

CODING FORMS FOR SRC INDEXING

Microfiche No.	OTS0001252		
New Doc ID	FYI-OTS-0794-1252	Old Doc ID	84940000309
Date Produced	05/17/84	Date Received	07/27/94
		TSCA Section	FYI
Submitting Organization	CHEM MFGS ASSN		
Contractor	BIO/DYNAMICS INC		
Document Title	INITIAL SUBMISSION: AN EVAL OF THE POTENTIAL ROLES OF SKIN IRRITATION, WEIGHT LOSS AND NONSPEC STRESS ON TESTICULAR ATROPHY IN THE RABBIT WITH ZINC DIALKYL DITHIOPHOSPHATE (VOL.1)		
Chemical Category	CMA-101; CMA-102		

FYI-0794-1252



INIT 87/27/94

Contains No CBI



04940800309

Bio/dynamics Inc.

Division of Biology and Safety Evaluation

RECEIVED
OPPT 0910
94 JUL 27 PM 12:42

PROJECT NO. 82-2701

AN EVALUATION OF THE POTENTIAL ROLES OF SKIN IRRITATION, WEIGHT LOSS
AND NONSPECIFIC STRESS ON TESTICULAR ATROPHY IN THE RABBIT

Final Report

VOLUME I: Appendices A - F

Submitted to: Chemical Manufacturers Association
Washington, D.C. 20037

Date: May 17, 1984

Abstract (cont.):

Four CMA-102 animals were described as being slightly or moderately emaciated on Day 15 of the study. Emaciation was observed in two CMA-102 and six fasted rabbits at Week 4. During the four-week extension period, the CMA-102 rabbits exhibited a higher incidence and/or severity of staining in the ano-genital area and emaciation than did either control group. Two fasted animals were slightly emaciated on Day 36 of the study.

Rabbits treated with CMA-101 (vehicle control), CMA-102, sodium hydroxide, hydrochloric acid and ultraviolet irradiation developed erythema, atonia and desquamation. The incidence and/or severity of these findings were most severe in the CMA-102 animals. Edema was evident in the vehicle control, sodium hydroxide, hydrochloric acid and ultraviolet groups. Fissuring was evident only in the CMA-102 animals. Eschar formation occurred only in the hydrochloric acid treated animals. Exfoliation occurred most severely and/or frequently in the CMA-102 rabbits. Exfoliation was also noted, to a lesser extent, in the sodium hydroxide, hydrochloric acid and ultraviolet irradiation groups.

Body weights of the vehicle control animals were lower than untreated control rabbits; while those of the rabbits dosed with sodium hydroxide, hydrochloric acid and ultraviolet irradiation were only slightly less than the untreated animals throughout the study. Rabbits dosed with CMA-102 exhibited the most drastic progressive reductions in body weight which paralleled those of the fasted animals, as evident by body weight decrements when compared to vehicle control and to untreated control values.

Food consumption of the CMA-102 animals was approximately half that of either control group during the first two weeks of the study. From Day 19 to Day 26, food consumption of these animals was generally comparable to or greater than that of the CMA-101 animals. Food consumption of the CMA-102 animals at Day 33 (2/4) and Day 36 (2/3) was reduced compared to the control. In general, body weight gain or loss of the CMA-102 animals paralleled food consumption.

Water consumption of the fasted animals was generally less than either control group up to Day 24.

Hemoglobin, hematocrit and erythrocyte counts of the rabbits treated with CMA-102 and of the fasted rabbits were statistically significantly lower than control values after four weeks of test substance administration. Hemoglobin values remained lower in the CMA-102 group than in either control group at the end of the extension period. Evaluation of the differential leukocyte counts revealed that nearly all rabbits receiving CMA-102 (Group III) and fasted (Group VII) rabbits displayed one or more of the following findings: anisocytosis, poikilocytosis, hypochromia and/or polychromia after four weeks on test. These findings were seen in only one or two control animals at this interval. Similar findings were also seen after the four-week extension period in the vehicle control group (Group I, CMA-101) as well as in the CMA-102 and fasted rabbits with approximately equal incidence and severity. These findings were not seen in any of the untreated control animals (Group II) at this interval. Animals treated with CMA-102 exhibited a shift in mature lymphocytes to segmented neutrophils after four weeks of treatment as well as at the end of the extension period.

Rabbits dosed with CMA-102 exhibited elevated triglyceride levels after four weeks of treatment as well as elevated blood urea nitrogen and cholesterol levels after the four week extension period. Cholesterol and triglyceride levels were elevated in the fasted rabbits while total protein, albumin, globulin and blood urea nitrogen levels were depressed at Week 4.

PROJECT NO. 82-2701

AN EVALUATION OF THE POTENTIAL ROLES OF SKIN IRRITATION, WEIGHT LOSS
AND NONSPECIFIC STRESS ON TESTICULAR ATROPHY IN THE RABBIT

ABSTRACT

A total of 105 male New Zealand White rabbits were randomly distributed into 7 groups of 15 animals/group. Animals in Group I served as the vehicle control group (100% (w/v) CMA-101, 2 ml/kg). Animals in Group II served as an untreated, uncollared and unshaved control group. Animals in Group III were treated with CMA-102 diluted to 25% (w/v) concentration with CMA-101. Animals in Group IV were treated with reagent grade Sodium Hydroxide dissolved in distilled water to yield a concentration of 2% (w/v). Animals in Group V were treated with Hydrochloric Acid dissolved in distilled water to yield a concentration of 5% (v/v). Each test substance was applied to the clipped dorsal surface of 15 rabbits/group at a dose volume of 2 ml/kg, once a day, 5 days/week, for four weeks. Animals in Group VI (15) were restrained in standard rabbit stocks and irradiated with U.V.B. light in an attempt to produce dermal irritation of approximate severity to that of Group III. Fifteen animals were fasted in an attempt to produce a body weight decrement of 25% of Day 1 body weights (Group VII). Estimates of fecal quantity, physical observations, body weights, food consumption and water consumption were performed at specified intervals throughout the study. Dermal observations were recorded 3x/week. Four animals/group were selected from the surviving animals in Groups I, II, and III to continue on treatment and four animals were selected from Group VII and presented with food ad libitum for an additional four weeks. Clinical laboratory studies (hematology and clinical chemistry) were performed on all animals pretest and on all survivors at Week 4 and at the end of the extension period. Selected organs were weighed and organ/body and organ/brain weight ratios were calculated. Complete gross postmortem examinations and histopathologic evaluations of selected tissues were performed on all animals in the study.

Five rabbits dosed with CMA-102 died spontaneously during the course of the study.

Animals treated with CMA-102, sodium hydroxide, hydrochloric acid and ultraviolet irradiation as well as the fasted rabbits exhibited a slightly higher incidence and/or severity of staining in the ano-genital area than was observed in either control group. The highest incidence and/or severity of this finding was seen in the CMA-102 group. CMA-102 and fasted

Abstract (cont.):

After four weeks of dosing, the following organ weight effects were evident in the rabbits treated with CMA-102 (Group III) when compared to the vehicle control group (CMA-101, Group I): lower absolute weights of the epididymides, testes and the prostate and higher absolute kidney weights. These changes in absolute organ weights were also accompanied by concomitant changes in relative (organ/body and organ/brain) weights. Therefore, they were considered to be indicative of treatment-related effects. After nine weeks of test substance administration the treatment-related effects on reproductive organ weights became more pronounced in the CMA-102 treated animals when compared to the vehicle control group as evidenced by reductions in the absolute and relative weights for: epididymides, seminal vesicles, testes and prostate. Kidney weights (absolute and relative) of the CMA-102 rabbits remained elevated at Week 9. Differences in these reproductive organ weights between the vehicle control and the untreated control animals at Week 9 were not as evident or as pronounced as they were at Week 4.

Gross postmortem examinations revealed that small testes and epididymides were most frequently observed in the animals in Group VII, followed by Group III. Also, the prostate gland was observed to be small most frequently in the animals in Group VII, followed by Groups I and III. With regard to the treated skin, various abnormalities tabulated previously were observed grossly. Various combinations of these occurred most frequently in the animals in Groups IV and V, followed by Group III.

Results of the histopathological evaluations of the tissues taken at the Week 4 necropsy revealed mild non-inflammatory changes present in the treated skins of the vehicle control (CMA-101) group and a few of the rabbits receiving U.V.B. irradiation. Slight to moderately severe changes were present in the treated skins of the rabbits receiving CMA-102, sodium hydroxide or hydrochloric acid. These changes were more severe in the sodium hydroxide and hydrochloric acid groups. No significant changes were observed in the skins of the untreated control and fasted groups of rabbits.

Testicular changes, characterized by a moderately severe diffuse tubular hypoplasia and aspermatogenesis, were present in two of the eleven rabbits treated with 25% CMA-102 (Group III). These two rabbits had slight and moderate hyperkeratosis and moderate epidermal hyperplasia of the treated skin. The other nine rabbits in this group had normal appearing testes and epididymides even though the skin lesions were more severe than observed in the two rabbits with testicular changes. Several of the nine rabbits had skin lesions characterized by severe hyperkeratosis, moderate to severe dermal hyperplasia and varying degrees of suppurative inflammation of the epidermis.

The groups of rabbits treated with sodium hydroxide (Group IV) and hydrochloric acid (Group V) had the most severe skin lesions of all of the treated animals in this study. These skin lesions included ulceration, increased dermal connective tissue and varying degrees of suppurative inflammation of the epidermis or dermis. All fifteen animals treated with sodium hydroxide and all fifteen rabbits treated with hydrochloric acid had normal appearing testes and epididymides and normal degrees of spermatogenesis.

