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CIBA-GEIGY CORP

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LITTON BIONETICS INC

Document Title

INITIAL SUBMISSION: 28-DAY TO 90-DAY SUBCHRONIC TOXICITY  
STUDY IN MICE WITH ATTACHMENTS AND COVER LETTER DATED 081992

Chemical Category

CGA-72651 TECHNICAL

Agricultural Division  
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CIBA-GEIGY

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INIT 08/24/92

Attention: Section 8(e) Coordinator  
(CAP Agreement)

SUBJECT: 8E CAP - 0024

Dear Section 8(e) Coordinator:

Enclosed are the original and two copies of a study  
CIBA-GEIGY Corporation is submitting pursuant to the TSCA  
Section 8(e) Compliance Audit Program and CAP Agreement  
number 8E CAP-0024. The information being submitted is not  
considered Confidential Business Information. We are sub-  
mitting the following information, as required by the CAP  
Agreement:

Company Name,  
Address and  
Telephone No.:

CIBA-GEIGY Corporation  
Attn.: Mr. Anthony Di Battista  
Toxicology, Regulatory Auditing  
and Compliance Department  
444 Saw Mill River Road  
Ardsley, New York 10502-2699  
Tel. No. 914-479-2776

Tested Chemical:

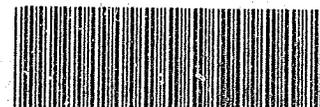
CGA-72651 Technical;  
N-formyl-4-chloro-o-toluidine  
(Currently a manufacturing intermedi-  
ate no longer in use)

CAS Registry No.:

87999-30-2

Report Title:

Lifespan (Chronic Toxicity and Carci-  
nogenicity) Feeding Study in Rats  
(Study Number 05688/1, June 16, 1980)



88920007206

Section 8(e) Coordinator  
August 19, 1992  
Page 2

Summary:

Albino rats were fed 0, 2, 20, 100, or 500 ppm N-formyl-4-chloro-o-toluidine in the diet for 105 weeks. Liver weights were increased at the high dose. There were increased incidences of hyperplasia of small biliary ducts and multiloculated cho-langiogenic biliary cysts in high-dose animals.

Category:

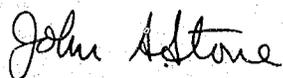
Unit II.B.2.b

Prior Reporting:

Not Applicable

Please call the undersigned at telephone number 919-632- 2179 if you have any questions about this submittal.

Very truly yours,



John A. Stone  
Manager, Environmental Issues

L201CRM0810LG.3/RD17

Enclosures (Two additional copies of this letter  
and three copies of the submitted study)

cc: Mr. A. Di Battista

"Contains NO CEL"

COMBINATION 28-DAY TO 90-DAY  
SUBCHRONIC DIETARY TOXICITY STUDY  
IN ALBINO MICE  
WITH CGA-143686 TECHNICAL  
FINAL REPORT  
VOLUME I

SUBMITTED TO:

CIBA-GEIGY CORPORATION  
P.O. BOX 18300  
GREENSBORO, NORTH CAROLINA 27419

SUBMITTED BY:

LITTON BIONETICS, INC.  
1330 PICCARD DRIVE  
ROCKVILLE, MARYLAND 20850

LBI PROJECT NO. 22245

DECEMBER 1984



Litton Bionetics

# TABLE OF CONTENTS

	<u>PAGE</u>
SUMMARY	
OBJECTIVE	iv
MATERIAL	1
EXPERIMENTAL DESIGN	1
RESULTS	2
CONCLUSION	6
TEXT TABLES	12
TABLE A - TABULATION OF MORTALITY STATUS	15
TABLE B - BODY WEIGHT (GRAMS)	16
FIGURE A - PLOT OF BODY WEIGHT ANALYSIS	20
TABLE C - FOOD CONSUMPTION (GRAMS)	22
FIGURE B - PLOT OF FOOD CONSUMPTION ANALYSIS	26
TABLE D - MEAN CLINICAL HEMATOLOGY	28
TABLE D-T - MEAN CLINICAL HEMATOLOGY BY TIME COURSE OF STUDY	59
TABLE E - MEAN CLINICAL CHEMISTRY	71
TABLE E-T - MEAN CLINICAL CHEMISTRY BY TIME COURSE OF STUDY	106
TABLE F - MEAN ORGAN WEIGHTS IN GRAMS	136
TABLE F-T - MEAN ORGAN WEIGHTS BY TIME COURSE OF STUDY	144
TABLE G - MEAN ORGAN TO BODY WEIGHT PERCENTAGES	158
TABLE G-T - MEAN ORGAN TO BODY WEIGHT PERCENTAGES BY TIME COURSE OF STUDY	166
TABLE H - MEAN ORGAN TO BRAIN WEIGHT PERCENTAGES	180
TABLE H-T - MEAN ORGAN TO BRAIN WEIGHT PERCENTAGES BY TIME COURSE OF STUDY	188

