CODING FORMS FOR SRC INDEXING

Microfiche No.	
OTS0001255	

New Doc ID	Old Doc ID
FYI-OTS-0794-1255	84940000312

Date Produced	Date Received	TSCA Section
02/04/82	07/27/94	FYI

Submitting Organization
CHEM MFGS ASSN

Contractor
BIO/DYNAMICS INC

Document Title
INITIAL SUBMISSION: A TWENTY-ONE DAY DERMAL TOXICITY STUDY IN RATS WITH THREE ZINC DIALKYLDITHIOPHOSPHATES (CMA-102, 103, 104) AND A VEHICLE CONTROL (CMA-101), FINAL REPORT

Chemical Category
ZINC DIALKYLDITHIOPHOSPHATES

47-C

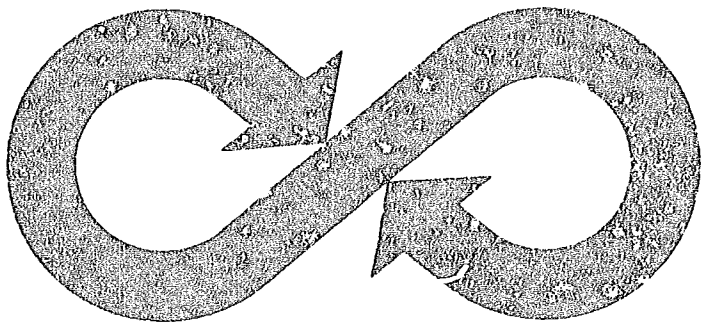
FYI-0794-1255

RECEIVED JUN 2 1982

Contains No CBI

FYI-91-001255
INIT 07/27/94

84940000312



Bio/dynamics Inc.

Division of Biology and Safety Evaluation

94 JUL 27 P12:44
RECEIVED

PROJECT NO. 81-2559

A TWENTY-ONE DAY DERMAL TOXICITY STUDY IN RATS WITH THREE ZINC DIALKYLDITHIOPHOSPHATES AND A VEHICLE CONTROL

Final Report

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

Date: February 4, 1982

Bio/dynamics Inc.

PROJECT NO.: 81-2559

A TWENTY-ONE DAY DERMAL TOXICITY STUDY IN RATS WITH THREE ZINC DIALKYLDITHIOPHOSPHATES AND A VEHICLE CONTROL

ABSTRACT

A total of 150 Sprague-Dawley CD® rats from two age groups (75 rats/age) were randomly distributed into five groups of 15 animals/age/group. Animals in Group I served as the vehicle control group (100% (w/v) CMA-101, 2 ml/kg). Animals in Groups II, III and IV were treated with test substances CMA-102, CMA-103 and CMA-104, respectively, diluted to 25% (w/v) concentration with CMA-101. Appropriate amounts of each of the test substance (CMA-102, CMA-103 and CMA-104) were dissolved in CMA-101 to yield concentrations of 25% (w/v), Groups II, III and IV. Animals in Group V were not dosed and were killed on Day 1 for testicular zinc determinations. Each substance was applied to the clipped dorsal surface of 15 rats/age/group at a dose volume of 2 ml/kg, once a day, five days a week for three consecutive weeks. Up to 10 animals/age/group were randomly selected from those surviving at the end of the third week of the study and sacrificed at the beginning of Week 4. The remaining animals, 5/age/group, continued on study for an additional 4 week untreated recovery period and then were sacrificed. Physical observations, testicular palpations, body weight and rectal temperatures were recorded weekly during the study. Dermal observations were graded during the Baseline week and at the Week 4 terminal sacrifice or the Week 8 recovery sacrifice. Complete gross postmortem examinations were performed on all animals in the study. Histopathological evaluations were performed on the left testicles of all animals killed at the scheduled terminal and recovery sacrifices. Testicular zinc determinations were performed on all animals in the study.

One treated young rat (CMA-103) and three treated mature rats (1: CMA-102 and 2: CMA-104) either died spontaneously or were killed in a moribund condition during the course of the study. The incidence and severity of alopecia, yellow staining in the ano-genital area and emaciation was greater in the treated animals than was observed in the control animals during the three week treatment period. With the exception of alopecia, these effects were not evident in those animals remaining during the four week untreated recovery period. Young and mature animals treated with CMA-102, CMA-103 and CMA-104 displayed a greater incidence and severity of dermal responses (erythema, edema, atonia, desquamation, fissuring, eschar formation and exfoliation) when compared to control responses at the end of the treatment period. The responses of the younger animals were slightly greater than the mature animals during the period of test substance administration. These effects were either not evident or minimal at the end of the recovery period. Body weights of the young and mature animals receiving CMA-102, CMA-103 and CMA-104 were statistically significantly lower when compared to control values,