In the group of rabbits fasted to achieve a 25% weight loss (Group VII, not treated with a test substance), nine of the eleven rabbits had moderately severe diffuse tubular hypoplasia and aspermatogenesis. The

Abstract (cont.):

remaining two rabbits had normal testes and epididymides but a reduction in the degree of spermatogenesis.

The eleven rabbits in each of the control groups and fourteen of fifteen rabbits exposed to U.V.B. irradiation had normal testes and epididymides with an active and normal spermatogenesis.

Skin treatment with sodium hydroxide and hydrochloric acid resulted in marked skin lesions but no testicular changes nor any effect on spermatogenesis, indicating that stress from severe skin lesions did not effect the spermatogenic cycle of these rabbits in this study. These animals did not exhibit a body weight loss. This same conclusion pertains to the effect of U.V.B. irradiation on rabbits.

Results of the histopathological evaluations of the tissues taken at the extension necropsy revealed testicular changes, characterized by a moderate multifocal or diffuse seminiferous tubular hypoplasia and reduced spermatogenesis, present in three of four rabbits treated with 25% CMA-102 (Group III). The testes and epididymides of the other rabbit in this group were normal with an active and normal spermatogenesis. All four rabbits in this group had moderate to moderately severe hyperkeratosis, moderate epidermal hyperplasia and slight suppurative inflammation of the treated skin. Three of the four rabbits with testicular changes were considered to be emaciated by gross examination and exhibited severe body weight loss.

All four rabbits in the group which was initially fasted to achieve a 25% weight loss (Group VII) and then given food ad libitum during the four-week extension period had microscopically normal testes and epididymides. The degree of spermatogenesis appeared to be normal in one rabbit and only slightly reduced in the other three rabbits in this group.

In conclusion, CMA-102 generally produced the most pronounced treatment-related effects as evident by the findings of the in-life physical and dermal observations, body weight, food consumption, clinical laboratory studies and organ weight data (with the exception of the fasted animals).

Fasting of rabbits to achieve a weight loss of approximately 25%, relative to their initial body weight on Day 1 of the study, was effective in disrupting the spermatogenic cycle of the rabbit and caused a diffuse seminiferous tubular hypoplasia or atrophy of the testes in nine of eleven rabbits with reduced spermatogenesis in the other two rabbits. The application of 25% CMA-102 to the skin of eleven rabbits resulted in similar testicular changes in two rabbits, both of which exhibited the most severe body weight loss among the eleven rabbits.

The results of this study have demonstrated that total or partial food deprivation and subsequent body weight loss during the study had significant effects on the testes and degree of spermatogenesis of the rabbit and that stress related to severe skin lesions alone appeared to have little effect on spermatogenesis.

The results of the histopathology indicate that returning rabbits to a normal feeding regime resulted in a return to almost normal spermatogenesis, while continued treatment with 25% CMA-102 and the associated body weight loss had an effect on the spermatogenic cycle of three of four rabbits which appeared to be proportional to the severe weight loss in the rabbits.

 TABLE OF CONTENTS

VOLUME I:

I.	INTRODUCTION.....	1
II.	MATERIALS AND METHODS	
	A. Test Substances.....	2
	B. Vehicle.....	3
	C. Test Animals.....	3
	D. Selection and Group Assignment.....	4
	E. Animal Identification.....	4
	F. Experimental Design.....	5
	G. Husbandry.....	6
	H. Test Substance Administration.....	6
	I. Observations	
	Physical.....	9
	Dermal.....	9
	J. Body Weights.....	9
	K. Food Consumption.....	9
	L. Water Consumption.....	9
	M. Laboratory Studies.....	9
	N. Postmortem.....	10
	O. Statistical Analysis.....	11
III.	RESULTS AND DISCUSSION	
	A. Mortality.....	12
	B. Physical Observations.....	12
	C. Dermal Responses.....	14
	D. Body Weight.....	16
	E. Food Consumption.....	16
	F. Water Consumption.....	16
	G. Clinical Laboratory Studies	
	1. Hematology.....	17
	2. Clinical Chemistry.....	18
	H. Terminal Organ and Body Weights and Organ/Body and Organ/Brain Weight Ratios.....	19
	I. Gross Postmortem Examinations and Histopathologic Evaluations.....	22
	Figure 1: Graph of Mean Body Weights - Groups I to VII: First 7 Weeks of Treatment.....	27
	Figure 2: Graph of Mean Body Weights - Groups I, II, III and VII: Extension Period.....	28
	Figure 3: Graph of Mean Body Weights (entire study).....	29
	Figure 4: Graph of Mean Body Weight Data - Days 1, 26 and 61.....	30

 TABLE OF CONTENTS (CONT.)

VOLUME I:

III. RESULTS AND DISCUSSION

Figure 5: Graph of Mean Food Consumption - Groups I to VII: First 4 Weeks of Treatment.....	31
Figure 6: Graph of Mean Food Consumption - Groups I, II, III and VII: Extension Period.....	32

APPENDICES

A. Methodology and References.....	A-1
B. Animal Termination History.....	B-1
C. Summary of Physical Observations.....	C-1
D. Summary of Dermal Responses.....	D-1
E. Body Weight	
Mean Values.....	E-1
Individual Values.....	E-20
Body Weight Change	
Mean Values.....	E-8
Individual Values.....	E-20
Food Consumption	
Mean Values.....	E-14
Individual Values.....	E-20
F. Water Consumption	
Mean Values.....	F-1
Individual Values.....	F-6

VOLUME II:

G. Hematology	
Mean Values.....	G-1
Individual Values.....	G-5
H. Individual Total and Differential Leukocytes.....	H-1
I. Clinical Chemistry	
Mean Values.....	I-1
Individual Values.....	I-8
J. Terminal Organ and Body Weights and Organ/Body and Organ/Brain Weight Ratios	
Mean Values.....	J-1
Individual Values.....	J-13
K. Gross Postmortem Examinations and Histopathologic Evaluations.....	K-1
L. Personnel.....	L-1
M. Quality Assurance Statement.....	M-1
N. Protocol.....	N-1

I. INTRODUCTION

This study, conducted for the Chemical Manufacturers Association, was designed to produce skin irritation, body weight loss, and nonspecific "stress" in the rabbit and to determine their potential effects on the testes. Animals in Group I served as a vehicle control group and received 100% (w/v) CMA-101. A 15 animal control group (Group II) was left untreated (not collared or shaved).

Animals in Group III were treated with CMA-102 diluted to 25% (w/v) with CMA-101. Animals in Group IV were treated with Sodium Hydroxide dissolved in distilled water to yield a concentration of 2% (w/v). Animals in Group V were treated with Hydrochloric Acid dissolved in distilled water to yield a concentration of 5% (v/v). Each substance was applied to the clipped dorsal surface of male New Zealand White rabbits (15/group) at a constant dose volume of 2 ml/kg, once a day, 5 days a week for 4 weeks. Animals in Group VI (15) were restrained in standard rabbit stocks and irradiated with U.V.B. light in an attempt to produce dermal irritation of the same approximate severity as that observed in Group III. Fifteen animals were fasted in an attempt to produce a body weight decrement of 25% of Day 1 body weights (Group VII). Four animals/group were selected from the surviving animals in Groups I, II and III to continue on treatment and four animals were selected from Group VII and were presented with food ad libitum for an additional four weeks.

Species and strain of the test system, dose levels and route of test substance administration were established by the sponsor. This study was performed at Bio/dynamics, Inc., Mettlers Road, East Millstone, New Jersey 08973. All raw data is stored at this testing facility.

II. MATERIALS AND METHODS

A. Test Substances:

1. CMA-102 - technical grade
2. Sodium Hydroxide - reagent grade
3. Hydrochloric Acid - reagent grade

Substance 1: CMA-102
Zinc dialkyl dithiophosphate

Concentration: As supplied.

Supplier: Chemical Manufacturers Association
2501 M Street, N.W.
Washington, D.C. 20037

Description: Formulation liquid

Date Received: 27 September 1982

Label Information: EXXON
CMA-102

Sampling: A representative 10 ml archival sample for study nos. 82-2678 and 82-2710 was retained in the archives of this testing facility.

Storage: Temperature monitored room (60° - 85°F)

Substance 2: Sodium Hydroxide

Concentration: 97%

Supplier: Aldrich Chemical Company, Inc.
Milwaukee, Wis. 53233

Description: White pellets

Label Information: NaOH
A.C.S. Reagent
Lot # 0912PH
Expires: 10/15/87

Storage: Temperature monitored room (60° - 85°)

A. Test Substances (cont.):

Substance 3: Hydrochloric Acid
Concentration: 37.2%
Supplier: J.T. Baker Chemical Co.
Phillipsburg, N.J. 08865
Description: Clear liquid
Label Information: 'BAKER ANALYZED'® Reagent
A.C.S. Reagent
Lot # 107067
Storage: Temperature monitored room (60° - 85°)

B. Vehicle:

CMA-101 Group III
Distilled Water Groups IV & V
(Methodology and References, Appendix A)
CMA-101
Concentration: As supplied.
Supplier: Chemical Manufacturers Association
2501 M Street, N.W.
Washington, D.C. 20037
Description: Clear viscous liquid
Liquid - Mineral oil of the Solvent 150
N type, motor oil basestock.
Dates Received: 27 September 1982 and 8 February 1983
Label Information: EXXON
CMA-101
Sampling: A representative 10 ml archival sample
for study nos. 82-2678 and 82-2701 was
retained in the archives of this testing
facility.
Storage: Temperature monitored room (60° - 85°F)

C. Test Animals:

Rabbits
Strain: New Zealand White
Justification: Rabbits have been historically used to
evaluate dermal irritation.
Supplier: Dutchland Laboratories, Inc.
Swampbridge Rd., Box 139A
Denver, Pennsylvania 17517

C. Test Animals (cont.):

Number of Animals:

- Purchased: 160 males total
- Placed on Test: 105 males (15/group)
- Date Received: 15 November 1982
- Desired Weight: Approximately 3.0 kg at receipt.

