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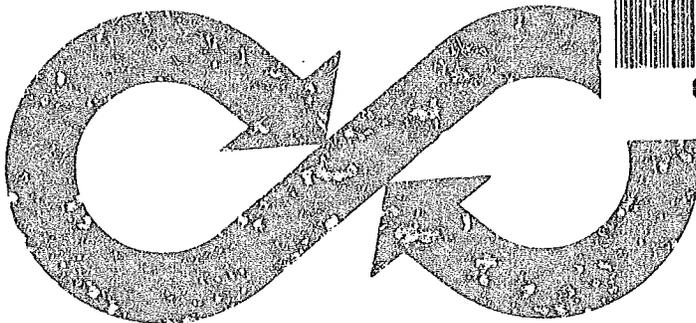
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Bio/dynamics Inc.

Division of Biology and Safety Evaluation

PROJECT NO. 81-2558

A TWENTY-ONE DAY DERMAL TOXICITY STUDY  
IN RABBITS WITH THREE ZINC DIALKYL DITHIOPHOSPHATES  
AND A VEHICLE CONTROL

Final Report

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

Date: February 4, 1982

PROJECT NO. 81-2558

A TWENTY-ONE DAY DERMAL TOXICITY STUDY  
IN RABBITS WITH THREE ZINC DIALKYLDITHIOPHOSPHATES  
AND A VEHICLE CONTROLABSTRACT

A total of 150 male New Zealand White rabbits of two age groups (75 rabbits/age) were randomly distributed into five groups of 15 animals/age/group (age defined by weight). 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 substances, CMA-102, CMA-103 and CMA-104, were dissolved in CMA-101 to yield concentrations of 25% (w/v) (Groups II, III and IV, respectively). Ten animals/age (Group V) were killed on Day 1 (untreated) and used for baseline testicular zinc determinations. Each substance was applied to the clipped dorsal surface of 15 rabbits/age/group at a dose volume of 2 ml/kg, once a day, five days a week for three weeks. Up to 10 rabbits/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. Where possible, the remaining animals, a maximum of 5/age/group, continued on study for an additional four week untreated recovery period. Physical observations, testicular palpations, body weights and rectal temperatures were performed 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. Histopathologic evaluations were performed on the left testicle of all animals killed at the scheduled terminal and recovery sacrifices. Testicular zinc determinations were performed on all animals in the study.

Fourteen treated young rabbits (8: CMA-102; 5: CMA-103 and 1: CMA-104) and twenty-two treated mature rabbits (6: CMA-102; 11: CMA-103 and 5: 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 young and mature animals than was observed in the control animals during the three week treatment period. This effect was still evident, to a lesser extent, at the end of the recovery period. The incidence and severity of dermal responses (erythema, edema, atonia, desquamation, fissuring, eschar formation and exfoliation) were greater in the young and mature animals receiving CMA-102, CMA-103 and CMA-104 when compared to control rabbits following the treatment period. These effects were somewhat more pronounced in the younger animals. Dermal responses of the treated young and mature rabbits were comparable to control following the recovery period. Body weights of the treated animals receiving CMA-102, CMA-103 and CMA-104 were slightly or statistically significantly lower than corresponding control values throughout the treatment period and became more severe over time. Although the body weights of the treated animals were either slightly or statistically significantly lower than control values at the

Abstract (cont.):

end of the recovery period, body weight decrements became less severe at each weekly interval. This was considered to be indicative of a recovery process. Young and mature rabbits treated with CMA-104 were considered to have recovered from this body weight effect by Week 7. Rectal temperatures taken from all animals at all intervals were normal. Marked reductions in the absolute and relative (organ/body and organ/brain weight ratios) weights of the testes were evident in the treated rabbits in all groups at the end of the treatment period. These effects were most severe in the younger animals receiving CMA-102 and CMA-103 and in the mature rabbits receiving CMA-103. The weights of the epididymides and prostates of the treated animals, particularly the younger rabbits, were slightly or statistically significantly lower than the control animals at the termination of dosing. The absolute and relative (organ/body and organ/brain) adrenal weights (young rabbits: CMA-103 and mature rabbits: CMA-102) were slightly or statistically significantly greater than the control animals at the end of the treatment and recovery periods. Marked reductions in all groups were still evident in the absolute and relative (organ/body and organ/brain) testes weights of the young and mature animals at the end of the recovery period. Reductions were also evident in the weights of the epididymides, prostates and seminal vesicles in all treated rabbits at the end of the recovery period. The zinc concentration of the testes of the young rabbits receiving CMA-102 and CMA-103 was approximately twice that of the control rabbits at the end of the treatment period. Slight to statistically significant elevations in the zinc concentration of the testes were also evident in the young animals treated with CMA-104 and in the mature rabbits in all groups at the end of the treatment period. The zinc concentration of the testes of the young and mature animals in all groups were comparable to control following the recovery period. Dermal responses observed in-life were confirmed at the gross postmortem examinations. The incidence of small testes subjectively observed at necropsy was greater, as compared to control animals, in the treated rabbits killed at the end of the treatment and recovery periods. Discoloration of the liver was observed in several of the treated animals. Other postmortem findings observed grossly occurred sporadically and were not considered to be related to the administration of the test substances. Test substance-related microscopic changes of the testes, consisting of diffuse tubular hypoplasia and aspermatogenesis, were present in many or all of the young and mature rabbits in Groups II, III and IV (CMA-102, CMA-103 and CMA-104, respectively). These changes did not appear to be completely reversed following the recovery period in the young and mature rabbits of these same groups.