Revised: William J. Tierney

Date: 5/22/82

Abstract (cont.):

throughout the treatment period. Body weights of the treated animals remained lower than control animals throughout the recovery period. However, trends indicative of a recovery process were evident. The mature animals displayed a nearly complete recovery by the end of Week 7. Food consumption of the young and mature treated rats in all groups was generally lower than control rats during the first two weeks of the study. Rectal temperatures taken from all rats at all intervals were normal. The absolute and/or relative (organ/body weight ratios) weights of the adrenals of the young and mature treated rats were slightly or statistically significantly greater than control values at the end of the treatment period. Weights of the prostates and seminal vesicles (absolute and/or relative) of the treated young and mature rats were slightly or statistically significantly lower than the control rats at the termination of the treatment period. At the end of the recovery period, the absolute and relative weights of the prostates of the mature rats treated with CMA-102 and CMA-104 were statistically significantly lower than control values. Total testicular zinc of the young and mature rats was considered to be comparable to control values at the end of the treatment and recovery periods. The zinc concentration of the testes on a ug/g of wet tissue basis of the young and mature rats was statistically significantly greater than control values at the end of the treatment period. Testicular zinc concentration in the young animals remained only slightly elevated, while those of the mature animals were comparable to control values at the end of the recovery period. Dermal responses observed in-life were confirmed at the gross postmortem examinations. There were no other grossly visible morphologic abnormalities which were considered related to the administration of the test substances. Overall microscopic evaluations of the testes from the rats in this study did not demonstrate any test substance-induced changes in the control and treated young and mature rats.

TABLE OF CONTENTS

I. INTRODUCTION..... 1

II. MATERIALS AND METHODS

A. Test Substances..... 1
B. Vehicle..... 3
C. Test Animals..... 3
D. Selection and Group Assignment..... 4
E. Animal Identification..... 4
F. Experimental Outline..... 5
G. Husbandry..... 6
H. Test Substance Administration..... 6
I. Observations
Physical..... 8
Dermal..... 8
J. Body Weight..... 8
K. Food Consumption..... 8
L. Rectal Temperature..... 9
M. Postmortem..... 9
N. Statistical Analysis..... 10

III. RESULTS AND DISCUSSION

A. Mortality..... 11
B. Physical Observations..... 11
C. Dermal Responses..... 11
D. Water Analysis and Test Substance Analysis..... 12
E. Body Weight..... 12
F. Food Consumption..... 12
G. Rectal Temperature..... 12
H. Organ and Body Weights and Organ/Body Weight Ratios..... 14
I. Analysis of Testicular Zinc..... 14
J. Gross Postmortem Examinations and Histopathological Evaluations..... 15

TABLE OF CONTENTS (CONT.)

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. Water Analysis and Test Substance Analysis..... E-1
F. Body Weights
Mean Values..... F-1
Individual Values..... F-11
F. Body Weight Change
Mean Values..... F-5
Individual Values..... F-11
F. Food Consumption
Mean Values..... F-7
Individual Values..... F-11
G. Rectal Temperature
Mean Values..... G-1
Individual Values..... G-2
H. Organ and Body Weights and Organ/Body Weight Ratios
Mean Values..... H-1
Individual Values..... H-21
Unscheduled Terminal Body Weights, Left and Right Testis Weights/Ratios and Testicular Zinc Determinations..... H-93
I. Analysis of Testicular Zinc Concentration
Mean Values..... I-1
Individual Values..... I-6
I. Analysis of Total Testicular Zinc
Mean Values..... I-25
Individual Values..... I-30
I. Testicular Zinc Determinations..... I-48
J. Gross Postmortem Examinations and Histopathological Evaluations..... J-1
K. Personnel..... K-1
L. Quality Assurance Statement..... L-1
M. Protocol..... M-1

I. INTRODUCTION

This study, conducted for the Chemical Manufacturers Association, was designed to evaluate the toxicity, with emphasis on the gonadal toxicity, of three zinc dialkyldithiophosphates (CMA-102, CMA-103 and CMA-104) and a vehicle control (CMA-101), when applied topically to the clipped dorsal surface of 120 Sprague-Dawley CD® rats (approximately 6 and 12 weeks of age; 15 males/age/test substance) at a constant dose volume of 2 ml/kg, once a day, 5 days a week for three weeks. Animals remaining after the Week 4 terminal sacrifice (5/age/test substance) remained on study for an additional 4 week untreated recovery period to assess the reversibility of any effects. 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 08873. All raw data is stored in the Archives at this testing facility.

