.006	0.67±0.01
	3	2.60±0.01	0.59±0.02	0.168±0.005		0.039±0.003	0.61±0.02
	10	2.58±0.08	0.60±0.08	0.180±0.012		0.047±0.007	0.66±0.02

Sex	% NC in Feed	Relative Organ Weights (gm/gm Brain Weight) Liver	Kidneys	Spleen	Testes	Ovaries
Male	0	7.8±0.5	1.83±0.06	0.40±0.02	1.52±0.07	
	10C	6.1±0.3	1.61±0.07	0.32±0.02	1.75±0.07	
	1	7.7±0.7	1.81±0.15	0.44±0.05	1.77±0.15	
	3	7.2±0.5	1.93±0.16	0.39±0.02	1.76±0.13	
	10	5.3±0.2[c]	1.42±0.07	0.32±0.01	1.61±0.02	
Female		4.2±0.2	1.10±0.04	0.30±0.01		0.076±0.015
	10C	4.0±0.1	0.73±0.12	0.28±0.00		0.064±0.003
	1	3.7±0.1	0.93±0.03	0.27±0.02		0.068±0.009
	3	4.3±0.1	0.97±0.02	0.28±0.01		0.064±0.007
	10	3.9±0.2	0.92±0.13	0.27±0.03		0.071±0.010

a/ Cotton control; fed 10% cotton linters.
b/ Mean ± standard error for rats.
c/ Significantly different from controls (Dunnett's multiple comparison procedure[3]).

TABLE 25

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

Lesions[a]	Dose (% in feed)											
	0				10[b]				1[illegible]			
Rat No.:	131	132	135	136	111	112	115	116	191	192	195	196
Eye												
Retinal rosettes										1		
Heart												
Myocarditis		1	1	1				1				
Lung												
Focal chronic murine pneumonia	1	1	2		1	1	2	1	1	1	1	1
Submaxillary salivary gland												
Focal chronic inflammation			1									
Liver												
Mononuclear cell infiltration	1	1	1		1		1	1	1	1	1	
Focal necrosis				3								
Large Intestine												
Pinworms in lumen							1		1			
Kidney												
Focal chronic interstitial nephritis	1	1	2		1						1	
Pelvic dilation		1	2	1	1					1		
Thyroid												
Cystic and hypoplastic	1											
Bone Marrow												
M/E ratio	1.5	1.3	1.4	1.5	1.6	1.5	1.5	1.3	1.4	1.5	1.4	1.4

Tissues not listed were normal.

a/ Severity of Lesions: 1 = mild; 2 = moderate; 3 = marked.

b/ Cotton control: fed 10% of cotton linters.

TABLE 26

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

Lesions[a]	Dose (% in feed)											
	0				10C[b]				10			
Rat No.:	231	232	235	236	211	212	215	216	291	292	295	296
Eye												
Retinal rosettes	1						1		1			
Epithelial hyperplasia							2					1
Corneal thickening												1
Chorioretinopathy							2					
Heart												
Myocarditis										1		
Lung												
Chronic murine pneumonia		1	1	1	1	1	1	1	1	1	1	1
Liver												
Mononuclear cell infiltration	1			1	1	1	1	1	1	1		1
Large Intestine												
Pinworms in lumen	1	1										
Kidney												
Focal chronic interstitial nephritis					1	1				1		
Pelvic dilation			1						1	1		
Microcalculi	1		1	1						1	1	1
Adrenal												
Mononuclear cell infiltration								1				
Bone Marrow												
M/E ratio	1.4	1.4	1.3	1.6	1.4	1.5	1.3	1.4	1.3	1.4	1.4	1.6

Tissues not listed were normal.

a/ Severity of Lesions: 1 = mild; 2 = moderate; 3 = marked.

b/ Cotton control; fed 10% of cotton linters.

TABLE 27

NUMERICAL DISTRIBUTION OF CHROSOMES FROM RATS FED 10% NC FOR 13 WEEKS

Treatment	Number of Rats	Chromosome Frequency ≤40	41	42	43	≥44	Tetraploids Per 100 Cells
Cotton Control[a]							
Lymphocyte	3	6[b]	2	41	1	0	0.17 ± 0.17[c]
Kidney	3	6	3	38	2	1	2.83 ± 0.93
NC							
Lymphocyte	3	4	4	41	1	0	0.17 ± 0.17
Kidney	3	4	6	38	2	0	1.17 ± 0.17

a/ Fed 10% of cotton linters.
b/ Mean
c/ Mean ± standard error.

TABLE 28

MORPHOLOGICAL ABERRATIONS OF CHROSOMES FROM RATS FED 10% OF NC FOR 13 WEEKS

Treatment	Number of Rats	Chromatid Breaks and Gaps per 50 Cells	Translocations Per 50 Cells	Total Aberrations Per 50 Cells
Cotton Control[a]				
Lymphocyte	3	0.67 ± 0.67[b]	0.50 ± 0.50	1.16 ± 0.60
Kidneys	3	1.00 ± 0.58	0.0 ± 0.0	1.00 ± 0.58
NC				
Lymphocyte	3	1.33 ± 0.33	0.33 ± 0.33	1.66 ± 0.33
Kidneys	3	0.67 ± 0.33	0.0 ± 0.0	0.67 ± 0.67

a/ Fed 10% of cotton linters.
b/ Mean ± standard error.

TABLE 29

SERUM IgE OF RATS FED 10% OF NC
FOR 13 WEEKS

Sex	Treatment	IgE (IU/ml)
Male	Cotton Control[a/]	1,431 ± 232[b/]
	NC	1,713 ± 316
Female	Cotton Control	2,081 ± 106
	NC	2,456 ± 183

a/ Fed 10% of cotton linters.
b/ Mean ± standard error of four rats.

III. MICE

TABLE OF CONTENTS

	Page
A. Subchronic Toxicity and Reversibility	51
1. Introduction	51
2. Material and Methods	51
3. Results	51
a. General Observations and Weight Gain	51
b. Feed Consumption	52
c. Blood Analysis	53
d. Organ Weights	53
e. Gross and Microscopic Examination of Tissues	53
4. Discussion	54
B. Other Studies	54
C. Summary	54
Tables 30 - 39	55

III. MICE

A. Subchronic Toxicity and Reversibility

1. Introduction

As for the dogs and rats, these studies were performed to define the nature and extent of effects of NC on the biological system at the biochemical and cellular levels and to elucidate the dose-response relationship in the mice fed NC for 13 weeks. The reversibility of any adverse effects was also studied in mice after the feeding of NC 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 40 male and 40 female young healthy albino swiss mice (National Laboratory Animals, O'Fallon, Missouri) were used for this study. They were divided into five groups, each consisting of eight males and eight females. The average weights of all groups were kept close. The various groups were fed the same diets as prepared for the rats: 1, 3 or 10% of NC in feed, the powdered standard rodent chow (Wayne Laboratory Meal) alone as normal control, or 10% of cotton linters as cotton control.

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

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

Deaths occurred during the first week; more followed rapidly. By the end of the second week, four cotton control males, four cotton control females, one low dosage male and six high dosage males had died. There was no apparent cause for the death of the low level male; the remaining deaths were due to intestinal impaction. A wad of fibers was collected, usually in the distal ileum or the colon.