# TABLE OF CONTENTS

	PAGE
APPENDIX A	
TABLE 1 - CLINICAL OBSERVATIONS	A 2
TABLE 1A - MORTALITY STATUS BY WEEK	44
TABLE 1B - MORTALITY STATUS BY DOSE GROUP	57
TABLE 2 - INDIVIDUAL BODY WEIGHT IN GRAMS - MALES AND FEMALES	60
TABLE 3 - INDIVIDUAL DAILY FOOD CONSUMPTION IN GRAMS - MALES AND FEMALES	92
TABLE 4 - INDIVIDUAL CLINICAL HEMATOLOGY - PRESTUDY, DAYS 14, 28, 45, 60 AND 75 - MALES AND FEMALES	120
TABLE 5 - INDIVIDUAL CLINICAL CHEMISTRY - PRESTUDY, DAYS 14, 28, 45, 60, 65, 75 AND 90 - MALES AND FEMALES	177
TABLE 6 - INDIVIDUAL ORGAN WEIGHTS - DAYS 28, 60, 75, 90 - MALES AND FEMALES	246
TABLE 7 - INDIVIDUAL ORGAN TO BODY WEIGHT PERCENTAGES - DAYS 28, 60, 75, 90 - MALES AND FEMALES	267
TABLE 8 - INDIVIDUAL ORGAN TO BRAIN WEIGHT PERCENTAGES - DAYS 28, 60, 75, 90 - MALES AND FEMALES	287
APPENDIX B - OPHTHALMOSCOPIC EXAMINATION	B 1
APPENDIX C - CLINICAL PATHOLOGY REPORT	C 1
APPENDIX D - LDHV/ASSAY REPORT	D 1
APPENDIX E - PATHOLOGY REPORT	E 1
APPENDIX F - CHEMISTRY REPORT	F 1

SPONSOR: Ciba-Geigy Corporation

MATERIAL: CGA-143686 Technical

SUBJECT: FINAL REPORT

Combination 28-Day to 90-Day Subchronic Dietary  
Toxicity Study in Albino Mice with CGA-143686 Technical  
LBI Project No. 22245

### SUMMARY

Seven groups of 40 CD-1 mice per sex per group were exposed to the test compound, CGA-143686 Technical, at the dose levels of 0, 1, 10, 100, 500, 1000 and 5000 ppm of the diet for Groups 1, 2, 3, 4, 5, 6 and 7, respectively. All mice were observed twice a day for mortality and any unusual signs or behavior. Body weights and food consumption were determined weekly. Detailed observations of individual mice, including palpation for tissue masses, were recorded weekly. Clinical pathology determinations were performed on 10 males and 10 females pre-study, on 5 mice per sex per dose at Day 14, on 10 mice per sex per dose at Day 28, and 5 mice per sex per dose on Day 45. These mice were subsequently killed at each interval as interim sacrifice animals.

After review of the Day 28 data, Groups 2, 3 and 5 were eliminated from further investigation. Prior to Day 60, 10 control mice (5 per sex) were taken off study to perform viral screens. Approximately one-third of the surviving animals at this time were killed on each of Days 60, 75 and 90. Clinical pathology determinations were repeated on these mice prior to their scheduled kill. (On Day 65, limited clinical pathology parameters - LDH, SGOT, SGPT and SAP - only were determined on approximately one-third of surviving mice in each group without kill.)

Gross necropsy examinations were performed on all animals found dead, killed moribund or sacrificed at the scheduled sacrifices. Organ weights were taken of all mice sacrificed at Days 28, 60, 75 and 90. Histopathological examination was performed on organs and tissues from all animals killed at Days 75 and 90, plus animals that died or were killed in a moribund condition. Only gross lesions were examined from animals sacrificed at Day 28. Eye examination was performed on all mice prior to the study start and again on all survivors prior to the Day 60 kill.

Compound related mortality occurred in 2 males and 5 females at the 5000 ppm level. No clinical signs were observed to indicate a specific toxic effect of the test compound. Body weights were depressed at the 5000 ppm level in both sexes. Food consumption was also depressed at the 5000 ppm level in both males and females.



SUMMARY (Continued)

Among the differences in organ weights or their ratios noted, decreased weights of kidneys (males and females of the 5000 ppm group) and thymus (5000 ppm males and 5000 and 1000 ppm females), increased liver to body weight ratios (males and females at 5000 ppm) and spleen to body weight ratio (males at 5000 ppm) at a later time period seem to correlate with pathological findings in these organs in male and female mice at those treatment levels.

There was an early transient increase in HCT not associated with a rise in HGB or RBC. Later there was a decrease in HCT, HGB and RBC exclusively in the 5000 ppm group. Total leukocytes were decreased due to a decrease in lymphocytes. Platelets were increased in the 5000 ppm group. BUN, cholesterol, total protein and albumin were decreased predominantly in the 5000 ppm group. Decrease in total protein appeared to be due to decrease in albumin. SAP was increased in the 5000 ppm group. Inorganic phosphorus was increased late in the study.

There were no neoplastic lesions encountered in this study. The histopathologic examinations revealed that the test compound had toxic effects on the kidneys (cortical atrophy), testes (atrophy of the seminiferous tubules and occasional necrosis) and livers (hepatocellular cytomegaly and necrosis) of mice killed at 75 and 90 days, mostly in the 5000 ppm group. The lymphoid atrophy and lymphocytolysis involving the thymus were probably non-specific stress related lesions.

The test compound in this study did not produce ocular changes in albino mice. The LDH assay proved this mouse colony to be free of LDH elevating virus.

From this study, no-observable-effect level of CGA-143686 Technical would be less than 100 ppm and the maximum tolerated level would be around 1000 ppm in the diet for male and female albino mice. The 5000 ppm level seems to be the toxic level of this compound.



SPONSOR: Ciba-Geigy Corporation

MATERIAL: CGA-143686 Technical

SUBJECT: FINAL REPORT  
Combination 28-Day to 90-Day Subchronic Dietary  
Toxicity Study in Albino Mice with CGA-143686 Technical  
LBI Project No. 22245

### OBJECTIVE

The purpose of this study was to determine the potential subchronic toxicity, identify target organs and provide a no-observable effect level, as well as the maximum tolerated level.

