

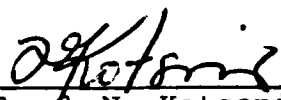
FOUR WEEK DIETARY ADMIX TOXICITY STUDY OF SC-19129  
IN THE DOG


James L. Allen, Curtis D. Port, and Robin C. Guy


Safety Assessment Project Number 2449


Department of Product Safety Assessment

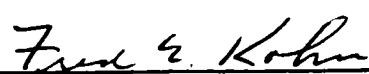
Approved:

 2/5/85  
\_\_\_\_\_  
Frank N. Kotsonis, Ph.D. Date  
Diplomate, A.B.T.  
Director, Toxicology  
Product Safety Assessment

 2/5/85  
\_\_\_\_\_  
Martin A. Sidor, D.V.M., M.S. Date  
Director,  
Laboratory Animal Resources  
Product Safety Assessment

 2.6.85  
\_\_\_\_\_  
David C. Dodd, B.V.Sc., M.A. Date  
Diplomate, A.C.V.P.  
Director, Pathology  
Product Safety Assessment

 2/8/85  
\_\_\_\_\_  
James Oppermann, Ph.D. Date  
Director, Drug Metabolism  
Product Safety Assessment

 2/8/85  
\_\_\_\_\_  
Fred E. Kohn, Ph.D. Date  
Senior Director,  
Product Safety Assessment

February 4, 1985

FOUR WEEK DIETARY ADMIX TOXICITY STUDY OF SC-19129  
IN THE DOG

TABLE OF CONTENTS

<u>Section</u>	<u>Page</u>
SUMMARY	1
INTRODUCTION	2
MATERIALS AND METHODS	2
Materials	2
Animals, housing, and feed	3
Experimental design	4
Clinical observations and physical, electro- cardiographic, ophthalmic examinations, water consumption, and urine volume	5
Clinical laboratory determinations	6
Test article bioavailability	8
Postmortem procedures	8
Statistical procedures	9
Data storage	10
Quality assurance	10
Professionals	10
RESULTS AND DISCUSSION	11
Quality and integrity of the data	11
Mortality and clinical observations	11
Body weights and feed and water consumption	11
Rectal temperatures	12
Clinical laboratory determinations	12
Electrocardiographic examinations	12
Ophthalmic examinations	12
Pathology	13
Organ weight and organ to body weight ratios	14
REFERENCES	15
Table 1    Mean male and female body weights	16
Table 2    Mean male and female body weight changes	18
Table 3    Mean male and female feed consumption	20
Table 4    Mean male, female, and pooled water consumption	32
Table 5    Mean weekly dosage	35

FOUR WEEK DIETARY ADMIX TOXICITY STUDY OF SC-19129  
IN THE DOG

TABLE OF CONTENTS (Cont.)

<u>Section</u>	<u>Page</u>
Table 6 Mean male, female and pooled rectal temperatures	36
Table 7.1 Mean male, female and pooled serum alanine aminotransferase activity	39
Table 7.2 Mean male, female and pooled serum aspartate aminotransferase activity	42
Table 7.3 Mean male, female and pooled serum alkaline phosphatase activity	45
Table 7.4 Mean male, female and pooled cholesterol concentration	48
Table 7.5. Mean male, female and pooled total bilirubin concentration	51
Table 7.6 Mean male, female and pooled serum glucose concentration	54
Table 7.7 Mean male, female and pooled serum urea concentration	57
Table 7.8 Mean male, female and pooled serum creatinine concentration	60
Table 7.9 Mean male, female and pooled serum sodium concentration	63
Table 7.10 Mean male, female and pooled serum potassium concentration	66
Table 7.11 Mean male, female and pooled serum chloride concentration	69
Table 7.12 Mean male, female and pooled serum calcium concentration	72
Table 7.13 Mean male, female and pooled serum total protein concentration	75
Table 7.14 Mean male, female and pooled serum albumin concentration	78
Table 7.15 Mean male, female and pooled serum globulin concentration	81
Table 7.16 Mean male, female and pooled serum albumin/globulin ratio	84
Table 8.1 Mean male, female and pooled hemoglobin	87
Table 8.2 Mean male, female and pooled hematocrit	90
Table 8.3 Mean male, female and pooled red blood cell count	93

FOUR WEEK DIETARY ADMIX TOXICITY STUDY OF SC-19129  
IN THE DOG

TABLE OF CONTENTS (Cont.)