Weight at Initiation of Treatment (grams):	<u>Mean</u>	<u>Range</u>
	3221	2548-3715

D. Selection and Group Assignment: More animals than required for the study were purchased and equilibrated. Animals considered unsuitable for study on the basis of pretest body weight and general health were eliminated from possible inclusion in the study. Animals considered suitable were randomly distributed (see Appendix A) into control and treatment groups in an attempt to equalize mean group body weights.

Four animals in Groups I, II, III and VII were selected to remain on test for an additional 4 weeks. Animals in each group were arbitrarily assigned, using the Day 26 body weights, to 2 blocks, one for an extension group and one for a necropsy group (see Appendix A).

E. Animal Identification: Each rabbit was identified with a monel ear tag bearing its unique identification number prior to testing. In addition, each cage was provided with a cage card which was color-coded for dose level identification and contained project number, animal number, and dose group information.

F. Experimental Design:

Group	Test Substance	Dose Level %	Initial	Number of Male Rabbits		Histopathology
				Laboratory Studies Heme & Cl. Chem. (Pretest & Term.)	Necropsy Days 29-32	
IA	CMA-101b	100 (w/v)	15	15	4	15
IIa	-	-	15	15	4	15
IIIa	CMA-102b	25 (w/v)	15	15	2C	15
IV	Sodium Hydroxide	2 (w/v)	15	15	-	15
V	Hydrochloric Acid	5 (v/v)	15	15	-	15
VI	U.V.B. Irradiation	d	15	15	-	15
VIIa	Fasted	e	15	15	4	15

aFour animals in Groups I, II, III and VII were continued on test for four weeks at the sponsor's request.
bDosing was based on 100% activity of the technical/formulated grade of the test substance, as supplied by the sponsor.
cFive animals died spontaneously during the course of the study (see Appendix B).
dAll animals in Group VI were restrained and irradiated (U.V.B.- ultraviolet light, 280 - 320 nm) with commercially available suntan lamps. The distance from the exposure site and the time of irradiation was varied in an attempt to produce dermal irritation of the same approximate severity as that observed in Group III.
eAnimals were fasted during the first 30 days of the study in an attempt to produce a body weight decrement of 25% of the Day 1 body weights. Animals were fed ad libitum from Day 31 to the end of the study at the sponsor's request.

G. Husbandry:

Acclimation Period: 55 days (15 November 1982 to 9 January 1983). All animals in Groups I, III, IV, and V were acclimated to Elizabethan collars for one week during the acclimation period.

Housing: Individually in suspended stainless steel cages.

Food: Purina High-Fiber Rabbit Chow®, #5326 - Groups I through VI, ad libitum; Group VII, fasted Days 1 to 30 and ad libitum from Day 31 to the end of the study.

Water: ad libitum via water bottles (Elizabeth-town Water Company).

Contaminants: There were no known contaminants in the feed or water which were expected to be capable of interfering with the results of this study.

Environmental Conditions: 12 hours light/dark cycle, 7 am - 7 pm via automatic timer

Temperature and humidity monitored twice daily.

Temperature:

Desired: 60° to 70°F
Actual: 64° to 76°F

Humidity:

Desired: 40% to 60%
Actual: 18% to 67%

H. Test Substance Administration:

Preparation of Animals: Prior to test substance application, an area equal to approximately 25% of the trunk was carefully and closely clipped (Oster Model A-2 Ang-ra Clipping Head) on the dorsal surface of all animals in Groups I, III, IV, V and VI; approximately 10 cm. wide and extending from the suprascapula area to the hind quarters. All rabbits were reclipped as needed.

Route: Topically to the dorsal surface.

H. Test Substance Administration (cont.):

Justification of Route of Administration:

This route represents a potential exposure pattern for humans.

Justification for Dose Level Selection:

Dose levels were selected by the sponsor on the basis of a dermal screen conducted with these test substances, Bio/dynamics Project No. 82-2678.

Method:

Dermally treated animals were given a volume of 2.0 ml/kg, containing the appropriate amount of test substance, which was applied to the clipped unabraded dorsal surface of the rabbits once daily, 5 days/week (Monday - Friday) up to the day prior to sacrifice. Dosing was based on the body weights from the first weighing of each week. The test substances were applied with a syringe and evenly distributed over the prescribed area with a glass stirring rod. The backs of these animals were gently wiped with paper towels approximately 6 hours after exposure to remove excessive test substance, if necessary. All rabbits in Groups I, III, IV, and V were fitted with Elizabethan collars to prevent oral ingestion of the test substances.

Group I:

Animals received 100% (w/v) CMA-101.

Group II:

Animals were left untreated and were not shaved.

Group III:

Animals received an appropriate amount CMA-102, diluted as supplied to 25% (w/v) in CMA-101; no correction for % active ingredient was made.

Group IV:

Animals received an appropriate amount of sodium hydroxide (reagent grade), dissolved in distilled water to yield a concentration of 2% (w/v).

Group V:

Animals received an appropriate amount of hydrochloric acid (reagent grade), dissolved in distilled water to yield a concentration of 5% (v/v).

Group VI:

Each rabbit was restrained in standard rabbit stocks and irradiated with ultra-violet light (U.V.B.), at a wavelength of 280 to 320 nm, using commercially available suntan lamps. The distance from the exposure site and the time of exposure was varied in an attempt to produce dermal irritation of approximate severity to that of Group III.

H. Test Substance Administration (cont.):

Method (cont):

Group VI - Exposure Dates, Distance and Time:

<u>Date of Exposure</u> (1983)	<u>Exposure Distance</u> (Inches)	<u>Exposure Time</u> (Hours)
1/10	12	.50
1/11	12	.50
1/12	12	.50
1/13	12	.50
1/14	12	.50
1/17	12	.50
1/18	12	.50
1/19	10	.50
1/20	10	.50
1/21	10	.50
1/24	10	.50
1/25	10	.50
1/26	10	.75
1/27	10	.75
1/28	10	.75
1/31	10	.75
2/01	10	.75
2/02	10	.75
2/03	10	.75
2/04	10	.75
2/07	10	.75
2/08	10	.75
2/09	10	.75

Group VII:

Animals were fasted during the first 30 days of the study to achieve a weight loss of approximately 25% relative to the initial body weight on Day 1 of the study. Weight loss was monitored and feed allotments of 50 g were presented to animal no. 7015 on Days 22 and 23 and to animal Nos. 7001 through 7015 on Days 24, 26, 28 and 30 to insure survival. Four animals in Group VII were continued on study for four weeks after the Week 4 necropsy. These rabbits were fed ad libitum from Day 31 to the end of the study.

H. Test Substance Administration (cont.):

Duration and Dates of Treatment: 31 days (10 January to 9 February 1983)- up to 11/group- Groups I, II, III, and VII and 15/group- Groups IV, V and VI. 64 days (10 January to 14 March 1983)- up to 4/group- Groups I, II, III and VII.

I. Observations:

For Mortality and Gross Signs of Toxicologic or Pharmacologic Effects: Twice daily, AM and PM - all animals. Estimates of fecal quantity were made daily.

Detailed Physical Examinations for Signs of Local or Systemic Toxicity and Pharmacologic Effects: Weekly - all animals.

Dermal: (Methodology and References, Appendix A) Recorded pretest and 3x/week, just prior to the first, third and fifth dose of each week - Groups I, III, IV, V and VI.

J. Body Weight: (Methodology and References, Appendix A) Three times pretest and Days 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, 24, 26, 29, 31, 33, 36, 38, 40, 43, 45, 47, 50, 52, 54, 57, 59 and 61.

K. Food Consumption: (Methodology and References, Appendix A) Groups I - VI: Pretest and at the same intervals that the body weight was recorded. Group VII: Fasted from Day 1 to 30; food consumption was recorded from Day 31 to the end of the study.

L. Water Consumption: (Methodology and References, Appendix A) Twice a week, calculated on a 24-hour/day basis - all animals.

M. Laboratory Studies: (Methodology and References, Appendix A) Blood was obtained from unanesthetized rabbits via the medial ear vein. Animals were fasted overnight (approximately 16 hours) prior to blood collections.

M. Laboratory Studies (cont.):

Parameters Evaluated

Time Interval and Number of Animals

Hematology:

- hemoglobin
- hematocrit
- erythrocyte count
- platelet count
- total and differential leukocytes
- erythrocyte morphology

Pretest (6/7 January 1983) - all animals.
 Termination, Days 29 - 32 (7 - 10 February 1983) - up to 11/Group, Groups I, II, III and VII; 15/Group, Groups IV - VI.
 Extension, Days 64/65 (14/15 March 1983) - all survivors.