### MATERIAL

One paper bag containing a powder (950 g net weight) and labeled:

CGA-143686 Technical, FL 830803

was received from Ciba-Geigy Corporation, by Litton Bionetics, Inc. (LBI) on April 6, 1983 and designated as LBI No. 8433. On June 20, 1983, a second shipment of the compound was received from Ciba-Geigy Corporation. It was labeled CGA-143686 Technical, FL 831398 and designated as LBI No. 8562. The test material was stored in the Rockville chemical storage room at 40°F.

Test diets containing the test article were prepared by the method as presented in Appendix F, Chemistry Report, Table 4, Formulation Batch Record. Prior to study initiation, a test to ensure homogeneity of the formulation at 1 and 5000 ppm was performed. A sample of each formulation to ensure its correctness was taken and analyzed at weekly intervals for the first 4 mixes and at the fourth mix after that. Any level found to be outside the set range of target concentration ( $\pm 10\%$  of target for the levels of 100, 500, 1000 and 5000 ppm, within 5 to 15 ppm of target for the 10 ppm level and within 0 to 2 ppm for the 1 ppm level) was remixed and analyzed. The better of the two mixes were to be used on study. To ensure stability of the test material in the formulated diet, samples were analyzed at a Day 0, after 3 weeks under refrigeration plus one week at room temperature and after 3 weeks under refrigeration plus 2 weeks at room temperature. This was done at the 10 and 1000 ppm dose levels.

The method of analysis and the findings of the LBI Chemistry Department are included in Appendix F, Chemistry Report. These results include validation of the analytical method, homogeneity attainment validation, stability and dose verification data.



## EXPERIMENTAL DESIGN

Six hundred forty (320 males and 320 females) mice of the CD-1 strain were ordered from Charles River Breeding Laboratories, Inc., Kingston, New York. The animals were quarantined for 14 days prior to dosing. The animals were received on 03/30/83 and housed in room 231E of the Rockville Toxicology Facility of LBI, 1330 Piccard Drive, Rockville, Maryland. The animals were individually housed in stainless steel hanging wire cages. The temperature in the room was set to maintain at  $23^{\circ}\text{C} \pm 2^{\circ}$  and the humidity at 30 to 70%. The light cycle was automatically controlled to provide 12 hours of fluorescent light and 12 hours of darkness each day. The animal rooms are designed for 12-15 changes of fresh air every hour. Acidified water (pH 2.5) and Purina Certified Rodent Chow #5002<sup>®</sup> were provided ad libitum. No significant deviations from prescribed environmental conditions occurred during the study. Animal identification was by ear punch, toe clip and cage card. No other species or test materials were under concurrent investigation in this animal room.

At the end of the quarantine period the animals were randomly assigned to experimental groups, as tabulated below, using a computer-generated random numbers table. Each group contained 40 animals/sex/group. Ten additional animals of each sex were used to establish baseline parameters for hematology and clinical chemistry prior to study start. All extra animals were discarded.

Group Number	Dose (ppm)	Number of Animals <sup>a</sup>			
		Males	Animal Nos.	Females	Animal Nos.
Pretest	-	10	---	10	---
1	0	40	6162-6201	40	6202-6241
2	1	40	6242-6281	40	6282-6321
3	10	40	6322-6361	40	6362-6401
4	100	40	6402-6441	40	6442-6481
5	500	40	6482-6521	40	6522-6561
6	1000	40	6562-6601	40	6602-6641
7	5000	40	6642-6681	40	6682-6721

Ophthalmological examinations were conducted by a board certified veterinarian ophthalmologist on all animals prior to dosing and at Day 60.

The first day of dosing was April 13, 1983. At that time, the animals were approximately 6 weeks of age. The weight variation of the animals (22.1-33.1 g for males and 16.5-24.8 g for females) prior to initiation did not exceed +20% of the mean weight for each sex. Each animal was observed twice daily, a.m. and p.m., 7 days per week, for mortality and changes in appearance, behavior, toxic signs or moribund condition. Any animal judged to be moribund was sacrificed and subjected to the same post mortem examination received by animals found dead or killed at scheduled sacrifices. Each animal was removed from its cage and identified, weighed and given a physical examination initially and weekly thereafter. This examination included palpation for tissue masses and observations of changes in skin and fur, eyes and mucous membranes. Respiratory, circulatory, autonomic and central nervous system signs, somatomotor activity and behavior pattern were also observed. Food consumption was determined for each animal weekly during the study period.

<sup>a</sup> Animal number "M" series.

## EXPERIMENTAL DESIGN (Continued)

Hematology and clinical chemistry determinations were made on 10 animals/sex prior to the start of the study. These determinations were also performed on 5/sex/group at Days 14 and 45 and on 10/sex/group at Day 28. On Days 60, 65, 75 and 90 an average of 6 animals/sex/group (approximately one-third of the surviving animals in each group) were also bled for these determinations. The parameters examined at these intervals are listed below.

On Days 65 (66)<sup>1</sup> and 90<sup>1</sup> approximately 6 animals/sex/group (approximately one-third of the surviving animals in each group) were bled for LDH, SGOT, SGPT and SAP determinations only. On Day 92<sup>1</sup> blood was submitted for conduct of required clinical chemistry tests that were not performed on Day 90<sup>1</sup>.