<u>Section</u>	<u>Page</u>
Table 8.4 Mean male, female and pooled mean corpuscular volume	96
Table 8.5 Mean male, female and pooled mean corpuscular hemoglobin	99
Table 8.6 Mean male, female and pooled mean corpuscular hemoglobin concentration	102
Table 8.7 Mean male, female and pooled white blood cell count	105
Table 8.8 Mean male, female and pooled immature neutrophils (%)	108
Table 8.9 Mean male, female and pooled mature neutrophils (%)	111
Table 8.10 Mean male, female and pooled lymphocytes (%)	114
Table 8.11 Mean male, female and pooled monocytes (%)	117
Table 8.12 Mean male, female and pooled eosinophils (%)	120
Table 8.13 Mean male, female and pooled basophils (%)	123
Table 8.14 Mean male, female and pooled platelet count	126
Table 8.15 Mean male, female and pooled activated partial thromboplastin time	129
Table 8.16 Mean male, female and pooled pro-thrombin time	132
Table 9 Mean male, female and pooled urine refractive index	135
Table 10 Mean male, female and pooled urine volume	138
Table 11 Lesion Incidence by Animal Number	141
Table 12.1 Mean male organ weights, organ to body weight ratios, and organ to brain weight ratios	145
Table 12.2 Mean female organ weights, organ to body weight ratios, and organ to brain weight ratios	151

FOUR WEEK DIETARY ADMIX TOXICITY STUDY OF SC-19129  
IN THE DOG

TABLE OF CONTENTS (Cont.)

<u>Section</u>	<u>Page</u>
Appendix A    Body weight values	A1-A4
Table 1    Body weights	A1
Table 2    Body weight changes	A3
Appendix B    Feed consumption	B1-B12
Appendix C    Water consumption	C1
Appendix D    Actual dosages	D1-D8
Appendix E    Rectal temperature values	E1
Appendix F    Serum clinicochemical values	F1-F16
Table 1    Alanine aminotransferase activity	F1
Table 2    Aspartate aminotransferase activity	F2
Table 3    Alkaline phosphatase activity	F3
Table 4    Cholesterol concentration	F4
Table 5    Bilirubin concentration	F5
Table 6    Glucose concentration	F6
Table 7    Urea concentration	F7
Table 8    Creatinine concentration	F8
Table 9    Sodium concentration	F9
Table 10   Potassium concentration	F10
Table 11   Chloride concentration	F11
Table 12   Calcium concentration	F12
Table 13   Total protein concentration	F13
Table 14   Albumin concentration	F14
Table 15   Globulin concentration	F15
Table 16   Albumin/globulin ratio	F16
Appendix G    Hematological values	G1-G16
Table 1    Hemoglobin	G1
Table 2    Hematocrit	G2
Table 3    Red blood cell count	G3
Table 4    Mean corpuscular volume	G4
Table 5    Mean corpuscular hemoglobin	G5

FOUR WEEK DIETARY ADMIX TOXICITY STUDY OF SC-19129  
IN THE DOG

TABLE OF CONTENTS (Cont.)