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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:**

**Rabbits**

**Strain:** New Zealand White (SPF)

**Justification for  
Animal Selection:** Standard laboratory rabbit available in  
good supply.

**Number of Animals:**

**Purchased:** 180 males total

**Placed on Test:** 120 males (15 animals/age/test substance -  
animals were purchased by weight; weights  
at initiation of treatment were: 16.8 -  
24.9 kg for young animals and 30.4 - 38.3  
kg for mature animals).

90 males, approximately 2.0 kg in body  
weight, were purchased initially. These  
animals were acclimated at Bio/dynamics,  
Inc. until they were approximately 3.2 kg.  
Then an additional 90 males, approximately  
2.0 kg in body weight, were purchased.  
These animals were quarantined and  
equilibrated in a separate room. After an  
equilibration period of approximately 2

C. Test Animals (cont.):

weeks and a treatment period of approximately 3 weeks, young recovery animals were consolidated in the animal room containing the mature recovery animals. Animals of both weight-age ranges began treatment on the same day (Day 1 of dosing).

Supplier: Hare Rabbit for Research  
Marland Breeding Farms, Inc.  
P.O. Box X  
Hewitt, New Jersey 07421

Date Received: Young Animals - 29 April 1981  
Mature Animals - 25 March 1981

Weight at Receipt: Approximately 1.7 to 2.5 kg.

Body Weights at Initiation  
of Treatment (grams):

	Mean	Range
Young Animals:	2136.3	1679.2 - 2489.2
Mature Animals:	3358.3	3042.7 - 3826.5

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 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 Outline:

Group <sup>a</sup>	Substance <sup>b</sup>	Dose Level % (w/v)	Initial		Number of Animals (Males)					
			2.0 kg <sup>d</sup>	3.5 kg <sup>d</sup>	Day 1 - Pretest Group V		Necropsy		Wk. 8 - Recovery	
					2.0 kg	3.5 kg	Wk. 4 <sup>c</sup> - Term.	Wk. 8 - Recovery		
I	CMA-101 (vehicle control)	100	15	15	-	-	10	10	5	5
II	CMA-102	25	15	15	-	-	5	5	2	4
III	CMA-103	25	15	15	-	-	5	4	5	0
IV	CMA-104	25	15	15	-	-	9	5	5	5
V	-	0	10	10	10	10	-	-	-	-

<sup>a</sup>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-weight) 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.

<sup>b</sup>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.

<sup>c</sup>Up to 10 animals/age-weight/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. Where possible, the remaining animals, a maximum of 5/age-weight/group, continued on study for an additional 4 week untreated recovery period and then were killed. Any group with 4 or less survivors was terminated at Week 4 and no animals continued on study during the recovery period.

<sup>d</sup>Approximate weight class; animals were purchased by weight - weights at initiation of treatment were 16.8-24.9 kg for young animals and 30.4-38.3 kg for mature animals.

G. Husbandry:

Acclimation Period: Young Animals - 19 days (29 April-17 May 1981)  
Mature Animals - 53 days (26 March - 17 May 1981)

Housing: Individually in elevated stainless steel cages.

Food: Certified Purina Rabbit Chow®, # 5322, ad libitum.

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: 60° - 70° F  
Actual: 61° - 72° F

Humidity:  
Desired: Not specified  
Actual: 23% - 78%

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; approximately 10 cm. wide and extending from the suprascapula area to the hind quarters.

H. Test Substance Administration (cont.):

**Route:** Topically to the dorsal surface.

**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. 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 weakly 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 6.5 x 5.0 cm. was held over the left suprascapular dorsal surface (6.5 x 5.0 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. All rabbits were fitted with Elizabethan collars on Day 1 to prevent oral ingestion of the test substances.

**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. 81-2556).

**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:** 22 days (Animals received 15/16 doses). (30 days - untreated recovery period)

**Dates of Treatment:** 18 May - 8 June 1981  
(6 June - 7 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. Rectal Temperature:

(Methodology and References, Appendix A)

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



L. 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:<sup>1</sup>

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

- adrenal (2)
- brain
- gonads
  - testes<sup>2</sup>
  - epididymides
- prostate
- seminal vesicles
- kidney (2)
- liver
- pituitary
- skin (treated and untreated - control dorsal: 6.5 x 5.0 cm. left suprascapular)
- 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 testicle 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. at Experimental Pathology Laboratories, Inc., Herndon Virginia.