### CLINICAL PATHOLOGY:

Hematology (Pretest, 14 (15)<sup>1</sup>, 28 (30)<sup>1</sup>, 45 (44)<sup>1</sup>, 60 (62)<sup>1</sup> and 75 (76)<sup>1</sup> days)

hemoglobin  
hematocrit (PCV)  
erythrocyte count  
total leukocyte count  
differential leukocyte count  
platelet count  
reticulocyte count

Clinical Chemistry (Pretest, 14 (15)<sup>1</sup>, 28 (30)<sup>1</sup>, 45 (44)<sup>1</sup>, 60 (62)<sup>1</sup>, 65 (66)<sup>1</sup>, 75 (76)<sup>1</sup> and 90 (90 and 92 days)<sup>1</sup>)

creatinine  
glucose  
total cholesterol  
blood urea nitrogen  
inorganic phosphorus  
serum alanine aminotransferase  
(serum glutamic-pyruvic transaminase)  
serum aspartate aminotransferase  
(serum glutamic-oxaloacetic transaminase)  
lactate dehydrogenase  
total proteins  
albumin  
globulin  
albumin/globulin ratio  
total bilirubin  
serum alkaline phosphatase  
gamma glutamyl transpeptidase

<sup>1</sup>The actual day of the study.



## EXPERIMENTAL DESIGN (Continued)

Gross necropsy examination were performed on all animals found dead, sacrificed moribund or those sacrificed at the scheduled sacrifices. Those sacrificed were exsanguinated. Five animals/sex/ group were sacrificed at Days 14 (15)<sup>1</sup> and 45 (44)<sup>1</sup> of the study. Ten animals/sex/group were sacrificed at Day 28 (30)<sup>1</sup> of the study. Three dose groups (Groups 2, 3 and 5) were eliminated after review of the 28 day kill data. Approximately one-third of the surviving animals were sacrificed at each three intervals, Day 60 (62)<sup>1</sup>, Day 75 (76)<sup>1</sup> and Day 90 (92)<sup>1</sup>.

The following organ weights were taken of all animals sacrificed at Days 28, 60, 75 and 90:

adrenal glands	liver
brain (including brainstem)	spleen
heart	thymus
kidneys	

Appropriate samples of each of the following organs and tissues from all animals were preserved in 10% neutral formalin for possible histopathologic examination.

all gross lesions (including adjacent tissue)	stomach (cardia, fundus and pylorus)
brain (cerebrum, cerebellum, and brainstem)	duodenum
spinal cord (three levels)	jejunum
eyes	ileum
pituitary	cecum
salivary glands	colon
heart	rectum
aorta	adrenal glands
thymus	pancreas
thyroid (if possible with parathyroid)	liver (at least two lobes)
lungs (with mainstem bronchi)	kidneys
trachea	urinary bladder
spleen	ovaries/testes
femur (with marrow)	epididymides
tongue	prostate
lymph nodes (two)	seminal vesicles
mandibular, mesenteric	uterus
sciatic nerve	vagina
esophagus	skin (mammary area)
	skeletal muscle

Histopathologic examination was performed on tissues from all animals sacrificed at 28 days which appeared abnormal at necropsy (gross lesions) and on all of the above tissues and organs from all animals sacrificed at Day 75 and termination and from all animals that died or were killed in extremis during the study.

<sup>1</sup>The actual day of the study.

EXPERIMENTAL DESIGN (Continued)

Body weight, food consumption, hematology, clinical chemistry and organ weight data were analyzed using Dunnett's t-test<sup>2</sup>. Statistical testing was based on both 95% confidence ( $p < 0.05$ ) and 99% confidence ( $p < 0.01$ ).

Thirty days after transmittal of this report, original data and a copy of the final report will be transferred to the LBI Archivist, 1330 Piccard Drive, Rockville, Maryland for distribution to the proper repositories. A copy of this report and underlying data were reviewed by the LBI Quality Assurance Unit prior to submission to the sponsor.

2C.W. Dunnett, Biometrics 20, p. 482, (1964).



## RESULTS

### Compound Effects on Mortality, Clinical Signs, Body Weights and Food Consumption

The cumulative mortality status of animals from the start of the study to termination is shown in Text Table A, and details regarding group number, sex, dosage, week of death and the circumstance of death are listed in Appendix A, Table 1A (by weeks of study) and Table 1B (by dose group). The list of animals that were sacrificed at the scheduled intervals are also tabulated in these tables.

Unscheduled deaths occurred in one control female (Week 6) and in seven 5000 ppm group mice (2 males and 5 females). There were no compound related mortalities in any other group. The majority of these deaths occurred during Weeks 5 to 8 of the study. Based on the frequency, time and dose distribution, the deaths in the 5000 ppm group appeared to be compound related. The mortality plot which graphically depicts cumulative mortalities per dose group (adjusted to the percentages of total survivors) at each week is not included in this report, since it will not give any additional information.

Clinical signs for the individual animal recorded each week during the entire study are appended in Appendix A, Table 1. Only deviations from normal appearance and behavior are recorded. The clinical signs that were observed were quite low in incidence and were those common and expected in laboratory rodents. They included stains on coat and scabs. The exception was the "weight loss" which was observed starting early in the study, and mostly in the 5000 ppm group.

Body weight data for the male and female mice are summarized in Text Table B. These data are presented graphically in Figure A. Individual body weight data are presented in Appendix A, Table 2.

In males, body weights were statistically significantly depressed (either at 0.05 or 0.01 levels) only at the 5000 ppm level throughout the 13 week study period as compared to its control group.