Additional fibers packed to form almost entirely white pellets. Gas frequently collected proximal to the blockage. The mice showed no unusual signs until they were found dead in the morning. It was usually difficult to obtain useful histopathological data. Although this effect was a purely mechanical one, not due to the toxicity of NC itself but rather to its fibrous nature, it was possible that no or not enough mice would be alive by the end of 13 weeks feeding. Therefore, a number of mice from the chronic study were added to this subchronic study. The mice used in the chronic study were from the same shipment and identical levels of NC in the feed had been started simultaneously.

Total deaths included eight normal control male mice in weeks 2, 4, 5, 9 (three mice) and 11 (two mice). In the low dosage mice, two males died in weeks 1 and 8. In the middle dosage mice, five males died in weeks 2 (two mice), 8, 9 and 10. In all these cases, there were no deaths among the females. The greatest number of deaths was among the high dosage mice, where 17 males died in weeks 1, 2 (eight mice), 3 (five mice), 4 (two mice) and 5; 14 females died in weeks 2, 3 (four mice), 4 (two mice), 5(four mice), 8 (two mice) and 9. In the cotton control group, nine males died in weeks 1, 2 (three mice), 10 (three mice), 11, and 12; 14 females died in weeks 1, 2 (three mice), 6, 11 and 12 (eight mice).

The average body weight of the mice fed cotton linters or various levels of NC are shown in Table 30. The other mice, nonsurvivors and surplus, had similar weights. The normal control mice generally gained weight throughout the study; some had small weight losses in the first few weeks of study. The body weights of mice fed the low (1%) or middle (3%) level of NC were similar to those of the normal controls. Mice fed the high level of NC (10%) had a severe weight loss in the first weeks. The survivors then began gaining weight; some had come close to normal control weight by week 13. The cotton control mice lost weight in the first weeks, and regained a little weight in the later weeks. During the recovery period, most mice gained weight.

b. Feed Consumption

Feed consumption of the mice fed linters or various levels of NC are shown in Table 31. Male mice fed the control diet consumed an average of 4.9 gm/mouse/day, while the corresponding females averaged 4.2 gm/mouse/day. The mice fed the low level (1%) or middle level (3%) of NC consumed slightly more feed. Feed consumptions of mice fed 10% of NC or cotton linters were considerably more, averaging 9.7 to 19.7 and 12.2 to 13.6 gm/mouse/day, respectively. As with the rats, these data reflected the scattering of cotton fibers and feed about the cage.

c. Blood Analysis

The hematology results from the control male mice and the male mice fed cotton linters or various levels of NC for 13 weeks or for 13 weeks and allowed to recover for 4 weeks are summarized in Tables 32 and 33. The peripheral blood elements were not apparently altered by NC. When compared to the normal control males, there were occasional significant differences. The differences were slight, inconsistent and of no toxicological significance.

The hematology results from the female mice are similarly summarized in Tables 34 and 35. As with the males, the few significant differences when compared with those of the normal control females were toxicologically insignificant.

d. Organ Weights

The organ weights of the mice fed cotton linters or various levels of NC for 13 weeks are summarized in Table 36. The absolute and/or relative spleen weights of mice fed 10% of NC or cotton linters were significantly smaller than those of the normal control mice. Other differences including liver and brain weights were not consistent.

The organ weights of the mice after feeding for 13 weeks and allowing to recover for 4 weeks are summarized in Table 37. The differences in spleen weights observed in mice fed 10% of NC or cotton linters for 13 weeks were not seen in these mice when the feeding of NC or cotton linters was discontinued for 4 weeks.

e. Gross and Microscopic Examination of Tissues

At necropsy, the control mice and mice fed cotton linters or various levels of NC for 13 weeks were in good nutritional condition. No gross lesions were identified in the survivors. The result of microscopic examinations are summarized in Tables 38 and 39. A number of lesions occurred in the normal control mice and mice fed 10% of NC or cotton linters. The lesions were usually mild and included focal ulceration and inflammation of the skin on the back; mononuclear cell infiltration and extramedullary hematopoiesis in the liver; mononuclear cell infiltration in the pancreas; chronic interstitial nephritis, perivasculitis, tubular basophilia and/or mononuclear cell infiltration in the kidney; a few lymphoid nodules in the submucosa of the urinary bladder; focal vacuolation (probably fatty change) of the zona reticularis of the adrenal cortex; focal tubular degeneration in one testis; and/or extramedullary hematopoiesis in the spleen. These lesions were spontaneous and were not caused by NC. The tissue slides prepared from the mice fed the lower levels of NC and from the recovery mice were not examined.

4. Discussion

The male and female mice fed the low, middle or high levels of NC showed no adverse effects from the chemical nature of the NC. There were weight losses and deaths in mice fed the high level (10%) of NC due to its fibrous physical nature. There were similar weight losses and similar deaths in mice fed 10% of cotton linters. Deaths were due to the blocking of the lower part of the gastrointestinal tract by masses of the fibers, particularly in the regions where the water was removed from the chyme. Rats did not suffer from these deaths (see Part II.A.3.a.). Their intestines were probably sufficiently large, relative to the fiber length, to allow continued passage of the dewatered balls of the fiber.

Apparent feed consumption of the male mice fed the low, middle or high levels of NC or of cotton linters averaged 4.9, 5.4, 19.2 and 13.6 gm/mouse/day, respectively. Feed consumption of the females averaged 4.7, 5.6, 9.7, and 12.2 gm/mouse/day. As with the rats, the excessive amounts of feed consumption for mice fed 10% of NC or cotton linters were due to its scattering about the cage.

Feeding of cotton linters or various levels of NC for 13 weeks did not cause any effects on peripheral blood elements or any lesions in mice. However, 10% of NC or cotton linters in the feed decreased the body weight, apparently due to decreased intake of nutritive values.

B. Other Studies

Mutagenic and immunologic studies were not performed in mice.

C. Summary

Mice fed 1 or 3% of NC in the feed for 13 weeks consumed slightly more feed without any adverse effects. High level (10%) of NC or cotton linters did not cause any changes in peripheral blood elements or any lesions. However, a number of mice died during the study due to impaction of fibers in their lower intestinal tract. The survivors fed these doses lost body weight due to insufficient nutritional intake.