II. MATERIALS AND METHODS

A. Test Substances: CMA-102, CMA-103 and CMA-104
Three Zinc dialkyldithiophosphates

$$(RO)_2\ P\text{-}S\text{-}Zn\text{-}S\text{-}P\ (OR)_2$$

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

Substance: CMA-102
Zinc dialkyldithiophosphate
R = 2-ethylhexyl

Concentration: As supplied.

Description: Formulation liquid

Date Received: 3 April 1981 (Container 1)

Label Information: New Formulation Sample
CMA-102

A. Test Substances (cont.):

Sampling:	A 50 ml archival sample was retained in the archives of this testing facility. In addition, a sample of approximately 50 ml was taken upon receipt and after the termination of the study.
Storage:	Temperature monitored room (60°-85°F)
Substance:	CMA-103 Zinc dialkyldithiophosphate R = mixture of related alkyl analogs
Concentration:	As supplied.
Description:	Liquid
Date Received:	24 April 1981 (Containers 1 and 2)
Lot. No.:	FCA-6371
Label Information:	CMA-103 Lot # FCA-6371 % AI 100% Exp. date - 4/82 Gross wt. - 6.905 kg Storage condition 60° - 85°F Skin and eye irritant
Sampling:	A 50 ml archival sample was retained in the archives of this testing facility. In addition, a sample of approximately 50 ml was taken upon receipt and after the termination of the study.
Storage:	Temperature monitored room (60° - 85°F)
Substance:	CMA-104 Zinc dialkyldithiophosphate R = mixture of related alkyl analogs
Concentration:	As supplied.
Description:	Liquid
Date Received:	15 April 1981 (Container 1)
Label Information:	CMA-104
Sampling:	A 50 ml archival sample was retained in the archives of this testing facility. In addition, a sample of approximately 50 ml was taken upon receipt and after the termination of the study.
Storage:	Temperature monitored room (60° - 85°F)

B. Vehicle:

Substance:	CMA-101
Concentration:	As supplied.
Supplier:	Chemical Manufacturers Association 2501 M Street, N.W. Washington, D.C. 20037
Description:	Liquid - Mineral oil of the Solvent 150N type, Motor oil basestock.
Date Received:	23 April 1981 (Container 1)
Label Information:	CMA-101 SN 22654 EC
Sampling:	A 50 ml archival sample was retained in the archives of this testing facility. In addition, a sample of approximately 50 ml was taken upon receipt and after the termination of the study.
Storage:	Temperature monitored room (60° - 85°F)

C. Test Animals: Rats

Strain:	Sprague-Dawley CD®
Justification for Animal Selection:	Standard laboratory rat available in good supply.
Number of Animals:	
Purchased:	184 males total
Placed on Test:	120 males (15 animals/age/test substance)

93 males, 4 weeks of age, were purchased initially. These animals were acclimated at Bio/dynamics, Inc. until they were 12 weeks of age. An additional 91 males, 4 weeks of age, were purchased. These animals were quarantined and equilibrated in a separate room. After an equilibration period of approximately 2 weeks, animals considered healthy from this shipment were transferred to the animal room containing the first shipment of animals. Animals of both age ranges began treatment on the same day (Day 1 of dosing).

C. Test Animals (cont.):

Supplier:	Charles River Breeding Laboratories, Inc. Kingston, New York 12484
Date Received:	Young Animals - 5 May 1981 Mature Animals - 24 March 1981
Age at Receipt:	28 days

Age at Initiation of Treatment:	Young Animals	Mature Animals
	41 days	33 days

Body Weights at Initiation of Treatment (grams):

	Mean	Range
Young Animals:	148	121 - 162
Mature Animals:	362	324 - 420

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.

E. Animal Identification: Each rat was identified with a metal ear tag bearing its unique identification number prior to testing. If the tag was lost, it was replaced or the rat was toe-clipped for identification. 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 Outline:

Group[a]	Test Substance[b]	Dose Level % (w/v)	Number of Animals (Males)							
			Initial		Necropsy					
					Day 1 - Pretest Group V		Wk. 4[c] - Term.		Wk. 8 - Recovery	
			6 Wks.	12 Wks.	6 Wks.	12 Wks.	6 Wks.	12 Wks.	6 Wks.	12 Wks.
I	CMA-101 (Vehicle control)	100	15	15	-	-	10	10	5	5
II	CMA-102	25	15	15	-	-	10	9	5	5
III	CMA-103	25	15	15	-	-	9	10	5	5
IV	CMA-104	25	15	15	-	-	10	8	5	5
V	-	0	10	10	10	10	-	-	-	-

[a]Animals considered suitable for study on the basis of pretest physical examinations were randomly assigned to treatment groups (Groups I-IV). An additional group of animals (Group V, 10/age) were randomly selected (from those considered suitable for study) and killed on Day 1, prior to test substance administration. Complete gross postmortem examinations were performed on these animals and tissues preserved. Testicular zinc determinations were performed on these animals.

