JUL 2 1991

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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JUL 2 1991

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Arsenal (Imazapyr) FAP#8H5547/8H5548 - Imazapyr in/on Palm Oil and Refined Sugar - Submission of Chronic Toxicology Studies: A 2-Generation Rat Reproduction Study, a 2-Year Rat Combined Chronic Feeding/Oncogenicity Study, an 18-Month Mouse Oncogenicity Study, and a 1-Year Dog Chronic Feeding Study

Caswell No.: 221G
Project No.: 9-1144
Record No.: 242,420/242,421
MRID Nos.: 410395-01, -02, -03, -04, -05

FROM: William Dykstra, Reviewer
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THRU: Roger Gardner, Acting Section Head
Review Section I
Toxicology Branch I - Insecticide, Rodenticide Support
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06/14/91 (for RG)

6/14/91

Petitioner/Registrant: American Cyanamid

Requested Action

Review chronic toxicology studies submitted in support of food additive tolerances for palm oil (8H5547) and refined sugar (8H5548).

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Conclusions and Recommendations.

1. For the 1-year dog study, the NOEL is 10,000 ppm (HDT) and the study is acceptable as Guideline data.

2. With respect to the 2-generation rat reproduction study, the NOEL is 10,000 ppm (HDT) and the study is acceptable as Guideline data.

3. With respect to the 18-month mouse oncogenicity study, historical control data are required to establish a NOEL for pulmonary edema, a non-neoplastic lesion, in female mice. Additionally, a more detailed description of subscapular adrenal gland "cell reaction" is required.

   The oncogenic potential is negative up to 10,000 ppm (HDT) which is also the MTD for the study.

   The study is classified as Core-Supplementary which can be upgraded after review of the historical control data and the establishment of a NOEL for non-neoplastic lesions (pulmonary edema in female mice).

4. With respect to the 2-year rat study, additional information is required. The registrant is required to submit complete statistical analyses (utilizing survival analyses) for female adrenal medullary neoplasms and male brain neoplasms.

   The issue of thyroid C-cell carcinoma is resolved.

   There were no compound-related toxic effects in male food consumption, body weight, clinical pathology, organ weights, and non-neoplastic lesions. There was a slight dose-related decrease in survival of high-dose male rats, but not female rats.

   There were no compound-related toxic effects in female rats with respect to body weight and food consumption (although food efficiency in female rats showed a marginal toxic effect). Additionally there were no compound-related toxic effects in clinical pathology and organ weights.

   The NOEL for non-neoplastic lesions in female rats is the mid-dose of 5000 ppm. The LEL is the high-dose of 10,000 ppm and the effects are an increased incidence of extramedullary hematopoiesis in the spleen and B-squamous cysts in the thyroid. Both of these lesions are not considered life-threatening.

The study is classified as Core-Supplementary which can be upgraded after review of additional data. The registrant needs to submit information relevant to the consideration of the MTD for this study. If it is concluded that the oncogenic potential is negative, the establishment of an MTD is needed to upgrade the study to an acceptable classification.

Reviewed By: William Dykstra 7/16/89
Section I, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Robert Zendzian 7/15/89
Section I, Toxicology Branch I - IRS (H7509C)
06/14/91 (for RG)

DATA EVALUATION REPORT

Study Type: 83-1 - Chronic Toxicity - Dog TOX Chem No.: 221G

Accession No.: N/A MRID No.: 410395-02 Vol. 1-5

Test Material: AC 243,997; Purity 99.5%; Lot No. AC 4866-62

Synonyms: Imazapyr, Arsenal

008426

Study Number(s): TL Project No. 86002

Sponsor: American Cyanamid

Testing Facility: Tegeris Laboratories

Title of Report: One-Year Dietary Toxicity Study in Purebred Beagle Dogs with AC 243,997.

Author(s): Thomas Shellenberger

Report Issued: May 2[illegible], 1987

Conclusions:

The NOEL is 10,000 ppm (HDT).

There were no compound-related effects in toxic signs, mortality, body weight, food consumption, ophthalmologic evaluations, hematology, clinical chemistry, urinalyses, gross pathology, organ weights, and non-neoplastic and neoplastic lesions.

Classification: Core-Guideline

Special Review Criteria (40 CFR 154.7): N/A

Review:

One-Year Dietary Toxicity Study in Purebred Beagle Dogs with AC 253,997 (Tegeris Laboratories Project No. 86002; May 29, 1987).

A. Materials:

1. Test Material - AC 243,997; Purity: 99.5%; Lot No. AC 4866-62; white powder.

2. Animals - 56 purebred beagle dogs (28 male and 28 female) were received from Laboratory Research Enterprises, Inc., Kalamazoo, MI. Upon arrival, the dogs were examined and found to be healthy. They were injected with Iron Dextran. All dogs received a fecal examination for ova and parasites and were found to be negative. The dogs were quarantined for 2 weeks, observed daily, and at the end of the quarantine, all dogs found healthy were placed on test. The dogs were approximately 5 to 6 months of age at initiation of dosing. Dogs were fed 400 g of Purina Certified Canine Chow, 5007 Meal. Males and females were housed in two rooms at 22 ± 3 °C, 30 to 70 percent humidity, and with fluorescent lighting on approximately a 12-hour light/dark cycle. The animals were housed individually.

B. Study Design:

Randomized groups of six male and six female purebred beagle dogs were fed dietary levels of 0 (control), 1000, 5000, and 10,000 ppm of the test material for 1 year as shown below:

1. Animal Assignment

Test Group	Dose in Diet (ppm)	Main Study 12 Months Male	Female
1 Control	0	6	6
2 Low (LDT)	1000	6	6
3 Mid (MDT)	5000	6	6
4 High (HDT)	10,000	6	6

2. Diet Preparation - Diet was prepared weekly and stored at room temperature. Samples of treated food were analyzed for stability and concentration at weekly intervals.

   Results - Initial samples of diet were analyzed for homogeneity at the top, middle, and bottom of the prepared feed. Concentrations of AC 243,997 from the 1000 ppm batch ranged from 927 to 959 ppm with a mean of 943 ppm. Initial samples at 10,000 ppm ranged from 8982 to 9891 ppm with a mean of 9275 ppm. It was concluded that the mixing of the diet was acceptable.

   With respect to stability, AC 243,997 concentrations in the diet after 1 and 24 hours in exposed feeders and after 7 and 24 days in lidded, opaque plastic pails were 957, 985, 940, and 974 ppm (range of 94.0 to 98.5%) for the 1000 ppm batch. At 10,000 ppm, the concentrations were 9638, 9371, 9560 and 10,500 ppm (range of 93.7 to 105.0%). It was concluded that stabilty was adequate.

   With respect to concentrations of AC 243,997 in weekly canine diets for 52 weeks, it was found that control diets were free of technical. At 1000 ppm, assays averaged 98.8 percent of nominal levels. At 5000 ppm, assays averaged 98.6 percent of nominal. At 1000 ppm, assays averaged 97.3 percent of nominal concentrations. It was concluded that AC 243,997 was stable in the diet during the 52-week study.

3. Animals received food (Purina-Certified Canine Chow) and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data: ANOVA using t-test for various comparisons and if means were significant, Dunnett's t-test at the 95 percent confidence limits.

   The following parameters were subject to this analysis: mean body weight data, mean feed consumption data, mean values for hematology and clinical chemistries, and mean organ weight, organ-to-body weight ratios, and organ-to-brain weight ratios.

5. Quality assurance was performed and signed by the QA officer.

C. Methods and Results:

1. Observations - Animals were inspected twice daily for signs of toxicity and mortality and a detailed observation was conducted weekly.

   Results - There were no deaths and no compound-related toxic signs.

   With respect to female dogs, there were four control animals, five low-dose animals, four mid-dose animals, and three high-dose animals with clinical signs reported. The most common clinical signs were alopecia, lesions (cuts and abrasions), and wart-like masses. Female dog #2446 of the high dose at week 9 had a light brown mucoid material found in feces with blood.

   With respect to male dogs, there were five control animals, two low-dose animals, three mid-dose animals, and two high-dose animals that were reported with clinical signs. Three control male dogs and one mid-dose dog had white mucoid material in feces. One mid-dose dog (#2435) had excessive salivation and one high-dose dog had a prolapsed penis.

   The most common clinical signs in all groups were alopecia and lesions (cuts and abrasions).

2. Body Weight - The animals were weighed weekly for the duration of the study.

   Results - Treated male dogs had about 3 to 14 percent higher body weight gains in comparison to control male dogs during the study. For males, the mean body weights at initiation of the study were 7.37, 7.50, 7.55, and 7.47 kg for the control, low-, mid-, and high-dose groups, respectively. At week 26 of the study, the mean body weights were 10.65, 11.97, 11.22, and 12.18 kg for the control, low-, mid-, and high-dose groups, respectively. At week 52, the mean body weights were 10.85, 12.17, 11.80, and 12.42 kg for the control, low-, mid-, and high-dose groups, respectively.

   Although the body weights of treated male dogs were higher than controls, this finding is not considered toxicologically significant. The increase in body weight gain at the high-dose was essentially due to male dog #2449 which weighed 14.5 kg on week 26, 15.4 kg at week 40, and 16.0 kg at week 52. In comparison, the other high-dose dogs had weight changes comparable to controls.

Female dogs at the high dose showed body weight gain decreases ranging from 7 to 13 percent in comparison to controls, although the differences were not statistically significant. However, the decreases in body weight for the high-dose females are not considered a compound-related toxic effect but are considered due to decreased food consumption (see below).

Body weight gain was comparable between control and mid-dose female dogs and slightly decreased (5 to 7%) in low-dose dogs in comparison to controls.

Therefore, the NOEL for body weight gain for male and female dogs is the high-dose of 10,000 ppm.

3. Food Consumption - Individual feed consumption was recorded daily and summarized on a weekly basis as mean daily feed consumption (g/dog/day).

Results - With respect to male dogs, treated groups showed 7 to 12 percent increases in feed consumption in comparison to controls. The increased mean food consumption values were statistically significant on several occasions for the treated male dogs in comparison to controls. However, the increases in food consumption were not dose-related, since low-dose dogs had the greatest increases followed for the most part by high-dose dogs and then mid-dose dogs. Additionally, increased food consumption is not considered a toxic effect.

Food consumption in high-dose female dogs was decreased 8 to 14 percent in comparison to controls up to week 26. Following week 26, the food consumption in high-dose female dogs exceeded the controls, were similar to controls or showed a 3 to 6 percent decrease in comparison to controls. The decreases in food consumption at the high-dose is not considered a toxic effect, but is due to a decrease in diet palatability during the first 6 months. Food consumption between control and low- and mid-dose female dogs was generally similar.

The NOEL for food consumption in male and female dogs is 10,000 ppm (HDT).

4. Ophthalmalogical Examinations were performed on all animals at pretest, 6 months, and at 1 year.

Results - At pretest, ocular examinations were conducted by L.F. Rubin. There were no compound-related ocular effects.

At 6 months, an ocular examination was conducted by S. Koch. Results show a granuloma in male control dog #2409, corneal scar in low-dose male dog #2423, and perinuclear rings in the lens of both eyes of high-dose male #2447.

At termination, a 12-month ocular examination was conducted by S. Koch. Results show that fullness to the optic nerve was observed in high-dose female #2440.

There were no compound-related effects in ophthalmological findings at pretest, 6 months, and 12 months.

5. Blood was collected before treatment and at 6 weeks and at 3, 6, and 12 months for hematology and clinical analysis from all animals. The checked (X) parameters were examined. All dogs were fasted approximately 18 hours prior to collection of blood samples.

a. Hematology

X	Hematocrit (Hct)*	X	Total plasma protein (TP)
X	Hemoglobin (HgB)*	X	Leukocyte differential count
X	Leukocyte count (WBC)*	X	Mean corpuscular HgB (MCH)
X	Erythrocyte count (RBC)*	X	Mean corpuscular HgB conc. (MCHC)
X	Platelet count*	X	Mean corpuscular volume (MCV)

Results

1) Males

Pretest and 6 Weeks - No statistically significant effects and no dose-related trends were observed, and values for control and treated male dogs were similar.

3 Months - No statistically significant effects and no dose-related trends were observed, and values for control and treated males dogs were similar.

6 Months - No statistically significant effects and no dose-related trends were observed, and values for control and treated males dogs were similar.

12 Months - No statistically significant effects, and no dose-related trends were observed, but mean hematocrit (Hct), RBC and hemoglobin (HgB) values in high-dose dogs were slightly decreased by 6 to 8 percent in comparison to controls (Hct in controls = 49.783%, Hct in high-dose = 46.800%). Individual values for Hct in control dogs ranged from 47.500 to 51.90 percent at 12 months in comparison to high-dose individual Hct values of 44.600 to 48.700 percent.

Additionally, there was also a slight decrease in mean RBC and HgB values in high-dose dogs in comparison to controls (mean values for RBC in controls = 7.490 x $10^6/mm^3$; mean values for RCB in high-dose = 7.012 x $10^6/mm^3$); (mean HgB in controls = 16.417 g/dL, mean HgB in high-dose = 15.350 g/dL).

Individual RBC values at 12 months in controls ranged from 7.260 to 7.720 x $10^6/mm^3$ in comparison to individual values in the high-dose dogs which ranged from 6.540 to 7.470 x $10^6/mm^3$.

Individual HgB values at 12 months in controls ranged from from 15.600 to 17.200 g/dL in comparison to individual values in the high-dose which ranged from 14.700 to 16.100 g/dL.

Although there were slighly lower individual values for Hct, RBC, and HgB in high-dose dogs at 12 months in comparison to control dogs, the individual values are all within the range of normal laboratory values for male dogs. Historical control data from the open literature are attached.

2) Females

Pretest and 6 Weeks - The values at pretest were comparable between control and treated dogs. At 6 weeks, high-dose females had a statistically significant mean increase in the band cells (%) in comparison to controls. Mean values in controls were 0.333 in comparison to 2.167 in high-dose. Individual control values for the bands ranged from 0.000 to 2.000 percent in comparison to the high-dose values which ranged from 0.000 to 4.000 percent. Additionally, individual control values for bands were all 0.000 percent except for female dog #2412 which had a 2.000 percent value. In

contrast, individual high-dose values for bands had only one 0.000 percent value and all other values were between 1.000 and 4.000 percent.

However, this distribution of band cells is essentially within the normal range of 1.7 ± 2.5% as shown in the attached open literature material (page 110 of Schalm's Veterinary Hematology (1986)).

Mean values for platelets were significantly increased at the mid-dose (385.833 x $10^3/mm^3$) in comparison to controls (307.500 x $10^3/mm^3$). The slight increase in mean values of platelets at the mid-dose was not dose-related and is not considered compound-related.

3 Months - At 3 months, as at 6 weeks, high-dose females had statistically significant increases in mean values for band cells (%) in comparison to controls. The means were 2.167 percent for the high-dose dogs in comparison to 0.500 percent for controls. Individual values at the high-dose ranged from 1000 to 5.000 percent whereas controls were 0.000 to 1.000 percent. These values are essentially within the normal range of 1.7 ± 2.5% (page 110, attached).

Also observed at 3 months in female dogs were ~ to 10 percent decreases in Hct and HgB at all dose levels.

The low-dose and mid-dose mean values were statistically significantly decreased for HgB and Hct.

The range of individual values for HgB were between 15.600 to 18.500 g/dL for controls, 13.900 to 15.000 g/dL for low-dose, 14.300 to 15.800 g/dL for mid-dose, and 14.00 to 15.600 g/dL for the high-dose. Although the HgB in treated female dogs appears to be slightly decreased, the values are within the normal range.

The range of individual values for Hct were between 45.100 to 56.300 percent for controls, 41.300 to 47.500 percent for low-dose, 42.000 to 47.200 percent for mid-dose and 42.400 to 49.800 percent for high-dose dogs. Although the Hct in treated female dogs appears to be slightly decreased, the values are within the normal range.

6 Months - At 6 months, mean HgB values for treated female dogs were decreased in comparison to controls and the mean low-dose and high-dose values were statistically significantly decreased.

The ranges of individual values for HgB were between 15.700 to 18.400 g/dL for control, 14.700 to 16.200 g/dL for low-dose, 14.100 to 16.900 g/dL for mid-dose, and 14.900 to 15.200 g/dL for the high-dose.

Although the HgB appears to be slightly decreased, the values are within the normal range.

12 Months - There were no statistically significant effects or dose-related trends for hematological parameters at 12 months. Mean and individual values were comparable between control and treated dogs. Historical control data from the open literature are attached.

b. Clinical Chemistry

X		X	
	Electrolytes:		Other:
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
X	Phosphorous*	X	Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	Enzymes	X	Total Bilirubin/direct bilirubin*
X	Alkaline phosphatase	X	Albumin/globulin ratio
	Cholinesterase	X	Total protein
X	Creatinine phosphokinase*		
X	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
X	Gamma-glutamyltranspeptidase (GGTP)		

Results

1) Males

Pretest and 6 Weeks - There were no compound-related effects. There were no statistically significant differences in treated male dogs in comparison to controls. They were no significant trends.

3 Months - Fasting blood sugar mean values were statistically significantly increased in low- and high-dose groups in comparison to controls. The mean values were 88.917, 98.067, 96.400, and 99.967 mg/dL in the control, low-, mid-, and high-dose groups, respectively.

Individual values ranged between 85 to 93 mg/dL in controls, 88 to 107 mg/dL in the low-dose, 92 to 107 mg/dL in the mid-dose, and 94 to 103 mg/dL in the high-dose. These individual values are within the normal range for dogs.

6 Months - Mean potassium values of the low-, mid-, and high-dose groups were statistically significantly increased in comparison to controls. Mean values were 4.497, 4.812, 4.822, and 4.898 meq/L for the . control, low-, mid-, and high-dose groups, respectively

Individual values for potassium ranged between 4.210 to 4.950 meq/L in the controls, 4.530 to 5.560 meq/L in the low-dose, 4.340 to 5.520 meq/L in the mid-dose, and 4.390 to 4.940 meq/L in the high-dose. These individual values are within the normal range for dogs.