Clinical Chemistry:

- serum glutamic oxaloacetic transaminase
- serum glutamic pyruvic transaminase
- blood urea nitrogen
- fasting glucose
- total protein
- albumin
- globulin (calculated)
- triglycerides
- cholesterol
- sodium
- potassium
- chloride

Pretest (6/7 January 1983) - all animals.
 Termination, Days 29 - 32 (7 - 10 February 1983) - up to 11/Group, Groups I, II, III and VII; 15/Group, Groups IV - VI.
 Extension, Days 64/65 (14/15 March 1983) - all survivors.

Bleeding time was recorded.

Additional blood samples (approximately 25 ml) were collected from animals at the time of the terminal and extension necropsies. The serum was separated, frozen and retained at Bio/dynamics for possible additional analysis by the sponsor.

N. Postmortem:

Complete gross postmortem examinations were performed on all animals in the study. The gross postmortem examinations included examinations of the external surface; all orifices; the cranial cavity; carcass; the external surface of the brain and spinal cord, the thoracic abdominal and pelvic cavities and their viscera; and the cervical tissues and organs.

Necropsy:

Termination, Days 29 - 32 (7 - 10 February 1983) - up to 11/Group, Groups I, II, III and VII; 15/Group, Groups IV - VI.
 Extension, Days 64/65 (14/15 March 1983) - all survivors.

II. Postmortem (cont.):

Sacrifice Method: I.V. overdose of sodium pentobarbital via the marginal ear vein.

Organs Weighed and Organ/Body and Organ/Brain Weight Ratios Calculated: All animals.

- | | |
|---------------|------------------|
| liver | kidneys* |
| brain | adrenals |
| testes* | seminal vesicles |
| epididymides* | prostate |
| pituitary | |

* left and right organs were weighed individually.

Tissues Preserved (All Animals)

- | | |
|-------------------------------------|------------------|
| liver | kidneys (2) |
| brain | adrenals (2) |
| testes (2) | seminal vesicles |
| epididymides (2) | prostate |
| pituitary | |
| treated skin (2 sections) | |
| all gross lesions and tissue masses | |

Preservations: Testes and epididymides - Bouin's solution. All tissues - 10% neutral buffered formalin.

Stain: Hematoxylin and Eosin.

Tissues Examined Histopathologically:

- | | |
|-------------------------------------|---------------------|
| treated skin (2 sections) | testes (both) |
| all gross lesions and tissue masses | epididymides (both) |

Slides of all tissues listed above were prepared and examined microscopically for all animals in the study by Larry J. Ackerman, V.M.D. of Experimental Pathology Laboratories, Herndon, Virginia.

O. Statistical Analysis: (Meth. and Ref., Appendix A)

Body weight, body weight change, food consumption, water consumption, hematology and clinical chemistry parameters and terminal organ and body weights and organ/body and organ/brain weight ratios were analyzed. Mean values of all dose groups were compared individually to each control group at each time interval. Statistically significant differences from control are indicated on mean tables in appendices.

III. RESULTS AND DISCUSSION:

A. Mortality (Appendix B):

Five animals receiving CMA-102 (Group III) died spontaneously during the course of the study. Three animals died during the first four weeks of treatment and two died during the extension period. The cause of death could not be determined from the results of the gross postmortem examinations performed on these animals.

All other animals survived the duration of the study.

B. Physical Observations (Appendix C):

During the first four weeks of the study, animals treated with sodium hydroxide (Group IV), hydrochloric acid (Group V), ultraviolet irradiation (Group VI) and fasted animals (Group VII) exhibited a slightly higher incidence and/or severity of staining in the ano-genital area than was observed in the animals in either control group. Rabbits dosed with CMA-102 exhibited a higher incidence and/or severity of this finding from Day 15 to the end of the study.

CMA-102 (Group III) and fasted (Group VII) animals exhibited little or no sign of stool from Day 8 to the Week 4 necropsy. Stools were not present under the cages of many rabbits in other treatment groups at termination; however, this was due to fasting for necropsy.

Four CMA-102 animals were described as being slightly or moderately emaciated on Day 15 of the study. Emaciation was observed in two CMA-102 and six fasted rabbits at Week 4.

Necrosis of the dorsal surface was evident in most of the sodium hydroxide treated rabbits (Group IV) from Day 5 through termination and in a few of the hydrochloric acid treated rabbits (Group V) from Day 15 through termination (see Table 1, page 13). Necrosis ranged in size from 0.5 to 17.0 x 5.0 in the Group IV animals and from 0.5 to 10.0 x 2.5 in the Group V

III. RESULTS AND DISCUSSION (cont.):

B. Physical Observations (cont.):

Table 1: Necrosis of the Dorsal Surface - Groups IV and V
(% Area Involved)

Animal Number	Day													TERM
	0	1	3	5	8	10	12	15	17	19	22	24	26	
Group IV - 2% (w/v) NaOH														
4001	0	0	0	1	1	1	1	1	2	1	4	1	1	1
4002	0	0	0	1	1	1	1	1	1	1	1	1	1	1
4003	0	0	0	1	1	1	1	1	2	1	1	2	0	0
4004	0	0	0	1	1	1	1	2	2	1	4	4	4	4
4005	0	0	0	1	1	1	1	1	2	2	4	2	1	1
4006	0	0	0	1	1	1	1	2	1	1	1	2	1	1
4007	0	0	0	0	4	1	1	1	1	1	1	2	2	1
4008	0	0	0	1	1	1	1	1	2	1	1	1	1	1
4009	0	0	0	1	1	1	1	2	2	1	2	1	2	2
4010	0	0	0	0	1	1	1	1	1	1	2	1	1	1
4011	0	0	0	1	1	1	1	1	1	1	1	1	1	1
4012	0	0	0	4	4	1	2	1	1	1	1	4	4	4
4013	0	0	0	4	1	1	1	2	2	2	2	1	1	1
4014	0	0	0	4	1	1	1	1	1	1	1	4	4	4
4015	0	0	0	1	1	1	1	1	1	1	1	1	1	1
Group V - 5% (v/v) HCl														
5001	0	0	0	0	0	0	0	1	1	1	1	1	1	2
5002	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5003	0	0	0	0	0	0	0	0	0	2	0	0	0	0
5004	0	0	0	0	0	0	0	0	2	2	2	2	2	2
5005	0	0	0	0	0	0	0	0	0	1	1	1	1	1
5006	0	0	0	0	0	0	0	0	0	1	1	1	1	1
5007	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5008	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5009	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5010	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5011	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5012	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5013	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5014	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5015	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Key:	%	Code
		0
1 -	25	1
26 -	50	2
51 -	75	3
76 -	100	4

III. RESULTS AND DISCUSSION (CONT.):

B. Physical Observations (cont.):

animals. Necrosis was not evident in any other group in the study.

During the four-week extension period, the CMA-102 rabbits exhibited a higher incidence and/or severity of staining in the ano-genital area and emaciation than did either control group. Two fasted animals were slightly emaciated on Day 36 of the study.

C. Dermal Responses (Appendix D):

Erythema, generally slight to moderate, was observed in Groups I (vehicle control), III (CMA-102), IV (sodium hydroxide), V (hydrochloric acid) and VI (ultraviolet irradiation) during the first four weeks of the study. A few instances of extreme erythema were evident in the rabbits receiving CMA-102 and ultraviolet exposure during this interval. Slight erythema was evident in the vehicle control animals while slight to extreme erythema was noted in the CMA-102 rabbits throughout the extension period.

Edema was present in several Group I animals during Week 4, in several Group IV animals during the first two weeks of the study and in one Group V rabbit during Week 1. Most of the animals in Group VI exhibited edema at least once during treatment. Edema was not evident in Group I or III during the four-week extension period.

Slight to moderate atonia became evident in the vehicle control (Group I) animals during the second two weeks of treatment and throughout the extension period. The incidence and severity of atonia was most marked in the rabbits treated with CMA-102 from Day 3 through the end of the study. This finding was slight to moderate in most of the CMA-102 rabbits during the first week and generally moderate to extreme in most to all animals thereafter. Atonia was generally slight to moderate in the rabbits in Groups IV and VI from Day 10

III. RESULTS AND DISCUSSION (CONT.):

C. Dermal Responses (cont.):

through termination. Only a few instances of slight atonia were evident in the Group V rabbits during this period.

Slight to moderate desquamation was present in approximately one-third of the vehicle control (Group I) animals from Day 15 through 26 while slight desquamation was generally evident in most of these animals during the extension period. Most of the CMA-102 (Group III) treated animals exhibited slight to moderate desquamation during the first two weeks which progressed to moderate to extreme in all animals during the next two weeks and throughout the four-week extension period. Slight to extreme, but generally slight to moderate, desquamation was also evident in most of the rabbits treated with sodium hydroxide (Group IV) and U.V.B. (Group VI) from Week 1 through termination; this finding was generally slight in the animals treated with hydrochloric acid (Group V) over this interval.

Slight to moderate fissuring was present in many CMA-102 animals from Day 8 to the termination of the first four weeks of the study. Fissuring progressed to moderate and extreme in most of these animals throughout the extension period. Fissuring was not evident in any of the rabbits in any other treatment group.

Eschar formation was evident in most of the rabbits in Group V from Day 8 through termination. There were only sporadic instances of this finding in the rabbits in Groups III and IV.

Exfoliation occurred on six CMA-102 rabbits on Days 15 and 17 and on three CMA-102 rabbits during the extension period. Exfoliation was also evident to a lesser extent in the rabbits in Groups IV and V during the second two weeks of dosing and in one Group VI rabbit at termination.