TABLE 30

BODY WEIGHTS OF MICE FED NC

	% NC In Feed	Body Weights (gm) Initial	4 Weeks	8 Weeks	13 Weeks	17 Weeks
Male	0	30.0 ± 1.7[b]	28.5 ± 2.8	35.3 ± 1.3	35.0 ± 1.9	
	10C[a]	29.3 ± 1.0	27.3 ± 1.3	28.0 ± 1.5	26.8 ± 1.9	
	1	26.5 ± 2.3	27.5 ± 1.7	33.8 ± 1.8	31.8 ± 1.3	
	3	29.0 ± 0.9	30.8 ± 0.6	33.0 ± 1.0	32.0 ± 0.4	
	10	29.8 ± 1.7	21.3 ± 1.2[c]	24.7 ± 2.0[c]	27.3 ± 1.2[c]	
Female	0	22.8 ± 1.5	25.0 ± 0.6	28.0 ± 0.8	26.8 ± 0.6	
	10C	22.5 ± 1.3	22.3 ± 0.9	24.0 ± 1.6	24.5 ± 2.2	
	1	24.8 ± 1.0	27.0 ± 0.6	29.0 ± 0.7	28.3 ± 1.8	
	3	21.8 ± 1.0	24.3 ± 0.3	25.3 ± 0.8	21.8 ± 1.3	
	10	23.8 ± 1.2	17.8 ± 1.1	22.0 ± 1.2	21.8 ± 1.1	
Male	0	29.3 ± 0.8	31.0 ± 1.3	36.5 ± 1.5	36.0 ± 2.2	25.3 ± 2.3
	10C	28.8 ± 0.4	27.3 ± 1.3	28.8 ± 2.5	27.8 ± 1.0[d]	32.3 ± 1.4
	1	28.0 ± 2.3	26.5 ± 1.7	31.5 ± 1.9	29.8 ± 1.3[d]	30.3 ± 0.9
	3	30.8 ± 1.1	30.5 ± 1.9	34.8 ± 2.0	33.3 ± 1.3[d]	36.8 ± 2.3
	10	25.5 ± 0.3	18.7 ± 0.7[c]	30.7 ± 0.3[c]	32.0 ± 0.6[c,d]	30.0 ± 1.0[c]
Female	0	23.3 ± 1.1	27.5 ± 1.9	27.8 ± 1.3	30.5 ± 0.3	27.8 ± 0.3
	10C	25.0 ± 1.5	24.0 ± 0.7	28.0 ± 0.8	25.0[d,e]	26.0[e]
	1	24.3 ± 1.0	24.5 ± 0.3	27.3 ± 0.5	28.8 ± 0.8[d]	27.0 ± 0.9
	3	23.0 ± 1.3	26.3 ± 1.4	28.5 ± 0.3	24.3 ± 0.3[d]	28.3 ± 0.5
	10	22.0 ± 0.7	20.0 ± 0.4	18.3 ± 0.6	27.8 ± 0.6[d]	26.8 ± 0.6

a/ Cotton control; fed 10% of cotton linters.
b/ Mean ± standard error of four mice.
c/ Three mice; one other died in week 4.
d/ NC or linters in feed discontinued thereafter.
e/ One mouse; three others died in weeks 11, 12 and 12 respectively.

TABLE 31

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

% NC in Feed	Males				
	1 - 4[b]	5 - 8	9 - 13	1 - 13	14 - 17[c]
0	4.4	4.5	5.7	4.9	5.2
10C[a]	13.4	9.7	17.7	13.6	6.9
1	4.7	5.2	4.8	4.9	5.2
3	5.1	5.6	5.7	5.4	5.9
10	8.6	24.4	24.5	19.2	6.0
	Females				
	1 - 4	5 - 8	9 - 13	1 - 13	14 - 17[c]
0	4.2	3.8	4.6	4.2	4.5
10C	9.1	11.1	16.4	12.2	3.9
1	4.5	4.4	5.2	4.7	4.4
3	5.5	5.1	6.3	5.6	5.2
10	6.3	10.5	12.4	9.7	4.7

a/ Cotton control; fed 10% of cotton linters.
b/ Weeks.
c/ Recovery period; all mice fed control feed.

TABLE 32

LABORATORY DATA OF MALE MICE FED NC FOR 13 WEEKS

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

DOSE: % IN FEED	0 (C, 4)	10C[a/] (C, 4)	1 (T, 4)	3 (T, 4)	10 (T, 3)
ERYTHROCYTES ($X10^6$ /MM^3)	7.00 ± .59	6.56 ± .27	7.30 ± .35	7.86 ± .03	6.96 ± .09
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	3.05 ± 1.59	3.06 ± 1.18	1.29 ± .09	.76 ± .18	1.02 ± .05
HEMATOCRIT, VOL. %	45.0 ± .4	42.5 ± 1.3	45.5 ± 1.3	48.8 ± .5[b/]	46.3 ± .3
HEMOGLOBIN, GM. %	16.6 ± .8	15.5 ± .8	15.7 ± .4	16.8 ± .3	15.6 ± .4
METHEMOGLOBIN, %	0.0 ± 0.0	.7 ± .7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	65.5 ± 5.2	65.0 ± 1.9	62.5 ± 1.3	62.0 ± .6	66.6 ± .7
MCHB, MICRO MICROGMS.	24.0 ± .9	23.8 ± 1.1	21.6 ± .5	21.3 ± .3	22.5 ± .5
MCHBC, GM %	36.9 ± 1.4	36.5 ± .7	34.6 ± .3	34.4 ± .6	33.7 ± .6
PLATELETS ($X10^5$ /MM^3)	4.9 ± 1.2	4.7 ± 1.3	4.9 ± .7	5.2 ± .5	5.0 ± .9
LEUKOCYTES ($X10^3$ /MM^3)	8.5 ± 2.0	5.2 ± .4	10.9 ± 1.0	11.6 ± 1.4	8.2 ± 2.1
NEUTROPHILS, %	13.5 ± 2.9	34.3 ± 7.5	24.3 ± 7.1	24.3 ± 4.2	21.7 ± 1.5
LYMPHOCYTES, %	85.3 ± 3.1	64.8 ± 7.2	72.0 ± 8.4	74.3 ± 4.7	77.7 ± 1.8
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.5 ± .3	.5 ± .5	3.8 ± 1.7	.5 ± .5	.3 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.8 ± .3	.5 ± .5	0.0 ± 0.0	1.0 ± .4	.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/ Cotton controls; fed 10% of cotton linters.
b/ Significantly different from the controls (Dunnett's multiple comparison procedure[3/]).

TABLE 33

LABORATORY DATA OF MALE MICE FED NC FOR 13 WEEKS AND ALLOWED TO RECOVER FOR 4 WEEKS

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

DOSE: % IN FEED	0 (C, 4)	10[a] (C, 4)	1 (T, 4)	3 (T, 4)	10 (T, 3)
ERYTHROCYTES ($X10^6$ /MM^3)	7.07 ± .81	8.39 ± .14	7.71 ± .21	7.48 ± .13	8.09 ± .16
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	8.38 ± 6.96	1.18 ± .11	1.09 ± .20	1.22 ± .46	1.57 ± .32
HEMATOCRIT, VOL. %	45.5 ± 4.0	50.3 ± .3	48.8 ± 1.7	49.3 ± 1.1	49.3 ± .9
HEMOGLOBIN, GM. %	14.3 ± 1.5	16.3 ± .2	15.6 ± .4	[illegible] ± .2	16.0 ± .4
METHEMOGLOBIN, %	4.3 ± 1.5	3.1 ± 1.3	0.0 ± 0.0	[illegible] ± 0.0	3.2 ± 1.8
MCV, CUBIC MICRONS	65.1 ± 2.1	59.9 ± 1.0	63.2 ± 1.0	[illegible] ± 1.6	61.0 ± 1.9
MCHB, MICRO MICROGMS.	20.3 ± .4	19.5 ± .3	20.3 ± .3	21.3 ± .3	19.7 ± .7
MCHBC, GM %	31.3 ± .8	32.5 ± .2	32.1 ± .4	32.3 ± .4	32.4 ± .2
PLATELETS ($X10^5$ /MM^3)	6.2 ± 1.4	7.3 ± .7	7.4 ± 1.2	6.5 ± .6	6.1 ± .2
LEUKOCYTES ($X10^3$ /MM^3)	12.7 ± .9	5.8 ± .7[b]	6.8 ± 1.1[b]	10.2 ± .9	7.8 ± .7
NEUTROPHILS, %	18.0 ± 2.9	27.5 ± 6.9	23.0 ± 3.3	14.8 ± 1.9	24.0 ± 5.7
LYMPHOCYTES, %	80.3 ± 3.5	71.8 ± 7.1	74.8 ± 2.9	84.8 ± 1.7	73.0 ± 5.0
BANDS, %	.5 ± .5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.3 ± .8	.8 ± .5	1.5 ± .9	.5 ± .3	2.3 ± 1.5
BASOPHILS, %	0.0 ± 0.0	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	.3 ± .3	0.0 ± 0.0	.7 ± .7
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Cotton controls; fed 10% of cotton linters.
b/ Significantly different from the controls (Dunnett's multiple comparison procedure[3]).