12 Months - Mean total protein was statistically significantly decreased in high-dose dogs in comparison to controls. Mean values were 7.4, 7.2, 7.2, and 6.8 g/dL in the control, low-, mid-, high-dose groups, respectively.

Individual values for total protein ranged between 7.0 to 7.9 g/dL in the controls, 6.8 to 7.9 g/dL in the low-dose, 6.9 to 7.5 in the mid-dose, and 6.5 to 7.2 g/dL in the high-dose. These individual values are within the normal range for dogs.

Mean potassium values were statistically significantly decreased in high-dose dogs in comparison to controls. The mean value for controls was 4.5 meq/L in comparison to 4.1 meq/L in the high-dose. Individual potassium values ranged between 4.250 to 4.920 meq/L in the controls in comparison to a range of 3.780 to 4.370 meq/L in the high-dose. These individual values are within the normal range for dogs.

2) Females

Pretest and 6 Weeks - In the pretest, the mean values for A/G ratio were statistically significantly increased in high-dose dogs in comparison to controls. This finding is considered random and unrelated to treatment, since it occurred before dietary exposure to the test material was initiated.

Mean cholesterol values for mid-dose female dogs were increased in comparison to controls. The mean values were 153, 146, 197, and 148 for the control, low-, mid-, and high-dose groups, respectively.

Since this increase in cholesterol values occurred at the mid-dose only and is not dose-related, it is not considered compound-related.

3 Months - Mean albumin values were statistically significantly decreased in mid-dose dogs in comparison to controls. Mean values were 3.6, 3.3, 3.2, and 3.4 g/dL in the control, low-, mid-, and high-dose groups, respectively. Since this slight decrease in albumin values occurred at the mid-dose and was not dose-related, it is not considered compound-related. Also observed at 3 months in female dogs was a statistically significant decrease in the low-dose mean values of potassium in comparison to controls.

Mean values were 4.993, 4.362, 4.838, and 4.442 meq/L for the control, low-, mid-, and high-dose groups, respectively.

Since this slight decrease in potassium level occurred in the low-dose only, and was not dose-related, it is not considered compound-related.

6 Months - Mean values for cholesterol were statistically significantly increased in mid-dose dogs in comparison to controls. The mean values were 166, 155, 206, and 148 for control, low-, mid-, high-dose groups, respectively. Since this finding occurred only at the mid-dose and was not dose-related, it is not considered compound-related.

Mean values for calcium were statistically significantly decreased in low-dose females in comparison to controls. The mean values were

11.3, 10.8, 11.1, and 10.9 mg/dL for the control, low-, mid-, and high-dose groups, respectively.

Since the slight decrease in calcium levels occurred only in the low-dose, and was not dose-related, it is not considered compound-related.

12 Months - There were no statistically significant differences between control and treated mean values for female dogs at 12 months. Additionally, there were no significant trends in any parameter which was suggestive of a compound-related effect.

6. Urinalysis - Urine was collected from fasted animals at pretest, 6 weeks, 3 months, 6 months, and 12 months. The CHECKED (X) parameters were examined.

X		X	
|X|	Appearance*	|X|	Glucose*
| |	Volume*	|X|	Ketones*
|X|	Specific gravity*	|X|	Bilirubin*
|X|	pH	|X|	Blood*
|X|	Sediment (microscopic)*	| |	Nitrate
|X|	Protein	|X|	Urobilinogen

Results

a. Males - There were no compound-related effects in urinalyses parameters at any sampling interval. Mean values for specific gravity and pH were comparable between control and treated dogs and no significant trends were observed.

b. Females - There were no compound-related effects in urinalyses parameters at any sampling interval. Mean values for specific gravity and pH were comparable between control and treated dogs and no significant trends were observed.

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X	Digestive system	X	Cardiovasc./Hemat.	X	Neurologic
X	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	XX	Pituitary*
X	Duodenum*	X	Spleen*	X	Eyes (optic n.)*
X	Jejunum*	XX	Thymus*		Glandular
X	Lleum*		Urogenital	X	Adrenals*
X	Cecum*	XX	Kidneys*		Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Mammary gland
X	Rectum*	XX	Testes*	XX	Parathyroids*
XX	Liver	X	Epididymides	XX	Thyroids*
X	Gallbladder*	X	Prostate		Other
X	Pancreas*		Seminal vesicle	X	Bone*
	Respiratory	X	Ovaries	X	Skeletal muscle*
X	Trachea*	XX	Uterus*	X	Skin
XX	Lung			X	All gross lesions and masses

Results:

a. Organ Weight - There were no compound-related effects in organ weight, organ-to-body weight or organ-to-brain weight in male and female dogs. Additionally, there were no statistically significant differences between control and treated animals and no significant trends were observed.

b. Gross Pathology - There were no compound-related gross lesions in male and female dogs. The gross findings observed occurred at comparable incidences between control and treated animals and no significant trends were observed.

The most frequent gross lesions were observed in the lymph nodes but were observed with comparable incidences among control and treated groups.

Mammary gland congestion was seen in one control, one mid-dose, and three high-dose female dogs but is considered incidental to the age of the females rather than a compound-related effect.

c. Microscopic Pathology

1) Non-neoplastic - There were no compound-related microscopic effects observed in male and female dogs.

In males, microscopic evaluations most frequently observed were one mid-dose male with interstitial pneumonia; two control and one high-dose male with atrophy of the mesenteric lymph nodes; two high-dose dogs with pituitary Rathke's pouch cyst; one low-dose male dog with organizing hemorrhage of the brain; one control, two low-dose, two mid-dose, and two high-dose male dogs had pigment in the cervical lymph nodes.

In female dogs, microscopic evaluations most frequently observed were one control dog with acute periadenitis of the mediastinal lymph nodes; one control dog with interstitial nephritis; two mid-dose dogs with atrophy of mesenteric lymph nodes; one high-dose dog with chronic cholecystitis of the gallbladder; one control, one low-dose, and one mid-dose dog with simple cysts of the ovaries; one mid-dose dog with pituitary Rathke's pouch cyst; one high-dose dog with simple cysts of the pituitary; and a high-dose dog with organizing hemorrhage of the spinal cord; one control dog with metastatic calcification of the brain; and one control, three low-dose, one mid-dose and one high-dose dogs with pigment cervical lymph nodes.

2) Neoplastic - There were no compound-related neoplastic lesions in male and female dogs.

In males, one high-dose dog had a small parasitic granulomata of the lungs, and one mid-dose dog had a subpleural parasitic granulomata of the lung.

In females, one mid-dose and one high-dose dog had small parasitic granulomata of the lungs, and one mid-dose dog had a subpleural granuloma of the lung.

Attachments

Schalm's
VETERINARY HEMATOLOGY

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School of Veterinary Medicine
University of California
Davis, California

FOURTH EDITION

Lea & Febiger *Philadelphia* *1986*

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4

The Dog: Normal Hematology with Comments on Response to Disease

Normal blood values for the dog, as reported in the older literature, were commonly developed from small numbers of dogs used in the study of physiologic, pharmacologic, or medical problems. More recently, extensive use of the beagle dog in biomedical research and the use of the electronic cell counter and the microhematocrit have provided more reliable values (Andersen and Schalm, 1970). Mongrel dogs obtained from the pound are less reliable for normal data, unless carefully selected for freedom from occult disease and conditioned for several months before data collection (Porter and Canaday, 1971; Soave and Boyle, 1965). Similarly, red cell parameters may be lower in undernourished (but not anemic) dogs than in well-nourished dogs (Oduye, 1978). The veterinarian should be cognizant that normal values from a cross section of the population (see chart) may be too wide, while those from control animals or closed colonies used in experimental investigations may be too narrow for application to clinical situations (Tvedten, 1981).

Normal blood values for basenji (Table 4-1) and beagle (Table 4-2) dogs of various age groups are given. These values, although based on a limited number of observations, compare favorably with the more extensive reports on the beagle dogs. Blood values, particularly red cell parameters (RBC, Hb, and PCV), have been found to vary with age and nutritional status of the animal, and diurnal and seasonal variations may occur (Andersen and Gee, 1958; Andersen and Schalm, 1970; Tsessarskaya and Burkovskaya, 1976). Data on hematologic changes to 60 days of age in clinically normal beagles have been published (Earl et al., 1973; Shifrine et al., 1973). Selected recent references on normal hematologic values in the dog are given (Brunk and Becker-Berger, 1980; Konrad et al., 1980; Lumsden et al., 1979).

THE ERYTHROCYTES

Erythrocyte Morphology

Canine erythrocytes are typically biconcave discs about 7.0 μm in diameter and 1.7 μm

Normal Blood Values for the Dog

Erythrocyte Series	*Range*	*Ave*
Erythrocytes (× 10⁶/μl)	5 5 - 8 5	6 8
Hemoglobin (g/dl)	12 0 18 0	15 0
PCV (%)	37 0 55 0	45 0
MCV (fl)	60 0 77 0	70 0
MCH (pg)	19 5 24 5	22 8
MCHC (%)		
Wintrobe	31 0-34 0	33 0
Microhematocrit	32 0-36 0	34 0
Reticulocytes (%)	0 0- 1 5	0 8
ESR (see Table 2-6) varies with PCV		
RBC diameter (μm)	6 7 - 7 2	7 0
RBC life span (days)	100 120	
Resistance to hypotonic saline (%)		
Min	0 40 0 50	0 46
Max	0 32 -0 42	0 33
Myeloid erythroid ratio	0 75–2 5 1 0	1 2 1 0
Other Data		
Thrombocytes (× 10⁵/μl)	2 5	
Icterus index (units)	2 5	
Total plasma proteins (g/dl)	6 0 8 0*	
Plasma fibrinogen (g/dl)		
Narrow range	0 2 0 4	
Broad range	0 1 0 5	

Leukocyte Series	*Range*	*Ave.*
Leukocytes/μl	6,000–17,000	11,500
Neutrophil (band)	0- 300	70
Neutrophil (mature)	3,000-11,500	7,000
Lymphocyte	1,000 4,800	2,800
Monocyte	150 - 1,350	750
Eosinophil	100 - 1,250	550
Basophil	rare	0
Percentage Distribution		
Neutrophil (band)	0- 3	0 8
Neutrophil (mature)	60-77	70 0
Lymphocyte	12-30	20 0
Monocyte	3-10	5 2
Eosinophil	2-10	4 0
Basophil	rare	0 0

*Varies with age, see Table 4-1

in thickness. They exhibit a distinct central pallor and slight anisocytosis (Plate XII-1). Minor artifactual crenation occurs frequently, but other shape transformations are abnormal. Slight rouleau formation is common. An occasional polychromic erythrocyte is a common finding that correlates well with the normal number (less than 2.0%) of reticulocytes in peripheral blood. An occasional nucleated erythrocyte and a rare red cell with a Howell-Jolly body may be encountered. Increased numbers of erythrocytes with Howell-Jolly bodies and, less commonly, nucleated erythrocytes appear in the blood of dogs on continuous corticosteroid therapy. These findings are a reflection of suppressed splenic function (Chapter 13; Fig. 13-21). Occasionally, hemoglobin crystals may be seen within and outside the red cells in the blood of young dogs (Lund, 1974; Spurling, 1977). This finding has been attributed to the immaturity of the mononuclear phagocyte system. Abnormalities of erythrocyte shape (Chapter 20) during disease are more often encountered in the dog and cat than in other animal species. Stomatocytic erythrocytes were found in Alaskan malamutes with hereditary hemolytic anemia (Pinkerton et al., 1974), and spheroechinocytes were observed in a basenji dog with anemia due to hereditary deficiency of pyruvate kinase (Chandler et al., 1975).

Erythrocyte Parameters and Wintrobe Indexes in Relation to Age

At birth, the erythrocytes of fetal origin are large; MCV is 95–100 fl (Andersen and Schalm, 1970; Ederstrom and DeBoer, 1946). As fetal erythrocytes are replaced by cells of smaller size, MCV becomes reduced, so that by 2–3 months of age, erythrocyte size is representative of the normal adult dog. Similarly, MCH is about 33 pg at birth and decreases to about 22 pg by 2 months of age (Shifrine et al., 1973). MCHC varies only slightly with age, being about 35% at birth and 33% at 2 months of age, and remaining constant at 32% irrespective of changes in PCV (Shifrine et al., 1973). MCHC may fluctuate within the normal range by 3–5% due to physiologic and technical variations.

Erythrocyte number, Hb, and PCV values are high at birth, but fall rapidly as the pup begins to nurse. Reduction of these values continues during the first month of life. These changes are related to increased destruction

Table 4-1. Influence of Age on the Canine Hemogram in Basenji Dogs* (means and 1 SD)

	Age				
	6–8 weeks	9–12 weeks	4–6 months	1–2 years	>2 years
Number of dogs	24	21	9	13	7
RBC (× 10⁶/μl)	4.73 ± 0.38	5.43 ± 0.54	6.56 ± 0.46	6.91 ± 0.60	7.19 ± 0.64
Hemoglobin (g/dl)	10.4 ± 0.58	11.8 ± 0.81	14.4 ± 0.82	15.9 ± 1.2	16.6 ± 1.1
PCV (%)	33.1 ± 2.2	37.2 ± 2.9	44.0 ± 2.4	49.3 ± 3.4	49.8 ± 3.4
MCV (fl)	70.1 ± 2.9	68.6 ± 2.9	67.2 ± 2.9	71.1 ± 4.0	69.6 ± 4.1
MCH (pg)	22.1 ± 1.4	21.8 ± 1.5	21.9 ± 0.9	23.0 ± 0.8	23.2 ± 1.8
MCHC (%)	31.5 ± 1.4	31.8 ± 1.3	32.7 ± 0.6	32.3 ± 1.2	33.3 ± 0.41
Icterus index units	1.3 ± 0.9	2.8 ± 1.9	0.66 ± 0.94	2.2 ± 2.3	2.0 ± 2.1
Plasma proteins (g/dl)	5.33 ± 0.29	5.87 ± 0.46	6.6 ± 0.25	7.03 ± 0.33	7.5 ± 0.24
Fibrinogen (g/dl)	0.18 ± 0.07	0.20 ± 0.08	0.22 ± 0.07	0.22 ± 0.08	0.20 ± 0.06
Reticulocytes (%)	3.4 ± 1.0	2.6 ± 1.8	0.24 ± 0.41	0.84 ± 0.9	0.34 ± 1.12
WBC/μl	13,433 ± 2,045	15,033 ± 2,077	13,389 ± 1,751	14,031 ± 2,270	12,157 ± 1,987
Percentage distribution of WBC					
Band neutrophils	0 63 ± 0.77	0.57 ± 1.1	0 61 ± 0.81	0.27 ± 0.42	0 07 ± 0.17
Segmenters	58.8 ± 10.9	56.4 ± 7.8	52.4 ± 5.5	58.1 ± 7.3	66.4 ± 6.7
Lymphocytes	30.1 ± 8.1	33.5 ± 8.1	36.9 ± 5.5	28.6 ± 7.7	23.1 ± 4.8
Monocytes	6.9 ± 2.6	6.7 ± 2.7	6.0 ± 1.8	5.2 ± 2.1	4.0 ± 1.4
Eosinophils	3.3 ± 1.9	2.3 ± 1.6	4 1 ± 1.9	7.3 ± 3.5	6.3 ± 2.3
Basophils	0.08 ± 0.24	0.07 ± 0.23	0 0 ± 0.0	0.12 ± 0.4	0 14 ± 0.52
Absolute numbers of WBC/μl					
Band neutrophils	85 ± 101	94 ± 186	86 ± 117	38 ± 62	8 ± 21
Segmenters	8,013 ± 2,387	8,463 ± 1,638	7,196 ± 1,509	8,169 ± 1,732	8,107 ± 1,864
Lymphocytes	3,963 ± 1,059	5,099 ± 1,553	4,948 ± 510	4,023 ± 1,207	2,905 ± 634
Monocytes	912 ± 323	986 ± 405	818 ± 311	715 ± 266	495 ± 191
Eosinophils	426 ± 259	341 ± 243	538 ± 225	990 ± 455	727 ± 229
Basophils	10 ± 29	10 ± 33	0 ± 0	17 ± 58	15 ± 23

*Normal basenji dogs reared by several commercial breeders. Data gathered in cooperation with Dr. G.O. Ewing, formerly at the Veterinary Medical Teaching Hospital, University of California, Davis. For additional data, see Ewing et al., 1972.