III. RESULTS AND DISCUSSION (CONT.):

D. Body Weight (Appendix E and Figures 1, 2, 3 and 4):

Body weights of the vehicle control rabbits (CMA-101, Group I) were lower (approximately 5 to 16%) than those of the untreated control rabbits (Group II) throughout the study. Body weights of the rabbits dosed with sodium hydroxide, hydrochloric acid and ultraviolet irradiation (Groups IV, V and VI, respectively) were greater than (up to 10%) those of the vehicle control and slightly less than (up to 6%) those of the untreated control rabbits throughout the study. An effect on body weight during the first four weeks of the study was observed in the vehicle control group (CMA-101).

Animals in Group VII were fasted from Day 1 through Day 30 of the study. As would be expected, they exhibited drastic reductions (up to 27%) in body weight during this time. These animals were fed ad libitum from Day 31 to 64 and progressively gained in body weight during this interval. During the last week of the study, their body weights were equal to or slightly greater than those of the vehicle control rabbits.

Rabbits dosed with CMA-102 also exhibited drastic reductions in body weight which paralleled those of the fasted rabbits during the first 15 days of treatment. These animals had two periods of body weight gain, from Day 15 through 29 and from Day 50 to 54 (Figures 1 & 2). Animals in Group III (CMA-102) had body weight decrements of 5 to 26% (Day 5 to 61) when compared to the CMA-101 (Group I) rabbits and 7 to 33% (Day 3 to 61) when compared to the untreated control rabbits (Group II).

E. Food Consumption (Appendix E and Figures 5 and 6):

Food consumption of the rabbits receiving CMA-102 (Group III) was generally statistically significantly lower (approximately half) than that of the control groups during the first two weeks of test substance administration.

III. RESULTS AND DISCUSSION (CONT.):

E. Food Consumption (cont.):

From Day 19 to Day 26, food consumption of these animals was generally comparable to or greater than that of the CMA-101 animals. Food consumption of the CMA-102 animals at Day 33 (2/4 animals) and 36 (2/3 animals) was reduced compared to the control groups. In general, body weight gain or loss of the CMA-102 animals paralleled food consumption.

Fasted animals were given access to food ad libitum from Day 31 through termination. Their food consumption was greater than that of the controls from Days 33 to 43 and approximately comparable thereafter. Food consumption of the untreated control rabbits was generally statistically significantly lower than that of the vehicle control animals throughout the extension period.

Food consumption values of all other rabbits were considered to be comparable to control values.

F. Water Consumption (Appendix F):

Water consumption of the fasted rabbits was generally somewhat less than both control groups up to Day 24.

Although sporadic fluctuations existed, water consumption of the rabbits in all other treatment groups was generally comparable to control values at all intervals and no clear evidence of treatment-related effects was evident from this data.

G. Clinical Laboratory Studies (Appendices G, H and I):

1. Hematology (Appendices G and H):

Hemoglobin, hematocrit and erythrocyte counts of the rabbits treated with CMA-102 and of the fasted rabbits were statistically significantly lower than control values after four weeks of test substance administration. Hemoglobin values of the CMA-102 animals remained lower than either control group at the end of the extension period.

III. RESULTS AND DISCUSSION (CONT.):

G. Clinical Laboratory Studies (cont.):

1. Hematology (cont.):

Evaluation of the differential leukocyte counts revealed that nearly all rabbits receiving CMA-102 (Group III) and fasted (Group VII) rabbits displayed one or more of the following findings: anisocytosis, poikilocytosis, hypochromia and/or polychromia after four weeks on test. These findings were seen in only one or two control animals at this interval. Similar findings were also seen after the four-week extension period in the vehicle control group (Group I, CMA-101) as well as in the CMA-102 and fasted rabbits with approximately equal incidence and severity. These findings were not seen in any of the untreated control animals (Group II) at this interval. Animals treated with CMA-102 exhibited a shift in mature lymphocytes to segmented neutrophils after four weeks of treatment as well as at the end of the extension period.

Other statistically significant differences existed in some of the hematologic parameters evaluated; however, these differences were considered to be either within normal physiological limits or not of toxicologic significance.

2. Clinical Chemistry (Appendix I):

Rabbits dosed with CMA-102 exhibited elevated triglyceride levels after four weeks of treatment as well as elevated blood urea nitrogen and cholesterol levels after the four week extension period.

Cholesterol and triglyceride levels were elevated in the fasted rabbits while total protein, albumin, globulin and blood urea nitrogen levels were depressed at Week 4.

Other statistically significant differences existed in some of the clinical chemistry parameters evaluated; however, these differences were considered to be either within normal physiological limits or not of toxicologic significance.

III. RESULTS AND DISCUSSION (CONT.):

H. Terminal Organ and Body Weights and Organ/Body and Organ/Brain Weight Ratios (Appendix J):

After four weeks of dosing, the following organ weight effects were evident in the rabbits treated with CMA-102 (Group III) when compared to the vehicle control group (CMA-101, Group I): lower absolute weights of the epididymides (approximately 15%), testes (20 - 25%) and the prostate (22%) and higher absolute kidney weights (approximately 17%). These changes in absolute organ weights were also accompanied by concomitant changes in relative (organ/body and organ/brain) weights. Therefore, they were considered to be indicative of treatment-related effects. However, when compared to the absolute and relative weights of these organs in the untreated control group (Group II), it became evident that the the same reductions (epididymides, testes and prostate) were seen, but to a lesser extent, in the vehicle control group (Group I). It was therefore concluded that the vehicle control (CMA-101) and/or the physical manipulations involved in dosing the vehicle control group was somewhat influential in these reproductive organ weight effects (see Table 2, page 20).

After nine weeks of test substance administration, following the extension period, the treatment-related effects on reproductive organ weights became more pronounced in the CMA-102 treated animals when compared to the vehicle control group as evidenced by the following reductions in absolute and relative weights: epididymides (26 - 48%), seminal vesicles (21%), testes (29 - 38%) and prostate (34%). Reductions in organ/body and organ/brain weight ratios accompanied these changes in absolute weights (see Table 3, page 21). Kidney weights (absolute: 17%, and relative) of the CMA-102 rabbits remained elevated at Week 9. Differences in these reproductive organ weights between the vehicle control and the untreated control animals at Week 9 were not as evident or as pronounced as they were at Week 4.

III. RESULTS AND DISCUSSION (cont.):

H. Terminal Organ and Body Weights and Organ/Body and Organ/Brain Weight Ratios (cont.):

Table 2: Absolute and Relative Adrenal, Combined Testes, Combined Epididymides, Prostate and Seminal Vesicle Weights and % Difference from Control Terminal

Test Substance:	<u>CMA-101</u>	<u>Untreated Control</u>	<u>CMA-102</u>	<u>NAOH</u>	<u>HCL</u>	<u>UVB</u>	<u>FASTED</u>
Absolute Adrenal Weight:	0.4452	0.3461	0.4735	0.3851	0.3446	0.3568	0.4116
% Difference from CMA-101 Control:	-	-22.3	+6.4	+42.0	-13.5	-19.9	-7.5
% Difference from Untreated Control:	+28.6	-	+36.8	+11.3	-0.4	+3.1	+18.9
O/BW Ratio* ($\times 10^4$):	1.51	1.00	1.73	1.19	1.05	1.06	1.75
Absolute Testes Weight:	4.5585	5.3015	3.5190	5.2298	4.5691	5.3851	1.2260
% Difference from CMA-101 Control:	-	+16.3	-22.8	+14.7	+0.2	+18.1	-73.1
% Difference from Untreated Control:	-14.0	-	-33.6	-1.4	-13.8	+1.6	-76.9
O/BW Ratio* ($\times 10^4$):	15.24	15.21	12.53	16.20	13.84	15.84	5.16
Absolute Epididymides Weight:	1.5764	2.0164	1.3345	1.7267	1.6756	1.9162	0.8525
% Difference from CMA-101 Control:	-	+27.9	-15.3	+9.5	+6.3	+21.6	-45.9
% Difference from Untreated Control:	-21.8	-	-33.8	-14.4	-16.9	-5.0	-57.7
O/BW Ratio* ($\times 10^4$):	5.29	5.83	4.78	5.33	5.06	5.67	3.64
Absolute Prostate Weight:	0.9205	1.2174	0.7137	1.1365	1.0433	1.2968	0.5239
% Difference from CMA-101 Control:	-	+32.3	-22.5	+23.5	+13.3	+40.9	-43.1
% Difference from Untreated Control:	-24.4	-	-41.4	-6.6	-14.3	+6.5	-57.0
O/BW Ratio* ($\times 10^4$):	3.01	3.51	2.59	3.50	3.17	3.87	2.22
Absolute Seminal Vesicle Weight:	0.1959	0.2340	0.1790	0.2082	0.2043	0.2467	0.1434
% Difference from CMA-101 Control:	-	+19.4	-8.6	+6.3	+4.3	+25.9	-26.8
% Difference from Untreated Control:	-16.3	-	-23.5	-11.0	-12.7	+5.4	-38.7
O/BW Ratio* ($\times 10^5$):	6.48	6.82	6.37	6.41	6.16	7.41	6.11

*O/BW = Organ/Body Weight Ratio.