TABLE 34

LABORATORY DATA OF FEMALE MICE FED NC FOR 13 WEEKS

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

DOSE: % IN FEED	0 (C, 4)	10[a] (C, 4)	1 (T, 4)	3 (T, 4)	10 (T, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	8.04 ± .16	6.21 ± .46[b]	6.88 ± .41	8.07 ± .14	6.98 ± .21
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	1.97 ± .22	9.23 ± 3.34[b]	1.77 ± .37	1.56 ± .52	2.22 ± .53
HEMATOCRIT, VOL. %	48.3 ± 1.7	41.3 ± 3.2	44.5 ± 2.3	50.8 ± .5	43.8 ± .9
HEMOGLOBIN, GM. %	16.5 ± .4	14.0 ± .9[b]	15.4 ± .5	16.7 ± .2	14.5 ± .3
METHEMOGLOBIN. %	0.0 ± 0.0	2.0 ± 1.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	60.0 ± 1.6	66.7 ± 4.4	64.8 ± 1.1	63.0 ± 1.3	62.8 ± 1.9
MCHB, MICRO MICROGMS.	20.5 ± .3	22.6 ± .7[b]	22.5 ± .7[b]	20.7 ± .2	20.9 ± .3
MCHBC, GM %	34.3 ± .8	34.1 ± 1.1	34.8 ± 1.0	33.0 ± .5	33.3 ± .9
PLATELETS ($X10^5$ /MM^3)	3.8 ± .4	4.4 ± .9	4.8 ± .8	4.7 ± .5	6.0 ± .3
LEUKOCYTES ($X10^3$ /MM^3)	13.3 ± .5	10.9 ± 1.8	10.3 ± 1.4	11.9 ± .9	11.4 ± 1.9
NEUTROPHILS, %	15.5 ± 4.4	37.5 ± 3.9	20.0 ± 1.5	32.8 ± 13.0	35.8 ± 2.5
LYMPHOCYTES, %	82.8 ± 4.2	58.5 ± 4.2	76.0 ± 1.5	66.0 ± 12.4	62.5 ± 2.4
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.5 ± .3	4.0 ± .6	3.8 ± 1.0	1.3 ± .9	1.8 ± 1.4
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0 0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.3 ± .3	0.0 ± 0.0	.3 ± .3	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	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

a/ Cotton controls; fed 10% of cotton linters.
b/ Significantly different from the controls (Dunnett's multiple comparison procedure[3]).

TABLE 35

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

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

DOSE: % IN FEED	0 (C. 4)	10C[a] (C, 1)	1 (T. 4)	3 (T. 4)	10 (T. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	8.09 ± .13	8.96	8.11 ± .21	7.98 ± .21	8.61 ± .11
HEINZ BODIES, %	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	1.07 ± .19	1.00	.83 ± .27	.49 ± .03	.75 ± .13
HEMATOCRIT, VOL. %	50.0 ± 1.1	53.0	48.5 ± .6	48.8 ± .9	50.5 ± .5
HEMOGLOBIN, GM. %	16.1 ± .3	18.0	15.5 ± .2	15.9 ± .4	16.7 ± .2
METHEMOGLOBIN, %	2.3 ± 1.4	0.0	0.0 ± 0.0	0.0 ± 0.0	3.0 ± .0
MCV, CUBIC MICRONS	61.8 ± .5	59.2	59.9 ± 1.0	61.1 ± .5	58.7 ± .8[b]
MCHH, MICRO MICROGMS.	19.9 ± .1	20.1	19.2 ± .3	19.9 ± .2	19.4 ± .1
MCHSC, GM %	32.2 ± .1	34.0	32.0 ± .1	32.6 ± .3	33.1 ± .3[b]
PLATELETS ($X10^5$ /MM^3)	6.6 ± .7	7.7	5.7 ± .7	6.3 ± .6	8.3 ± 1.0
LEUKOCYTES ($X10^3$ /MM^3)	6.3 ± 1.4	10.5	4.0 ± .7	8.3 ± 1.0	11.9 ± .4[b]
NEUTROPHILS, %	36.3 ± 8.1	35.0	19.0 ± 1.5	21.8 ± 5.7	16.3 ± 2.6[b]
LYMPHOCYTES, %	63.0 ± 7.8	65.0	80.5 ± 1.8	78.0 ± 5.6	83.0 ± 2.9[b]
BANDS, %	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.5 ± .3	0.0	.3 ± .3	.3 ± .3	.8 ± .5
BASOPHILS, %	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	0.0	.3 ± .3	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 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Cotton controls; fed 10% of cotton linters.

b/ Significantly different from the controls (Dunnett's multiple comparison procedure[3]).

TABLE 36

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

Sex	% NC in Feed	Terminal Body Weight (gm)	Absolute Weight (gm)				
			Liver	Spleen	Kidneys	Heart	Brain
Male	0	35.0±1.9 [b]	1.60±0.12	0.16±0.04	0.54±0.04	0.21±0.01	0.45±0.01
	10C [a]	26.8±1.9 [c]	1.13±0.15 [c]	0.06±0.01 [c]	0.47±0.02	0.19±0.04	0.42±0.02
	1	31.8±1.3	1.47±0.07	0.13±0.01	0.57±0.04	0.22±0.01	0.43±0.03
	3	32.0±0.4	1.36±0.06	0.10±0.01	0.51±0.00	0.21±0.02	0.43±0.02
	10	27.3±1.2 [c,d]	1.28±0.05	0.08±0.03	0.51±0.00	0.21±0.01	0.45±0.02
Female	0	26.8±0.6	1.25±0.10	0.11±0.01	0.39±0.03	0.17±0.01	0.44±0.00
	10C	24.5±2.2	1.27±0.13	0.13±0.02	0.38±0.03	0.15±0.01	0.41±0.02
	1	28.3±1.8	1.35±0.05	0.08±0.01	0.41±0.01	0.16±0.01	0.45±0.01
	3	21.8±1.3	0.94±0.05	0.05±0.01 [c]	0.36±0.02	0.16±0.02	0 ..01
	10	21.8±1.1	1.04±0.07	0.06±0.01 [c]	0.34±0.00	0.17±0.01	0.39±0.01 [c]

Sex	% NC in Feed	Relative Organ Weight (gm/100 gm body weight)				
		Liver	Spleen	Kidneys	Heart	Brain
Male	0	4.6±0.2	0.45±0.08	1.55±0.05	0.61±0.04	1.31±0.06
	10C	4.2±0.4	0.21±0.01 [c]	1.78±0.[illegible]	0.71±0.11	1.57±0.06 [c]
	1	4.6±0.1	0.41±0.05	1.[illegible]±0.[illegible]	0.68±0.05	1.35±0.07
	3	4.2±0.2	0.32±0.04	1.[illegible]±0.02	0.65±0.06	1.34±0.05
	10 [d]	4.7±0.2	0.29±0.11	1.66±0.09	0.77±0.02	1.63±0.01 [c]
Female	0	4.7±0.5	0.43±0.05	1.44±0.11	0.64±0.04	1.66±0.04
	10C	5.2±0.1	0.53±0.10	1.54±0.05	0.62±0.05	1.71±0.11
	1	4.7±0.2	0.29±0.02	1.46±0.06	0.56±0.04	1.60±0.10
	3	4.3±0.3	0.23±0.03	1.67±0.06	0.76±0.08	1.98±0.10
	10	4.8±0.2	0.29±0.03	1.59±0.08	0.79±0.05	1.82±0.12