Table 4-2. Blood Values in Normal Beagles to 2 Months of Age

	Age				
	0–3 days	14–17 days	28–31 days	40–45 days	56–59 days
Number of dogs	40	46	48	44	42
RBC (× 10⁶ µl)	[illegible]	[illegible]	3.9 ± 0.4	[illegible]	[illegible]
Hemoglobin (g/dl)	[illegible]	[illegible]	9.6 ± 0.9	[illegible]	[illegible]
PCV (%)	[illegible]	[illegible]	28.4 ± 2.5	[illegible]	[illegible]
MCV (fl)	[illegible]	[illegible]	71 ± 3.5	[illegible]	[illegible]
MCH (pg)	32.7 ± 1.8	[illegible]	24.3 ± 1.0	[illegible]	[illegible]
MCHC (%)	34.0 ± 1.4	34.3 ± 1.6	33.5 ± 1.4	[illegible]	[illegible]
NucRBC/100 WBC	[illegible]	[illegible]	1.1 ± 1.5	[illegible]	[illegible]
Reticulocytes (%)*	6.5	[illegible]	5.8	4.5	[illegible]
WBC/µl	16,800 ± 5,700	13,600 ± 4,400	13,900 ± 3,300	15,300 ± 3,700	[illegible] ± 4,400
Absolute number of WBC/µl					
Band neutrophils	600 ± 500	200 ± 200	100 ± 200	300 ± 200	300 ± 300
Segmenters	9,200 ± 6,600	[illegible] ± 3,100	6,800 ± 2,000	[illegible] ± 2,400	[illegible]
Lymphocytes	3,700 ± 2,300	[illegible] ± 1,700	5,400 ± 1,600	[illegible] ± 1,900	[illegible]
Monocytes	1,400 ± 1,300	1,100 ± 600	1,100 ± 600	1,300 ± 600	[illegible]
Eosinophils	400 ± 400	500 ± 500	400 ± 400	300 ± 300	400 ± 400
Platelets/µl*	302,000	290,000	257,000	[illegible]	411,000
M:E ratio*	1.5:1	[illegible]	[illegible]	[illegible]	1.4:1

Data from Shifrine et al., 1973, except as noted.
*Data from Earl et al. (1973); values were approximated for various age groups shown here; 5 males and 5 females were studied in each group.

of fetal erythrocytes as well as rapid growth of the pup whereby circulating red cell mass is significantly reduced (Lee et al., 1976). At about the beginning of the second month of life a gradual increase in RBC, Hb, and PCV takes place and continues until adult levels are attained at about 1 year of age (Andersen and Gee, 1958). In beagles, PCV was found to increase 43% from 2–8 months of age, after which it remained quite uniform, with RBC and Hb following the same pattern (Bulgin et al. 1970). In other studies, Hb and PCV increased at 42–52 days (Earl et al., 1973; Shifrine et al., 1973) and continued to increase until 18 months of age (Weiner and Bradley, 1972). Peak values were found between 13 months and 2 years of age (Abel and Schneider, 1973) with a steady decline thereafter (Dougherty and Rosenblatt, 1965). In a study of 73 German shepherd dogs between 6 months and 7 years of age, no age differences were found in RBC, WBC and differential leukocyte counts, Hb, MCV, MCH, and MCHC (Konrad et al., 1980).

Average life span of canine erythrocytes is about 115 days (range 110–120 days) and half-life of ^{51}Cr-labeled red cells is about 21–30 days (Spurling, 1977).

Breed Differences

Our experience with clinical specimens suggests that certain breeds of dogs tend to have high RBC, Hb, and PCV values, which may sometimes exceed the normal range for the species. Breeds most frequently involved have been poodles, German shepherds, boxers, beagles, dachshunds, and Chihuahuas and occasionally members of other common breeds. Greyhounds were found to have higher PCV and Hb values than other dogs of similar age (Doxey, 1966; Heneghan, 1977; Porter and Canaday, 1971). Hematologic and biochemical values in 36 purebred dalmatians were similar to values for beagles (Mazue et al., 1977). It is highly probable that the PCV of these breeds of dogs, which is normally between 50% and 55%, becomes elevated as a result of apprehension or fear. The dog that becomes apprehensive when being examined by a veterinarian may experience contraction of the spleen, forcing a concentrated mass of erythrocytes into the circulation. Such a change is not seen in splenectomized dogs (Reece and Snodgrass, 1972). Hemoconcentration as a result of failure to drink water, in addition to excessive water loss when the dog is sick, will also result in increased red cell values.

The Japanese Akita generally exhibits MCV values of 55–65 fl, thereby indicating a tendency toward a smaller red cell than in other breeds. In contrast, certain poodles may normally have macrocytic (MCV over 80 fl) normochromic red cells and exhibit morphologic abnormalities such as nuclear fragmentation in nucleated erythrocytes and multiple Howell-Jolly bodies in mature red cells (Chapter 25).

Sex Differences

There is disagreement among investigators about sex differences in the circulating mass of erythrocytes. Slightly higher mean values for Hb in males (16.0 g/dl) than in females (15.6 g/dl) were found in a study of 46 male and 68 female beagle dogs (Michaelson et al., 1966). Similarly, higher levels of RBC, Hb, and PCV were found in male beagles under optimum conditions of nutrition and management (Andersen and Gee, 1958). It was also observed that during gestation PCV became gradually reduced from a mean of 53% to 32% at term, then increased to 42% during the next 6 weeks, and returned to normal level by the ninth week. In another study of 101 beagle dogs of each sex, the mean values of PCV were found to be 44.6% in females and 42.5% in males; RBC and Hb followed the same pattern (Robinson and Ziegler, 1968). The latter authors concluded that the differences between the sexes were of little practical value. In a more recent study involving 382 male and 382 female beagle dogs between 8 and 16 months of age, age-related changes in RBC, Hb, and PCV were seen, but no sex influence was noted (Brunk and Becker-Berger, 1980).

Interrelationship of PCV, Hb, and RBC

In a normal animal, the Hb occupies one-third of the volume of the red cell. Therefore it is possible to predict the Hb by dividing the PCV by 3 or to predict the PCV by multiplying the Hb by 3. A rough estimate of RBC number

in millions can be obtained by dividing the PCV by 6 (Schalm and Wood, 1954, Ughaloro and Alder, 1957). This relationship does not hold during disease states and when laboratory errors occur in determination of various red cell parameters. In dwarfism in the Alaskan malamute, the PCV:Hb ratio is regularly 4:1 or 5:1 instead of 3:1 (Fletch et al., 1973). It also does not apply for certain animals (e.g., the family Camellidae).

Reticulocytes

Canine reticulocytes are mostly of the "aggregate" type, i.e., with strings and clumps of reticular material, and so they are easy to count. Their number correlates well with polychromatic red cells in Romanowsky-stained blood films (Laber et al., 1974). Circulating reticulocyte number is commonly less than 2% in the adult dog. In puppies, the reticulocyte count may be close to 7% (Table 4-2). Rapid replacement of fetal red cells and increased need to compensate for growth result in increased erythropoietic activity and release of more reticulocytes. In dogs younger than 16 months of age the reticulocyte count may be lower in females than in males (Brunk and Becker Berger, 1980). In the adult dog, reticulocytes are released in blood with a periodicity of 14 days (Morley and Stohlman, 1969), and their average maturation time in circulation ranges from 19-43 hours with a mean of 31 hours (Nizet and Robschet-Robbins, 1950). Increased erythropoietic response to anemia is associated with an elevation in reticulocyte numbers in the bone marrow, which precedes increase in the peripheral blood reticulocyte count by about 3 days (Sjöberg, 1978). The oscillatory nature of reticulocyte release, the release rate, and the intravascular maturation time may all change considerably during response to anemia. In such instances, a corrected reticulocyte count or reticulocyte production index (RPI) should be calculated before interpretations are made (Chapters 2 and 21). An absolute number of reticulocytes may be calculated by multiplying the percentile fraction by RBC count. A reticulocyte count of greater than 60,000/μl of blood is indicative of an erythropoietically more active marrow, and moderate to marked erythropoietic activity is indicated by counts of 150,000-600,000 or more per μl (Weiser, 1981). Empirical observations suggest a normal RPI of 1.0 or less, and an RPI of 2.0 or more is indicative of responsive anemia.

Erythrocyte Sedimentation Rate

Erythrocyte sedimentation rate (ESR) may be performed on canine blood to gain some useful information for clinical evaluation of the patient. ESR is usually performed with the Wintrobe tube, although Westergreen (Spurling, 1977) and microhematocrit (Jain and Kono, 1975) tubes can also be used. The extent of ESR varies with the method used and is affected by a variety of factors. Most important, ESR varies inversely with the number of red cells or PCV. Hence, for proper evaluation of the influence of disease upon ESR, the observed ESR value must be corrected by subtracting from it the anticipated ESR due entirely to ratio of red cells to plasma. Table 2-6 provides anticipated ESR values obtained with the Wintrobe method for a wide range of PCV to correct observed ESR values, and a similar chart has been developed for correcting Westergreen ESR values (Spurling, 1977). No correction table or chart is available for microhematocrit ESR values. See Chapter 2 for factors affecting ESR and interpretation of ESR in various clinical situations in the dog.

Erythrocyte Osmotic Fragility

In a study of the pathogenesis of a hemolytic disease, determination of erythrocyte osmotic fragility may be helpful. Among common domestic animals, canine red cells are least susceptible to osmotic changes (Coldman et al., 1969), but they are more prone to lysis by change in pH than human or ovine red cells (Cruz and Baumgarten, 1957; Iampietro et al., 1967). Hence, osmotic fragility determinations should always be made with buffered NaCl solution (see Chapter 2 for technique). Maximum and minimum resistance to hypotonic NaCl solution has been measured, and although differences are apparent in published values, broad ranges are generally similar. In 34 determinations on 9 normal beagle dogs, initial hemolysis was seen at 0.450 ± 0.022% NaCl concentration, and complete hemolysis at 0.358 ± 0.025%. Observations on 26 male and 25 female dogs

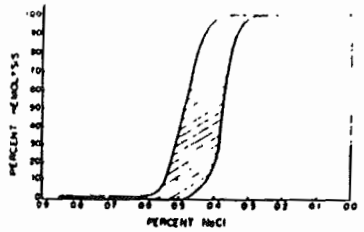

Fig. 4-1. Osmotic fragility curve for erythrocytes from 51 clinically normal dogs.

of various breeds and ages indicated beginning hemolysis (less than 5%) at a NaCl concentration of 0.45-0.55% and complete hemolysis at 0.4% or lower concentration (Fig. 4-1; Jain, 1973). The range and mean for the mean corpuscular fragility were, respectively, 0.36-0.48 and 0.43. Erythrocyte osmotic fragility essentially remains unchanged for canine blood kept for 3 days at 4°C. Mechanical fragility of canine erythrocytes is increased during lipemia (Jasper and Jain, 1964). The observation that newly formed red cells during response to anemia are osmotically more fragile than older red cells (Stewart et al., 1950) needs to be reconciled with common experience that reticulocytes are osmotically more resistant because of their greater surface area (Jain, 1973). See Chapter 20 for comments on changes in erythrocyte osmotic fragility of canine patients with hemolytic anemia.

THE LEUKOCYTES

The differential leukocyte count is expressed in both percentage and absolute number of each cell type per μl of blood. Responses to disease are more critically evaluated from absolute values (Schalm, 1963). Normal values of total leukocyte (WBC) and differential counts for the dog are summarized in Table 4-1 (basenji) and Tables 4-2 and 4-3 (beagles). Normal values are lower and the ranges are narrower for dogs maintained in colonies developed for research purposes than are similar values for small groups of dogs or the canine population at large.

There is little sex difference in WBC counts, but significant age differences are seen in both total and differential leukocyte counts. Some breed, diurnal, seasonal, and physiologic variations have been noted (Andersen and Gee, 1958; Andersen and Schalm, 1970). Beagles have somewhat higher lymphocyte numbers, and higher neutrophil numbers may be seen in older beagles than in other breeds (Spurling, 1977). Young beagles were found to have low WBC counts at 6 A.M. and gradually increasing counts during the day and night with highest level at 2 A.M. (Andersen and Schalm, 1970). These changes were related to physical activity of the animals. Adult dogs did not reveal such a pattern. A seasonal variation amounting to about 2,500 leukocytes/μl of blood was seen in beagles, with highest counts in early summer and lowest during fall and winter. Leukocyte counts increased, mainly due to changes in neutrophil numbers, during pregnancy to about 20,000/μl at term. Then a decline to normal level was seen after weaning.

Influence of Age

The normal range of 6,000-17,000 leukocytes/μl of blood for WBC count includes the effects of age and normal activity. WBC count is highest in young dogs and gradually decreases with age. This decrease is primarily due to changes in lymphocyte and neutrophil numbers. In a study of age-related hematologic changes in beagles up to 60 days in age, mean WBC, neutrophil, and lymphocyte numbers at birth were, respectively, 16,800, 9,200, and 3,700/μl of blood (Shifrine et al., 1973). The WBC count decreased during the first three weeks (12,300) and then gradually increased (15,700) until the eighth week. Neutrophil numbers followed a similar pattern. Lymphocyte numbers increased gradually to 6,100 by the sixth week and then declined to 4,000 by the sixtieth day. Observations on 193 beagles between 2 months and 4½ years in age, revealed a decrease in WBC count with age from a mean of 13,000/μl at 60 days to a mean of 10,000/μl at 4½ years, owing to decrease in both neutrophil and lymphocyte numbers (Bulgin et al., 1970). It was concluded that although a WBC count of 7,000 may be found in a clinically normal older dog, it would be representative of leukopenia in a dog less than 18 months of age. In other stud-

Table 4–3. Total and Differential Leukocyte Counts in Beagle Dogs (means ± 1 SD)

Reference	No. of Dogs	Age	Sex	Total WBC (× 10³/µl)	Neutrophils: Bands Absolute No./µl	Bands %	Segmenters Absolute No./µl	Segmenters %	Lymphocytes Absolute No./µl	Lymphocytes %	Monocytes Absolute No./µl	Monocytes %	Eosinophils Absolute No./µl	Eosinophils %	Basophils Absolute No./µl	Basophils %
Michaelson (1966)	46	6 mo to	Male	14.2 ± 3.3	—	2.0 ± [illegible]	—	57.5 ± 8.5	—	28.8 ± 7.0	—	4.5 ± 1.7	—	7.1 ± 5.2	—	—
	68	9 yr	Female	14.8 ± 3.4	—	1.6 ± 1.2	—	57.3 ± 7.3	—	31.9 ± 7.0	—	3.7 ± 1.5	—	5.5 ± 4.6	—	—
Robinson (1968)	101	6–12 mo	Male	13.8 ± 3.5	294 ± 388	2.1 ± 2.7	7,224 ± 2,652	52.5 ± 12.1	5,007 ± 1,964	37.3 ± 13.2	563 ± 449	4.1 ± 3.0	590 ± 658	4.2 ± 4.2	20 ± 73	0.1 ± 0.5
	101	6–12 mo	Female	12.7 ± 3.5	247 ± 600	1.7 ± 2.5	6,623 ± 3,288	51.3 ± 12.9	4,942 ± 2,536	39.3 ± 13.2	529 ± 434	4.2 ± 3.3	388 ± 422	3.0 ± 3.3	17 ± 68	0.1 ± 0.5
Andersen and Schalm (1970)	51	18 mo	Male	12.9	500	—	9,600	—	2,900	—	300	—	500	—	—	—
	47	18 mo	Female	12.1	400	—	7,900	—	2,900	—	400	—	600	—	—	—
Earl et al. (1973)	—	Adults	—	6.6–17.4 (12.0)	50	0.24%	1,700–12,200 (5,600)	36–69 (47)	1,700–12,200 (5,800)	25–71 (48)	[illegible] (200)	0–6 (2.0)	0–1,700 (400)	0–10 (3.0)	0	0–0.32

ies conducted over a longer period (Andersen and Gee, 1958; Bulgin et al., 1970; Dougherty and Rosenblatt, 1965; Weisse et al., 1971), neutrophil and lymphocyte numbers were found to decrease from 6 months to 4 years of age and remain constant between 4 years and 7 years. Thereafter, an increase was seen in neutrophils. In a 10-year study that included 433 beagles (Dougherty and Rosenblatt, 1965), an age-dependent decrease in WBC, lymphocyte, and eosinophil counts and a slight increase in monocyte numbers were seen. The extent of decrease in lymphocytes is useful in the interpretation of the degree of stress imposed by the disease. It is our experience that lymphopenia may be said to exist when the absolute lymphocyte count is less than 2,000 in dogs 3–6 months of age, less than 1,500 in dogs 8–24 months of age, and less than 1,000 in dogs over 2 years of age.

Effects of Corticosteroids

The release of corticosteroids from the adrenal cortex in response to disease or stressful situations produces marked changes in the total number and differential distribution of circulating leukocytes. A hemogram developed from blood of an animal under treatment with corticosteroids may be misinterpreted when the effects due to the corticosteroids per se are not taken into consideration.

In the dog, in addition to the triad of neutrophilia, lymphopenia, and eosinopenia, a monocytosis results from exposure to synthetic corticosteroids or the injection of ACTH (Fig. 4–2; Jasper and Jain, 1965). Diurnal variations in WBC counts were determined 24 hours prior to exposure to 10 units of ACTH given intramuscularly and to 20 mg of prednisolone given orally (2.2 mg/kg body weight) 5 days later (Table 4–4). After ACTH administration, maximum changes in WBC counts occurred at the eighth hour. The WBC count increased threefold as a result of three- to fourfold increase in neutrophil and monocyte numbers. A marked decrease in lymphocyte and eosinophil numbers occurred by the fourth hour, with complete disappearance of eosinophils by the eighth hour. A normal pattern was reestablished by the twenty-fourth

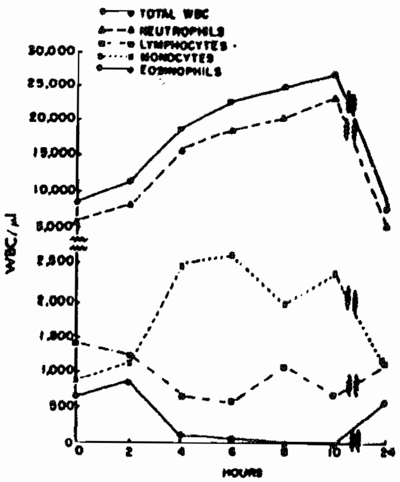

Fig. 4–2. Effect of 20 mg of oral prednisolone on leukocyte numbers in blood.

hour. Effects of prednisolone were similar to those of ACTH. In both instances, the neutrophil increase was accomplished essentially with mature cells. This increase is attributable to release of mature neutrophils from the marrow reserve and decrease in diapedesis of circulating neutrophils into the tissues (see Chapter 26 for details).

The occurrence of corticosteroid-induced monocytosis in the dog is significant in view of the opposite effect in the human, mouse, and rabbit (Fauci et al., 1976; Tompkins, 1952). Its cause is unknown. It probably involves: (*a*) a shift of marrow monocytes, although the existence of a marrow reserve of monocytes in the dog, unlike in the human and mouse, remains to be shown, or (*b*) a shift of monocytes from the marginal pool into the circulating pool, unlike the neutrophilic response. Some species differences can be anticipated in the effect of corticosteroids on circulating monocytes, as in the cat, horse, and cow.