<u>Section</u>	<u>Page</u>
Table 6 Mean corpuscular hemoglobin concentration	G6
Table 7 White blood cell count	G7
Table 8 Immature neutrophils (%)	G8
Table 9 Mature neutrophils (%)	G9
Table 10 Lymphocytes (%)	G10
Table 11 Monocytes (%)	G11
Table 12 Eosinophils (%)	G12
Table 13 Basophils (%)	G13
Table 14 Platelet count	G14
Table 15 Activated partial thromboplastin time	G15
Table 16 Prothrombin time	G16
Appendix H Urinalysis values	H1-H14
Table 1 Refractive index	H1
Table 2 Urine volume	H2
Table 3 pH	H3
Table 4 Glucose	H4
Table 5 Bilirubin	H5
Table 6 Protein	H6
Table 7 Ketones	H7
Table 8 Occult blood	H8
Table 9 Urobilinogen	H9
Table 10 Red blood cells	H10
Table 11 White blood cells	H11
Table 12 Casts	H12
Table 13 Crystals	H13
Table 14 Bacteria	H14
Appendix I Ophthalmic examination report	I1-I3
Appendix J Interim analytical summary	J1-J3
Appendix K Pathology	K1-K37
Explanatory notes for pathology tables	K1
Individual gross and microscopic findings	K2

FOUR WEEK DIETARY ADMIX TOXICITY STUDY OF SC-19129  
IN THE DOG

TABLE OF CONTENTS (Cont.)

<u>Section</u>	<u>Page</u>
Table 1 Individual male organ weights, organ to body weight ratios, and organ to brain weight ratios	K26
Table 2 Individual female organ weights, organ/body weight ratios, and organ to brain weight ratios	K32
Appendix L Protocol and amendments	L1-L16
Appendix M Quality assurance statement	M1

DEPARTMENT OF PRODUCT SAFETY ASSESSMENT

G. D. Searle & Co., Skokie, IL

Title: Four Week Dietary Admix Toxicity Study of SC-19129  
in the Dog

Author(s): James L. Allen, Curtis D. Port, and Robin C. Guy

Study No.: S.A. 2449

Date: February 4, 1985

Type of Report: Final

Summary:

SC-19129 was administered by dietary admix for four weeks to Beagle dogs (3/sex/dosage group) at intended dosages of 0, 250, 500, and 1000 mg/kg. The actual dosages based on body weight and average weekly consumption were generally 70-95% of the intended low dosage, 61-91% of the intended medium dosage, and 84-99% of the intended high dosage.

None of the animals died. There were no meaningful treatment-related clinical signs or changes in body weights, feed consumption, water consumption, rectal temperatures, clinical laboratory determinations, electrocardiograms, or ophthalmic examinations.

There were no treatment related gross or microscopic findings. No significant differences were found in organ weights or organ to body weight ratios of treated versus control animals.



## FOUR WEEK DIETARY ADMIX TOXICITY STUDY OF SC-19129 IN THE DOG

### INTRODUCTION

SC-19129 was administered by dietary admix for four weeks to Beagle dogs (3/sex/dosage group) at intended dosages of 0, 250, 500, and 1000 mg/kg. Control animals received untreated diet. The purpose of this study was to determine toxic effects as evidenced by clinical signs and organ damage and to provide a basis for selecting dosages for longer studies.

### MATERIALS AND METHODS

#### Materials

The chemical name of SC-19129 is N-L- $\beta$ -aspartyl-L-phenylalanine, 1-methyl ester.

The test article was administered in the diet at concentrations of 0, 1.25, 2.5, and 5%. The identity, strength, purity, and composition of SC-19129 (lot number 84K-047-101 (840413)) were determined. Samples were taken to determine the stability of neat chemical and the stability, homogeneity, and concentration of test article in carrier. These samples were frozen and will be analyzed at a future date. The results of these analyses will be provided as a separate report by the Product Development Analytical Department. A summary of the analytical data is in Appendix J.

The test article was stored in well-closed, light-resistant containers at controlled room temperature. The test article diet admixtures were stored in plastic bags in plastic containers at controlled room temperature during the course of the study.

SC-19129 was mixed with Purina Certified Canine Meal 5007. Premix concentrates were first prepared using a Turbula mixer and the final concentrations prepared with a Patterson-Kelly cross-flow blender. Diet admixtures were used within 1 week of preparation.

The amount of diet provided to each animal was based on individual body weights determined on the day prior to the start of the respective dosing week. The appropriate amount of test article or control diet (20 g/kg body weight) was provided to each animal for two hours in the morning. The animals then received approximately 200-300g of untreated diet for one hour. The actual dosages based on body weight and average weekly feed consumption were generally 70-95% of the intended low dosage, 61-91% of the intended medium dosage, and 84-99% of the intended high dosage. The actual dosages are presented in Table 5 and Appendix D.