[b]The technical (formulated) grade of each test substance was diluted as supplied to 25% (w/v) in CMA-101. No correction for % active ingredient was made.

[c]Up to 10 animals/age/group were randomly selected from those surviving at the end of the third week of the study and sacrificed at the beginning of Week 4. The remaining animals, 5/age/group, continued on study for an additional 4-week untreated recovery period and then were killed.

G. Husbandry:

Acclimation Period:	Young Animals - 13 days (5-17 May 1981) Mature Animals - 55 days (24 March - 17 May 1981)
Housing:	Individually in elevated stainless steel wire mesh cages.
Food:	Certified Purina Rodent Chow®, # 5002, ad libitum. Fresh food presented weekly.
Analysis of Feed:	Three samples (approximately 100g each) were taken from each batch of feed and were stored frozen at this testing facility.
Water:	ad libitum. By automated water system (Elizabethtown Water Company).
Analysis of Water:	A sample of water from the animal room was analyzed prior to initiation of dosing (see Appendix E).
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 hour light/dark cycle Temperature and humidity monitored twice daily.
Temperature:	
Desired:	67° - 76° F
Actual:	68° - 76° F
Humidity:	
Desired:	Not specified
Actual:	20% - 77%

H. Test Substance Administration:

Preparation of Animals:	Prior to test substance application, an area equal to approximately 25% of the total body surface area was carefully and closely clipped (Oster Model A-2 Ang-ra Clipping Head) on the dorsal surface of all animals; extending from the suprascapula area to the hind quarters.
Route:	Topically to the dorsal surface.

H. Test Substance Administration (cont.):

Justification of Route of Administration: The dermal route is the expected route of human exposure.

Method: Appropriate amounts of each of the test substances were dissolved in a vehicle (CMA-101) to yield a concentration of 25% (w/v) at a dose volume of 2.0 ml/kg for Groups II, III and IV. Group I animals received 100% (w/v) CMA-101, at a dose volume of 2.0 ml/kg. The technical (formulated) grade of each test substance was diluted as supplied to 25% (w/v) in CMA-101. No correction for % active ingredient was made. Dosing was based on the most recent weekly body weight data. A volume of 2.0 ml/kg containing CMA -101 (Group I) or the appropriate amount of test substances in CMA-101 was applied to the clipped, unabraded dorsal surface, once daily, 5 days/week. The test substances were applied with a syringe and evenly distributed over the prescribed area with a glass stirring rod. A control untreated dorsal area was used as a visual comparison of normal skin while grading dermal reactions and/or a contralateral section when histopathological evaluations were required. In an attempt to preclude the possibility of applying the test substance to the control dorsal area, a paper towel folded to the approximate dimensions of 4.0 x 4.0 cm. was held over the left suprascapular dorsal surface (4.0 x 4.0 cm.) of all animals during the application of vehicle and test substances. The backs of all animals were gently wiped with paper towels approximately 6 hours after exposure to remove excessive test substance, if necessary.

Justification of Dose Level Selection: Doses were selected on the basis of a previous Dermal Irritation Screen conducted with these test substances (Bio/dynamics Project No. 81-2557).

Frequency: Once daily, 5 days per week: Monday through Friday, and up to the day prior to sacrifice excluding animals alive during the 4 week untreated recovery period.

Duration: 25 days (Animals received 17/18 doses)
(31 days - untreated recovery period)

Dates of Treatment: 18 May - 11 June 1981
(6 June - 8 July 1981 - untreated recovery period)

H. Test Substance Administration (cont.):

Sampling:

Homogeneity/Stability:

Duplicate 10 ml samples of the first weeks batches of dosing solutions were taken in triplicate from the top middle and bottom of each batch. Both sets of samples were stored at Bio/dynamics, Inc. at room temperature.

Analytical Confirmation of Dosing Solution Concentration:

Duplicate 10 ml samples were taken from each weekly batch of dosing solutions prepared. Both sets of samples were stored at Bio/dynamics, Inc. at room temperature.

I. Observations:

For Mortality and Gross Signs of Toxicologic or Pharmacologic Effects:	Twice daily, AM and PM - All animals.
Detailed Physical Examinations for Signs of Local or Systemic Toxicity, Pharmacologic Effects and Palpation of Testes:	Weekly - All animals.
Dermal:	(Methodology and References, Appendix A)
	Recorded pretest and the day of the scheduled necropsies - All animals.
J. Body Weight:	(Methodology and References, Appendix A)
	Twice pretest, weekly thereafter, and terminally - All animals.
K. Food Consumption:	(Methodology and References, Appendix A)
	Weekly during the study - All animals.