Physiologic and "Emotional" Leukocytosis

Muscular activity, excitement, apprehension, and emotional stress may have a significant influence on total and differential leu-

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Table 4–4. Leukocyte Response in the Dog to ACTH- and Prednisolone

Clock Time	Elapsed Time (hr)	PCV (%)	Plasma Proteins (g/dl)	WBC /µl	Neutrophils Band	Neutrophils Mature	Lymphocytes	Monocytes	Eosinophils	Other
					Absolute Leukocyte Number/µl					
Control Hemograms										
8 a.m.	0	43	6.6	9,150	47	5,640	1,924	846	893	0
10 a.m.	2	40	6.3	9,800	0	6,125	1,568	1,078	931	98
2 p.m.	6	39	6.5	9,750	0	6,338	1,268	1,414	780	0
4 p.m.	8	42	6.9	9,500	0	6,412	1,140	1,188	760	0
6 p.m.	10	42	6.9	13,400	0	9,380	1,608	1,340	1,072	0
8 a.m.	24	40	6.9	11,400	0	7,980	1,482	798	798	342
10 Units ACTH										
8 a.m.	0	40	6.9	11,400	0	7,980	1,482	798	798	342
10 a.m.	2	42	7.0	10,600	0	7,738	1,113	1,113	636	0
Noon	4	39	7.2	17,900	0	14,857	895	1,880	179	99
2 p.m.	6	39	7.1	32,900	329	28,130	1,645	2,796	0	0
4 p.m.	8	39	6.6	20,400	102	17,748	1,020	1,530	0	0
6 p.m.	10	39	6.9	8,500	42	5,440	1,232	1,020	724	42
8 a.m.	24	40	6.9	8,500	42	5,440	1,488	935	595	0
20 mg of Prednisolone, Orally										
8 a.m.	0	42	6.9	8,500	42	5,440	1,488	935	595	0
10 a.m.	2	40	6.8	11,400	0	8,151	1,254	1,197	798	0
Noon	4	40	6.8	19,800	297	16,038	693	2,574	198	0
2 p.m.	6	41	7.1	22,700	227	19,068	567	2,724	114	0
4 p.m.	8	45	7.1	25,300	759	21,508	1,012	2,024	0	0
6 p.m.	10	45	7.4	27,000	405	23,490	675	2,430	0	0
8 a.m.	24	39	6.9	8,100	40	5,265	1,134	1,134	527	0

Table 4–5. Physiologic Leukocytosis in a Clinically Normal 3-Month-Old Male Beagle; Comparison of the Hemogram at Rest (9 A.M.) and after Normal Activity in an Outside Run (4 P.M.)

	9 A.M.	4 P.M.
PCV%	36	46
Hemoglobin (g/dl)	11.9	15.4
ESR/1 hour (corrected)	14 –	4 –
Icterus index units	2	5
WBC/µl	13,100	18,900
Neutrophils	8,777	12,096
Lymphocytes	3,668	4,914
Monocytes	393	1,418
Eosinophils	262	472
Plasma proteins (g/dl)	5.6	5.9

kocyte numbers. In dogs at rest, the number of neutrophils sequestered in the capillary beds (marginal pool) on an average nearly equals the number circulating (see Chapter 26) and, similarly, lymphocytes are probably distributed in an extravascular pool of considerable magnitude (see Chapter 30). With increasing activity, circulation of blood and lymph increases leading to washing of sequestered leukocytes into large-vessel blood. An example of the effects of normal muscular activity on blood composition is presented in Table 4–5.

Another form of leukocytosis, which may have an emotional basis, is seen in young dogs under conditions of hospitalization or incarceration at a dog pound. There is an increase in number of several leukocyte types which may mask the leukocyte pattern commonly anticipated as a result of disease. Total and differential leukocyte counts of pound dogs are high, vary widely, and require several days or weeks for normalization (Soave and Boyle, 1965).

Table 4–6 presents data on several young dogs in which the normal pattern of lymphopenia and eosinopenia in response to stress of disease did not occur. Lymphocytosis or persistence of lymphocytes at the high normal range associated in some dogs with eosinophilia resulted in differential leukocyte patterns not common to disease. The dachshund developed a significant lymphocytosis during nine days of hospitalization, while the basset hound revealed a reduction in lymphocytes and monocytes during hospitalization. One might speculate that the dachshund failed to adjust to the hospital environment, while the basset hound became less "emotionally" disturbed under hospitalization. A lost dog (shepherd type) was found on necropsy to be positive for canine distemper, but the marked lymphopenia and eosinopenia common to distemper were masked by eosinophilia and persistence of lymphocytes in the high normal range. Thus significantly increased total leukocyte counts in young dogs, associated with persistence at high levels or increases above the maximum normal ranges of neutrophils, lymphocytes, monocytes, and eosinophils, is considered suggestive of an emotional crisis.

The Neutrophil

Canine neutrophils are produced in the bone marrow over a period of 3–5 days. Their release into the general circulation is regulated by neurohormonal mechanisms as well as need in body tissues. Once in blood, neutrophils distribute into the circulating and marginal pools almost equally or sometimes preferentially into the latter. They leave the circulation randomly, with a half-life of about 7 hours. Further information on granulokinetics is given in Chapter 26.

Mature Neutrophil

The nucleus of the mature neutrophil is irregularly lobed with rounded prominences. Filaments joining two lobes are occasionally seen, but simple narrowing between lobes without true filament formation is the rule. The chromatin is clumped or plaqued into large, deeply staining masses separated by lighter-staining ground substance (Plate VIII–8). The nuclear membrane is irregular or "moth-eaten." The cytoplasm is a faint pale gray with generally indistinct, diffuse, pinkish granulation.

Band Neutrophil

Band neutrophils are present in peripheral blood only in small numbers in health. The nucleus is a curved band having a smooth nuclear membrane and parallel sides for an appreciable length. The chromatin is somewhat less clumped than in the mature cell (Plate XII–3). The cytoplasm is characteristic of the mature neutrophil.

Table 4-6. Examples of Leukocytosis in Young Dogs, Possibly Resulting from Emotional Stress

Breed	Age (mo)	Sex	Clinical Entry	TWBC*	Neutrophils		Lymphocytes	Monocytes	Eosinophils	Basophils
					WBC/μl*	Band	Segmenter				
Dachshund	3	F	Sneezing and coughing (admission)	44	34,200	0	26,334	4,275	3,078	513	0
			4th day of hospitalization	42	42,900	214	24,338	13,514	4,719	214	0
Basset	2	F	Treated by owner for intestinal parasites	29	47,500	1,193	26,712	14,787	5,009	0	0
			After 4 days of hospitalization	32	24,300	1,337	19,926	2,430	486	121	0
Mongrel	2	F	Depression and anorexia. No distemper in-dications found	39	24,800	0	14,260	6,076	3,100	1,364	0
Mongrel	8	M	Fever of unknown origin	42	31,900	0	16,429	11,005	3,190	1,117	159
Shepherd type	8	F	Lost dog. Positive for distemper at necropsy	31	32,900	0	17,766	5,736	6,086	3,290	0

*All differential leukocyte values in absolute numbers per μl of blood.

Metamyelocyte Neutrophil

The metamyelocyte neutrophil is not found in the peripheral blood in health. Its nucleus varies in outline from round, with a slight indentation, to a kidney-bean shape. The chromatin is more diffuse and stains less intensely than that of the mature neutrophil. The cytoplasm tends to be bluer, but still essentially pale, and has indistinct granulation (Plate V–3). When metamyelocytes appear in peripheral blood, they are associated with a definite increase in the number of band neutrophils. The presence of more immature cells, myelocytes and promyelocytes, is an abnormal finding.

Shift to the Left and "Toxic" Neutrophils

The dog responds dramatically to the need for neutrophils to counteract bacterial infections and to participate in the inflammatory process. When need for neutrophils is greater than ability of the bone marrow pool to supply mature forms, bands, metamyelocytes, occasionally myelocytes, and, less frequently, promyelocytes appear together with mature neutrophils in peripheral blood. This is called "a shift to the left."

In severe toxemic states, granulopoiesis becomes suppressed and neutrophil morphology is altered by maturation defects. The most common alteration is inappropriate development of cytoplasmic granules, while the cytoplasm retains the bluish staining characteristic of the myelocyte. The primary granules retain their azurophilia through various maturative stages and become visible in mature neutrophils as reddish purple granules. This process is generally referred to as "toxic" granulation. In more severe toxemias, the cytoplasm stains a deeper blue and may be extensively vacuolated (Plate VIII–12). These characteristics permit the designation "toxic" neutrophils. Toxic granulation is not a common feature of canine neutrophils. Sometimes the nucleus of the neutrophil precursors may undergo beginning maturation without cell division (polyploidy). This results in the formation of occasional giant band forms and mature neutrophils presenting large, twisted, and bizarre nuclei. The nucleus undergoing maturation may divide, resulting in some instances in a cell with double nuclei. Infrequently, nuclear maturation begins with the formation of a central hole, leading to release of a giant cell with a doughnut-shaped nucleus (Plate XV–11).

In dogs, the nitroblue tetrazolium (NBT) reduction test is a useful indicator of the presence of bacterial infection or endotoxemia (Banas, 1974; Hallet and Wilson, 1973). NBT reduction is also increased after infection with *Dirofilaria immitis* (Farnes et al., 1972) and nonspecifically after blood transfusion (Hallet and Wilson, 1973).

Shift to the Right

Five or more lobes of the nucleus in the mature neutrophil commonly represent aging of the cell in the circulation. Corticosteroids have the effect of reducing neutrophil diapedesis into tissues. The circulating neutrophils may then become hypersegmented as a result of longer stay in the circulation. Hypersegmentation is also a feature of deficiency of vitamin B_{12} and folate. Reduced cell mitosis leads to larger definitive neutrophils with hypersegmentation (Plate XI–7). Hypersegmentation of leukocytes may occasionally be an artifact in stored blood (Figs. 4–3, 4–4).

Female Sex Chromatin

The nuclei of certain neutrophil leukocytes of the female characteristically reveal a chromatin appendage called the "drumstick" or "Barr body" (Fig. 4–5; Plate XI–7). Porter (1957) applied the criteria described for drumsticks in human neutrophils to the leukocytes of the dog. The typical drumstick was found in the female on an average of 1 in 22 cells (4.5%). Occasionally, the nucleus of the eosinophil will show a sex lobe. Most of the neutrophils in the blood films from male dogs showed nothing resembling a true drumstick. Occasionally, however, minor lobes and sessile nodules (Fig. 4–6) caused confusion on superficial examination. Small chromatin clumps, often multiple, were fairly frequent. Three hundred neutrophils were found to be a sufficient number to examine before concluding that the blood under study came from a male dog. In another study involving 77 female dogs (Irfan, 1961), the neutrophil with typical sex lobe occurred on an average only

Fig. 4–3. Degenerating monocyte in old blood

Fig. 4–4. Degenerating lymphocyte and neutrophil in old blood

Fig. 4–5. Sex chromatin or "drumstick" lobe in a neutrophil of a female

Fig. 4–6. Sessile nodules on the nucleus of a neutrophil of a male

Fig. 4–7. Eosinophils in Pelger-Huët anomaly

Fig. 4–8. Neutrophils in Pelger-Huët anomaly

once in 51 cells, although in one female dog the sex lobe was present in 1 out of every 15 neutrophils and in another female in only 1 cell out of 95

Pelger-Huet Anomaly

This is a hereditary trait in humans and is characterized by failure of the nucleus of granulocytes, especially the neutrophil leukocyte, to undergo normal maturation to the segmented form. This results in an apparent "pseudo" left shift in neutrophil leukocytes. Observations on the condition as it appears in humans have been briefly reviewed along with findings in a strain of rabbits discovered in Switzerland (Nachsheim, 1950; see also Chapter 12). In rabbits, the anomaly is lethal when a homozygous state is produced by selective breeding. The homozygous rabbit tends to die in fetal life, or if it survives to parturition, it usually dies within the first month of life. Surviving homozygous rabbits were stunted and presented a marked skeletal deformity. The Pelger Huet anomaly in humans is benign when the condition is heterozygous. The total leukocyte count, phagocytic activity, and survival time are normal.

The first observation of the Pelger-Huët anomaly in the dog was as follows. A 6-year-old redbone hound was presented with a complaint of hind-leg stiffness. Radiographs revealed a chronic arthritis. Five hemograms developed between August 10 and December 13, 1961, revealed total leukocyte count to be in the normal range and the existence of a "permanent" shift to the left. This was not a true shift to the left, however, as the cytoplasm of the neutrophils and eosinophils appeared mature, while the nuclei were essentially nonsegmented and deeply stained as a result of the greater than normal condensation of chromatin (Figs. 4–7 and 4–8). A differential leukocyte count from the December 13 hemogram is presented in Table 4–7 to show the unusual morphologic features of the neutrophils and eosinophils. The existence of the anomaly did not appear to have produced any effect on general health. An unsuccessful search was made for littermates for a study of their blood. Few other cases have been described in the literature (Bowles et al., 1979, Feldman and Ramans, 1976, Kiss and Kómár, 1957, Pace, 1977).

Blood from the redbone hound in question was transfused into a healthy female Walker hound of A-negative blood type to study in vivo survival time of neutrophils and eosinophils (Carper and Hoffman, 1956). Disappearance of the Pelger-Huët neutrophils from the blood of the recipient was exponential with a $T_{1/2}$ value of 4.8 hours. Transfused Pelger-Huët eosinophils disappeared with a $T_{1/2}$ value of 30 minutes, indicating that the circulating time is shorter for eosinophils than for neutrophils in the dog. Pelger-Huët canine neutrophils have chemotactic properties similar to normal neutrophils (Latimer and Prasse, 1982).

Acquired hyposegmentation of the granulocyte nucleus is referred to as pseudo-Pelger-Huet anomaly. It may occur during disturbed granulopoiesis from severe infection, leukemia, or idiopathic causes. Such cells were seen for 9 months in a dog with intermittent signs of prostatitis and were thought to have resulted from an idiosyncrasy to a chemotherapeutic agent (Shull and Powell, 1979).

Cyclic Hematopoiesis in Silver-Gray Collie Dogs

Canine cyclic hematopoiesis, previously known as canine cyclic neutropenia, is an autosomal recessive, semilethal condition of silver-gray collies (see reviews by Jones and

Table 4–7. Nuclear Abnormalities in Leukocytes of a Dog with Pelger-Huët Anomaly

	Number of Cells with Different Nuclear Shapes					Differential Count (%)
Cell	Round	Metamyelocyte type	Band type	2-lobed*	3-lobed*	
Neutrophils	5	15	40	5	4	69
Eosinophils	2	5	5	2	0	14
Lymphocytes						8
Monocytes						9

*No true filament formation, only slight indentation of the nuclear membrane.

Lange, 1983, and Lange and Jones, 1980). It was originally characterized by neutropenic cycles occurring in both sexes and resulting in premature deaths most often from sudden overwhelming infection during a period of neutropenia (Ford, 1969; Lund et al., 1970). The affected pups are stunted or weak and less active at birth than their normal littermates. The first episode of neutropenia is observed within 12 days after birth. Observations on 92 affected pups showed that 62 pups died at birth or during the first week of life and only two pups lived to the age of one year (Ford, 1969). Survival of a female to 2½ years and a male to 3 years has been reported (Lund et al., 1970). The syndrome is remarkably similar to that of human cyclic neutropenia which cycles at approximately 21 days.

Data on blood and bone marrow cytology in cyclic hematopoiesis have been reported (Cheville, 1968; Dale et al., 1972; Jones et al., 1974; Lange et al., 1976; Lund et al., 1967). A cyclic maturation arrest of granulocytes was demonstrated so that at times the bone marrow samples were nearly devoid of mature neutrophils. The conclusion was that the peripheral blood neutropenia resulted from a cyclic maturation arrest at the level of differentiation of the stem cell. The peripheral blood was characterized by a marked cyclic neutropenia at 11- to 14 day intervals; a monocytosis peaking just as the neutropenia abates, a variable degree of thrombocytosis occurring 1–2 days before the neutropenia; and reticulocytosis (1.5–10%) occurring during the neutropenic phase. Neutrophil life span was normal, and so was the red cell survival. Erythrocyte parameters were often normal in mature dogs, although the affected pups may have had a mild anemia. Thrombocytopenia and anemia complicated the neutropenic episodes in terminal cases. Fever, septicemia, pneumonia, and enteritis may occur during neutropenic phases.

Recent studies have shown that the cytologic defect resides at the pluripotential stem cell level and is presumably due to an intrinsic marrow defect (Jones and Jolly, 1982). Because cyclic activity of other blood cells, including reticulocytes, platelets, monocytes, and lymphocytes, also appears to occur, the defect is termed canine cyclic hematopoiesis. Levels of colony stimulating factor (Yang et al., 1974), erythropoietin (Jones et al., 1975a), and thrombopoietin (McDonald et al., 1976) also show cyclic patterns in relation to changes in blood neutrophil, reticulocyte, and platelet numbers, respectively. Cyclic effect on erythrocyte parameters is not seen, probably because of the longer red cell life span (Lange et al., 1976). An abnormality of the lymphoid system has been demonstrated (Angus et al., 1978), and a periodic variation in lymphokine production has been observed (Lange and Jones, 1980). Functional abnormalities of platelets, e.g., defective adhesiveness and clot retractions, have also been detected (Reese et al., 1976).