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

Body weight, 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.

<sup>1</sup>Numbers in parentheses indicate number of organs/sections preserved.

<sup>2</sup>After weighing, the left testicle was 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):

Fourteen treated young rabbits (8:CMA-102; 5:CMA-103 and 1:CMA-104) and twenty-two treated mature rabbits (6:CMA-102; 11:CMA-103 and 5: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 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 young and mature animals than was observed in the control animals during the three week treatment period. This effect was still evident, to a lesser extent, 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 rabbit and were not considered related to the administration of the test substances.

#### C. Dermal Responses (Appendix D):

The incidence and severity of dermal responses (erythema, edema, atonia, desquamation, fissuring, eschar formation and exfoliation) were greater in the young and mature animals receiving CMA-102, CMA-103 and CMA-104 when compared to control responses following the treatment period. These effects were somewhat more pronounced in the younger animals. Dermal responses of the treated animals, young and mature, were comparable to control following the four week untreated recovery period.

III. RESULTS AND DISCUSSION (cont.):

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

E. Body Weight (Appendix F):

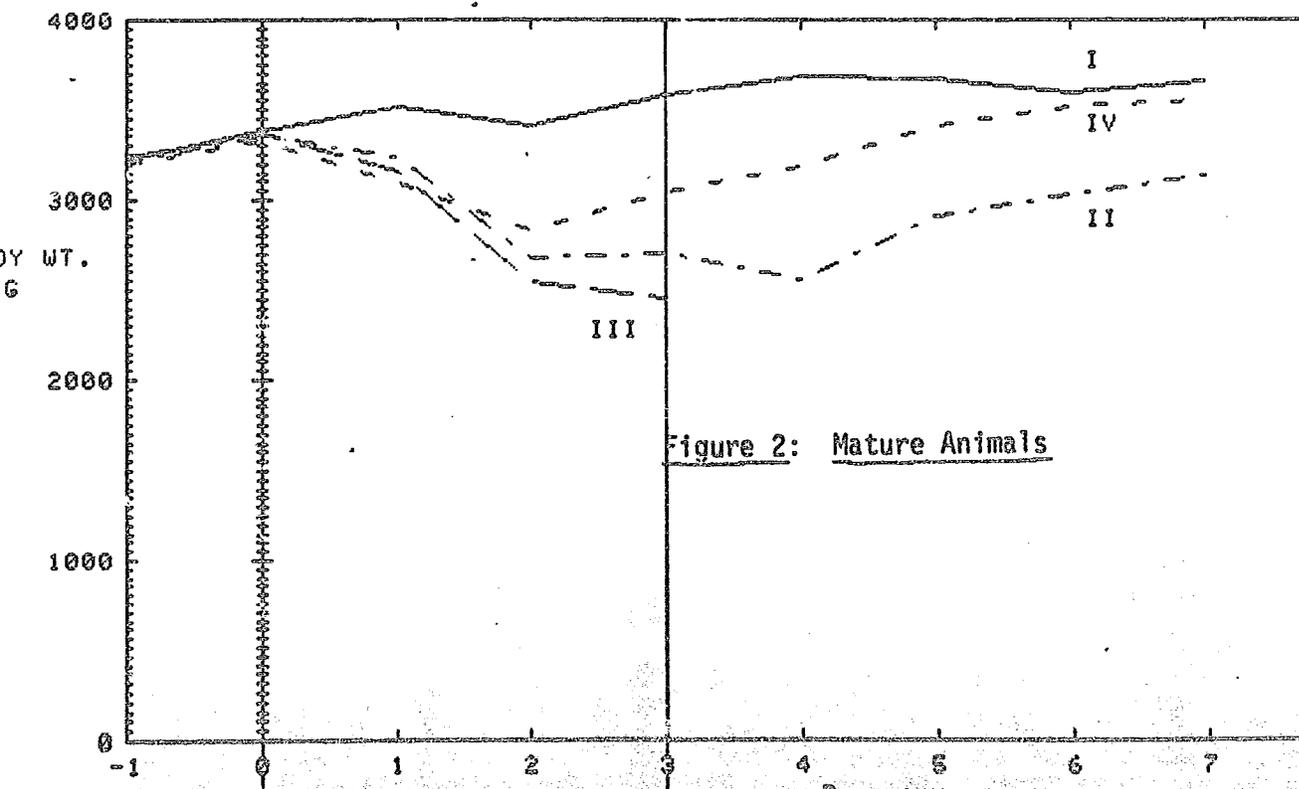
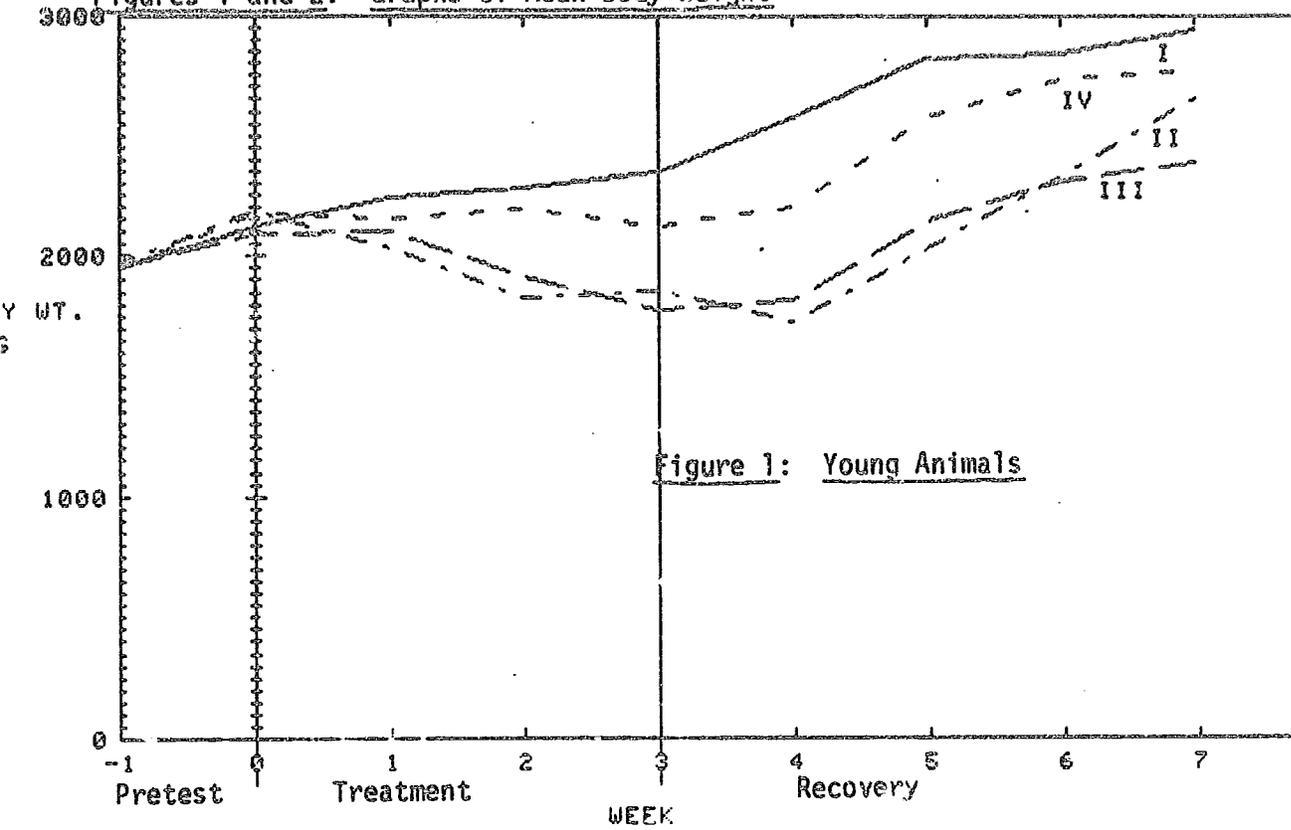
Body weights of the young and mature animals receiving CMA-102, CMA-103 and CMA-104 were slightly or statistically significantly lower than corresponding control values throughout the treatment period. These body weight effects became more severe over time and were evident in the young animals by body weight decrements of approximately -21% (CMA-102), -24% (CMA-103) and -10% (CMA-104) and in the mature animals by decrements of approximately -24% (CMA-102), -31% (CMA-103) and -15% (CMA-104) when compared to control values at the end of Week 3.

Although the body weights of the treated young and mature animals were either slightly or statistically significantly lower than control values at the end of the recovery period, body weight decrements became less severe at each weekly interval (Figures I and II). This was considered to be indicative of a recovery process. Young and mature rabbits treated with CMA-104 were considered to have recovered from this body weight effect by the end of the recovery period.

RESULTS AND DISCUSSION (cont.)

E. Body Weight (cont.):

Figures 1 and 2: Graphs of Mean Body Weight



### III. RESULTS AND DISCUSSION (cont.):

#### F. Rectal Temperature (Appendix G):

Rectal temperatures taken from all animals at all intervals were within the normal physiological limits for rabbits<sup>a</sup> and no indications of treatment-related effects were evident.