III. RESULTS AND DISCUSSION (cont.):

H. Terminal Organ and Body Weights and Organ/Body and Organ/Brain Weight Ratios (cont.):

Table 3: Absolute and Relative Adrenal, Combined Testes, Combined Epididymides, Prostate and Seminal Vesicle Weights and % Difference from Control - Recovery

Test Substance:	<u>CMA-101</u>	<u>Control</u>	<u>CMA-102</u>	<u>FASTED</u>
Absolute Adrenal Weight:	0.463	0.371	0.492	0.335
% Difference from CMA-101 Control:	-	-19.9	+6.3	-27.6
% Difference from Untreated Control:	+24.8	-	+32.6	-9.7
O/BW Ratio* ($\times 10^4$):	1.40	1.01	1.86	0.96
Absolute Testes Weight:	5.105	5.583	3.388	2.935
% Difference from CMA-101 Control:	-	+9.4	-33.6	-42.5
% Difference from Untreated Control:	-8.6	-	-39.3	-47.4
O/BW Ratio* ($\times 10^4$):	15.26	14.93	12.75	8.46
Absolute Epididymides Weight:	1.9706	2.1029	1.2421	1.2849
% Difference from CMA-101 Control:	-	+6.7	-37.0	-34.8
% Difference from Untreated Control:	-6.3	-	-40.9	-38.9
O/BW Ratio* ($\times 10^4$):	5.89	5.67	4.71	3.72
Absolute Prostate Weight:	1.304	1.552	0.863	0.904
% Difference from CMA-101 Control:	-	+19.0	-33.8	-30.7
% Difference from Untreated Control:	-16.0	-	-44.4	-41.8
O/BW Ratio* ($\times 10^4$):	3.90	4.27	3.21	2.62
Absolute Seminal Vesicle Weight:	0.1717	0.2181	0.1354	0.2170
% Difference from CMA-101 Control:	-	+27.0	-21.1	+26.4
% Difference from Untreated Control:	-21.3	-	-37.9	-0.5
O/BW Ratio* ($\times 10^5$):	5.13	6.07	5.14	6.22

*O/BW = Organ/Body Weight Ratio.

III. RESULTS AND DISCUSSION (CONT.):

H. Terminal Organ and Body Weights and Organ/Body and Organ/Brain Weight Ratios (cont.):

Rabbits in Group VII were fasted from Day 1 to Day 30 of the study. Marked reductions were evident in the absolute and relative weights of the epididymides, seminal vesicles, testes, prostates, kidneys and livers of these animals killed after four weeks of test substance administration when compared to either control group. The absolute and/or relative weights of the epididymides, testes, prostates and kidneys of the Group VII animals remained markedly lower than either of the control groups at the end of the extension period, after having access to food ad libitum for 32 to 33 days and recovering from a body weight effect.

The absolute and relative pituitary weights of the Group III (CMA-102) and VII (fasted) animals were greater than those of the Group I (CMA-101) animals.

The absolute and relative adrenal weights of the vehicle control animals (Group I, CMA-101) and of the treated animals in all groups were slightly or statistically significantly greater than those of the untreated control rabbits (Group II) at Week 4. The absolute and relative adrenal weights of the CMA-101 and CMA-102 rabbits remained greater than those of the untreated control rabbits; while those of the fasted animals were found to be comparable at the end of the extension period. This was most probably a combination of handling and/or physical manipulations and a treatment-related effect.

I. Gross Postmortem Examinations and Histopathologic evaluations (Appendix K):

Gross postmortem examinations revealed that small testes and epididymides were most frequently observed in the animals in Group VII, followed by

III. RESULTS AND DISCUSSION (CONT.):

I. Gross Postmortem Examinations and Histopathologic evaluations (Appendix K):

Group III. Also, the prostate gland was observed to be small most frequently in the animals in Group VII, followed by Groups I and III. With regard to the treated skin, various abnormalities tabulated previously were observed grossly. Various combinations of these occurred most frequently in the animals in Groups IV and V, followed by Group III.

Other gross postmortem findings occurred sporadically; their relationship, if any, to the type of treatment to which the animals were subjected was not known.

Results of the histopathological evaluations of the tissues taken at the Week 4 necropsy revealed mild non-inflammatory changes present in the treated skins of the vehicle control (CMA-101) group and a few of the rabbits receiving U.V.B. irradiation. Slight to moderately severe changes were present in the treated skins of the rabbits receiving CMA-102, sodium hydroxide or hydrochloric acid. These changes were more severe in the sodium hydroxide and hydrochloric acid groups. No significant changes were observed in the skins of the untreated control and fasted groups of rabbits.

Testicular changes, characterized by a moderately severe diffuse tubular hypoplasia and aspermatogenesis, were present in two of the eleven rabbits treated with 25% CMA-102 (Group III). These two rabbits had slight and moderate hyperkeratosis and moderate epidermal hyperplasia of the treated skin. One of these two rabbits was noted grossly to be emaciated. The other nine rabbits in this group had normal appearing testes and epididymides even though the skin lesions were more severe than observed in the two rabbits with testicular

III. RESULTS AND DISCUSSION (CONT.):

I. Gross Postmortem Examinations and Histopathologic evaluations (cont.): changes. Several of the nine rabbits had skin lesions characterized by severe hyperkeratosis, moderate to severe dermal hyperplasia and varying degrees of suppurative inflammation of the epidermis.

The groups of rabbits treated with sodium hydroxide (Group IV) and hydrochloric acid (Group V) had the most severe skin lesions of all of the treated animals in this study. These skin lesions included ulceration, increased dermal connective tissue and varying degrees of suppurative inflammation of the epidermis or dermis. All fifteen rabbits treated with sodium hydroxide and all fifteen rabbits treated with hydrochloric acid had normal appearing testes and epididymides and normal degrees of spermatogenesis.

In the group of rabbits fasted to achieve a 25% weight loss (Group VII), nine of the eleven rabbits had moderately severe diffuse tubular hypoplasia and aspermatogenesis. The remaining two rabbits had normal testes and epididymides but a reduction in the degree of spermatogenesis. Examination of ten of eleven skins from the rabbits in this group showed normal skins in nine of ten rabbits.

The eleven rabbits in each of the control groups and fourteen of fifteen rabbits exposed to U.V.B. irradiation had normal testes and epididymides with an active and normal spermatogenesis.

Skin treatment with sodium hydroxide and hydrochloric acid resulted in marked skin lesions but no testicular changes nor any effect on spermatogenesis, indicating that stress from severe skin lesions did not effect the spermatogenic cycle of these rabbits in this study. These animals did not exhibit a body weight loss. This same conclusion pertains to the effect of U.V.B. irradiation on rabbits.

III. RESULTS AND DISCUSSION (CONT.):

1. Gross Postmortem Examinations and Histopathologic evaluations (cont.):

Results of the histopathological evaluations of the tissues taken at the extension necropsy revealed testicular changes, characterized by a moderate multifocal or diffuse seminiferous tubular hypoplasia and reduced spermatogenesis, present in three of four rabbits treated with 25% CMA-102 (Group III). The testes and epididymides of the other rabbit in this group were normal with an active and normal spermatogenesis. All four rabbits in this group had moderate to moderately severe hyperkeratosis, moderate epidermal hyperplasia and slight suppurative inflammation of the treated skin. Three of the four rabbits with testicular changes were considered to be emaciated by gross examination and exhibited a severe body weight loss.

All four rabbits in the group which was initially fasted to achieve a 25% weight loss (Group VII) and then given food ad libitum during the four-week extension period had microscopically normal testes and epididymides. The degree of spermatogenesis appeared to be normal in one rabbit and only slightly reduced in the other three rabbits in this group.

III. RESULTS AND DISCUSSION (CONT.):

1. Gross Postmortem Examinations and Histopathologic evaluations (cont.):

In conclusion, the results of these evaluations:

1.) indicate that fasting of rabbits to achieve a weight loss of approximately 25%, relative to their initial body weight on Day 1 of the study, was effective in disrupting the spermatogenic cycle of the rabbit and caused a diffuse seminiferous tubular hypoplasia or atrophy of the testes in nine of eleven rabbits with reduced spermatogenesis in the other two rabbits. The application of 25% CMA-102 to the skin of eleven rabbits resulted in similar testicular changes in two rabbits, both of which exhibited the most severe body weight loss among the eleven rabbits.

2.) have demonstrated that total or partial food deprivation and body weight loss during the study had significant effects on the testes and degree of spermatogenesis of the rabbit and that stress related to severe skin lesions alone appeared to have little effect on spermatogenesis.

and 3.) indicate that returning rabbits to a normal feeding regime resulted in a return to almost normal spermatogenesis, while continued treatment with 25% CMA-102 and the associated weight loss had an effect on the spermatogenic cycle of three of four rabbits which appeared to be proportional to the severe weight loss in these rabbits.