L. Rectal Temperature: (Methodology and References, Appendix A)

Three times pretest, weekly thereafter (on Friday), and terminally - All animals.

M. Postmortem: Complete gross postmortem examinations were performed on up to 10/age/test substance (randomly selected) at termination and on 5/age/test substance at the end of the 4 week recovery period as well as on all animals either dying spontaneously or killed in extremis during the course of the study.

The gross postmortem examinations included examination of the external surface, all orifices the cranial cavity, carcass, the external and cut surface of the brain and spinal cord, the thoracic, abdominal and pelvic cavities and their viscera, and the cervical tissues and organs.

Necropsy: Day 1 (18 May 1981), Group V (10/age) - Pretest sacrifice.
Day 25 and 26 (June 11 and 12, 1981) - Terminal sacrifice.
Day 52 (July 8, 1981) - Untreated Recovery sacrifice.

Number of Animals: Pretest, Group V (10/age).
Terminal, up to 10/age/test substance.
Recovery, 5/age/test substance.

Sacrifice Method: Animals were exsanguinated via the abdominal aorta under light ether anesthesia.

Organs Weighed and Organ/Body Weight Ratios Calculated: (at all intervals) (Methodology and References, Appendix A)

adrenals	kidneys
brain	liver
gonads	pituitary
testes	prostate
(weighed separately)	seminal vesicles
epididymides	thyroid with
(weighed separately)	parathyroid weighed post-fixation

Prior to weighing, all organs were carefully dissected and properly trimmed to remove fat and other contiguous tissue in a uniform manner. Organs were weighed as soon as possible after dissection to avoid drying.

M. Postmortem (cont.):

Organs, with the exception of testes, were not weighed from animals dying spontaneously or killed in extremis during the course of the study. The testes were weighed from any animals either dying spontaneously or killed in extremis during the course of the study (for zinc determinations) but were excluded from organ weight and organ/body weight ratio data.

Tissues Preserved:[1]

The following tissues were taken from all animals in the study and preserved for possible future histopathologic evaluations.

adrenal (2)
brain
gonads
 testes[2]
 epididymides
prostate
seminal vesicles
kidney (2)
liver
pituitary
skin (treated and untreated - control dorsal: 4.0 x 4.0 cm. left superscapular)
thyroid (with parathyroid)
all gross lesions, tissue masses and any grossly abnormal tissues

Preservative:	All tissues: 10% neutral buffered formalin.
Stain:	Hematoxylin and Eosin.
Tissue Examined Histopathologically:	Slides of the left testicles were prepared and examined microscopically for all animals killed at the scheduled necropsies (terminal and recovery). Slides were prepared and examined by Larry J. Ackerman, V.M.D. Experimental Pathology Laboratories, Inc., Herndon, Virginia.

N. Statistical Analysis: (Methodology and References, Appendix A)

Body weight, food consumption, terminal organ and body weights and organ/body weight ratios and testicular zinc levels were analyzed. Mean values of all dose groups were compared to control at each time interval. Statistically significant differences from control are indicated in appendices.

[1]Numbers in parentheses indicate number of organs/sections preserved.
[2]After weighing, the left testicle preserved in formalin. The right testicle of each animal was analyzed for zinc content by the Metabolic and Analytical Chemistry Division of Bio/dynamics, Inc.

III. RESULTS AND DISCUSSION:

A. Mortality (Appendix B):

One treated young rat (CMA-103) and three treated mature rats (1: CMA-102 and 2: CMA-104) either died spontaneously or were killed in a moribund condition during the course of the study. The cause of death could not be determined from the results of the gross postmortem examinations performed on these animals. There were no deaths in the control animals (CMA-101) and all other treated animals survived the duration of the study.

B. Physical Observations (Appendix C):

The incidence and severity of alopecia, yellow staining in the anogenital area and emaciation was greater in the treated animals than was observed in the control animals during the three week treatment period. With the exception of alopecia, these effects were not evident in those animals remaining during the four week untreated recovery period.

All other daily and weekly physical observations for signs of pharmacologic and toxicologic effects were of the type commonly seen in the laboratory rat and were not considered related to the administration of the test substances.

C. Dermal Responses (Appendix D):

Young and mature animals treated with CMA-102, CMA-103 and CMA-104 displayed a greater incidence and severity of dermal responses (erythema, edema, atonia, desquamation, fissuring, eschar formation and exfoliation) when compared to control responses at the end of the treatment period. The responses of the younger animals were slightly greater than the mature animals during the period of test substance administration.