#### Animals, housing, and feed

Twenty-four (12/sex) Beagle dogs (Hazleton Research Laboratories, Inc., Cumberland, VA), approximately 7-8 months of age and weighing 7-14 kg, were used. To ensure that healthy animals were selected, animals were screened within the 3 weeks before chemical administration.

Pretreatment data (PT) included body weights, physical examination findings (including rectal temperatures), electrocardiograms, urinalysis, hematological, and serum clinicochemical determinations.

During the dosing period, the animals were housed individually in stainless steel cages in a temperature ( $72^{\circ}\text{F} \pm 5^{\circ}\text{F}$ ) and humidity (25% or greater) controlled room maintained on a 12-hour light, 12-hour dark cycle. Each animal was identified by an ear tattoo applied by the supplier and a tag with a unique identification number attached to a collar. Purina Certified Canine Meal 5007 (Purina Lab Chows, St. Louis, MO) was provided to all animals. The feeding period was approximately 3 hours each day. Tap water from the municipal water supply was available ad libitum. Special analyses of food and water were not performed since no contaminants known to be capable of interfering with this study were reasonably expected to be present.

#### Experimental design

<u>Group</u>	<u>Intended Dosage (mg/kg)</u>	<u>Animals/Sex</u>	<u>Sacrifice Animals/Day</u>
1	0	3	3
2	250	3	3
3	500	3	3
4	1000	3	3

To distribute animals of each sex to dosage groups, the 12 animals were ranked by body weight. The ranked list was divided into 3 blocks of 4 animals and one animal

was randomly selected from each block and assigned to the first group. The same procedure was followed for the remaining 3 groups. The randomization achieved was evaluated by testing the significance of intergroup differences for each quantitative clinicochemical and hematological variable (except immature neutrophil, basophil, eosinophil, and monocyte percentages) and for urinary refractivity. Balance among the groups was achieved in 22 iterations for males and four iterations for females. The 4 groups were then assigned to dosage groups using a random number table. Permanent identification numbers were assigned to animals within each group according to the order which appeared on the randomization print out. During week 4, animals were randomly assigned to either the day 29 or 30 sacrifice.

Dosing began on October 18, 1984 and concluded on November 15, 1984. The sacrifices were on November 15 and 16, 1984. A copy of the protocol and amendments is Appendix L.

Clinical observations and physical, electrocardio-graphic, ophthalmic examinations, water consumption, and urine volume

Each animal was observed before and after feeding on weekdays and before feeding on weekends.

Physical examinations were performed before feeding on day 1 and after feeding on days 12 and 28. The physical examination consisted of the measurement of rectal temperature, observation of gait and general demeanor, palpation of the head, thorax, and abdomen, examination of

eyes, ears, and body orifices, and testing of the following reflexes: pupillary, patellar, front and rear hopping, and righting.

Body weights were determined on days 7, 14, 21, 29 and 30.

Food consumption was determined daily beginning within 3 weeks before initiation of dosing.

Electrocardiographic examinations (Leads II, aVL, V<sub>10</sub>) on restrained prone animals were recorded before feeding on day 1, and after feeding on days 12 and 28.

Ophthalmic examinations were performed on day 26. The direct and consensual pupillary light response was evaluated. The eyelids, membrana nictitans, conjunctiva, cornea, and iris were examined with the focal light source and 2.5X magnification. The pupils were then dilated with Mydriacyl (Alcon Laboratories, Inc., Fort Worth, TX). The adnexa, cornea, anterior chamber, and lens were examined under reduced illumination by biomicroscopy. The fundus was also evaluated.

Water consumption and urine volume were determined for approximate 24 hour periods during week 4.