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

Marked reductions in the absolute and relative weights (organ/body weight ratios) of the testes were evident in the young and mature treated rabbits in all groups at the Week 4 sacrifice; Table 1:

Table 1: Absolute and Relative Adrenal, Combined Testes, Combined Epididymides, Prostate and Seminal Vesicle Weights and % Difference from Control - Terminal

Test Substance: Age Group:	CMA-101		CMA-102		CMA-103		CMA-104	
	Young	Mature	Young	Mature	Young	Mature	Young	Mature
Absolute Adrenal Weight:	0.193	0.259	0.193	0.365	0.221	0.268	0.119	0.276
% Difference from Control:	-	-	0	+40.9	+14.5	+3.5	+3.1	+6.6
O/BW Ratio* (x10 <sup>3</sup> ):	0.84	0.73	1.16	1.36	1.36	1.15	0.95	0.95
Absolute Testes Weight:	1.930	4.348	0.392	2.109	0.410	1.573	0.875	2.174
% Difference from Control:	-	-	-79.7	-51.5	-78.8	-63.8	-54.7	-50.0
O/BW Ratio* (x10 <sup>3</sup> ):	8.25	12.25	2.34	7.68	2.46	6.00	4.06	7.19
Absolute Epididymides Weight:	0.687	1.434	0.283	1.098	0.357	1.122	0.446	1.036
% Difference from Control:	-	-	-58.8	-23.4	-48.0	-21.8	-35.1	-27.8
O/BW Ratio* (x10 <sup>3</sup> ):	3.01	4.01	1.71	4.08	2.16	4.75	2.10	3.49
Absolute Prostate Weight:	0.480	0.887	0.201	0.498	0.361	0.587	0.225	0.479
% Difference from Control:	-	-	-58.1	-43.9	-24.8	-33.8	-53.1	-46.0
O/BW Ratio* (x10 <sup>3</sup> ):	2.14	2.49	1.17	1.88	2.42	2.47	1.07	1.65
Absolute Seminal Vesicle Weight:	0.120	0.208	0.142	0.196	0.116	0.132	0.110	0.128
% Difference from Control:	-	-	+18.3	-5.8	-3.3	-36.5	-8.3	-38.5
O/BW Ratio* (x10 <sup>4</sup> ):	5.07	5.94	8.31	7.36	7.45	5.64	5.27	4.39

\*O/BW = Organ/Body Weight Ratio.

<sup>a</sup>C. Kozma, et al in The Biology of the Laboratory Rabbit. ed. S.H. Weisbroth: Academic Press, p. 58, 1974.

## III. RESULTS AND DISCUSSION (cont.):

## G. Organ and Body Weights and Organ/Body Weight Ratios (cont.):

These effects were most severe in the younger animals receiving CMA-102 and CMA-103 (approximately equal in severity), and in the mature rabbits receiving CMA-103. The weights of the epididymides and prostates of the treated animals, especially the younger rabbits, were slightly or statistically significantly lower than the control animals at the termination of dosing. The absolute and relative (organ/body and organ/brain) adrenal weights (young animals: CMA-103 and mature animals: CMA-102) were slightly or statistically significantly greater than those of the control animals at the end of the treatment and recovery periods.

At the end of the four week untreated recovery period, marked reductions in all groups were still evident in the absolute and relative testes weights of the young and mature rabbits; Table 2:

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

Test Substance: Age Group:	CMA-101		CMA-102		CMA-103		CMA-104	
	Young	Mature	Young	Mature	Young	Mature	Young	Mature
Absolute Adrenal Weight:	0.269	0.383	0.243	0.478	0.282	-	0.305	0.370
% Difference from Control:	-	-	-9.7	-24.8	+4.8	-	+13.4	-3.4
O/BW Ratio* (x10 <sup>3</sup> ):	0.90	1.05	0.88	1.55	1.17	-	1.10	1.06
Absolute Testes Weight:	3.735	6.432	1.490	2.63	1.260	-	2.818	4.225
% Difference from Control:	-	-	-60.1	-59.0	-66.3	-	-24.6	-34.3
O/BW Ratio* (x10 <sup>3</sup> ):	12.42	17.44	5.34	8.43	5.03	-	10.20	11.71
Absolute Epididymides Weight:	1.404	2.101	0.707	1.074	0.653	-	1.167	1.592
% Difference from Control:	-	-	-49.6	-48.9	-53.5	-	-16.9	-24.2
O/BW Ratio* (x10 <sup>3</sup> ):	4.69	5.69	2.57	3.45	2.65	-	4.19	4.46
Absolute Prostate Weight:	0.911	1.519	0.327	0.598	0.313	-	0.489	1.254
% Difference from Control:	-	-	-64.6	-60.6	-65.6	-	-46.3	-17.4
O/BW Ratio* (x10 <sup>3</sup> ):	2.99	4.12	1.19	1.91	1.25	-	1.79	3.48
Absolute Seminal Vesicle Weight:	0.159	0.402	0.134	0.223	0.094	-	0.122	0.344
% Difference from Control:	-	-	-15.7	-44.5	-40.9	-	-23.3	-14.4
O/BW Ratio* (x10 <sup>4</sup> ):	5.19	10.81	4.85	7.14	3.83	-	4.37	9.34

\*O/BW = Organ/Body Weight Ratio.