Sex	% NC in Feed	Relative Organ Weight (gm/gm brain weight)			
		Liver	Spleen	Kidneys	Heart
Male	0	3.5±0.2	0.35±0.08	1.20±0.08	0.47±0.02
	10C	2.7±0.3	0.14±0.01 [c]	1.14±0.01	0.46±0.08
	1	3.4±0.1	0.30±0.03	1.36±0.13	0.51±0.03
	3	3.2±0.2	0.24±0.03	1.19±0.04	0.49±0.02
	10 [d]	2.9±0.1	0.16±0.04 [c]	1.14±0.06	0.47±0.01
Female	0	2.8±0.2	0.26±0.03	0.87±0.06	0.38±0.02
	10C	3.1±0.3	0.32±0.06	0.91±0.07	0.36±0.01
	1	3.0±0.1	0.18±0.02	0.91±0.04	0.35±0.01
	3	2.2±0.1	0.12±0.02 [c]	0.85±0.04	0.38±0.04
	10	2.7±0.2	0.16±0.01	0.88±0.02	0.43±0.02

[a] Cotton control; fed 10% of cotton linters.
[b] Mean ± standard error of four mice.
[c] Significantly different from controls (Dunnett's multiple comparison procedure[3]).
[d] Mean ± standard error of three surviving mice.

TABLE 37

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

Sex	% NC in Feed	Terminal Body Weight (gm)	Absolute Weight (gm)				
			Liver	Spleen	Kidneys	Heart	Brain
Male	0	35.3±2.3[b]	1.61±0.02	0.16±0.03	0.67±0.05	0.28±0.02	0.48±0.00
	10C[a]	32.3±1.4	1.48±0.11	0.10±0.01	0.66±0.05	0.23±0.01	0.50±0.01
	1	30.3±0.9	1.34±0.07	0.14±0.00	0.61±0.01	0.20±0.01	0.45±0.01
	3	36.8±2.3	1.70±0.08	0.20±0.02	0.67±0.02	0.25±0.03	0.47±0.03
	10	30.0±1.0[d]	1.40±0.10	0.10±0.01	0.65±0.02	0.25±0.01	0.50±0.01
Female	0	27.8±0.3	1.29±0.10	0.12±0.02	0.46±0.02	0.19±0.02	0.50±0.03
	10C	26.0[e]	1.23	0.07	0.43	0.19	0.48
	1	27.0±0.9	1.22±0.05	0.11±0.01	0.41±0.02	0.16±0.01	0.51±0.02
	3	28.3±0.5	1.43±0.06	0.12±0.02	0.46±0.04	0.21±0.03	0.49±0.03
	10	26.8±0.6	1.25±0.06	0.10±0.01	0.45±0.00	0.19±0.02	0.46±0.01

Sex	% NC in Feed	Relative Organ Weight (gm/100 gm Body Weight)				
		Liver	Spleen	Kidneys	Heart	Brain
Male	0	4.6±0.0	0.42±0.07	1.90±0.07	0.81±0.11	1.36±0.09
	10C	4.6±0.2	0.31±0.03	2.05±0.08	0.73±0.06	1.55±0.07
	1	4.4±0.1	0.47±0.02	2.01±0.02	0.67±0.06	1.49±0.06
	3	4.7±0.2	0.53±0.05	1.84±0.12	0.69±0.07	1.29±0.05
	10[d]	4.7±0.2	0.34±0.02	2.17±0.15	0.83±0.05	1.66±0.06[c]
Female	0	4.7±0.3	0.44±0.07	1.65±0.05	0.69±0.07	1.80±0.12
	10C[e]	4.7	0.33	1.64	0.73	1.86
	1	4.5±0.2	0.39±0.03	1.53±0.07	0.59±0.03	1.38±0.02
	3	5.1±0.2	0.44±0.06	1.64±0.13	0.74±0.10	1.74±0.11
	10	4.7±0.2	0.35±0.03	1.67±0.05	0.73±0.08	1.73±0.06

Sex	% NC in Feed	Relative Organ Weight (gm/gm Body Weight)			
		Liver	Spleen	Kidneys	Heart
Male	0	3.4±0.0	0.33±0.05	1.41±0.09	0.59±0.05
	10C	3.0±0.2	0.20±0.02	1.33±0.08	0.47±0.03
	1	3.0±0.2	0.31±0.01	1.35±0.04	0.45±0.03
	3	3.6±0.1	0.41±0.03	1.42±0.05	0.53±0.04
	10[d]	2.8±0.2	0.20±0.02	1.31±0.05	0.50±0.02
Female	0	2.6±0.3	0.25±0.04	0.93±0.05	0.39±0.03
	10C[e]	2.6	0.18	0.88	0.39
	1	2.4±0.1	0.21±0.02	0.82±0.04	0.31±0.02
	3	2.9±0.1	0.25±0.03	0.94±0.04	0.43±0.04
	10	2.7±0.1	0.21±0.01	0.97±0.02	0.42±0.04

a/ Cotton control; fed 10% of cotton linters.
b/ Mean ± standard error of four mice.
c/ Significantly different from controls (Dunnett's multiple comparison procedure[3]).
d/ Mean ± standard error of three surviving mice.
e/ One surviving mouse.

TABLE 38

SUMMARY OF TISSUE LESIONS OF MALE MICE FED NC FOR 13 WEEKS

Lesions[a]	Dose (% in feed)										
	0				10C[b]				10		
Mouse No.:	301	302	303	304	51	52	55	56	376	377	378
Skin											
Focal ulceration and dermatitis		1									
Liver											
Mononuclear cell infiltration		1	1	1	1		1	1	1		1
Extramedullary hematopoiesis											1
Pancreas											
Mononuclear cell infiltration				1			1				
Cecum											
Pinworms in lumen			+				+				
Kidney											
Chronic interstitial nephritis	1		1			1					1
Focal tubular basophilia	1										
Chronic perivasculitis						1			1		
Mononuclear cell infiltration		1			1						
Testis											
Focal tubular degeneration			1								1
Spleen											
Extramedullary hematopoiesis		1		2							1
Bone Marrow											
M/E ratio	1.5	1.3	1.1	1.3	1.4	1.3	1.4	1.3	1.4	1.4	c/

Tissues not listed were normal.

a/ Severity of Lesions: + = present; ± = minimal; 1 = mild; 2 = moderate; 3 = marked; 4 = markedly severe.

b/ Cotton control; fed 10% of cotton linters.

c/ Not readable; poor staining.