#### Clinical laboratory determinations

Venous blood was collected before feeding on day 1 and after feeding on days 12, 15, and 28. All animals had slightly elevated urea and bilirubin concentrations on

day 12. To confirm this finding, the day 12 serum was reanalyzed and samples were collected on day 15 to analyze for urea and bilirubin concentrations. Samples were collected in evacuated tubes containing EDTA and evacuated tubes containing citrate for hematological determinations and in plain evacuated tubes for clinicochemical determinations. The following hematological parameters were determined: white blood cell count, red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, activated partial thromboplastin time, and prothrombin time. A differential smear evaluation also was done.

The following serum clinicochemical parameters were determined: alanine aminotransferase activity, aspartate aminotransferase activity, urea concentration, creatinine concentration, glucose concentration, alkaline phosphatase activity, cholesterol concentration, total bilirubin concentration, sodium concentration, potassium concentration, chloride concentration, calcium concentration, total protein concentration, albumin (A) concentration, globulin (G) concentration (calculated), and A/G ratio (calculated).

Urine was collected during weeks 2 and 4. The following urinary parameters were determined: pH, refractive index, glucose, bilirubin, protein, ketones, occult blood, and urobilinogen. Microscopic examination of the centrifuged sediment was also done.

Smears of bone marrow collected at necropsy were prepared but not examined.

#### Test article bioavailability

Venous blood samples (approximately 7 ml) were collected in evacuated tubes containing heparin from each animal before feeding on day 1, and 2, 4, 6, and 24 hours after the initiation of the feeding period on days 1, 15, and 28. Blood samples were centrifuged and plasma samples were frozen for future determination of SC-19129 concentrations. The results of these analyses will be provided as a separate report by the Department of Drug Metabolism.

#### Postmortem procedures

The dogs were killed by an overdose of sodium pentobarbital and examined immediately. The following organs were weighed in their fresh state: adrenal, brain, epididymis, heart, kidney, liver, ovary, pituitary gland, prostate, salivary gland, stomach (although not specified in the protocol), testis, thymus, thyroid, and uterus. Representative samples of the following organs and tissues were taken for microscopic examination: adrenal, aorta, bone, sternum, brain, epididymis, esophagus, eye, gallbladder, heart, duodenum, jejunum, ileum, cecum, colon, rectum, kidney, larynx, liver, lung, lymph node, mammary gland (females only), ovary, pancreas, peripheral nerve, pituitary gland, prostate, salivary gland, skeletal muscle, skin, spinal cord, spleen, stomach, testis, thymus, thyroid gland, parathyroid (only if included in the section of thyroid), tongue, trachea, urinary bladder, uterus, and vagina. Costal bone marrow was taken from each dog and submitted to hematology for preparation of a smear and

microscopic examination, if necessary. The testes were fixed in Bouin's solution and the eyes in Zenker's solution. All other tissues were fixed in Carson's solution (buffered 10% formalin). Any gross lesion that required microscopic examination was also placed in fixative.

After fixation, the tissues were embedded in paraffin, sectioned and stained with hematoxylin and eosin. Tissues of all dogs from all dosage groups were examined microscopically.

#### Statistical procedures

Means and standard deviations were calculated for all quantitative variables for each sex and for pooled sexes except those involving body weights, feed consumption, and organ weights. Analyses of these variables were done separately for each sex. Body weight, body weight change, water consumption, urine volume, clinicochemical, hematological (except immature neutrophil, monocyte, basophil, and eosinophil percentages), urinary refractivity, rectal temperature, organ weight, organ/body weight ratio, and organ/brain weight ratio data were analyzed by one-way analysis of variance (Winer, 1971) at each observation period. If the F-test among all groups was significant ( $p < 0.05$ ), two-tailed t-tests of each SC-19129-treated group versus the control group were done using the pooled error term from the one-way analysis of variance. If the F-test among all groups was not significant at the 5% level, the t-tests were not done. A homogeneity-of-variance test using the Bartlett-Box method (Box, 1949) was done on all variables mentioned above. Significances of the various tests are indicated in the tables.



### Data storage

The protocol and related documents, raw data, specimens, and final report are stored at G. D. Searle & Co. Skokie, Illinois.

### Quality assurance

The Quality Assurance Statement is in Appendix M.