These effects were either not evident or minimal at the end of the four week untreated recovery period.

III. RESULTS AND DISCUSSION (cont.):

D. Water Analysis and Analysis of Test Substances are presented in Appendix E.

E. Body Weight (Appendix F):

Body weights of the young and mature animals receiving CMA-102, CMA-103 and CMA-104 were statistically significantly lower when compared to control values throughout the treatment period. These body weight effects were evident by body weight decrements of approximately -19% (CMA-102), -21% (CMA-103) and -16% (CMA-104) in the young animals and decrements of -24% (CMA-102), -22% (CMA-103) and -20% (CMA-104) in the mature animals at the end of Week 3.

Body weights of the treated animals remained lower than control animals throughout the recovery period. However, trends indicative of a recovery process were evident by body weight decrements of approximately -13% (CMA-102), -14% (CMA-103) and -11% (CMA-104) in the young animals and decrements of -6% (CMA-102), -6% (CMA-103) and -4% (CMA-104) in the mature animals when compared to control values at the end of Week 7. The mature animals displayed a nearly complete recovery by this time (Figures 1 and 2).

F. Food Consumption (Appendix F):

Food consumption of the young and mature treated rats in all groups was generally lower than control rats during the first two weeks of the study and greater than control rats from Weeks 3 through 7.

G. Rectal Temperature (Appendix G):

Rectal temperatures taken from all animals at all intervals were within the normal physiological limits for rats and no indications of treatment-related effects were evident.