TABLE 39

SUMMARY OF TISSUE LESIONS OF FEMALE MICE FED NC FOR 13 WEEKS

Lesions[a]	Dose (% in feed)											
	0				10C[b]				10			
Mouse No.:	401	402	403	404	76	80	81	84	476	477	478	479
Liver												
Mononuclear cell infiltration	1	1	1	1	1	1		1				
Extramedullary hematopoiesis								1				
Pancreas												
Mononuclear cell infiltration									±	1		
Kidney												
Chronic interstitial nephritis					1							
Mononuclear cell infiltration			1	1								
Urinary Bladder												
Lymphoid nodules in submucosa				1								
Adrenal												
Vacuolization of zona reticularis		3		2								
Spleen												
Extramedullary hematopoiesis	2		1	2	1		1	3				
Bone Marrow												
M/E ratio	c/	1.4	1.2	1.4	1.2	1.3	1.[illegible]	1.1	1.5	1.5	1.3	1.4

Tissues not listed were normal.

a/ Severity of lesions: + = present; ± = minimal; 1 = mild; 2 = moderate; 3 = marked; 4 = markedly severe.

b/ Cotton control; fed 10% of cotton linters.

c/ Not readable; poor staining.

IV. ABSORPTION

TABLE OF CONTENTS

	Page
A. Introduction	67
B Methodology	67
C. Results and Conclusions	68
Table 40	69

IV. ABSORPTION

A. Introduction

As part of our subchronic toxicity study, we studied the absorption of nitrocellulose in the rat. ^{14}C-Labeled nitrocellulose was given orally to see if it was absorbed and how rats handled the compound.

B. Methodology

1. Radiolabeled nitrocellulose: The ^{14}C-labeled nitrocellulose was prepared from cotton which was grown in the presence of D-glucose-UL-^{14}C and furnished by Dr. C. R. Benedict of Texas A & M University, College Station, Texas. The ^{14}C-cotton was nitrated by standard procedures.[10] The ^{14}C-nitrocellulose was assayed for its nitrogen content[11] and found to contain 12.9% nitrogen by weight, identical to the average value of that used in the nitrocellulose toxicity studies above.

2. Preparation of ^{14}C-nitrocellulose for oral dosing: The ^{14}C-nitrocellulose dose was prepared by cutting the fibers with scissors and grinding an aqueous suspension in a mortar and pestle. The dose was concentrated by sedimentation and only fibers small enough to go through an 18-gauge dosing needle were used. For the initial experiment the dose was suspended in distilled water. For a second experiment the dose was suspended in 0.2% methyl cellulose-0.4% Tween 80 (MC-TW80), to obtain a better suspension.

3. Experimental procedure: Two Charles River CD® male rats weighing 715 and 607 gm were used for this study. Each rat was fasted overnight before being given 1 ml/100 gm (about 20,000 dpm/ml) of either the aqueous or MC-TW80 suspension orally. After dosing, each rat was placed immediately in a "Roth-Delmar" metabolism chamber. The chamber was vented continuously with CO_2-free air at a rate of 250 ml/min. Expired CO_2 was collected by passing the air through three absorption columns connected in series. Each column contained 100 ml of 5% NaOH. Feces and urine were collected separately in the apparatus. To ensure that sufficient radioactivity was administered, the dosing was repeated daily for 4 days. Twenty-four hours after the last dose, the rat was anesthetized with ether and aortic blood collected in a heparinized syringe. Liver, spleen, kidneys, brain, lungs and thigh muscle were removed, weighed, and representative samples taken for analysis of radioactivity. The stomach, small intestine, cecum and large intestine were removed and weighed. These sections and the feces were homogenized in water and representative samples assayed for radioactivity.

4. Radioactive assays: Aliquots of whole blood and tissue samples were digested in 2N NaOH. Blood samples were decolorized by dropwise addition of hydrogen peroxide. Samples of tissue digests were neutralized with Beckman BBS-2, solubilized in Beckman BBS-3, and counted in a toluene-PPO-dimethyl POPOP cocktail using a Packard Tricarb 3375 liquid scintillation spectrometer. Samples of plasma, urine, and ^{14}C-nitrocellulose were solubilized directly in BBS-3 and counted. $^{14}CO_2$ samples from the air traps were spotted on filter paper, dried, and counted. All data were corrected for background and quenching.

C. Results and Conclusions

The result after repeated oral doses of radiolabeled nitrocellulose is summarized in Table 40. No detectable radioactivity was found in any tissue or body fluid. Radioactivity was recovered only in the various components of the gastrointestinal tract plus contents and in the feces. From these results, we can conclude that the nitrocellulose molecule is not absorbed by the rat.

TABLE 40

DISTRIBUTION AND EXCRETION OF RADIOACTIVITY AFTER ORAL ADMINISTRATION OF ^{14}C-NITROCELLULOSE

	Total dpm Recovered	
	Rat No. 1[a/]	Rat No. 2[b/]
Gastrointestinal Tract Plus Contents		
Stomach	169,575	6,867
Small intestine	4,979	0
Cecum	60,735	0
Large intestine	3,222	0
Feces	168,579	488,720
Expired Air	0	0
Blood	0	0
Urine	0	0
Liver	0	0
Spleen	0	0
Kidneys	0	0
Lungs	0	0
Muscle	0	0

a/ Rat No. 1 received the ^{14}C-nitrocellulose as an aqueous suspension.

b/ Rat No. 2 received the ^{14}C-nitrocellulose as a suspension in 0.2% methylcellulose - 0.4% Tween 80.

V. GENERAL SUMMARY AND CONCLUSIONS

TABLE OF CONTENTS

	Page
A. Summary and Conclusions.	73
B. Additional Research.	73

V. GENERAL SUMMARY AND CONCLUSIONS

A. Summary and Conclusions

There is no evidence that nitrocellulose is a "toxic chemical." The only effects seen (increased feed consumption, decreased weight gain, intestinal impaction) were also seen in animals fed a similar concentration of cotton linters, the material which is nitrated to form NC. Since dogs, rats and mice, like humans, cannot digest cellulose, the NC and linters passed straight through the gastrointestinal tract, as confirmed in the absorption study. The fibrous nature of the NC and linters produced the observed effect of impaction in the relatively small intestine of mice; the feed and weight effects were due to non-nutritive bulk of the fibers.

B. Additional Research

Although no effects were seen in these subchronic feeding studies, it is possible that the lifetime feeding of these fibers may have some adverse effects. Possible mechanisms include continual irritation, predisposing to tumors, and possible denitrification of the NC by intestinal bacteria, followed by absorption of the nitrate and potential toxicity from it or reaction products, such as nitrosamines. Nitrocellulose should be given a chronic toxicity study before the subject of toxicity is dismissed.

REFERENCES

1. Lee, C. C., J. V. Dilley, J. R. Hodgson, D. N. Roberts, D. O. Helton and W. J. Wiegand. Mammalian Toxicity of Munition Compounds: Phase I. Acute Oral Toxicity, Primary Skin and Eye Irritation, Dermal Sensitization and Disposition and Metabolism. USAMRDC Contract No. DAMD-17-74-C-4073. Report No. 1:1-100, 22 July 1975.

2. Seligson, D., J. Marino, and E. Dodson: Determination of Sulfobromophthalein in Serum. Clin. Chem., 3:638 (1957).

3. Dunnett, C. W.: A Multiple Comparison Procedure for Comparing Several Treatments with a Control. J. Am. Stat. Assoc. 50:1096 (1955).

4. Reisman, R. E., and C. E. Arbesman: Systemic Allergic Reactions Due To Inhalation of Penicillin. J. Am. Med. Assoc., 203:986 (1968).

5. Mancini, G., A. O. Carbonara, and J. F. Heremans: Immunochemical Quantitation of Antigens by Single Radial Immunodiffusion. Immunochemistry, 2:235 (1964).