Blood cell cycling can be induced in a normal gray collie dog by irradiation and infusion of marrow cells from an affected gray collie (Jones et al., 1975a; Weiden et al., 1974). Conversely, the disorder in an affected gray collie can be eliminated by total body irradiation followed by bone marrow transplantation from a normal gray collie (Dale and Graw, 1974; Jones et al., 1975b). Two of the bone marrow transplant chimeras were reported to be alive for 6 years without detectable signs of graft-versus-host reaction in the absence of any immunosuppressive therapy (Matsas and Yang, 1980). In this regard, it is noteworthy that bone marrow transplantation similarly corrected anemia in 3 basenji dogs with autosomal recessive deficiency of pyruvate kinase (Weiden et al., 1976). Cyclic changes in blood neutrophil, platelet, and reticulocyte numbers can be eliminated by daily administration of endotoxin over a period of several weeks (Hammond et al., 1979; Hammond et al., 1982). Also, treatment with lithium carbonate eliminated the recurrent neutropenia and normalized the other blood cell counts, probably by affecting the most primitive hematopoietic precursor cells (Hammond and Dale, 1980).

The Eosinophil

The granulation of the canine eosinophil is extremely variable. The granules may be numerous, small, and regular, or few and large, e.g., 3–4 μm in diameter (Plates XII-1, XII-2). Cells with extremely large granules are found infrequently. The granules have a weak affinity for the eosin stain. A better staining can be achieved with Wright-Leishman or May-Grünwald-Giemsa stain. Granule color is normally little more intense than that of the erythrocytes in the same blood film but is difficult to capture in color photographs. The nucleus may be partially obscured by granules, but commonly the granules are confined to the cytoplasm. The cytoplasm between the granules takes a light blue stain. Occasionally a few small vacuoles are seen in the cytoplasm, giving the impression of granule lysis; they correspond to electron-lucent, membrane-bound structures seen in electron microscopy (Fig. 27–2A). The eosinophil of the adult greyhound contains more vacuoles, which measure about 2 μm in diameter, than granules (Jones and Paris, 1963).

The Basophil

The granules in basophils vary in number and size. They are never enough to fill the cytoplasm, which stains a gray blue (Plate XII-1). Basophil granules are water-soluble and tend to disappear in unfixed films stained with new methylene blue. In methanol-fixed films stained with Wright stain, basophil granules stain metachromatically (reddish purple), appear as partially vacuolated structures, and may become less numerous from dissolution. Basophils occur rarely in normal canine blood. They tend to appear in appreciable numbers in association with eosinophilia (Chapter 27). Sometimes they are seen in small numbers in the absence of eosinophils, as in Cushing's disease (Chapter 36).

The Lymphocyte

This leukocyte varies in size from small to large. The small lymphocyte is the most common. The nucleus of the small lymphocyte almost fills the cell, with only a narrow rim or crescent of cytoplasm being visible. The nuclear chromatin is clumped and stains deeply. The cytoplasm varies in color, but is usually pale blue. In the medium and large lymphocytes, the cytoplasm is more voluminous and encircles the nucleus. Occasionally a small lymphocyte with intensely blue-stained cytoplasm is found. An infrequent lymphocyte may present a cluster of a few small, reddish azurophilic granules in the cytoplasm.

Persistent lymphopenia in chronic disease is either a sign of direct action of the disease agent on lymphocytic tissue, as in canine distemper, or a sign of continuation of the depressing effect of corticosteroids. The return of lymphocytes in normal numbers to the circulation generally can be interpreted as a favorable sign.

The Monocyte

The monocyte is generally the largest of the mature leukocytes in the blood. Its most characteristic feature is a basophilic or blue-gray ground-glass cytoplasm. The cytoplasm commonly presents vacuoles that vary in size and frequently are clustered at one side of the cell (Plates VIII-5, VIII-9). An occasional vacuole may be within the nucleus. Another identifying feature, when present, is the occurrence of numerous dust-like, pinkish, azurophilic granules (Plates VII-9, XI-8). The nucleus is extremely variable and can assume any shape. It may at times resemble that of the early band neutrophil or late metamyelocyte (Plates VII-10, VIII-5 to VIII-8). However, the ends of the band-like nucleus of the monocyte are enlarged and knob-like. The nuclear chromatin pattern is characteristically diffuse or mesh-like; when clumping or condensation is present, it generally does not assume a uniform pattern seen in cells of the neutrophilic series. Because the nucleus tends to be ameboid in outline, slender processes suggesting pseudopodia represent another variation sometimes encountered in the monocyte nucleus.

This cell increases in number in peripheral blood in the dog in response to endogenous release of corticosteroids or exogenous administration of ACTH or synthetic corticosteroids (Fig. 4–2). Numbers are generally increased in all acute and chronic diseases of the dog, as shown in a majority of the appendix cases relating to this species.

THE PLATELETS

Platelets are cytoplasmic fragments of megakaryocytes (Plate III-5). They are pleomorphic, exhibiting noticeable variation in

size and shape. Most commonly, they appear as roundish structures presenting a central cluster of fine, purplish azurophilic granules surrounded by a pale blue matrix enclosed in a delicate membrane (Plate VIII 2). Some platelets may appear agranular or have only a few granules. Occasionally, long, thread-like processes project outward in several directions. Round platelets measure about 2.5 μm in diameter, and oval forms are about 3.5 μm in length (Belleville et al., 1966). Clumping of platelets may occur in blood in which the clotting mechanism started to function before anticoagulation was complete. Giant forms, as large as the erythrocyte, may appear during remission from thrombocytopenia. Granular platelets are believed to be young platelets and often appear in increased numbers following blood loss (Ingram and Coopersmith, 1969).

Morphology of canine platelets has been studied with the transmission (Schultz, 1968) and scanning (Jain, 1975) electron microscopes. Their surface features are generally similar to those of other domestic animals, although there are species variations in shape and size and also variations within individuals of the same species. In critical point dried specimens prepared for scanning electron microscopy, canine platelets measure about 2.2–3.7 μm (mean 2.98) in diameter and about 0.5 μm in thickness. They present two morphological forms (Fig. 4-9). The majority are discoid, oval, or elongated, slightly biconvex or flat platelets with even contour. Their surface is less smooth or at times slightly rough when compared to that of mature erythrocytes, and sometimes exhibits few small shallow depressions or tiny protuberances. Few short pseudopods are seen on some platelets. The other variety is composed of irregular spheroidal platelets having many surface buds and few long pseudopods. Platelet morphology is markedly affected by temperature and time of storage of EDTA-anticoagulated blood; ideal storage time is less than 1 hour at 37°C.

The normal range of platelet count in the dog is 200,000–500,000/μl of blood. Venous blood has relatively less numerous platelets than the arterial blood, respective counts being 468,000 and 550,000/μl (Tocantins, 1938). In young beagle dogs, platelet counts are lower (280,000/μl at 6 months) than in adults (446,000/μl at 4 years) (Andersen and Gee, 1958). In another study on beagle dogs, the average platelet count was found to be 400,000/μl at 18 months of age and 300,000/μl at 10 years of age (Dougherty and Rosenblatt, 1965). These findings may be a reflection of reduction in adrenocortical activity with advancing age since administration of corticosteroids generally stimulates thrombopoiesis.

Fig. 4-9. Scanning electron photomicrograph of canine platelets. ×5,600.

Table 4-8. Differential Cell Counts in Bone Marrow Aspirated from the Iliac Crest of the Same Normal Dog on Three Separate Days, Compared with Bone Marrow from a Dog with an Elevated Total Leukocyte Count in Blood

	Percentage Distribution, 500 Cells Differentiated			
	Normal Dog			Dog with Otitis Externa
	April 2	April 3	April 7	
Erythrocytic Cells				
Rubriblasts	0.2	0.0	0.4	0.2
Prorubricytes	0.6	1.2	0.8	1.0
Basophilic rubricytes	11.6	9.6	8.2	5.2
Polychromatophilic rubricytes	17.0	19.0	13.6	11.6
Metarubricytes	9.6	9.8	8.2	1.8
Total erythrocytic cells	39.0	39.6	31.2	19.8
Granulocytic Cells				
Myeloblasts	0.8	1.2	1.2	1.0
Progranulocytes	0.6	2.0	1.6	2.6
Neutrophilic myelocytes	4.8	4.4	5.4	5.8
Eosinophilic myelocytes	2.0	0.8	1.0	1.0
Neutrophilic metamyelocytes	5.8	5.0	5.0	7.8
Eosinophilic metamyelocytes	1.6	1.4	1.2	0.6
Neutrophilic bands	17.0	10.4	10.6	14.2
Eosinophilic bands	1.4	1.2	2.4	1.4
Neutrophils	21.8	22.8	28.0	27.6
Eosinophils	1.6	1.4	0.8	0.4
Basophils	0.0	0.2	0.0	0.0
Total granulocytic cells	57.4	50.8	57.2	62.4
M:E ratio	1.5:1.0	1.3:1.0	1.8:1.0	3.27:1.0
Other Cells				
Hematogones	0.6	1.0	0.8	1.2
Lymphocytes	0.6	2.0	2.6	4.8
Monocytes	0.4	1.8	0.8	1.0
Mitotic cells	0.0	0.8	0.4	0.2
Plasma cells	0.2	0.4	2.2	5.0
Unclassified cells	0.2	0.0	0.0	0.2
Degenerated cells	1.8	1.8	2.8	2.4
Nuclei of RE cells	0.4	1.8	2.0	3.0
Peripheral Blood Values				
PCV	45.0	47.0	44.0	47.0
WBC (total leukocyte count/μl)	9,900	11,000	16,300	25,800

Canine platelets are produced over a period of 3–4 days (Craddock et al., 1955). Their half-life in circulation was estimated at 2.2 days (Adelson et al., 1961) and exponential survival between 1.7 and 5.3 days (Rowsell et al., 1967). Platelet survival was prolonged by moderate doses of heparin, but with progressively increased doses it attained a maximum value and then declined. Recent studies suggest normal life span of canine platelets of about 5–6 days (see Chapter 15).

Functional studies of canine platelets have been performed, and some species differences have been found with regard to responses to aggregating agents. Canine platelets exhibit variable aggregation with adrenaline and marked and usually irreversible aggregation with thrombin (Calkins et al., 1974; Hall, 1972; MacMillan and Sim, 1970; Mason and Read, 1967), adenosine diphosphate (ADP), and collagen. Snake venom and ristocetin produce fine aggregation visible macroscopically. Dipyridamole inhibits ADP-induced aggregation of canine platelets as in humans (Calkins et al., 1974). Noradrenaline and serotonin do not induce platelet aggregation, but the latter potentiates the effect of ADP (Sinakos and Caen, 1967). Secondary response to adrenaline is also variable, and it is slower and follows disaggregation

Table 4-9 Representative Differential Cell Counts in Marrow Aspirated from the Iliac Crest of the Dog in Normal and Disease States

	Percentage Distribution 500 Cells Differentiated				
	Normal	Iron Deficiency Anemia in Remission (Blood + Iron Therapy)	Chronic Ulcerative Colitis (Blood Loss)	End Stage Kidney Disease	Lymphocyte Leukemia
Erythrocytic Cells					
Rubriblasts	0.2	1.3	0.0	0.2	0.0
Prorubricytes	3.9	2.7	2.3	5.2	1.1
Rubricytes	27.0	19.0	29.6	3.0	0.7
Metarubricytes	15.3	43.0	18.2	1.0	0.0
Total erythrocytic cells	46.4	66.0	50.1	9.4	1.8
Granulocytic Cells					
Myeloblasts	0.0	0.4	1.7	0.2	0.7
Promyelocytes	1.7	0.5	0.3	1.0	3.8
Neutrophilic myelocyte	9.0	1.1	3.3	0.2	9.8
Eosinophilic myelocyte	0.0	0.1	0.3	0.2	0.0
Neutrophilic metamyelocyte	7.5	2.4	6.0	7.0	25.5
Eosinophilic metamyelocyte	2.4	0.0	1.0	0.4	0.7
Neutrophilic bands	13.6	9.3	12.0	20.4	17.5
Eosinophilic bands	0.9	0.3	0.0	0.0	0.2
Neutrophils	18.4	8.8	23.0	34.4	11.8
Eosinophils	0.3	0.1	0.0	0.4	0.0
Basophils	0.0	0.0	0.0	0.2	0.0
Total granulocytic cells	53.4	23.0	47.6	64.4	70.0
M:E ratio	1.15:1.0	0.35:1.0	0.95:1.0	6.85:1.0	38.8:1.0
M:E including degenerated cells		0.44		7.1	—
Other Cells					
Prolymphocytes	0.0	0.0	0.0	0.0	21.6
Lymphocytes	0.2	4.1	1.1	11.6	6.6
Monocytes	0.0	0.2	1.0	10.6	0.0
Unclassified cells	0.0	0.7	0.0	1.4	0.0
Degenerated cells	0.0	6.0	0.0	2.6	0.0
Peripheral Blood Values					
Total blood leukocytes/μl	12,000	9,100	19,850	13,500	58,000
Blood PCV (%)	45	29	15.5	22.5	21.3

(MacMillan and Sim, 1970) Adherence of canine platelets to glass bead column has been studied (Dodds, 1974); also see Chapter 16.

THE PLASMA

Icterus Index

The icterus index of the dog in health seldom exceeds 5 units. Plasma color equal to 7.5 units may occur in severe dehydration and always requires interpretation, while a reading of 10 units or greater is distinctly abnormal and clinically significant.

Total Plasma Proteins

Plasma protein concentration is lowest at birth, being commonly less than 5.0 g/dl, and is between 6.0 and 7.0 g/dl in dogs 4–6 months in age (Table 4–1). Dogs 1 year of age or older commonly have plasma proteins between 7.0 and 8.0 g/dl. The increase in plasma proteins with age is primarily due to the globulin fraction, which increases in response to foreign antigens. Influence of dehydration and disease on the plasma protein concentration is discussed in Chapter 34. Clinical significance of plasma protein concentration has been discussed (Schalm, 1970).

Plasma Fibrinogen

Plasma fibrinogen is not influenced by age. It normally ranges between 0.1 and 0.5 g/dl, but is most commonly between 0.2 and 0.4 g/dl (Table 4–1). Fibrinogen concentration increases in inflammatory and tissue-destroying diseases (see Chapter 34). An increase in plasma fibrinogen concentration, with total and differential leukocyte counts within the normal range, may be found in some diseased animals (Sutton and Johnstone, 1977). A two- to threefold increase in plasma fibrinogen may be seen in the pregnant bitch. This may be due partly to increases in plasma progesterone since its injection elevates plasma fibrinogen level (Gentry and Liptrap, 1981).

THE BONE MARROW

The Myeloid:Erythroid Ratio

The M:E ratio in clinically normal dogs is generally between 1.0 and 2.0:1. Published values have included a range of 0.75–2.53:1, with a mean of 1.25:1 (Bloom and Meyer, 1944) and a mean value of 1.20:1 from a study involving 187 dogs (Albritton, 1952). A comparison was made of marrow from the rib, femur, tibia, and humerus (Rekers and Coulter, 1948). Mean M:E ratio for the rib marrow from 30 normal dogs, 9–24 months of age and representing both sexes, was 1.89:1, while the M:E ratio for the long bones varied between 1.39 and 2.99:1. Similarly, a comparison of the cytology of aspirates from the sternum and ribs of 5 beagle dogs, 1–1½ years of age, revealed a mean M:E ratio of 1.66 ± 0.38 for sternal marrow and 1.53 ± 0.23 for rib marrow (Melveger, 1969).

Bone marrow was aspirated from the iliac crest of the same normal dog on three separate occasions (Table 4–8). The M:E ratios were 1.5, 1.3, and 1.8:1, demonstrating repeatability of results with aspirates made on different days from the same dog. Included in the study was a dog with a peripheral blood leukocytosis of 25,800/μl in response to otitis externa; the M:E ratio for that dog was 3.27:1 reflecting the intensification of granulopoiesis in response to infection.

Influence of a variety of disease entities on the M:E ratio is shown in Table 4–9. In iron deficiency in remission, the intensification of erythrogenesis is indicated by an M:E ratio considerably less than 1.0:1.0. In chronic ulcerative colitis with blood loss, an M:E ratio of 0.95:1.0 revealed a somewhat higher than normal erythrogenesis. In end stage kidney disease, a depression of erythrogenesis was evident by a PCV of 22.5% and an M:E ratio of 6.85:1. The mean M:E ratio of 38.8:1 in lymphocytic leukemia indicates a depression of erythrogenesis, possibly as a result of massive infiltration of the bone marrow by neoplastic lymphocytes.

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Section I, Toxicology Branch I - IRS (H7509C) 7/31/91
(Jmaves 06/14/91 (for RG))

DATA EVALUATION REPORT

Study Type: 83-2 - Oncogenicity, Mouse TOX Chem No.: 221G

Accession No.: N/A MRID No.: 410395-04; Vol. 1-6

Test Material: AC 243,997 technical

008426

Synonyms: Imazapyr; Arsenal

Study No.: 86-3074

Sponsor: American Cyanamid

Testing Facility: Bio/dynamics

Title of Report: A Chronic Dietary Toxicity and Oncogenicity Study with AC 243,997 in Mice.

Author: Carol Auletta

Report Issued: November 3, 1988

Conclusions:

The oncogenic potential is negative up to 10,000 ppm (HDT). The HDT exceeds the 7500 parts per million (ppm) limit dose for mouse oncogenicity studies and is therefore the maximum tolerated dose (MTD).

There were no compound-related effects in toxic signs, mortality, body weight, food consumption, hematology, organ weights, and tumors. Historical control data are required to establish the NOEL for pulmonary edema in female mice. Additionally, a more detailed description of subscapular adrenal gland cell reaction is required.

Following the submission of the historical control data and descriptive material, a NOEL for the study will be determined.

Classification:

Core-Supplementary, which can be upgraded after review of historical control data.

Special Review Criteria (40 CFR 154.7): N/A

A. Materials:

1. Test Compound - AC 243,997, Description: Off white powder, Batch No. AC 4866-062, Purity 99.5%.

2. Test Animals - Species: mice, Strain: CD-1, Age: 42 days, Average Weight: males, 27 g, females, 21 g, Source: Charles River, Kingston, NY.