In females, body weights were statistically significantly (at 0.01 level) depressed at the 5000 ppm level throughout the study period (except at Week 11), as in males, compared to its control group. At Week 1 only, both 500 ppm and 1000 ppm females weighed significantly less than control; however, these depressions were judged to be due to the smaller body weights in these groups at the start of the study (Week 0) compared to the control females.

Tables for body weight gain are not included in this report, since they will not give any additional information.

The food consumption values for male and female mice are summarized in Text Table C. These data are presented graphically in Figure B. Individual food consumption values are presented in Appendix A, Table 3.

Compound Effects on Mortality, Clinical Signs, Body Weights and Food Consumption (Continued)

The trend in food consumption was similar to that for body weight changes. There were significant decreases in food consumption at the 5000 ppm level in both male and female mice when compared to their respective controls (statistically significant in males 5 out of 12 weeks and in females 10 out of 12 weeks). Earlier in the study, particularly during the first 2 weeks of the study, other groups (mostly males) had food consumptions significantly greater than controls.

It appears that the depressed body weight seen in the 5000 ppm groups (both males and females) is partly due to the decreased food intake in these groups as compared to their controls.

Effects on Organ Weights

The organ weights taken at Days 28, 60, 75 and 90 kill were compared as absolute weights and as percentages of body weights and brain weights. The group mean values with statistical analysis are summarized in Text Tables F, G and H, respectively. These values are also tabulated against the time of determinations in Text Tables F-T, G-T and H-T, respectively. Individual organ weights and organ to body weight and brain weight percentages are presented in Appendix A, Tables 6, 7 and 8, respectively.

The depressed body weight of the 5000 ppm group in male and female mice accounted for most of the organ weights being smaller and the organ to body weight ratios being greater or similar, and the organ to brain weight ratios being smaller or similar compared to their respective control values at this level. Below, only organs of which weights, or body weight or brain weight-ratios differed statistically significantly (either at 0.01 or 0.05 level) from the controls are discussed.

At 28 days, kidney weight was decreased, while brain and liver body weight ratios were increased in the 5000 ppm males. Brain and heart weights were decreased, while body weight ratios of brain, kidneys and liver were increased in the 5000 ppm females.

At 60 days, in the 5000 ppm males, kidneys, heart and liver weights were decreased, while body weight ratios of brain, spleen and liver were increased. The significant increase in kidney to brain weight ratio in the 100 ppm males appears to be incidental. In the 5000 ppm females, adrenal weight was decreased, body weight ratios of brain, kidneys and liver were increased.

At Day 75, in the 5000 ppm males, body weight ratios of spleen and liver are increased. In the 5000 ppm females, thymus weight was decreased, while liver to body weight ratio was increased.

### Effects on Organ Weights (Continued)

At Day 90, kidneys and thymus weights were decreased in the 5000 ppm males and females. Thymus weight was also decreased in the 1000 ppm females in dose-related manner. In males, body weight ratios of brain and spleen were increased at the 5000 ppm level, and brain weight ratios of kidneys and thymus were decreased at the 5000 ppm level. In females, body weight ratios of brain and liver were increased at the 5000 ppm level. Thymus to body weight ratios were decreased at the 5000 and 1000 ppm levels. Also, brain weight ratios of kidneys and thymus were decreased both at the 5000 and 1000 ppm levels in females. Thymus to brain weight ratio was also decreased at the 100 ppm level, as well, in females.

Decreased weights of kidneys (males and females of the 5000 ppm level) and thymus (5000 ppm males and 5000 and 1000 ppm females), increased liver to body weight ratios (males and females at 5000 ppm) and spleen to body weight ratio (males at 5000 ppm) at a later time period seem to correlate with pathological findings in these organs in male and female mice at the treatment level mentioned above.

### Clinical Pathology

The results of the clinical pathology determinations at pretest, Days 14, 28, 45, 60, 65, 75 and 90 are summarized in Text Tables D and E, they are also tabulated against the times of determinations in Text Tables D-T and E-T, and individual data are included in Appendix A, Tables 4 and 5, Clinical Hematology and Clinical Chemistry, respectively. All of these data were evaluated by our clinical pathologist. His review is appended in Appendix C, Clinical Pathology Report.

Hematologic studies were done pretest and at 14, 28, 45, 60 and 75 days. Clinical chemistry studies were done pretest and at 14, 28, 45, 60, 65, 75 and 90 days. Chemistry determinations performed at pretest, Days 14, 28, 45 and 60 were performed on the Technicon SMAC® using serum diluted 1:5, except for all GGT and pretest creatinine which were performed on the Centrifichem®. 1:5 dilution of serum reduces the sensitivity and precision of the test by a factor of 5.

At Day 65, only LDH, SGPT, SGOT and SAP were performed on the Centrifichem® on serum diluted 1:5. At Day 75, chemistry determinations were performed on the Centrifichem® using undiluted serum. On Days 90-92, mice were bled twice, on Day 90 for LDH, SGPT, SGOT and SAP performed on the Centrifichem® on undiluted serum and again on Day 92, for all remaining chemistry determinations performed on the Centrifichem® on undiluted serum. Our in-house studies suggested that bleeding the mice was followed by intravascular hemolysis which was present in 3 minutes and persisted at least 24 hours. This resulted in an increase in LDH, SGOT, SGPT and sometimes inorganic phosphorus.

Clinical Pathology Report should be consulted for details of comments on data points and some interpretations on significant differences which existed in the treated groups compared to the controls. Below, only statistically significant clinical pathologic changes which appear to be treatment related are summarized.