6. Moorhead, P. S., P. C. Nowell, W. J. Mellman, D. M. Battips, and D. A. Hungerford: Chromosome Preparations of Leukocytes Cultured from Human Peripheral Blood. Expt. Cell Res., 20:613 (1960).

7. Fernandes, M. V.: The Development of a Human Ammion Strain of Cells. Texas Repts. Biol. Med., 16:48 (1958).

8. Vogt, M., and R. Dulbecco: Virus-Cell Interaction with Tumor Producing Virus. Proc. Nat. Acad. Sci., 46:365 (1960).

9. Moorhead, P. S., and P. C. Nowell: Chromosome Cytology. In Methods in Medical Research (H. N. Eisen, Editor). Year Book Medical Publ. Inc., Chicago, 10:310 (1964).

10. Shafizadeh, F., M. L. Wolfrom and P. McWain: Controlled Thermal Decomposition of Cellulose Nitrate. V. Carbon-14 Tracer Experiments. J. Amer. Chem. Soc., 81:1221 (1959).

11. Selig, W.: Microdetermination of Aromatic Nitrocompounds, Nitrocellulose and Cyclic Nitramines. AEC Report No. UCRL-6639, Lawrence Radiation Laboratory, Livermore, Cal. (1961).

Procedure from

DISTRIBUTION LIST

No. of Copies	
4	HQDA (SGRD-AJ) Ft. Detrick Frederick, MD 21701
25	U.S. Army Medical Bioengineering Research and Development Laboratory Attn: SGRD-UBG-R Fort Detrick Frederick, MD 21701
12	Defense Documentation Center (DDC) Attn: DDC-TCA Cameron Station Alexandria, VA 22314
1	Superintendent Academy of Health Sciences, U.S. Army Attn: AHS-COM Fort Sam Houston, TX 78234
1	Dean School of Medicine Uniformed Services University of the Health Sciences Office of the Secretary of Defense 6917 Arlington Road Bethesda, MD 20014

APPENDIX I

MANUAL FOR

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

Cheng-Chun Lee
Chuen-Bin Hong
Jagdish C. Bhandari
Judith D. Girvin
John J. Kowalski

Midwest Research Institute

January 1977

TABLE OF CONTENTS

		Page
I.	Hematology and Clinical Laboratory Tests	1
	A. Hematology	1
	B. Clinical Blood Chemistry	2
	C. Urinalysis	3
	D. Occult Blood in Feces	4
	E. Precision of Hematology and Clinical Blood Chemistry Tests	4
	1. Reproducibility	4
	2. Reproducibility Within a Test Day	4
	3. Proficiency Test Service	5
II.	Histopathology	5
	A. Necropsy and Gross Examination	5
	B. Organ Weights	5
	C. Tissues for Microscopic Examination	6
	D. Fixation and Staining of Tissues	6
III.	Statistical Analysis	5
IV.	Normal Values	7
	A. Hematology, Clinical Laboratory Tests and Bone Marrow	7
	B. Absolute and Relative Organ Weights	7
	C. Presence of Various Substances in the Urine	7
	D. Occult Blood in Feces	8
V.	References	8
Tables A - O		10 - 24

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 manual 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%, 27.6 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 0.

V. REFERENCES

1. Brecher, G., M. Schneiderman, and C. Z. William: Evaluation of the Electronic Red Cell Counter. Am. J. Clin. Path., 26: 1439, 1956.

2. Selegson, D.: Standard Methods of Clinical Chemistry, Academic Press, Inc., New York, Vol. 2, p. 52, 1958.

3. Dubowski, K. M.: Measurement of Hemoglobin Derivatives in Hemoglobin, Its Precusors and Metabolites. (F. W. Sunderman and F. W. Sunderman, Jrs., eds.), J. B. Lippincott Company, Philadelphia, p. 29, 1964.

4. Brecher, G., and M. Schneiderman: A Time-Saving Device for the Counting of Reticulocytes. Am. J. Clin. Path., 20: 1079, 1950.

5. Bull, B. S., M. A. Schneiderman, and G. Brecher: Platelet Counts With the Coulter Counter. Am. J. Clin. Path., 44: 678-688, 1965.

6. Brecher, G., M. Schneiderman, and E. P. Cronkite: The Reproducibility and Constancy of the Platelet Count. Am. J. Clin. Path., 23: 15, 1953.

7. Hepler, O. E.: Manual of Clinical Laboratory Methods, p. 83, Charles C. Thomas, Springfield, Illinois, 1935.

8. Slein, M. W.: Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), p. 117, Academic Press, New York, 1963.

9. Amador, E., and W. E. C. Wacker: Serum Glutamic-Oxaloacetic Transaminase Activity: A New Modification and an Analytical Assessment of Current Assay Technics. Clin. Chem., 8: 343, 1962.

10. Henry, R. J., N. Chiamori, O. J. Golub, and S. Berkman: Revised Spectrophotometric Methods for the Determination of Glutamic-Oxaloacetic Transaminase, Glutamic-Pyruvic Transaminase, and Lactic Dehydrogenase. Am. J. Clin. Path., 34: 381, 1960.

11. Bowers, G. N., Jr., and R. B. McComb: A Continuous Spectrophotometric Method for Measuring the Activity of Serum Alkaline Phosphatase. Clin. Chem., 12: 70, 1966.

12. Chaney, A. L., and E. P. Manback: Modified Reagents for Determination of Urea and Ammonia. Clin. Chem., 8: 130, 1962.

13. Lustgarten, J. A.: A Simple, Rapid, Kinetic Method for Creatinine Concentration. Clin. Chem., 18: 1419, 1972.

14. Wacker, W. E. C., D. D. Ulmer, and B. L. Vallee: Metalloenzymes and Myocardial Infarction. II. Malic and Lactic Dehydrogenase Activities and Zinc Concentrations in Serum. New Eng. J. Med., 225, 449, 1956.

15. Rosalki, S. B., and J. H. Wilkinson: Re[illegible]ion of α-Ketobutyrate by Human Serum. Nature (London), 188, 11[illegible]960.

16. Rosalki, J. B.: An Improved Procedure for Serum Creatine Phosphokinase Determination. J. Lab. Clin. Med., 69, 696, 1967.

17. Oliver, I. T.: A Spectrophotometric Method for the Determination of Creatine Phosphokinase and Myokinase. Biochem. J., 61, 116, 1955.

18. Dunnett, C. W.: A Multiple Comparison Procedure for Comparing Several Treatments with a Control. J. Am. Stat. Assoc., 50: 1096-1121, 1955.

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	69.6 - 117.3
MCHb ($\mu\mu$g)	108	3.74 ± 0.50	24.4 ± 1.8	21.0 - 33.6
MCHbC (mg %)	108	3.74 ± 0.50	31.4 ± 1.3	27.2 - 34.1
Platelets (x 10^5/mm^3)	99	3.74 ± 0.50	3.08 ± 0.45	0.80 - 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 ± 7.5	12 - 65
Proth. Time (sec)	62	3.91 ± 0.44	10.2 ± 0.7	9.3 - 11.9
Serum Creat. (mg %)	100	3.76 ± 0.51	1.1 ± 0.3	0.6 - 1.8
Bilirubin				
Total (mg %)	62	3.91 ± 0.44	0.1 ± 0.2	0.0 - 0.8
Direct (mg %)	62	3.91 ± 0.44	0.0 ± 0.0	0.0 - 0.0
BSP 15 min (% ret.)	62	3.91 ± 0.44	18.0 ± 7.4	2 - 34
Na (mEq/ℓ)	62	3.91 ± 0.44	154.0 ± 19.1	144 - 179
K (mEq/ℓ)	62	3.91 ± 0.44	4.8 ± 0.6	3.9 - 5.7
Cl (mEq/ℓ)	62	3.91 ± 0.44	109.0 ± 6.4	93 - 118
Ca (mEq/ℓ)	62	3.91 ± 0.44	5.2 ± 0.4	4.2 - 6.3
Mg (mEq/ℓ)	62	3.91 ± 0.44	1.6 ± 0.1	1.2 - 1.8
Bone Marrow				
Myeloid/erythroid ratio	15	3.65 ± 0.41	1.5 ± 0.3	1.5 - 2.1

a/ Data collected between June 1971 and December 1976.