B. Study Design:

1. Animal Assignment - Animals were assigned randomly to the following test groups:

Test Group	Dose in Diet (ppm)	Main Study 18 Months Male	Main Study 18 Months Female	Interim Sac. 12 Months Male	Interim Sac. 12 Months Female
1. Control	0	65	65	10	10
2. Low (LDT)	1000	65	65	10	10
3. Mid (MDT)	5000	65	65	10	10
4. High (HDT)	10,000	65	65	10	10

2. Diet Preparation - Diet was prepared weekly and stored at room temperature. Samples of treated food were analyzed for stability and concentration at weekly intervals.

Results - The test material was stable in the diet for the required 2-week interval based on analyses of the 1000 and 10,000 ppm batches.

The concentration of the test material in the diet for 80 weeks ranged from 888 to 1026 ppm for the low dose, 4753 to 5479 ppm for mid dose, and 9178 to 11,273 ppm for the high dose.

At the low dose, assays averaged 97.6 percent with a coefficient of variation (CV) of 4.8 percent of nominal. At the mid dose, assays averaged 100.4 percent with a CV of 4.6 percent of nominal. At the high dose, assays averaged 99.6 percent with a CV of 4.9 percent of nominal. Technical material was stable for the duration of the study.

3. Animals received food (Purina Certified Rodent Chow No. 5002) and water ad libitum.

4. Statistics - The following level was utilized in analyzing the numerical data: $p < 0.05$, $p < 0.01$.

5. Quality Assurance was performed and the report was signed by the Study Director.

C. Methods and Results:

1. Observations - Animals were inspected twice daily for signs of toxicity and mortality. Detailed examinations were performed weekly.

Results:

a. Toxicity Signs - There were no compound-related toxic signs during the study. The most frequently observed toxic signs were, in males, ear problems, yellow stains on fur and genital area, scabs and alopecia. The frequency of these findings was comparable between control and treated male groups.

The most frequently observed toxic signs in females were ear problems, scabs, and alopecia. The incidence of these toxic signs were comparable between control and treated female mice.

b. Mortality - There were no compound-related effects in mortality between control and treated male and female groups.

The mortality at 18 months for males and females, excluding the 10 mice/sex/group sacrificed at 12 months, was as shown below:

Mortality (%)

Group	I	II	III	IV
Dose (ppm)	0	1000	5000	10,000
Males	27/55	19/54[a]	20/54[b]	22/55
Percent	(49%)	(35%)	(37%)	(40%)
Females	19/55	18/55	27/55	24/54[b]
Percent	(35%)	(33%)	(49%)	(44%)

a/Excludes one animal which escaped and was missing for more than 24 hours, found and killed.
b/Excludes one animal which died accidently.

As can be noted from the data, total survival among male treated groups was better than the control

group. In females, total survival in the mid- and high-dose groups was slightly decreased in comparison to controls. None of the differences in survival between control and treated male and female groups were statistically significant and in females, the decreased survival was not dose-related.

There was no compound-related effect in the time course of cumulative mortality in treated males and the slight increase in mortality in high-dose females is not considered compound-related.

Total Cumulative Mortality

Group (ppm)	No. of Mice	Month 1	4	8	12[a/]	15	18
		Males					
I 0	65	0	0	2	4	5	27
II 1000	65	0	0	2	2	3	19
III 5000	65	0	0	1	2	8	20
IV 10,000	65	0	0	3	4	8	22
		Females					
I 0	65	0	0	0	2	7	19
II 1000	65	0	0	1	3	6	18
III 5000	65	0	0	2	4	8	27
IV 10,000	65	0	2	4	5	12	24

[a/] Ten mice/sex/group were sacrificed at 12 months.

These mortality data indicate that the greatest number of mice dying in each group occurred between month 15 and at termination in month 18.

2. Body Weight - They were weighed weekly for 14 weeks, then biweekly for 10 weeks, and monthly, thereafter.

Results - There were no compound-related adverse effects in body weight gain between control and treated male and female groups. Occasionally, there were statistically significant differences in body weight between the control and treated groups but the treated mice had gained more weight than the controls. An increase in body weight gain by the treated mice is not considered a toxic effect. The following table shows the mean body weights during the study:

Males (Body Weight in Grams)

Dose (ppm)	0	1000	5000	10,000
Week				
0	27.4	27.2	27.3	27.7
4	31.3	31.6	31.3	31.7
8	33.8	34.1	33.6	34.1
16	37.4	37.7	37.0	37.3
30	38.4	38.5	38.1	38.4
64	40.0	40.4	39.3	39.6
77	41.3	40.7	41.0	41.4

Females (Body Weight in Grams)

Dose (ppm)	0	1000	5000	10,000
Week				
0	20.7	21.5*	20.7	21.8**
4	25.0	25.3	24.8	25.5
8	27.2	27.0	26.9	28.3**
16	28.6	29.0	29.4	29.1
30	32.4	32.4	33.2	32.1
64	33.4	34.6	34.0	33.1
77	33.9	35.7	33.5	35.6

* $p < 0.05$
** $p < 0.01$

3. Food Consumption and Compound Intake - Consumption was determined and mean daily diet consumption was calculated. Compound intake was calculated from the consumption and body weight gain data.

a. Food Consumption - There were no compound-related adverse effects in food consumption between control

and treated male and female mice. The occasionally statistically significant increases and decreases observed between control and treated groups were, for the most part, not consistent in a time or dose-related fashion and, in frequent instances, the treated mice consumed more food than the controls. The following table shows the mean food consumption during the study:

Males (Food Consumption: mg/kg/day)

Dose (ppm)	0	1000	5000	10,000
Week				
0	229.0	241.8	223.8	212.9
4	214.8	220.1	218.0	205.4
8	225.0	220.9	221.0	216.6
16	190.6	195.4	150.6**	155.6**
30	160.5	158.8	158.0	152.1
64	123.8	125.7	137.1**	135.3*
77	138.5	149.5	163.4**	148.4

Females (Food Consumption: mg/kg/day)

Dose (ppm)	0	1000	5000	10,000
Week				
0	306.1	296.5	315.8	285.4
4	269.5	269.9	279.7	285.1
8	293.0	294.7	300.1	282.2
16	287.8	264.6**	203.0*	210.8**
30	192.1	175.5*	163.3**	184.7
64	155.5	159.1	155.3	180.6**
77	197.4	182.7	185.0	171.3**

* $p < 0.05$
** $p < 0.01$

b. Compound-Intake - The range of test material intake in mg/kg/day as presented in the report is shown below:

Dose (ppm)	1000	5000	10,000
Males	126-254	674-1194	1301-2409
Females	151-303	776-1501	1639-3149

4. Ophthalmological Examinations - The mice were not examined in life by an ophthalmologist for ocular lesions.

5. Blood was collected at 12 and 18 months for hematology analysis from 10/sex/group animals. The CHECKED (X) parameters were examined.

- Hematology

X		X	
X	Hematocrit (HCT)*		
X	Hemoglobin (HGB)*	X	Leukocyte differential count
X	Leukocyte count (WBC)*		Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)*		Mean corpuscular HGB conc. (MCHC)
X	Platelet count*		Mean corpuscular volume (MCV)

Results - There were no compound-related effects in mean hematological values at 12 and 18 months in treated male and female mice in comparison to controls. Additionally, there were no statistically significant differences.

The following tables show the results of the 12-month and 18-month hematological findings:

12 Month Analysis

	Males					Females			
Dose ppm Mean values	0	1000	5000	10,000	Dose ppm Mean values	0	1000	5000	10,000
HgB (g/dL)	13.8	13.6	13.9	14.3	HgB (g/dL)	14.2	14.7	14.9	14.5
HCT (%)	37	36	37	38	HCT (%)	37	38	37	37
RBC (mil/uL)	6.95	7.06	7.16	7.31	RBC (mil/uL)	7.35	7.63	7.54	7.42
Plat (100T/uL)	15.65	16.58	15.02	16.32	Plat (100T/uL)	12.67	14.69	12.78	13.66
WBC (thous/uL)	4.9	4.2	4.0	7.9*	WBC (thous/uL)	3.9	4.6	3.9	4.6

*Male mouse No. 4031 of the high-dose group had a WBC count of 34.1 (thous/uL) due to an increased segmented neutrophil count. This isolated finding was not considered treatment-related.

18 Month Analysis

Dose ppm Mean values	Males 0	1000	5000	10,000	Dose ppm Mean values	Females 0	1000	5000	10,000
HgB (g/dL)	14.3	13.5	14.0	14.7	HgB (g/dL)	14.3	13.4	15.0	13.2
HcT (%)	41	39	41	43	HcT (%)	42	40	44	38
RBC (mil/uL)	7.85	7.44	7.72	7.95	RBC (mil/uL)	7.82	7.42	8.15	7.21
Plat (100T/uL)	25.83	20.23	28.25	25.55	Plat (100T/uL)	20.74	20.24	16.62	21.05
WBC (thous/uL)	4.7	3.8	4.5	3.6	WBC (thous/uL)	4.0	4.6	5.6	3.0

6. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X	Digestive system	X	Cardiovasc./Hemat.	X	Neurologic
X	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (2 levels)*
X	Stomach*	X	Lymph nodes*	XX	Pituitary*
X	Duodenum*	XX	Spleen*	X	Eyes (optic n.)*
X	Jejunum*	X	Thymus*		Glandular
X	Ileum*		Urogenital	XX	Adrenals*
X	Cecum*	XX	Kidneys*		Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Mammary gland*
	Rectum*	XX	Testes*	XX	Parathyroids*
XX	Liver*	XX	Epididymides	XX	Thyroids*
X	Gallbladder*	X	Prostate		Other
X	Pancreas*	X	Seminal vesicle	X	Bone*
	Respiratory	XX	Ovaries	X	Skeletal muscle*
X	Trachea*	X	Uterus*	X	Skin (mammary area)
XX	Lung*			X	All gross lesions and masses

Results:

a. Organ Weight at 12 Months - There were no compound-related effects in organ weight, organ-to-body weight, and organ-to-brain weight ratios for male and female mice sacrificed at 12 months. Additionally, there were no statistically significant differences between control and treated groups of male and female mice.

b. Organ Weight at 18 Months - At the terminal sacrifice, there were no compound-related effects in organ weight, organ-to-body weight, and

organ-to-brain weight ratios for male and female mice sacrificed terminally at 18 months.

The statistically significant differences observed between control and treated mean values in absolute and relative organ weight observed occasionally were not dose-related, and therefore, were not considered compound-related.

These statistically significant differences in means included for males: increased absolute liver weight at mid dose.

For females, the following data were observed: decreased brain-to-body weight ratio at low dose, increased heart-to-brain weight ratio at low dose, increased kidney-to-brain weight ratio at low dose, and increased liver-to-brain weight ratio at low dose.

c. Gross Pathology

1) Twelve Months - There were no compound-related or toxicologically significant effects in gross necropsy findings at 12 months. Uterine cysts occurred at an increased incidence in treated female mice (2/10, 3/10, 7/10, and 6/10 for the control, low-, mid-, and high-dose groups, respectively). This lesion was not considered toxicologically significant since the incidence of this gross lesion in female mice dying on study and mice terminally sacrificed was randomly distributed without any relationship to dose.

2) All Unscheduled Deaths - There were no compound-related or toxicologically significant effects or dose-trends in groups or pathological findings in mice which were unscheduled deaths.

3) Terminal Sacrifice - There was one possible compound-related effect in mice terminally sacrificed.

The incidence of enlarged seminal vesicles in male mice was 3/28 (11%), 6/35 (17%), 9/34 (27%), and 10/33 (30%) for the control, low-, mid-, and high-dose groups, respectively. Histologically, there was no increase in microscopic lesions in terminally sacrificed male mice which correlated with the gross findings. The report states that the incidence of the seminal vesicle gross

findings were not statistically significant, but a probability value was not presented.

Toxicology Branch (TB) does not consider the gross findings incidence in the seminal vesicles to be toxicologically significant.

Additionally, there was an increased incidence of kidney cysts in high dose male mice in comparison to controls. The incidence was 2/28 (7%), 0 (0%), 3/34 (9%), and 5/33 (15%) in control, low-, mid-, and high-dose groups, respectively. Histologically, there were no microscopic increases in kidney lesions which correlated with the gross findings.

Although the finding is dose related, it is not considered toxicologically significant.

d. Microscopic Pathology

1) Non-neoplastic

a) 12 Month Sacrifice - The most frequently observed microscopic lesion observed at 12 months was amyloidosis and included the kidney, heart, mesenteric lymph nodes, stomach and intestines, tongue, ovaries, liver, thyroid gland, and adrenal gland. Although there were occasional increased incidences in treated mice in comparison to controls, the increases were usually less than twice the control level at the high dose. Additionally, the incidences of amyloidosis in these and other organs in mice dying on study or terminally sacrificed were distributed in a similar random pattern. Also, the grades of the lesion were comparable among control and treated groups.

Therefore, the occurrence of amyloidosis is not considered compound-related in mice.

Another 12-month microscopic finding that occurred at slightly higher incidences in treated mice in comparison to controls was unilateral (but not bilateral) subscapular cell reaction of the adrenal gland in male and female mice. The incidence of the unilateral lesion was 0/10, 1/10, 2/10, and 5/10

in male mice of the control, low-, mid-, and high-dose groups, respectively. Additionally, in female mice the incidence was 4/10, 3/10, 1/10, and 7/10 in the control, low-, mid-, and high-dose groups, respectively.

The occurrence of the unilateral (and bilateral) subscapular cell reaction of the adrenal gland in treated mice that died on study or were terminally sacrificed, did not show any treatment-related distribution. Also, the grades of the lesion were comparable among control and treated groups. A more detailed description of this lesson is required.

In light of these findings, the occurrence of unilateral subscapular cell reactions of the adrenal is not considered compound-related.

b) Unscheduled Deaths - There was an increased incidence at the high-dose group in females of congestion of the brain. The incidences were 3/19 (16%), 2/17 (12%), 3/27 (11%), and 7/25 (28%) in the control, low-, mid-, and high-dose groups, respectively.

There were no occurrences of congestion in the brain in the control and treated groups of both sexes at 12 months and terminal sacrifice. The grades of the lesion were comparable between control and treated females in the unscheduled deaths.

Additionally, the increased occurrence of this lesion in high dose females is not greater than 2X of the controls.

The incidence, but not the grade, of edema in the alveoli of the lungs in female mice occurred in an increased manner. The incidence was 2/19 (11%), 4/18 (22%), 5/27 (19%), and 6/25 (24%) for the control, low-, mid-, and high-dose groups, respectively, in the unscheduled deaths. There was no alveoli edema in mice sacrificed at 12 months and no compound-related increase in female mice sacrificed terminally. The

incidence of this lesion in all female mice on study was 3/65, 5/65, 5/65, and 7/65 in the control, low-, mid-, and high-dose groups, respectively.

The increased incidence at the high dose may be compound-related.

The registrant is required to provide historical control data to resolve this issue.

The incidence and grades of other lesions in the tissues and organs of both sexes of mice were comparable between control and treated groups.

c) Terminal Sacrifice - There was an increased incidence, but not increased grade, of erythrocytes in the sinus of mediastinal lymph nodes in treated female mice in comparison to controls. The incidences were 3/29 (10%), 5/33 (15%), 2/22 (9%), and 10/28 (36%) in the control, low-, mid-, and high-dose groups, respectively. For all female animals on study, the incidence was 5/48 (10%), 9/48 (18%), 10/46 (21%), and 12/52 (23%) for the control, low-, mid-, and high-dose groups, respectively.

The increased incidence of this lesion at the high dose in terminally sacrificed female mice is not considered compound-related.

There was an increased incidence, but not grade, of brown pigment in the Harderian gland of female mice sacrificed terminally. The incidence was 7/14 (50%) in the controls compared to 18/21 (85%) in the high-dose group. For all female mice on study, the incidence of this lesion was 8/15 in controls compared to 20/25 at this high dose.

Since the incidence of this lesion at the high dose is not 2X greater than controls, the lesion is not considered compound-related.

The incidences and grades of lesions in other tissues and organs of both sexes of mice were comparable between control and treated groups.

2) Neoplastic Lesions - There were no compound-related increases in benign or malignant neoplasms in the various tissues and organs of both sexes of mice and no decrease in latency for any tumors.

The most frequently observed neoplasms were in the lungs. The overall incidences for all mice on study are shown below:

	Lung							
	Males				Females			
Dose (ppm)	0	1000	5000	10,000	0	1000	5000	10,000
No. examined	65	65	65	65	65	65	65	65
Adenoma	12	9	12	9	9	5	14	6
Percent (%)	18	14	18	14	14	8	22	9
Carcinoma	3	1	1	0	1	0	0	0
Percent (%)	5	2	2	0	2	0	0	0

The distribution of the adenomas and carcinomas between mice of the 12-month sacrifice, the unscheduled deaths, and terminal kill are shown below:

	Lung							
	Males				Females			
Dose (ppm)	0	1000	5000	10,000	0	1000	5000	10,000
Adenomas								
12 Months	2	1	2	0	1	1	1	1
Unsch. Deaths	4	4	2	4	0	1	2	1
Term. Kill	6	4	8	5	8	3	11	4
Carcinomas								
12 Months	1	0	0	0	0	0	0	0
Unsch. Deaths	1	1	1	0	1	0	0	0
Term. Kill	1	0	0	0	0	0	0	0

It can be concluded from the data that there is no compound-related decrease in latency for lung adenoma and carcinoma.

R:57217:Dykstra:C.Disk:KENCO:6/19/89:CL
R:55897:Dykstra:C.Disk:KENCO:7/26/89:CL:VO:EK:AS

D. Discussion:

The oncogenic potential is negative up to 10,000 ppm (HDT). The HDT exceeds the 7000 ppm limit dose for mouse oncogenicity studies. A NOEL for various non-neoplastic gross and microscopic lesions could not be established. Historical control data for these lesions are required to be submitted to resolve these issues. The historical control data required are the following: 1) incidence of gross pathological findings of enlarged seminal vesicles in 18-month old male mice and a statistical analysis of this lesion in male mice in the study; 2) incidence of congestion of brain in female mice; 3) incidence of edema of alveoli in female mice; and 4) incidence of erythrocytes in the sinus of mediastinal lymph nodes in female mice.