## RESULTS (Continued)

### Clinical Pathology (Continued)

At 14 days, males at 100, 500, 1000 and 5000 ppm and females at 500 and 1000 ppm had elevated packed cell volumes. The test compound seems to induce a transient increase in packed cell volume perhaps due to a change in water distribution.

Packed cell volumes were decreased in 5000 ppm males at 45, 60 and 75 days, in 5000 ppm females at 45 and 75 days. Hemoglobin was decreased in 10, 100, 1000 and 5000 ppm females at 14 days, in 5000 ppm males and females at 28, 45, 60 and 75 days. Erythrocyte count (RBC) was reduced in 5000 ppm males at 28, 45, 60 and 75 days, in 5000 ppm females at 28, 45 and 75 days. The 5000 ppm group had significantly decreased body weight gain and food consumption. The hematologic changes seen in the 5000 ppm group could be the result of this effect.

Leukocyte count (WBC) was reduced in 5000 ppm males at 45 and 60 days, in 5000 ppm females at 75 days, in 100 ppm males at 14 and 60 days, in 100 and 1000 ppm females at 75 days. Each group with decreased WBC correlates with decreased lymphocyte count.

Platelets were increased in 5000 ppm males at 14, 28, 45, 60 and 75 days, in 5000 ppm females at 45 days, in 1000 ppm males at 45 days. Total lymphocytes were decreased in 5000 ppm males at 45 and 60 days, in 100 ppm males at 14 and 60 days.

In serum chemistry, BUN was decreased in 5000 ppm males at 28, 45 and 60 days, in 1000 ppm females at 14, 45 and 90 days, in 100 ppm females at 45 days, in 10 ppm females at 28 days and increased in 100 ppm males at 14 days.

Cholesterol was decreased in 5000 ppm males at 28, 60, 75 and 90 days and in 100 ppm males at 75 days.

These decreases in BUN and cholesterol along with albumin discussed below are likely to be treatment effect on the decreased hepatic synthesis.

Inorganic phosphorus was increased in 100 ppm males at 60 days, in 1000 ppm males at 60 days, in 5000 ppm males at 90 days, in 1000 ppm and 5000 ppm females at 90 days.

SAP was increased in 5000 ppm males and females at 28, 45, 60, 65, 75 and 90 days, in 1000 ppm females at 60 days.

Total protein was decreased in 100 ppm females at 60 days and in 5000 ppm females at 60 and 90 days. Albumin was decreased in 5000 ppm females at 60 and 90 days, in 100 ppm females at 60 days, in 5000 ppm males at 75 days, in 1000 ppm males at 75 days. Globulin and A/G ratio were accordingly affected. The decreases in total protein represent decreases in albumin component. Either decreased food consumption or decreased hepatic synthesis would explain these decreases.

Other changes, although statistically significant, were judged to be incidental and not treatment related.

## RESULTS (Continued)

### Clinical Pathology (Continued)

In summary, treatment induced numerous changes in clinical laboratory parameters. There was an early transient increase in packed cell volume not associated with a rise in HGB or RBC. Later there was a decrease in packed cell volume, HGB and RBC, exclusively in the 5000 ppm group. Total leukocytes were decreased due to a decrease in lymphocytes. Platelets were increased in the 5000 ppm group. Blood urea nitrogen (BUN), cholesterol, total protein and albumin were decreased predominantly in the 5000 ppm group. Decrease in total protein appeared to be due to the decrease in albumin. Serum alkaline phosphatase (SAP) was increased in the 5000 ppm group. Serum inorganic phosphorus was increased late in the study. Serum bilirubin may have been decreased.

### Pathology

Details of all of the pathologic findings are in Appendix E, Pathology Report. This includes gross and microscopic observations of individual animals along with a summary of histologic findings and dose related incidence tables for tumor and non-tumor pathology of scheduled, unscheduled and combined deaths.

All animals, with the exception of those from Group 1, that were withdrawn from study, received a complete gross examination. Complete histopathologic examination of the organs and tissues listed in the study design was performed only on animals from Groups 1, 4, 6 and 7, killed at Days 75 and 90 plus animals that died or were killed in a moribund condition. Only gross lesions were examined from animals sacrificed at the 28 day kill.

There were no neoplastic lesions encountered in this study. The histopathologic observations revealed that the test compound had toxic effects on the kidneys, testes and livers of mice killed at 11 and 13 weeks.

The renal lesions consisted principally of cortical atrophy which was observed in both sexes of Group 7 mice. The severity and incidence of the lesions tended to be greater at 13 weeks.

In the testes, atrophy of the seminiferous tubules and occasional necrosis were observed in Group 7 males and again the lesions tended to be more prevalent and more severe at the later time period.

Compound related hepatic changes were observed in a few males from Group 6 and most of the males and females from Group 7 killed at both 11 and 13 weeks. Those changes consisted principally of hepatocellular cytomegaly and necrosis. These changes were also seen in most of the Group 7 animals that died or were killed in a moribund condition.

Extramedullary hematopoiesis encountered in several males from Groups 4, 6 and 7 may have been compound related.

### Pathology (Continued)

The lymphoid atrophy and lymphocytolysis principally involving the thymus and encountered in many animals of both sexes in Group 7, and a lesser number of Group 6 animals, were probably non-specific stress related lesions resulting from other compound effects such as hepatorenal alterations.