TABLE E

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

	Female Rhesus Monkeys			
	Number Studied	Body Weight (kg) Mean ± S.D.	Observed Results Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)	81	3.51 ± 0.48	5.33 ± 0.40	4.25 - 6.03
Reticulocytes (%)	81	3.51 ± 0.48	1.07 ± 0.54	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 (mg %)	81	3.51 ± 0.48	92.1 ± 15.3	57 - 116
SGOT (IU/ℓ)	81	3.51 ± 0.48	32.1 ± 7.6	20 - 70
SGPT (IU/ℓ)	81	3.51 ± 0.48	30.1 ± 7.6	12 - 39
Alkaline Phosphatase (IU/ℓ)	81	3.51 ± 0.48	349.9 ± 112.3	148 - 572
BUN (mg %)	81	3.51 ± 0.48	17.3 ± 4.2	13 - 29
Proth. Time (sec)	59	3.56 ± 0.43	10.5 ± 0.9	9.7 - 12.3
Serum Creat. (mg %)	81	3.51 ± 0.48	1.1 ± 0.3	0.6 - 1.7
Bilirubin				
Total (mg %)	81	3.51 ± 0.48	0.1 ± 0.1	0.0 - 0.8
Direct (mg %)	81	3.51 ± 0.48	0.0 ± 0.0	0.0 - 0.0
BSP 15 min (% ret.)	59	3.56 ± 0.43	16.4 ± 8.3	5 - 36
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.	Absolute 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.	Absolute 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				
	Number Studied	Age (months)	Body Weight (kg) Mean ± S.D.	Observed Results Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)	276	4 - 7	8.3 ± 1.7	5.55 ± 0.73	3.62 - 7.60
Reticulocytes (%)	284	4 - 7	8.3 ± 1.7	0.72 ± 0.46	0.04 - 4.35
Hematocrit (vol %)	276	4 - 7	8.3 ± 1.7	41.6 ± 3.5	31 - 50
Hemoglobin (gm %)	276	4 - 7	8.3 ± 1.7	13.5 ± 1.4	10.0 - 16.9
MCV (μ^3)	276	4 - 7	8.3 ± 1.7	75.6 ± 8.3	56.7 - 127.1
MCHb ($\mu\mu$g)	276	4 - 7	8.3 ± 1.7	24.6 ± 3.0	17.1 - 41.7
MCHbC (mg %)	276	4 - 7	8.3 ± 1.7	32.5 ± 1.5	28.1 - 40.3
Platelets (x 10^5/mm^3)	270	4 - 7	8.4 ± 1.7	2.91 ± 1.02	0.93 - 6.35
Leukocytes (x 10^3/mm^3)	284	4 - 7	8.3 ± 1.7	11.9 ± 3.5	4.6 - 24.6
Neutrophils I (%)	284	4 - 7	8.3 ± 1.7	0.55 ± 1.06	0 - 6
Neutrophils M (%)	284	4 - 7	8.3 ± 1.7	56.81 ± 9.47	22 - 80
Lymphocytes (%)	284	4 - 7	8.3 ± 1.7	37.94 ± 9.26	13 - 71
Eosinophils (%)	284	4 - 7	8.3 ± 1.7	2.76 ± 2.93	0 - 16
Monophils (%)	284	4 - 7	8.3 ± 1.7	1.78 ± 1.84	0 - 11
Basophils (%)	284	4 - 7	8.3 ± 1.7	0.01 ± 0.10	0 - 2
Atypical cells (%)	284	4 - 7	8.3 ± 1.7	0.11 ± 0.37	0 - 2
Nucleated RBC (%)	284	4 - 7	8.3 ± 1.7	0.02 ± 0.10	0 - 2
Fasting Glucose (mg %)	284	4 - 7	8.3 ± 1.7	100.9 ± 12.6	66 - 134
SGOT (IU/ℓ)	276	4 - 7	8.3 ± 1.7	23.2 ± 7.4	11 - 59
SGPT (IU/ℓ)	276	4 - 7	8.3 ± 1.7	25.7 ± 7.9	8 - 46
Alkaline Phosphatase (IU/ℓ)	276	4 - 7	8.3 ± 1.7	73.3 ± 18.5	21 - 133
BUN (mg %)	284	4 - 7	8.3 ± 1.7	12.1 ± 3.3	4 - 23
Bone Marrow					
Myeloid/erythroid ratio	34	5 - 9	9.4 ± 1.6	1.6 ± 0.4	1.1 - 3.0

a/ Data collected between September 1971 and December 1976.

TABLE I

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

	Female Beagle Dogs				
	Number Studied	Age (months)	Body Weight (kg) Mean ± S.D.	Observed Results Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)	257	4 - 7	6.9 ± 1.3	5.59 ± 0.73	3.27 - 7.75
Reticulocytes (%)	265	4 - 7	6.9 ± 1.3	0.74 ± 0.52	0.04 - 5.05
Hematocrit (vol %)	257	4 - 7	6.9 ± 1.3	42.3 ± 3.5	32 - 51
Hemoglobin (gm %)	257	4 - 7	6.9 ± 1.3	13.7 ± 1.3	11.0 - 18.6
MCV (μ^3)	257	4 - 7	6.9 ± 1.3	76.7 ± 9.7	55.8 - 128.4
MCHb ($\mu\mu$g)	257	4 - 7	6.9 ± 1.3	24.8 ± 3.3	17.1 - 41.6
MCHbC (mg %)	257	4 - 7	6.9 ± 1.3	32.3 ± 1.6	28.7 - 40.4
Platelets (x 10^5/mm^3)	227	4 - 7	6.9 ± 1.3	3.08 ± 1.15	1.08 - 7.95
Leukocytes (x 10^3/mm^3)	265	4 - 7	6.9 ± 1.3	10.9 ± 3.4	3.8 - 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 ± 0.35	0.49 - 1.65
Thyroids (gm)	0.88 ± 0.25	0.55 - 1.91
Ovaries (gm)	0.74 ± 0.24	0.38 - 1.27
	Relative (per kg body weight)	
	Mean ± S.D.	**Range**
Liver (gm)	28.2 ± 5.0	20.7 - 38.8
Spleen (gm)	6.0 ± 2.3	3.1 - 10.9
Kidneys (gm)	5.5 ± 0.9	3.7 - 7.9
Adrenals (mg)	135 ± 35	67 - 215
Thyroids (mg)	112 ± 31	75 - 219
Ovaries (mg)	96 ± 33	54 - 222

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

TABLE L

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

	Male Rats			Observed Results	
	Number Studied	Age (weeks)	Body Weight (gm) Mean ± S.D.	Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)	527	5 - 7	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.	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)	[illegible] ± 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).