III. RESULTS AND DISCUSSION (cont.):

Figures 1 and 2: Graphs of Mean Body Weight

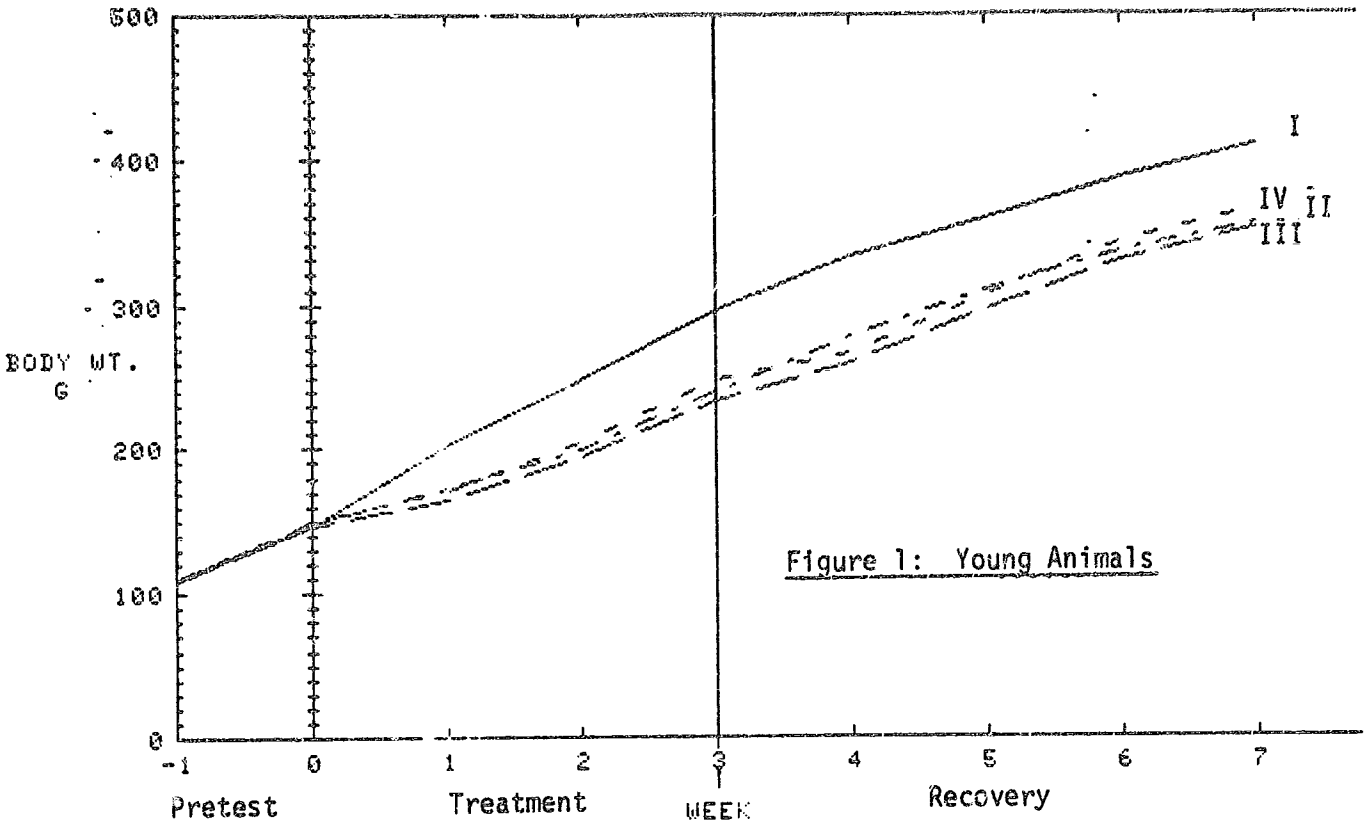


Figure 1: Young Animals

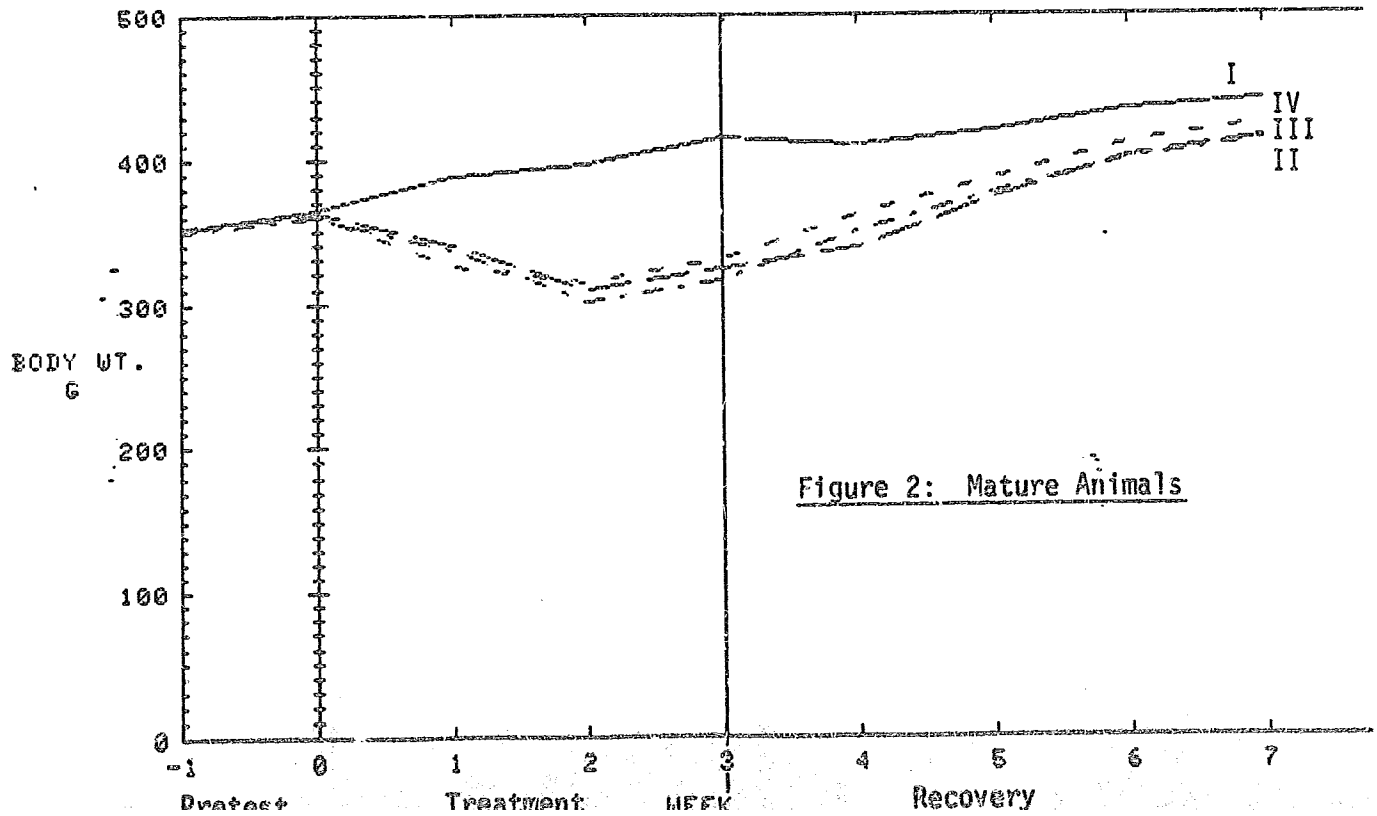


Figure 2: Mature Animals

Revised: William J. Kennedy

Date: 5/27/82

III. RESULTS AND DISCUSSION (cont.):

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

The absolute and/or relative (organ/body weight ratios) weights of the adrenals of the young and mature treated rats were slightly or statistically significantly greater than control values at the end of the treatment period. Weights of the prostates and seminal vesicles (absolute and/or relative) of the treated young and mature rats were slightly or statistically significantly lower than the control rats at the termination of the treatment period.