The ophthalmoscopic examination was done on all mice prior to the start of the study and again on all survivors prior to the Day 60 kill, which was originally scheduled as terminal kill of this study. These results are presented in Appendix B, Ophthalmoscopic Examination. The test compound in this study did not produce ocular changes in albino mice.

During the course of this study, very high LDH values in some of the mice, aroused a suspicion of an infection of the mice colony with LDH elevating virus. Five males and 5 females from the control group were taken off study on Day 56 (Week 8 of the study) and sent to Microbiological Associates for LDHV Assay. The cover letter sent to Microbiological Associates and their report are included in Appendix D. The results were negative.

Thirty days after transmittal of the final report, original data and a copy of the final report will be transferred to the LBI Archivist, 1330 Piccard Drive, Rockville, Maryland for distribution to the proper repositories. A copy of this report and underlying data were reviewed by the LBI Quality Assurance Unit prior to submission to the sponsor.



CONCLUSION

Compound related mortality occurred in 2 males and 5 females at the 5000 ppm level. No clinical signs were observed to indicate a specific toxic effect of the test compound. Body weights were depressed at the 5000 ppm level in both sexes. Food consumption was also depressed at the 5000 ppm level in both males and females.

Among the differences in organ weights or their ratios noted, decreased weights of kidneys (males and females of the 5000 ppm group) and thymus (5000 ppm males and 5000 and 1000 ppm females), increased liver to body weight ratios (males and females at 5000 ppm) and spleen to body weight ratio (males at 5000 ppm) at a later time period seem to correlate with pathological findings in these organs in male and female mice at those treatment levels.

There was an early transient increase in HCT not associated with a rise in HGB or RBC. Later there was a decrease in HCT, HGB and RBC exclusively in the 5000 ppm group. Total leukocytes were decreased due to a decrease in lymphocytes. Platelets were increased in the 5000 ppm group. BUN, cholesterol, total protein and albumin were decreased predominantly in the 5000 ppm group. Decrease in total protein appeared to be due to decrease in albumin. SAP was increased in the 5000 ppm group. Inorganic phosphorus was increased late in the study.

There were no neoplastic lesions encountered in this study. The histopathologic examinations revealed that the test compound had toxic effects on the kidneys (cortical atrophy), testes (atrophy of the seminiferous tubules and occasional necrosis) and livers (hepatocellular cytomegaly and necrosis) of mice killed at 75 and 90 days, mostly in the 5000 ppm group. The lymphoid atrophy and lymphocytolysis involving the thymus were probably non-specific stress related lesions.

The test compound in this study did not produce ocular changes in albino mice. The LDH assay proved this mouse colony to be free of LDH elevating virus.

From this study, no-observable-effect level of CGA-143686 Technical would be less than 100 ppm and the maximum tolerated level would be around 1000 ppm in the diet for male and female albino mice. The 5000 ppm level seems to be the toxic level of this compound.

Submitted by:

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2-2-84  
Date

Reviewed by:

Douglas K. Craig  
Douglas K. Craig, Ph.D.  
Director  
Department of Toxicology

2/2/84  
Date

Q.A. Inspection Statement  
 [reference 21 CFR 58.35(b)(7)]

PROJECT 22245 LBI COMPOUND NO. 8433, 8552  
 TYPE OF STUDY Combination 28-Day to 90-Day Subchronic Dietary Toxicity Study  
in Albino Mice

This final study report was reviewed by the LBI Quality Assurance Unit on January 27, 1984. A report of findings was submitted to the study director and to management on January 30, 1984.

This study was inspected by the LBI Quality Assurance Unit and findings were reported to the study director and to management. Dates of inspection and reporting were as follows:

<u>DATE OF INSPECTION</u>	<u>DATE OF REPORT TO MANAGEMENT AND STUDY DIRECTOR</u>	<u>DATE OF INSPECTION</u>	<u>DATE OF REPORT TO MANAGEMENT AND STUDY DIRECTOR</u>
03/30/83	04/05/83	06/10/83	06/10/83
04/04/83	04/04/83	06/10/83	06/11/83
04/05/83	04/06/83	06/10/83	06/21/83
04/06/83	04/06/83	06/10/83	*
04/19/83	*	06/15/83	06/15/83
04/20/83	04/20/83	07/05/83	07/06/83
04/20/83	04/20/83	07/11/83	07/12/83
04/20/83	04/20/83	07/13/83	07/14/83
04/22/83	04/20/83	07/13/83	07/15/83
04/25/83	*	08/01/83	08/02/83
04/27/83	04/26/83	08/01/83	08/02/83
04/27/83	05/05/83	08/03/83	08/03/83
04/27/83	04/29/83	08/03/83	08/04/83
05/02/83	04/29/83	08/09/83	08/09/83
05/02/83	05/05/83	09/27/83	09/27/83
05/23/83	05/23/83	11/18/83	11/21/83
05/25/83	05/26/83	01/26/84	01/26/84
05/26/83	05/31/83		

*Francis J. Klein*  
 Auditor, Quality Assurance Unit

\*No reportable findings.

## Personnel Involved in the Study

Study Director - Nobuko Hamada, Ph.D., D.A.B.T.

Department Director - Douglas K. Craig, Ph.D.

Technical Group Leader - M. J. Shander

Animal Services Supervisor - H. Eskildson

Analytical Chemistry Director - Jerry Fitzgerald, Ph.D.