### Professionals

The following professionals were involved in the conduct of this study:

Study Director/ Study Toxicologist	J. Allen
Study Pathologist	C. Port
Study Supervisor	R. Guy
Analytical Coordinator	K. Pilipauskas
Clinical Chemistry	J. North
Clinical Hematology	R. Leonard
Data Assessment	G. Kirby
Department of Drug Metabolism	E. Burton
Histology	P. Hemmer
Laboratory Animal Resources	J. Erickson
Ophthalmic Examinations	D. Vestre
Product Development Analytical Department	J. Jiu
Study Statistician	P. Sanders

## RESULTS AND DISCUSSION

### Quality and integrity of the data

There were no known circumstances that affected the quality or integrity of the data.

### Mortality and clinical observations

None of the animals died during the study.

Soft/watery stool was observed in all groups however, the incidence appeared to be slightly higher in the high dose group during weeks 3 and 4. The incidence of soft/watery stool at the high dosage is not unusual for laboratory Beagles. Other observations seen during the study were convulsions in a control animal on day 1, swollen vulva with a red discharge, emesis, and injected sclerae. These signs were of the type commonly seen in laboratory Beagles or occurred in a few instances with no particular pattern.

### Body weights and feed and water consumption

Mean values for body weights, body weight changes, food consumption, and water consumption are in Tables 1, 2, 3, and 4, respectively. Individual values are in Appendix A, B, and C.

There were no meaningful changes in body weights or feed and water consumption.

#### Rectal temperatures

Mean values for rectal temperatures are in Table 6 and individual values are in Appendix E.

There were no meaningful changes in rectal temperatures.

#### Clinical laboratory determinations

Mean values for clinical chemistry, hematology and urinalysis are in Tables 7, 8, and 9, respectively. Individual values are in Appendices F, G, and H.

There were increases in urea, total bilirubin concentrations, and globulin compared to pretreatment values in all groups. These changes may have been the result of the time of blood collection relative to feeding. That is, during pretreatment and on day 1 prior to SC-19129 administration, blood was collected from overnight fasted animals, while on days 12, 15 and 28 blood was collected after feeding. Other changes in clinical laboratory values were incidental and generally within the range of normal physiologic variation.

#### Electrocardiographic examinations

There were no meaningful electrocardiographic changes.

#### Ophthalmic examinations

The ophthalmic examination report is in Appendix I.

There were no compound related changes seen during the ophthalmic examination.

### Pathology

The incidence of microscopic findings is in Table 11. Individual animal findings are presented in Appendix K.

The only findings at necropsy were an abscess of the epididymis (84-2002) and petechial hemorrhage of the colon (84-2013). Dilated ventricles, annotated as hydrocephalus, were present when the brain of one dog was trimmed (84-2017). These were considered incidental and unimportant.

Microscopically the main findings were congenital tubular hypoplasia in two high dosage males and testicular germinal epithelial degeneration, considered to be due to immaturity, in one control dog. Various degrees of testicular immaturity were present in all the males. Because of the changes that are commonly seen in the testes of immature animals, identification and evaluation for possible chemical effects becomes difficult. The abscess of the epididymis was diagnosed as a sperm granuloma. The petechial hemorrhage in the colon was not confirmed microscopically, but this is not unexpected. Other microscopic findings, including valvular endocardiosis, cysts in the pituitary, thymus, thyroid, and parathyroid, as well as mineralization of the kidney medulla are known spontaneous conditions in dogs and are considered incidental and unimportant. Thus, there were no treatment related findings.

Organ weight and organ to body weight ratios

Mean organ weights and ratios are in Table 12. Individual organ weights and ratios are in Appendix K, Tables 1 and 2.

No significant differences were found in organ weights or ratios between control and compound treated animals.

## REFERENCES

- Box, G.E.P. (1949). A general distribution theory for a class of likelihood criteria, Biometrika, 36, pp. 317-346.
- Winer, B.J. (1971). Statistical Principles in Experimental Design, 2nd edition. McGraw-Hill, New York. pp. 149-185, 210-219.