At the end of the four week untreated recovery period, the absolute and relative weights of the prostates of the mature rats treated with CMA-102 and CMA-104 were statistically significantly lower than control values.

Other slight or statistically significant differences existed in organ weights and organ/body weight ratios between the young and mature control and treated animals at the terminal and recovery intervals. However, these differences were considered to be either reflective of body weight effects or not of toxicologic significance.

I. Analysis of Testicular Zinc (Appendix I):

Total testicular zinc of the young and mature rats was considered to be comparable to control values at the end of the treatment and recovery periods. The zinc concentration of the testes on a ug/g of wet tissue basis of the young and mature rats was statistically significantly greater than control values at the end of the treatment period. Testicular zinc concentration in the young animals remained only slightly elevated, while those of the mature animals were comparable to control values at the end of the recovery period.

III. RESULTS AND DISCUSSION (cont.):

J. Gross Postmortem Examinations and Histopathologic Evaluations (Appendix J):

Dermal responses observed in-life were confirmed at the gross postmortem examinations. The testes of all animals were palpated prior to necropsy. Testes were found to be undescended in the following young animals: 1024 & 1025 & 1030 (CMA-101); 2029 (CMA-102); 3017 & 3023 & 3027 (CMA-103); 4016 & 4019 & 4020 & 4022 (CMA-104) at the terminal sacrifice and in 1019 (CMA-101) and 3020 (CMA-103) at the recovery sacrifice. Testes were found to be undescended in animal 4004 (CMA-104; mature) at the terminal sacrifice. There were no other grossly visible morphologic abnormalities which were considered related to the administration of the test substances.

Overall microscopic evaluations of the testes in this study did not demonstrate any test substance-induced changes in the young or mature rats treated with 100% (w/v) CMA-101, or 25% (w/v) CMA-102, CMA-103 and CMA-104 for a three week period.

William J. Tierney 2/10/82.

William J. Tierney, Ph.D. Date
Associate Director of
Toxicology and Pharmacology

Geoffrey K. Hogan 2/11/82

Geoffrey K. Hogan, Ph.D. Date
Vice President of Toxicology

A Twenty-One Day Dermal Toxicity Study
in Rats with Four Test Materials

Preface to Appendices

Age	Group	Test Substance	Dose Level % (w/v)	Animal Numbers
Young	I	CMA-101 (Vehicle control)	100	1016 - 1030
	II	CMA-102[a]	25	2016 - 2030
	III	CMA-103[a]	25	3016 - 3030
	IV	CMA-104[a]	25	4016 - 4030
	V	-	0	5011 - 5020
Mature	I	CMA-101 (Vehicle control)	100	1001 - 1015
	II	CMA-102[a]	25	2001 - 2015
	III	CMA-103[a]	25	3001 - 3015
	IV	CMA-104[a]	25	4001 - 4015
	V	-	0	5001 - 5010

NOTE: Statistical analysis is presented on mean tables only.
[a]Zinc dialkyldithiophosphates.

A

Appendix A
A Twenty-One Day Dermal Toxicity Study
in Rats with Four Test Materials

Methodology and References - General

Parameter	Reference or Description
Body Weight	INTEC Automatic Data Acquisition System.
Terminal Body Weight (TBW)	Weight taken just prior to necropsy.
Food Consumption	INTEC Automatic Data Acquisition System. Animals were presented with full feeders weighing 570 grams (includes weight of feed, jar and lid). After 7 days feeders were reweighed and resulting weight was subtracted from the full feeder weight. Resulting value = g/7 days (labeled G/INT on tables). $g/kg/day = \frac{g/7\ Days}{average\ body\ weight\ (kg)} \div 7\ Days.$ $Average\ BW = \frac{Previous\ BW + Current\ BW}{2}$
Rectal Temperatures	Diatek, Inc., 3910 Sorrento Valley Blvd., San Diego, Ca. Pretest temperatures were recorded in Farenheit, Model #500 CID; all other temperatures were recorded in Centigrade, Model #500 CIE, Friday of each week at approximately 1:30 pm. Prior to each weekly monitoring, the instrument was calibrated using a procedure provided by the manufacturer. A sanitary probe cover was placed on the probe. The probe was inserted into the rectum approximately 1/4 inch and when a tone was heard, a digital display was read and recorded. After each reading, the instrument was cleared and reset.
Organ Weights	Mettler AK 160 - All organs.