Clinical Laboratory Personnel -

K. S. Sibinovic, Ph.D. - Technical Director

A. Scott - Clin Lab Supervisor

R. Heikkila - Chemistry

M. Wickum - Hematology

W. Loeb, Ph.D., V.M.D. - Clinical Pathologist

Pathology Personnel -

J. Langloss, D.V.M., Ph.D. - Pathologist

D. Boyd - Necropsy Supervisor

N. Knecht - Histology Supervisor

Consulting Veterinary Ophthalmologist - James M. Clinton, V.M.D.

Project Coordinator - Amy Tate

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PROJECT NO. 8228

TABLE A

EVALUATION OF MORTALITY STATUS OF ANIMALS  
FROM THE START OF THE STUDY THROUGH TERMINATION

CODE

FREQUENCY	FOUND DEAD	MORUND I KILL	ACCIDENT I ORATH	WITH I ORATH	INTERIM I KILL	TOTAL
0 PPM MALE	0	0	0	0	0	0
0 PPM FEMALE	1	0	0	5	4	10
1 PPM MALE	0	0	0	5	6	11
1 PPM FEMALE	0	0	0	25	0	25
10 PPM MALE	0	0	0	25	0	25
10 PPM FEMALE	0	0	0	25	0	25
100 PPM MALE	0	0	0	25	0	25
100 PPM FEMALE	0	0	0	25	0	25
500 PPM MALE	0	0	0	0	7	7
500 PPM FEMALE	0	0	0	0	33	33
1000 PPM MALE	0	0	0	25	0	25
1000 PPM FEMALE	0	0	1	24	0	25
5000 PPM MALE	1	1	0	0	7	9
5000 PPM FEMALE	1	1	0	0	34	36
TOTAL	3	5	1	159	48	196

TABLE 8

MEAN BODY WEIGHT IN GRAMS  
BY TEST TABLE

- PALS

6 PPM	4/13	4/20	4/27	5/ 4	5/11	5/18	5/25	6/ 1	6/ 8	6/15	6/22	6/29	7/ 6
DATE	4/13	4/20	4/27	5/ 4	5/11	5/18	5/25	6/ 1	6/ 8	6/15	6/22	6/29	7/ 6
WEEK	0	1	2	3	4	5	6	7	8	9	10	11	12
SAMPLE	40	40	35	35	35	25	25	20	16	9	9	11	12
MEAN	27.9	29.0	30.0	31.3	32.3	32.9	33.1	33.5	33.9	33.8	36.1	35.5	34.6
S.E.	0.3	0.3	0.3	0.4	0.3	0.4	0.4	0.5	0.6	0.6	0.9	0.7	1.2
R													
1 PPM	4/13	4/20	4/27	5/ 4	5/11	5/18	5/25	6/ 1	6/ 8	6/15	6/22	6/29	7/ 6
DATE	4/13	4/20	4/27	5/ 4	5/11	5/18	5/25	6/ 1	6/ 8	6/15	6/22	6/29	7/ 6
WEEK	0	1	2	3	4	5	6	7	8	9	10	11	12
SAMPLE	40	40	35	35	35	25	25	20	16	9	9	11	12
MEAN	28.0	29.0	30.6	30.8	31.9	32.0	32.5	32.0	32.0	32.0	32.0	32.0	32.0
S.E.	0.3	0.3	0.3	0.4	0.4	0.6	0.6	0.5	0.6	0.6	0.5	0.6	0.6
R													
10 PPM	4/13	4/20	4/27	5/ 4	5/11	5/18	5/25	6/ 1	6/ 8	6/15	6/22	6/29	7/ 6
DATE	4/13	4/20	4/27	5/ 4	5/11	5/18	5/25	6/ 1	6/ 8	6/15	6/22	6/29	7/ 6
WEEK	0	1	2	3	4	5	6	7	8	9	10	11	12
SAMPLE	40	40	35	35	35	25	25	20	16	9	9	11	12
MEAN	28.5	29.2	29.5	30.9	31.4	32.2	33.5	33.3	33.4	33.0	33.5	33.5	33.7
S.E.	0.4	0.4	0.5	0.5	0.5	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
R													
50 PPM	4/13	4/20	4/27	5/ 4	5/11	5/18	5/25	6/ 1	6/ 8	6/15	6/22	6/29	7/ 6
DATE	4/13	4/20	4/27	5/ 4	5/11	5/18	5/25	6/ 1	6/ 8	6/15	6/22	6/29	7/ 6
WEEK	0	1	2	3	4	5	6	7	8	9	10	11	12
SAMPLE	40	40	35	35	35	25	25	20	16	9	9	11	12
MEAN	28.9	29.6	30.4	31.8	32.0	32.7	33.5	33.0	33.4	33.0	33.5	33.5	33.7
S.E.	0.3	0.4	0.4	0.5	0.5	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.6
R													
100 PPM	4/13	4/20	4/27	5/ 4	5/11	5/18	5/25	6/ 1	6/ 8	6/15	6/22	6/29	7/ 6
DATE	4/13	4/20	4/27	5/ 4	5/11	5/18	5/25	6/ 1	6/ 8	6/15	6/22	6/29	7/ 6
WEEK	0	1	2	3	4	5	6	7	8	9	10	11	12
SAMPLE	40	40	35	35	35	25	25	20	16	9	9	11	12
MEAN	27.0	28.3	29.2	30.7	31.1	31.0	32.2	32.2	32.2	32.5	32.6	32.2	32.7
S.E.	0.3	0.3	0.3	0.4	0.4	0.4	0.4	0.5	0.5	0.6	0.6	0.7	0.7
R													

\*P<0.05 as compared to controls: Dunnett's t-test.

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