III. RESULTS AND DISCUSSION (cont.):

G. Organ and Body Weights and Organ/Body Weight Ratios (cont.):

Reductions were also evident in mean weights of the epididymides, prostates and seminal vesicles in all treated groups (young and mature rabbits) at the end of the recovery period.

Based on the findings of the organ and body weight and organ/body weight ratio of the adrenals, testes, epididymides, prostates and seminal vesicles, organ/brain weight ratios were calculated (Tables 3 and 4). Reductions were evident in the organ/brain weight ratios of the testes, epididymides and prostates of the young and mature rabbits at the end of the treatment period and in the organ/brain weight ratios of the seminal vesicles of the mature rabbits receiving CMA-103 and CMA-104 at the end of treatment. Reductions in organ/brain weight ratios of the testes, epididymides, prostates and seminal vesicles were also evident at the end of the four week untreated recovery period; however, these effects were considered minimal in the seminal vesicles.

The reductions in absolute and relative (organ/body and organ/brain) weight ratios of the testes, epididymides, prostates and seminal vesicles were considered to be indicative of treatment-related effects. However, since histopathologic evaluations were not performed on any tissues except the testes, a more definitive evaluation of the possible degree and severity of these apparent effects could not be made.

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

III. RESULTS AND DISCUSSION (cont.):

G. Organ and Body Weights and Organ/Body Weight Ratios (cont.):

Table 3: Mean Terminal Organ and Brain Weights and Organ/Brain Weight Ratios

Group Test Substance	Brain		Adrenal		Testes		Epididymis		Prostate		Seminal Vesicles	
	Weight g	Ratio %	Weight g	Ratio %								
I/CMA-101	9.096	0.193	2.1218	1.930	21.2181	0.687	7.5528	0.480	5.2770	0.120	1.3193	
II/CMA-102	8.653	0.193	2.2304	0.392	4.5302	0.283	3.2705	0.201	2.3229	0.142	1.6410	
III/CMA-103	8.673	0.221	2.5481	0.410	4.7273	0.357	4.1162	0.361	4.1623	0.116	1.3375	
IV/CMA-104	9.062	0.199	2.1960	0.875	9.6557	0.446	4.9217	0.225	2.4829	0.110	1.2139	
<u>Young Animals</u>												
I/CMA-101	9.965	0.259	2.5991	4.348	43.6327	1.434	14.3904	0.867	8.9012	0.208	2.0873	
II/CMA-102	9.885	0.365	3.6925	2.109	21.3354	1.098	11.1077	0.498	5.0379	0.196	1.9828	
III/CMA-103	9.975	0.268	2.6867	1.573	15.7694	1.122	11.2481	0.587	5.8847	0.132	1.3233	
IV/CMA-1014	10.215	0.276	2.7019	2.174	21.2824	1.036	10.1419	0.479	4.6892	0.128	1.2531	
<u>Mature Animals</u>												

III. RESULTS AND DISCUSSION (cont.):

G. Organ and Body Weights and Organ/Body Weight Ratios (cont.):

Table 4: Mean Recovery Organ and Brain Weights and Organ/Brain Weight Ratios

Group Test Substance	Brain		Adrenal		Testes		Epididymis		Prostate		Seminal Vesicles	
	Weight g	Ratio %	Weight g	Ratio %								
I/CMA-101	9.736	0.269	2.7629	3.735	38.3628	1.404	14.4207	0.911	9.3570	0.159	1.6331	
II/CMA-102	9.579	0.243	2.5368	1.490	15.5549	0.707	7.3807	0.327	3.4137	0.134	1.3989	
III/CMA-103	9.267	0.282	3.0431	1.260	13.5966	0.653	7.0465	0.313	3.3776	0.094	1.6144	
IV/CMA-104	9.454	0.305	3.2261	2.818	29.8075	1.167	12.3440	0.489	5.1724	0.122	1.2905	
<u>Young Animals</u>												
I/CMA-101	10.641	0.383	3.5993	5.432	60.4454	2.101	19.7444	1.519	14.2750	0.402	3.7778	
II/CMA-102	9.393	0.478	5.0889	2.634	28.0422	1.074	11.4340	0.598	6.3664	0.223	2.3741	
III/CMA-103	-	-	-	-	-	-	-	-	-	-	-	
IV/CMA-104	10.111	0.370	3.6594	4.225	41.7862	1.592	15.7452	1.254	12.4023	0.344	3.4022	
<u>Mature Animals</u>												

III. RESULTS AND DISCUSSION (cont.):

H. Analysis of Testicular Zinc (Appendix I):

Total testicular zinc concentrations of the young and mature rabbits were substantially lower than corresponding control values at the end of the treatment and recovery periods. However, the concentration of zinc on a ug/g of wet tissue basis in the testes of young rabbits receiving CMA-102 and CMA-103 was approximately twice that of the control animals at the end of the treatment period. Slight to statistically significant elevations in zinc concentration (ug/g) in the testes were also evident in the young rabbits treated with CMA-104 (+ 24%) and in the mature rabbits in all treatment groups at the end of the treatment period (+ 28% to + 52%). The zinc concentration (ug/g) of the testes of the young and mature rabbits in all groups were considered to be comparable to control values following the recovery period.