From: L.J. Slaughter 7-26-89

To: E.R. Dykstra

I dont think any request for historical control data is warranted at this time. The incedence of all neoplastic + non-neoplastic lesion spontaneously occur in CD, mice. However I would suggest that you get or give a more detailed description of the subcapsular adrenal gland cell reaction. Also, it would (maybe) important to know what other lesion (s) were associated with the pulmonary edema.

Reviewed By: William Dykstra William Dykstra 6/9/89
Section I, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Robert Zendzian
Section I, Toxicology Branch I - IRS (H7509C)

DATA EVALUATION REPORT

Study Type: 83-4 - Reproduction - Rat TOX Chem. No.: 221G

Accession No.: N/A MRID No.: 410395-05 Vol. 1-5

Test Material: AC 243,997; Lot No. AC 4866-062

008426

Synonyms: Arsenal; Imazapyr

Study No.: 82408

Sponsor: American Cyanamid

Testing Facility: Bio-Research Laboratories, Ltd.

Title of Report: A 2-Generation (2-Litter) Reproduction Study of AC 243,997 Administered in the Diet of the Rat.

Author: Keith Robinson

Report Issued: May 6, 1987

Conclusions:

The NOEL is 10,000 ppm (HDT).

There were no compound-related toxicological effects in treated rats in comparison to controls in toxic signs, mortality, parental body weight and food consumption, mating indices, fertility indices, conception rates, gestation indices, pup viability indices, pup survival indices, pup lactation indices, pup sex ratio, pup body weight, and gross and microscopic pathological findings.

Classification: Core-Guideline

Special Review Criteria (40 CFR 154.7): N/A

Review:

A 2-Generation (2-Litters) Reproduction Study of AC 243,997 Administered in the Diet of the Rat (Bio-Research Laboratories, Ltd; Report No. 82408; May 6, 1987).

A. Materials:

1. Test Material - AC 243,997 technical; Lot No. AC 4866-062; 99.5% purity; Appearance: fine, white powder; Storage conditions: airtight container at room temperature and in a dark, cool, and dry place.

2. Test Animals - Species: Rat; Strain: Sprague-Dawley; Age: 43 days old on first treatment day; Males: 187 to 240 g, females 128 to 166 g; Source: Charles River Breeding Laboratories, Kingston, NY.

B. Study Design:

1. Randomized groups of 25 male and 25 female Sprague-Dawley rats (the F_0 generation) were administered 0, 1000, 5000, and 10,000 ppm of test material in the diet. The rats were treated for a 64-day growth period, throughout the two mating periods and up to necropsy, which was approximately 3 weeks after the end of the second mating period. The females were also treated throughout gestation and lactation.

   On day 21 postpartum, the F_1b generation litters were weaned and randomized groups of 25 male and 25 female rats were selected to form the F_1 parental generation. These animals were fed their respective diets for at least 78 days prior to mating. The F_1 parents were mated twice to produce the F_2a and F_2b litters (F_2 generation). The F_1 adult females were fed the diet continuously throughout the mating, gestation, and lactation periods. The F_1 adult males were treated until necropsy, which was approximately 4 weeks after the end of the second mating period.

   The F_2 generation pups were killed following weaning.

2. Diet Preparation - Diet was mixed weekly by preparing a premix of basal diet and AC 243,997 for each dose level in a 5 kg capacity V-blender. The final concentrations were achieved by the addition of further basal diet and mixing in a 35 kg capacity V-blender. Prepared diets were stored in a plastic bag at room temperature. Samples of treated food were analyzed for stability and concentration each week for the first 4 weeks and monthly thereafter.

Results - Dietary analysis showed acceptable results. Dietary analysis for weeks 1 to 55 ranged from 919 to 1068 ppm for the 1000 ppm dose level, 4574 to 5115 ppm for the 5000 ppm dose level, and 9024 to 10,330 pm for the 10,000 ppm dose level.

Representative samples of AC 243,997 technical used during the study were assayed with an average of 99.5 percent purity.

Control diets were free of detectable technical material.

3. Animals received food (Certified Purina Rodent Chow, No. 5002) and water ad libitum.

4. Statistics - Appropriate statistical analyses were performed on parental food consumption and pup body weight data, parental mating index, fertility index, conception rate, and gestation index, and pup viability index, survival index, lactation index, and pup body weight with $p < 0.05$ being significant.

5. Quality Assurance - Quality assurance and GLP compliance were undertaken. The report was signed by the authors.

C. Methods and Results:

1. Observations - All animals were inspected daily for signs of toxicity and mortality. In addition, a detailed physical examination was performed once each week.

Results:

F_0 Generation - There were no deaths in the male and female rats of the F_0 generation. Individual animal data for clinical signs were not submitted. According to the summary data, there were no compound-related clinical signs.

During weeks 18 and 19, most animals had infection with sialadacryoadenitis (SDAV) which delayed the second mating by 1 week.

There was no compound-related clinical effect on the second mating due to the viral infection.

F_1 Generation - One 5000 ppm male (No. 3118) was found dead during week 21 with lymphoid leukemia and multifocal erosions of the gastric mucosa. There were no other deaths in the F_1 parental animals.

There were no compound-related clinical findings during the F_1 generation.

2. Body Weight - Body weight was measured weekly for all of the F_0 and F_1 animals and mated females were weighed on days 0, 7, 14, and 21 of gestation and days 0, 7, 14, and 21 postpartum.

Results:

F_0 Parents - There were no compound-related effects in body weight or body weight gain in males during the F_0 treatment period. At week 18, both control and treated male groups showed a weight loss due to the SDAV infection. Statistically significant differences in body weight gain between control and treated male groups did not occur in a dose-related manner.

There were no compound-related effects in body weight or body weight gain in females during the growth phase or gestation and lactation phases of both the F_1a and F_1b reproductive phases. Additionally, there were no statistically significant differences between control and treated female groups during all phases of the F_0 generation.

F_1 Parents - There were no compound-related effects in body weight or body weight gain between control and treated male groups during the 24-week F_1 period. Statistically significant differences between control and treated male groups did not occur in a dose-related or time-related pattern and were considered unrelated to treatment.

The body weights and body weight gains between control and treated female groups did not display any compound-related effects. Statistically significant differences between control and treated female groups were not dose-related or did not occur in a consistent time-related pattern and were considered unrelated to treatment.

3. Food Consumption - Food consumption was determined weekly during the treatment periods, but not during mating, gestation, or lactation. Compound intake was calculated from the food consumption and body weight gain.

Results:

F_0 Generation - Food consumption (grams/animal) was comparable between control and treated male and female groups during the 20-week period. During week 18, food

consumption in males was decreased in all groups due to SDAV infection.

F_1 Generation - Food consumption in males was comparable between control and treated groups except during week 19 where the high-dose group males consumed significantly less food in comparison to controls. Food consumption in females did not show any compound-related effects during the F_1 generation and there were no statistically significant differences between control and treated female groups.

4. Compound Intake

F_0 Generation - The mean compound intake for males was 74.2, 380.5, and 738.0 mg/kg/day for the low-, mid-, and high-dose groups, respectively. For females, the mean compound intake was 94.3, 471.2, and 933.3 mg/kg/day for the low-, mid-, and high-dose groups, respectively.

F_1 Generation - The mean compound intake for males was 83.8, 418.4, and 849.9 mg/kg/day for the low-, mid-, and high-dose groups, respectively. For females, the mean compound intake was 102.0, 515.3, and 1026.4 mg/kg/day for the low-, mid-, and high-dose groups, respectively.

5. Evaluation of Mating and Reproductive Indices - Analysis of the mating and reproductive performance consisted of determining the following indices:

Mating Index = [Females mated]/[Females placed for mating] x 100

Fertility Index = [Pregnant females]/[Females placed for mating] x 100

Conception Rate = [Pregnant females]/[Females mated] x 100

Maternal performance was determined as:

Gestation Index = [Rats with live pups]/[Pregnant rats] x 100

Litter data was assessed by the following indices:

Viability Index = [Live pups on day 4*]/[Live pups on day 0] x 100

*Postpartum day.

Survival Index = [Live pups on day 7* or 14*]/[Live pups on day 4*] x 100

Lactation Index = [Live pups on day 21*]/[Live pups on day 4*] x 100

Additionally, pup body weight and sex ratio were also evaluated.

Results:

F_0 Generation - For the first mating phase (F_1a litters), the mating indices were comparable between control and treated groups. The mating indices were 92.0, 100.0, 100.0, and 100.0 percent for the control, low-, mid-, and high-dose groups, respectively. For the fertility indices, the high-dose group value was decreased in comparison to the controls. The fertility indices were 88.0, 84.0, 84.0, and 72.0 percent for the control, low-, mid-, and high-dose groups, respectively. Similarly, the high-dose groups had a lower conception rate in comparison to controls. The conception rates were 95.7, 84.0, 84.0, and 72.0 percent for the control, low-, mid-, and high-dose groups, respectively. The decreased conception rate in the high-dose was statistically significant.

The day of mating in the control and treated groups for the first mating phase was comparable. The mean number of days to mating were 2.70, 3.40, 3.61, and 2.44 for control, low-, mid-, and high-dose groups, respectively.

With respect to maternal performance for the first mating phase, there were no compound-related effects in gestation indices, length of gestation, length of parturition, number of live pups at birth, and sex ratio of pups. For the first mating phase, the gestation indices were 100 percent for all groups. The length of gestation was 21.6, 21.8, 21.7, and 21.7 days for the control, low-, mid-, and high-dose groups, respectively; the length of parturition was 2.21, 2.52, 2.40, and 2.87 hours for the control, low-, mid-, and high-dose groups, respectively; the number of live pups at birth was 13.5, 14.8, 13.2, and 13.0 for the control, low-, mid-, and high-dose groups, respectively. The incidence of dead pups and number of litters with dead pups (in parentheses) was 6(4), 5(3), 3(3), and 4(4) for the control, low-, mid-, and high-dose groups, respectively. The percentage of male pups in the litters was 45.3, 46.9,

*Postpartum day.

52.2, and 51.6 for the control, low-, mid-, and high-dose groups, respectively.

The decreases in fertility index and conception rate in the high-dose group during the first mating phase is not considerered compound-related, since evaluation of the number of fertile males and females for both the first and second mating phase of the F_0 generation did not reveal any compound-related effects.

The number of males producing at least one pregnancy was 22 out of 25 for all groups and the number of males producing two pregnancies was 16, 19, 16, and 16 for the control, low-, mid-, and high-dose groups, respectively. Similarly, the number of females pregnant at least once was 22, 23, 22, and 22 and the number of females pregnant twice was 16, 19, 17, and 16 for the control, low-, mid-, and high-dose groups, respectively.

There were no compound-related effects in the F_{1a} pups with respect to viability, survival, and lactation indices and pup body weight. The viability indices were 99.7, 98.4, 97.4, and 98.6 percent for the control, low-, mid-, and high dose groups, respectively. Survival indices for both days 4 to 7 and days 4 to 14 were 100 percent for all groups. Lactation indices were 100, 99.4, 100, and 100 percent for the control, low-, mid-, and high-dose groups, respectively.

There were no compound-related gross pathological findings in F_{1a} pups.

For the second mating phase (F_{1b}) of the F_0 generation, there were no compound-related effects in mating indices, fertility indices, or conception rates.

The mating indices were 92.0, 92.0, 96.0, and 96.0 percent for the control, low-, mid-, and high-dose groups, respectively. For the high-dose groups, the fertility indices and conception rates were increased in comparison to controls. The fertility indices were 68.0, 84.0, 72.0, and 84.0 percent and the conception rates were 73.9, 91.3, 75.0, and 87.5 percent for the control, low-, mid-, and high-dose groups, respectively.

The differences in these findings are not considered compound-related.

With respect to maternal performance for the second mating phase, there were no compound-related effects in gestation indices, length of gestation in days, length

of parturition in hours, number of live pups at birth, and sex ratio.

For the control, low-, mid-, and high-dose groups, respectively, the gestation indices were 100.0, 100.0, 100.0, and 95.2 percent; the length of gestation in days was 21.7, 21.8, 21.8, and 21.8; the length of parturition in hours was 1.92, 1.93, 2.46, and 1.81; the number of live pups at birth was 13.5, 13.2, 13.4, and 13.7; and the sex ratio expressed as percent males was 45.1, 50.0, 48.6, and 50.1 percent - all values are for the control, low-, mid-, and high-dose groups, respectively.

The incidence of dead pups at birth was statistically significantly increased in the high-dose in comparison to controls. The dead pup incidences were 3/219, 5/282, 4/286, and 15/288 for the control, low-, mid-, and high-dose groups, respectively.

The increase at the high-dose was attributed primarily to the large litter loss for dam No. 455 of the high-dose group. Since the number of litters with dead pups was 2, 3, 4, and 4 for the control, low-, mid-, and high-dose groups, respectively, the increased incidence in the number of dead pups at the high dose was not considered compound-related.

There were no compound-related effects in the F_1b pups with respect to viability, survival and lactation indices, and pup body weight. The viability indices were 98.8, 99.2, 100.0, and 94.5 percent; the day 4 to 7 postpartum survival indices were 100.0, 100.0, 98.6, and 100.0 percent; the day 4 to 14 postpartum survival indices were 100.0, 100.0, 100.0, and 95.0 percent; and the lactation indices were 100.0, 100.0, 100.0, and 95.0 percent - all values are for the control, low-, mid-, and high-dose groups, respectively.

There were no compound-related gross pathological findings in the F_1b pups.

F_1 Generation - For the first mating phase (F_2a litters), there were no compound-related effects in mating indices, fertility indices, conception rates, or the mean number of days to mating. The mating indices were 88.0, 96.0, 100.0, and 96.0 percent; the fertility indices were 88.0, 88.0, 84.0, and 80.0 percent; the conception rates were 100.0, 91.7, 84.0, and 83.3 percent; and the mean number of days to mating were 2.38, 2.39, 2.38, and 2.71 days - all values are for the control, low-, mid-, and high-dose groups, respectively.

With respect to maternal performance, there were no compound-related effects in gestation indices, the length of gestation in days, the length of parturition in hours, both the number of pups and the number of litters with live and dead pups, and the sex ratio of the pups.

The gestation indices were 100 percent for all groups; the length of gestation was 21.6, 21.7, 21.7, and 21.6 days; the length of parturition was 1.66, 2.38, 2.15, and 1.77 hours; the number of live pups at birth was 12.8, 13.7, 12.0, and 13.0; the incidence of dead pups (litters in parentheses) was 8(3), 4(4), 5(5), and 2(1); and the sex ratio, expressed as percent males, was 52.2, 49.1, 52.7, and 53.9 percent - all values are for the control, low-, mid-, and high-dose groups, respectively.

There were no compound-related effects in the F_2a pups with respect to viability indices, day 4 to 7 postpartum survival indices, day 4 to 14 postpartum survival indices, lactation indices, and pup body weight.

The viability indices were 98.4, 99.1, 98.9, and 99.2 percent; the day 4 to 7 postpartum survival indices were 100 percent for all groups; the day 4 to 14 postpartum survival indices were 99.4, 100, 100, and 100 percent; and the lactation indices were 99.4, 100, 100, and 100 percent - all values are for the control, low-, mid-, and high-dose groups, respectively.

There were no compound-related lesions in the gross pathological findings of F_2a pups.

For the second mating phase (the F_2b litters), there were no compound-related effects in the mean number of days to mating, the mating indices, the fertility indices, and conception rates.

The mean number of days to mating was 2.91, 2.47, 2.72, and 2.96; the mating indices were 88.0, 76.0, 100.0, and 92.0 percent; the fertility indices were 80.0, 72.0, and 92.0, and 76.0 percent; and the conception rates were 90.9, 94.7, 92.0, and 82.6 percent - all values are for the control, low-, mid-, and high-dose groups, respectively.

With respect to maternal performance, there were no compound-related effects in gestation indices, length of gestation in days, length of parturition in hours, number and incidence of live and dead pups at birth and sex ratio. The gestation indices were 100 percent for all groups; the length of gestation in days was 21.8, 21.9,

21.9, and 21.8; the length of parturition in hours was 1.75, 1.81, 1.97, and 1.96; the number of live pups at birth was 13.0, 15.0, 13.1, and 13.3; the incidence of dead pups at birth (litters in parentheses) was 13(5), 4(4), 2(2), and 8(6); and the sex ratio expressed as percent of males was 45.3, 44.8, 53.5, and 53.2 - all values are for the control, low-, mid-, and high-dose groups, respectively.

The decreases in the incidence of dead pups at birth in the low- and mid-dose groups in relation to controls were statistically significant. The incidence of litters with dead pups was not statistically different in these groups in comparison to controls. These intergroup variations in dead pups at birth are not considered compound-related, since the decreases in dead pups were not dose-related (no high-dose effect was observed) and the litter incidence was similar between control and treated groups.

The combined parental performance for males and females for the F_1 generation (both F_2a and F_2b litters) did not reveal any treatment related findings. The number of males producing at least one pregnancy was 23, 21, 23, and 22; the number of females pregnant at least once was 23, 22, 23, and 22; the number of males producing two pregnancies was 19, 18, 21, and 17; and the number of females pregnant twice was 19, 18, 21, and 17 - all values are for the control, low-, mid-, and high-dose groups, respectively.