William J. Tierney 5/17/84
Date
William J. Tierney, Ph.D., D.A.B.T.
Study Director
Associate Director of Toxicology

Ira W. Daly 5/17/84
Date
Ira W. Daly, Ph.D., D.A.B.T.
Director of Toxicology

Figure 1: Graph of Mean Body Weights - Groups I to VII
First 4 weeks of Treatment

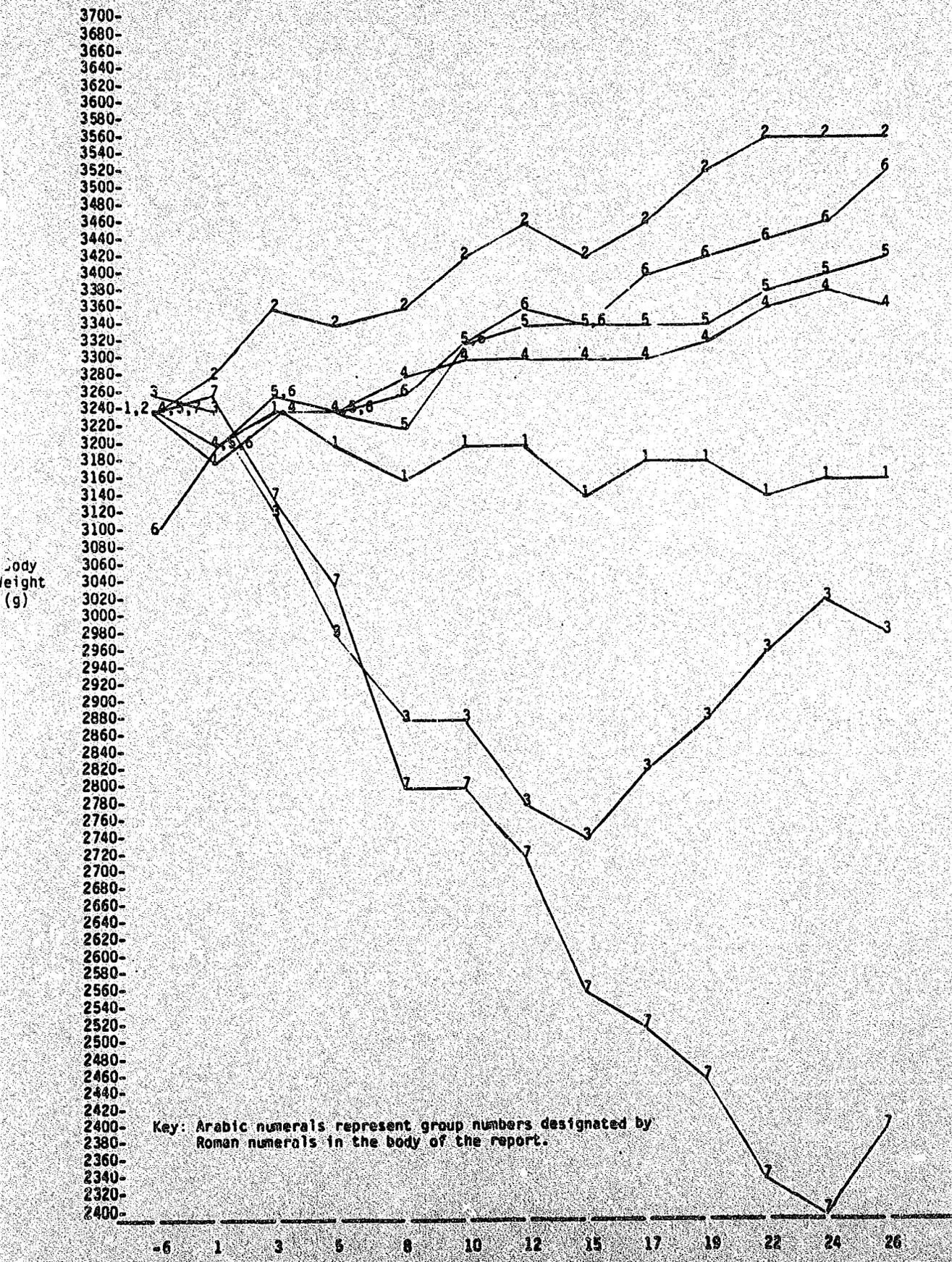


Figure 2: Graph of Mean Body Weights - Groups I, II, III and VII Extension Period

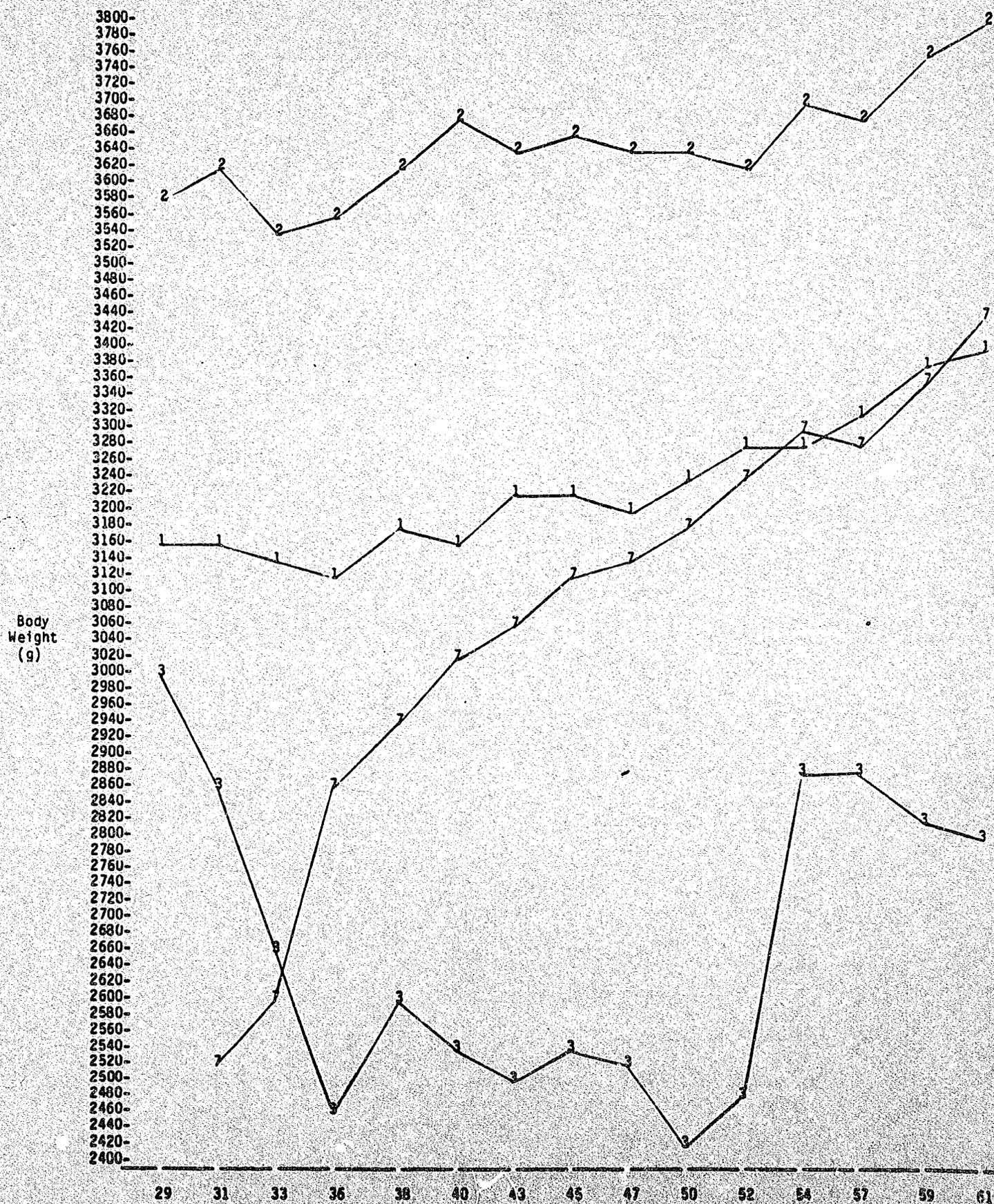
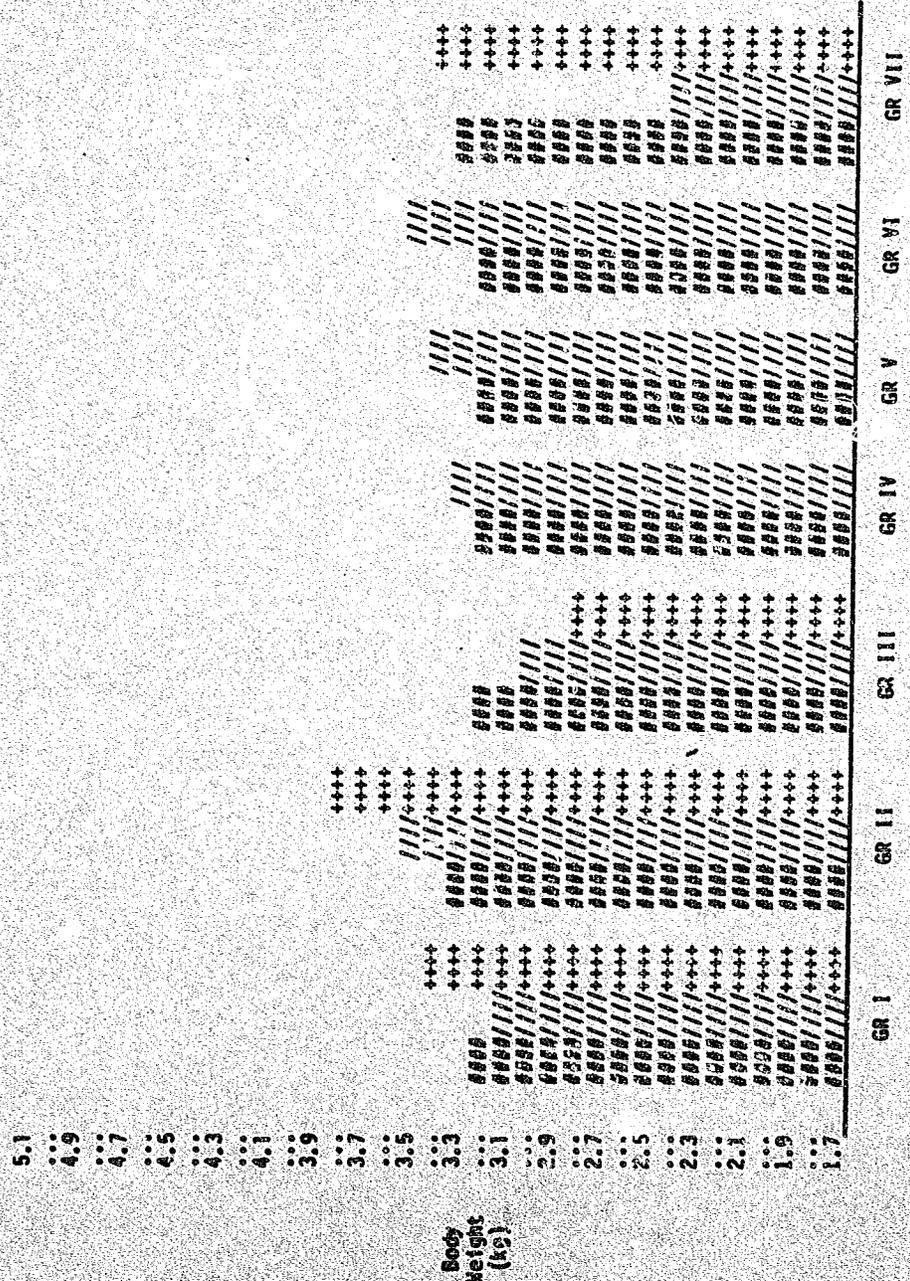
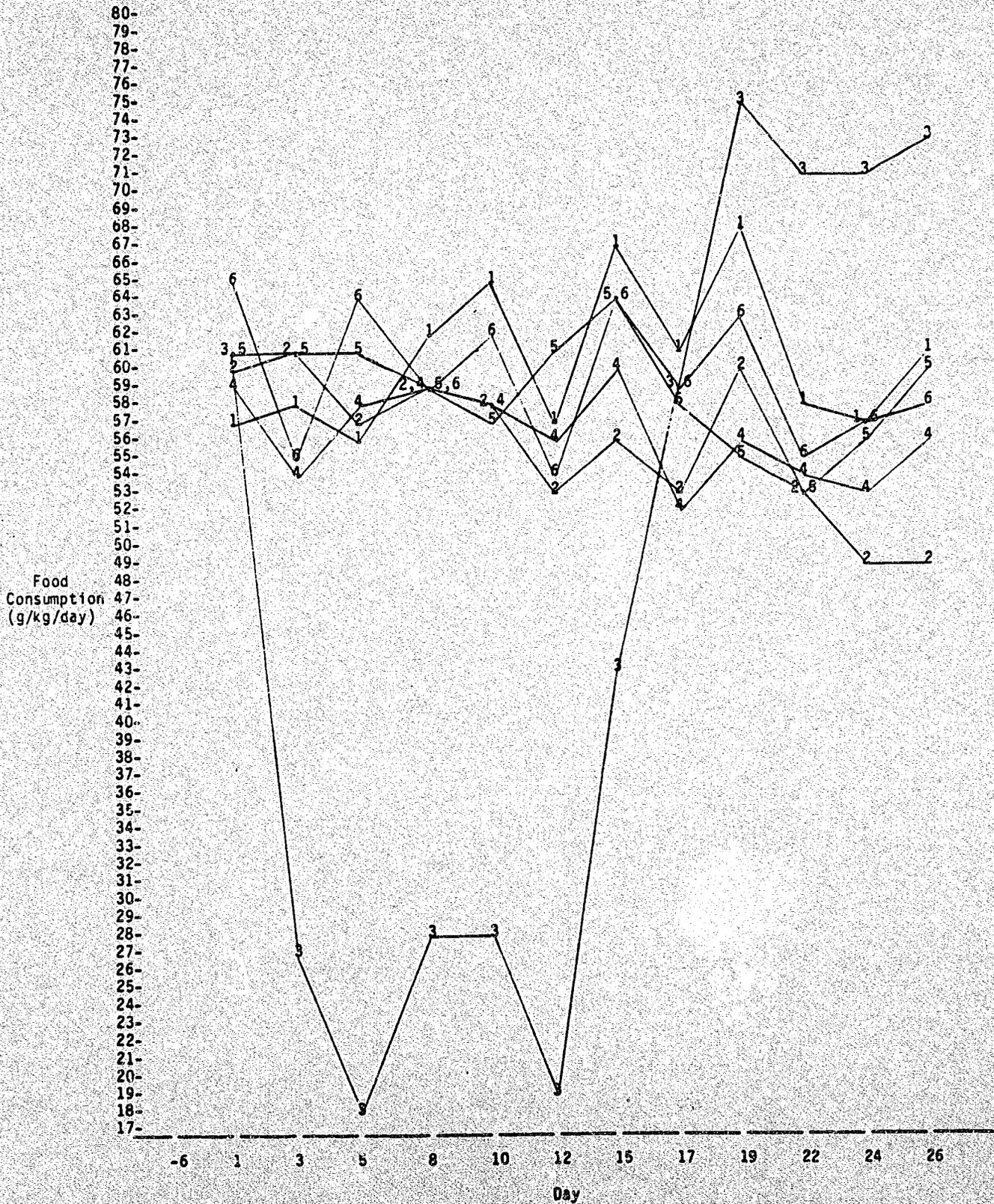