The results are difficult to interpret. Treatment of rabbits with CMA-102, 103 and 104 does cause an increase in testicular zinc concentration (ug/g) when compared with the vehicle-treated control group (CMA-101). However, is the increase in zinc concentration due to increased zinc accumulation in the testes or due to a loss of testicular cells which do not contain zinc? The total zinc level actually declined compared with the vehicle treated group.

III. RESULTS AND DISCUSSION (cont.):

H. Analysis of Testicular Zinc (cont.):

Regardless of how the data are interpreted, the test substances did have an effect upon testicle weight and testicular zinc concentrations. Removal of test substances during the recovery period showed zinc concentrations per gram of testes returned to control values in the vehicle-treated group, CMA-101. Based upon these results it may be concluded that the CMA test substances do not affect testes weight and testicular zinc content. It cannot be determined if increased zinc concentrations are a causation factor in the weight loss of the testes or is a result of testicular weight loss caused by other factors.

I. Gross Postmortem Examinations and Histopathological Evaluations (Appendix J):

Dermal responses observed in-life were confirmed at the gross postmortem examinations. The incidence of small testes subjectively observed by the prosectors at necropsy was greater, as compared to control animals, in the young and mature rabbits killed at the end of the treatment and recovery periods. In nearly all instances this observation of small testes was confirmed by the organ weight data and histopathologic evaluations and was, therefore, considered to be a treatment-related effect (Tables 5 and 6).

## III. RESULTS AND DISCUSSION (cont.):

I. Gross Postmortem Examinations and Histopathological Evaluations  
(cont.):Table 5: Correlation of Gross Pathological Findings, Testes Weights and Microscopic Findings - Terminal

Group % (w/v)	# Animals Sacrificed	An. No.	Gross Necropsy Observations of Testes		Testes Weights - grams		Gross/Micro Correlation*
			Left	Right			
<u>Mature Animals</u>							
I 100 CMA-101	10	1004	Both Small	0.989	1.001	1+	
II 25 CMA-102	5	2002	Both Flaccid	1.146	0.866	1+	
		2003	Both Small	0.738	0.819	1+	
		2014	Both Small	0.371	0.573	1+	
		2015	Both Small	1.305	0.867	2+	
III 25 CMA-103	4	3003	-	0.777	0.809	1+	
		3004	Both Flaccid	0.421	0.402	1+	
		3015	Both Small	0.678	0.698	1+	
IV 25 CMA-104	5	4001	Both Small	0.611	0.539	1+	
		4008	Both Small	0.731	0.663	1+	
		4009	Both Small	0.389	0.464	1+	
<u>Young Animals</u>							
I 100 CMA-101	10	1016	Both Small	0.480	0.478	1+	
		1017	-	1.245	1.230	1+	
		1023	Both Small	0.264	0.264	1+	
		1024	Both Small	0.492	0.520	1+	
II 25 CMA-102	5	2016	Both Small	0.221	0.253	1+	
		2017	Both Small	0.137	0.133	1+	
		2019	Both Small	0.195	0.191	1+	
		2025	Both Small	0.225	0.209	1+	
		2029	Both Small	0.209	0.187	1+	
III 25 CMA-103	5	3016	Both Small	0.206	0.195	1+	
		3021	Left Small	0.249	0.225	1+	
		3023	Both Small	0.163	0.166	1+	
		3025	Both Small	0.131	0.126	1+	
IV 25 CMA-104	9	4016	Both Small	0.293	0.287	1+	
		4019	Both Small	0.827	0.905	2+	
		4021	Both Small	0.363	0.334	1+	
		4024	Both Small	0.504	0.327	1+	
		4025	Both Small	0.342	0.331	1+	
		4026	Both Small	0.198	0.221	1+	
		4027	Both Small	0.473	0.565	1+	
		4029	Both Small	0.533	0.709	1+	
		4030	Both Small	0.326	0.332	1+	

1+ = Diffuse Tubular Hypoplasia; 2+ = Multifocal Tubular Hypoplasia.

\* Appendix J, pp. J-59 to J-75.