Figure 4: Graph of Mean Body Weight Data- Days 1, 26 and 61
Groups I - VII



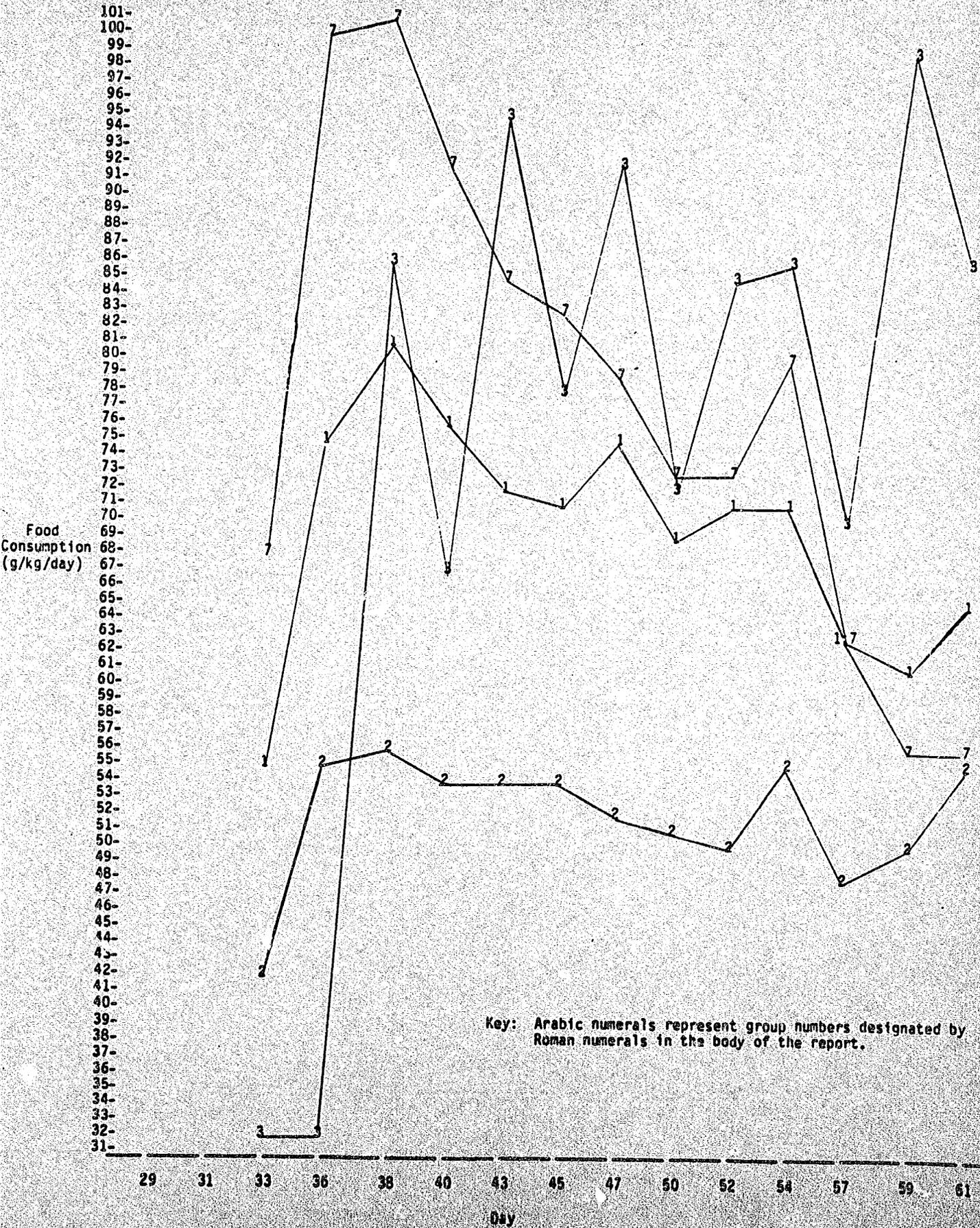
KEY: + = Day 1
/ = Day 26 (end of first 4 weeks of treatment)
+ = Day 61 (end of extension period)

Figure 5: Graph of Mean Food Consumption Groups I to VI
First 4 Weeks of Treatment



Key: Arabic numerals represent group numbers designated by Roman numerals in the body of the report.

Figure 6: Graph of Mean Food Consumption - Groups I, II, III and VII
Extension Period



APPENDIX 43