III. RESULTS AND DISCUSSION (cont.):

I. Gross Postmortem Examinations and Histopathological Evaluations (cont.):

Table 6: Correlation of Gross Pathological Findings, Testes Weights and Microscopic Findings - Recovery

Group % (w/v)	# Animals Sacrificed	An. No.	Gross Necropsy Observations of Testes		Testes Weights - grams		Gross/Micro Correlation*
			Left	Right	Left	Right	
<u>Mature Animals</u>							
I 100 CMA-101	5	-	-	-	-	-	-
II 25 CMA-102	4	2001	Both Small		1.629	1.664	1+
		2009	Left Small		1.770	1.727	-
		2012	Both Small		0.736	0.761	1+
		2013	Both Small		1.083	1.167	1+
IV 25 CMA-104	5	4003	Both Small		1.371	1.586	1+
		4011	Both Small		1.281	2.410	1+
<u>Young Animals</u>							
I 100 CMA-101	5	1022	Left Small		0.333	2.870	1+
		1030	Left Small		0.534	2.302	1+
II 25 CMA-102	2	2021	Both Small		0.540	0.575	1+
		2024	Left Small		0.996	0.870	1+
III 25 CMA-103	5	3018	Both Small		0.366	0.359	1+
		3019	Both Small		0.545	0.415	1+
		3022	Both Small		0.516	0.538	1+
		3026	Both Small		0.562	0.554	1+
		3027	Both Small		1.133	1.312	2+
IV 25 CMA-104	5	4018	-		1.312	1.262	1+
		4022	Both Small		1.085	1.066	1+
		4023	Both Small		1.344	1.318	-
		4028	Both Small		1.614	1.698	-

1+ = Diffuse Tubular Hypoplasia; 2+ = Multifocal Tubular Hypoplasia.  
\* Appendix J, pp. J-59 to J-75.

III. RESULTS AND DISCUSSION (cont.):

I. Gross Postmortem Examinations and Histopathological Evaluations (cont.):

The testes of all animals were palpated and found to be descended at each weekly palpation as well as prior to necropsy. The testes of the following animals were not descended at necropsy: young animals - 1017 (CMA-101), 2016 (CMA-102), 3021 (CMA-103) and 4029 (CMA-104) at the terminal sacrifice and in 1022 and 1030 (CMA-101), 2021 (CMA-102) and 4022 (CMA-104) at the recovery sacrifice; mature animals - 1002 and 1015 (CMA-101), 2004 (CMA-102), 3003 (CMA-103) and 4009 and 4012 (CMA-104) at the terminal sacrifice and 2009 (CMA-102) and 4013 (CMA-104) at the recovery sacrifice. Several animals from Group III (CMA-103), killed at the end of the recovery period, also had small epididymides, seminal vesicles and/or prostate glands.

Discoloration of the liver was observed in several of the treated animals.

Other postmortem findings observed grossly occurred sporadically and were not considered to be related to the administration of the test substances.

Test substance-related microscopic changes of the testes, consisting of diffuse tubular hypoplasia and aspermatogenesis, were present in many or all of the young and mature rabbits in Groups II (25%, CMA-102), III (25%, CMA-103) and IV (25%, CMA-104). These changes did not appear to be completely reversed following the recovery period in the young and mature rabbits of these

III. RESULTS AND DISCUSSION (cont.):

I. Gross Postmortem Examinations and Histopathological Evaluations (cont.):

same groups. The testicular changes in the young and mature rabbits in Group I (100%, CMA-101) appeared to be more related to the age of the rabbits than to the treatment with 100% CMA-101.

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

Preface to Appendices

<u>Age</u>	<u>Group</u>	<u>Test Substance</u>	<u>Dose Level</u> % (w/v)	<u>Animal Numbers</u>
Young	I	CMA-101 Vehicle Control	100	1016 - 1030
	II	CMA-102 <sup>a</sup>	25	2016 - 2030
	III	CMA-103 <sup>a</sup>	25	3016 - 3030
	IV	CMA-104 <sup>a</sup>	25	4016 - 4030
	V	-	0	5011 - 5021 <sup>b</sup>
Mature	I	CMA-101 Vehicle Control	100	1001 - 1015
	II	CMA-102 <sup>a</sup>	25	2001 - 2015
	III	CMA-103 <sup>a</sup>	25	3001 - 3015
	IV	CMA-104 <sup>a</sup>	25	4001 - 4015
	V	-	0	5001 - 5010

NOTE: Statistical analysis is presented on mean tables only.

<sup>a</sup>Zinc dialkyldithiophosphates.

<sup>b</sup>Animal number differs due to the replacement of an animal.

A

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

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Methodology and References - General

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Parameter	Reference or Description
Body Weight	Sartorius Scale
Terminal Body Weight (TBW)	Weight taken just prior to necropsy.
Rectal Temperatures	Diatek, Inc., 3910 Sorrento Valley Blvd., San Diego, Ca. Pretest temperatures were recorded in Fahrenheit, Model #500 C1D; all other temperatures were recorded in Centigrade, Model #500 C1E, Friday of each week at approximately 9:30 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.