With respect to the F_2b group viability results, there were no compound-related effects in viability indices, day 4 to 7 postpartum survival indices, day 4 to 14 postpartum survival indices, lactation indices and pup body weight. The viability indices were 99.7, 96.7, 98.0, and 98.5 percent; the day 4 to 7 postpartum survival indices were 98.7, 100.0, 99.5, and 100.0 percent; the day 4 to 14 postpartum survival indices were 98.7, 100.0, 98.8, and 99.3 percent; and the lactation indices were 98.7, 100.0, 98.8, and 99.3 percent - all values are for the control, low-, mid-, and high-dose groups, respectively.

5. Sacrifice and Pathology

a. Parental Rats - All F_0 and F_1 adult animals found dead or sacrificed in extremis were given a detailed internal gross examination. Additionally, adult males and females of the F_0 and F_1 generations were

examined grossly by a pathologist, and histopathology was done on the following tissues and organs:

Epididymides*	Seminal vesicles*
Liver	Testes*
Mammary glands	Uterus*
Ovaries*	Vagina
Orostate*	Abnormal lesions**

*Histopathology for control and high dose.
**Histopathology for all groups.

Results - There were no compound-related gross findings in the F_0 adults. Histologically, there was an increased incidence of prostatitis in high-dose F_0 male rats (7/25 in controls, 0/4 at low-dose, 2/7 at mid-dose, and 12/25 at high-dose) but this slight increase was not considered toxicologically significant. Female F_0 rats were histologically unremarkable.

There were no compound-related gross pathological findings in F_1 adult rats. Histologically, the male and female F_1 adults were comparable between control and treated groups.

b. Pups and Weanlings - Dead and abnormal pups were given a gross pathological examination. One male and one female weanling from each litter of the F_1b and F_2b generations were given a gross necropsy examination. Abnormal tissues observed in pups and weanlings were preserved in 10% formalin. Complete histopathological evaluation of forty tissues was conducted on all F_2b weanlings which received gross pathological examination. The following number of F_2b weanlings pups) were examined histologically: males: 19, 19, 23, and 20- for control, low-, mid-, and high-dose groups, respectively; females: 22, 17, 22, and 18 from control, low-, mid-, and high-dose groups, respectively.

Tissues Examined Histologically

Adrenals	Lungs (samples of 2 lobes	Spinal cord (thoracic and lumbar)
Aorta (thoracic)	Lymph nodes (mandibular and mesenteric)	Spleen
Bone and marrow sternum)		Stomach

Brain (3 levels)	Mammary gland (inguinal)+	Testes*
Cecum	Optic nerve*+	Thymus
Colon	Ovaries	Thyroid (and parathyroids)+
Duodenum	Pancreas	Tongue
Epididymides*	Pituitary	Trachea
Esophagus	Prostate	Urinary bladder
Eyes*	Salivary gland (sub-maxillary)	Uterus (corpus and cervix)
Heart	Sciatic nerve	Vagina
Ileum	Seminal vesicles	Any other tissue(s) with gross lesions
Jejunum	Skeletal muscle	
Kidneys	Skin	
Liver (sample of 2 lobes		

Results - There were no compound-related gross or microscopic pathological findings in F_2b generation pups.

*Fixed with Zenker's fluid (sacrificed animals only).
+Only examined histologically where present in routine sections of eyes (optic nerves), thyroid (parathyroids), or skin (mammary gland).

57211:I:Dykstra:C.Disk:KENCO:06/05/89:DD:vo:de
R:57214:Dykstra:C.disk:KENCO:06/08/89:de:vo:rw

008425

Reviewed By: William Dykstra (William Dykstra 11/25/90)
Section I, Toxicology Branch I, IRS (H7509C)
Secondary Reviewer: Roger Gardner, Acting Section Head (by Gardner 06/14/91 (for RG))
Section I, Toxicology Branch I, IRS (H7509C)

DATA EVALUATION REPORT

Study Type: 83-5: Combined Chronic Toxicity/Oncogenicity Study Study, Rats

TOX Chem. No. 221G
MRID No. 410395-03
Vol. 1-9

Accession No.: N/A

008426

Test Material: AC 243,997 Technical
99.5% a.i.

Synonyms: Arsenal (Imazapyr)

Study No.: 84-2862

Sponsor(s): American Cyanamid Company

Testing Facility: Bio/dynamics, Inc.

Title of Report: A Chronic Dietary Toxicity and Oncogenicity Study With AC 243,997 in Rats.

Author(s): Ira Daly

Report Issued: April 6, 1988

Conclusion:

Additional information is required. The registrant is required to submit complete statistical analyses for female adrenal medullary neoplasms and male brain neoplasms.

The issue of thyroid C-cell carcinoma is resolved.

There were no compound-related toxic effects in male food consumption, body weight, clinical pathology, organ weights, and non-neoplastic lesions. There was a slight dose-related decrease in survival of high-dose male rats, but not female rats.

There were no compound-related toxic effects in female rats with respect to body weight and food consumption (although food efficiency in female rats showed a marginal toxic effect). Additionally there were no compound-related toxic effects in clinical pathology and organ weights. The NOEL for non-neoplastic lesions in female rats is the mid-dose of 5000 ppm. The LEL is the high-dose of 10,000 ppm and the effects are an increased incidence of extramedullary hematopoiesis in the spleen and B-squamous cysts in the thyroid. An MTD may not be established in the study.

Classification: Core-Supplementary

Special Review Criteria (40 CFR 154.7): N/A

Review:

A. Materials:

1. Test Compound - AC 243,997; Description: Off-white chunky powder; Batch No.: AC 4866-062; Purity: 99.5%; Contaminants: List in CBI Appendix.

2. Test Animals - Species: Rat; Strain: Sprague-Dawley; Age: 29 days old; Weight (mean): Males 195 g, Females 148 g; Source: Charles River Breeding Labs, Kingston, NY.

B. Study Design:

1. Animal Assignment - Animals were assigned randomly to the following test groups:

Test Group	Dose in Diet (ppm)	Main Study Total Nos. Male	Main Study Total Nos. Female	Interim Sac.[a/] 12 Months Male	Interim Sac.[a/] 12 Months Female	Months24[b/] Necropsy and Histopathology Male	Months24[b/] Necropsy and Histopathology Female
1 Control	0	65	65	13	14	52	51
2 Low (LDT)	1000	65	65	13	10	52	55
3 Mid (MDT)	5000	65	65	12	12	53	53
4 High (HDT)	10,000	65	65	13	10	52	55

a/Includes unscheduled deaths prior to month 12.
b/Includes unscheduled deaths between month 12 and study termination at month 24.

2. Diet Preparation - Diet was prepared weekly and stored at room temperature. Samples of treated food were analyzed for stability and concentration.

Results - The dose levels of 1000 and 10,000 ppm were analyzed for homogeneity and found to have means of 100.9 and 106.2 percent, respectively, with coefficients of variation of 1.9 and 7.1 percent, respectively. The test material was stable in the diet for the 2-week period while it was exposed in the rat feeders. The low-dose batch lost an apparent 3.6 percent per week while the high-dose lost an apparent 4.0 percent per week. Additionally, diets prepared and dispensed to the rats weekly during the entire study were found to contain an average of 95.8 percent (1000 ppm), 96.0 percent (5000 ppm), and 96.8 percent (10,000 ppm) of nominal concentration. The coefficients of variation were 5.3 percent (low-dose), 4.5 percent (mid-dose), and 4.5 percent (high-dose).

3. Animals received food (Purina Certified Rodent Chow #5002) and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data: parametric and nonparametric. The following is quoted from the study report:

"Body weight, food consumption, hematology and clinical chemistry parameters, organ weights, organ/body, organ/brain weight ratios and survivorship and tumor onset analyses were analyzed. Mean values of all dose groups were compared to control at each time interval. Statistically significant differences from control are indicated on mean tables of appendices.

"Statistical evaluation of equality of means was made by the appropriate one way analysis of variance technique, followed by a multiple comparison procedure if needed. First, Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. The parametric procedures were the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly different from the control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summed rank test (Dunn) was used to determine which treatments differed from control.

"A statistical test for trend in the dose levels was also performed. In the parametric case (i.e., equal variance) standard regression techniques with a test for trend and lack of fit were used. In the nonparametric case Jonckheere's test for monotonic trend was used.

"The test for equal variance (Bartlett's) was conducted at the 1%, two-sided risk level. All other statistical tests were conducted at the 5% and 1%, two-sided risk level." [End of quotation.]

Life Table Analysis - The data on time to neoplastic lesion were analyzed for each sex separately by the series of programs included in the N.C.I. package for histopathologically proven tumors and time to tumor.

5. Quality assurance was performed and the report was signed.

C. Methods and Results:

1. Observations - Animals were inspected twice daily for signs of toxicity and mortality. Detailed physical examinations for signs of local or systemic toxicity, pharmacologic effects, and palpable masses were performed pretest and weekly thereafter.

Results:

a. Toxicity - There were no compound-related toxic signs. The most frequently observed in-life physical signs were chromodacryorrhea, opacity, lacrimation, teeth problems, and alopecia in males. In females excess lacrimation, chromodacryorrhea, ear problems, teeth problems, alopecia and stains were observed most frequently.

b. Survival - For males, there was a slight dose-response in the time to death. Based on Kaplan-Meier estimated median survival times, the days on study for the control, low-, mid-, and high-dose groups were 667, 689, 660, and 655 days, respectively. Using similar methods for the females, survival times were 726, 734+, 708, and 740 days for the control, low-, mid-, and high-dose groups, respectively.

As reported in the summary data, animal survival (%) at selected intervals during the study was as follows:

Group	An.	Dose Level (ppm)	% Survivorship*			
Months	6 M/F	12 M/F	18 M/F	Term M/F
I	65	0	98/100	95/94	85/84	36/45
II	65	1000	100/100	98/100	85/89	40/37
III	65	5000	100/100	97/97	76/78	36/38
IV	65	10,000	98/100	97/100	75/85	25/49

*Excludes a 12-month interim sacrifice of 10 animals/sex/group and one accidental death.

The slight effect in survivorship in high-dose males, which is also slightly dose-related, may support a position that an MTD was approached for males in the study. This position is supported by the fact that the major effect in survivorship occurred during the last year of the study when, in addition to aging, cumulative toxicity is more apparent in males.

2. Body Weight - Animals were weighed weekly for 14 weeks, then biweekly through 40 weeks, monthly thereafter, and terminally (after fasting).

Results - There were no compound-related toxic effects in body weight gain in treated male and female rats in comparison to controls. The following data shows the mean body weights of both sexes during the study. Slight increases in body weight gain were apparent for mid- and high-dose male rats in comparison to controls.

Mean Body Weight (grams)

	Males				Females			
	Dose				Dose			
	0	1000	5000	10,000	0	1000	5000	10,000
Month								
0	195	195	194	194	149	147	148	146
1	375	377	384	385	238	237	236	234
4	545	542	549	558	306	302	306	299
6	602	602	615	625	339	338	341	335
8	641	639	650	664	363	359	370	361
12	702	700	710	722	422	419	437	427
18	738	736	708	750	489	483	486	474
24	674	637	614	659	501	486	492	501

3. Food Consumption and Compound Intake - Consumption was determined and mean daily diet consumption was calculated. Efficiency and compound intake were calculated from the consumption and body weight gain data.

Results - Mean food consumption values (g/kg/day) were, on occasion, statistically significantly increased in treated male groups, although the differences were not usually dose-related. Additionally the differences seldom exceeded 5 percent at the high-dose where the differences were most pronounced. This slight increase in food consumption can be correlated with the slight increase in body weight in these high-dose males.

In females, increases in food consumption occurred in all treated groups at about a 5 to 7 percent increased rate above controls during about the first 60 weeks of the study. These increased levels of food consumption are considered compound-related, although they were not

always dose-related and, in contrast to the males, were not reflected in higher body weight gains. This observation suggests that food efficiency was reduced in treated females. This slight reduction in food efficiency represents a marginal toxic effect.

Mean test substance intake calculated over the 2-year study period, as calculated in the study report, was as follows:

	AC 243,997 (mg/kg/day)		
Group	Dose Level (ppm)	Male	Female
II	1000	49.9	64.2
III	5000	252.6	317.6
IV	10,000	503.0	638.6

4. Ophthalmalogical examinations were performed at 12 and 24 months on all animals.

Results - There were no compound-related ophthalmalogical abnormalities noted by Dr. L.F. Rubin, the examining veterinary ophthalomalogist (Appendix D).

5. Blood was collected at 3, 6, 12, 18, and 24 months for hematology and clinical analysis from 10/sex/dose animals. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*		Total plasma protein (TP)
X	Hemoglobin (HGB)*	X	Leukocyte differential count
X	Leukocyte count (WBC)*		Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)*		Mean corpuscular HGB conc. (MCHC)
X	Platelet count*		Mean corpuscular volume (MCV)
		X	Erythrocyte morphology

Results - The hematocrit of high-dose males was slightly, though significantly, increased (43% at high-dose vs. 40% in controls), at 3 months but not at later times. Inspection of individual values showed a generally higher percent hematocrit for most high-dose animals rather than the increase being the results of one or a few highly aberrant values. These findings in hematocrit in high-dose males are of no toxicological significance, since individual values ranged between 32 to 52 percent (Note: hematocrit values between 32-52% are 2 ± SD0 of mean

of 42%) which is within the historical control range for hematocrit values for young rats (strain unspecified) at IRDC. Other statistically significant hematological values observed during the 2-year period (and there were only a couple) did not occur at the high-dose and, therefore, were not dose-related. These statistically significant hematological findings were an increased percent hematocrit at the low-dose (42% vs. 40% control) at 3 months in females and an increased number of WBC (3.1 thousand/mL at mid-dose vs. 6.3 thousand/mL in control) in females at 12 months.

Since these aberrant values in hematology were not dose- or time-related, they were not considered compound-related.

b. Clinical Chemistry

X	Electrolytes:	X	Other:
X	Calcium*	X	Albumin*
X	Chloride*		Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
	Phosphorous*	X	Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	Enzymes	X	Total Bilirubin*
X	Alkaline phosphatase	X	Total Protein*
	Cholinesterase		Triglycerides
	Creatinine phosphokinase*	X	Direct Bilirubin
X	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
X	Gamma glutamyl transpeptidase		

Results:

3 Months - Slight, though significant, increase in BUN (mg/dl) in high-dose males (15.8 mg/dl vs. 13.3 mg/dl in controls). Significant increase in potassium in low-dose males (5.4 mEq/L vs. 4.9 mEq/L in control). There were no statistically significant differences between control and treated groups for any other parameters in males or for any parameters at all in females.

6 Months - No statistically significant differences between control and treated groups in males and females for any clinical chemistry parameter at all at 6 months.

12 Months - No statistically significant differences between control and treated groups in males and females for any clinical chemistry parameter at all at 12 months.

18 Months - A slight, though significant, decrease in total protein in mid-dose males (but not high-dose males). No statistically significant differences between control and treated groups in other clinical parameters in males or in any clinical parameter at all in females.

24 Months - No statistically significant differences between control and treated groups in males and females for any clinical chemistry parameter at all at 24 months.

Due to the absence of relationships to dose and time in the few abnormal clinical chemistry values, there were no compound-related effects in clinical chemistry.

6. Urinalysis - Urine was collected from fasted animals at 3, 6, 12, 18, and 24 months. The CHECKED (X) parameters were examined.

X		X	
X	Appearance*	X	Glucose*
	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein*		Urobilinogen

Results - There were no compound-related effects in urinalysis at 3, 6, 12, 18, or 24 months.

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X		X		X	
	Digestive system		Cardiovas./Hemat.		Neurologic
X	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	XX	Pituitary*
X	Duodenum*	XX	Spleen*	X	Eyes (optic n.)*
X	Jejunum*	XX	Thymus* (interim only)		Glandular
X	Ileum*		Urogenital	XX	Adrenals*

X	Cecum*	XX	Kidneys*		Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Mammary gland*
X	Rectum*	XX	Testes*	X	Parathyroids*
XX	Liver*	X	Epididymides	XX	Thyroids*
	Gall bladder*	X	Prostate	Other	
X	Pancreas*	X	Seminal vesicle	X	Bone*
Respiratory		XX	Ovaries	X	Skeletal muscle*
X	Trachea*	X	Uterus*	X	Skin
XX	Lung*			X	All gross lesions and masses

Results:

A. Organ Weights

12 Months - No statistically significant differences in means in organ weight or organ/body weight ratios or organ/brain weight ratios between control and treated male and female rats at 12 months in any weighed organs.

Terminal Sacrifice - No statistically significant differences in means in organ weights or organ/body weight ratios or organ/brain weight ratios between control and treated male and female rats at terminal sacrifice in any weighed organs.

B. Gross Pathology - There were no compound-related necropsy findings in male or female rats that died on study, or were sacrificed at 12 or 24 months.

C. Microscopic

1. Neoplastic

a. Brain Tumors

There was an increased incidence of astrocytomas (a brain tumor) in high-dose male rats in comparison to controls.

Male Rats

Dose (ppm)	Animal Number	Week Death	Tumor
0	1051	106	B-astrocytoma
1000	2010	53	B-astrocytoma
5000	3059	106	B-granular cell tumor
5000	3046	55	B-oligodendroglioma

Male Rats (cont'd)

Dose (ppm)	Animal Number	Week Death	Tumor
10,000	4005	106	M-astrocytoma
10,000	4028	106	B-astrocytoma
10,000	4037	106	B-astrocytoma
10,000	4051	106	B-astrocytoma

With respect to the incidence for the number examined by effective proportion, without survival disparity analyses, the summary of brain tumors for males, is presented below:

		Male			
	Groups:	1	2	3	4
Brain:	No. Examined:	51	52	51	51
M-astrocytoma		0	0	0	1
B-astrocytoma		1	1	0	3
B-oligodendroglioma		0	0	1	0
B-granular cell tumor		0	0	1	0
Percentages		2.0	1.9	3.9	7.8

Since there was a survival disparity among the various groups of male rats, a complete statistical analysis of the data is necessary and is being performed by HED statisticians.

Historical controls for brain tumors (all types) was provided by Ira Daly of Bio/dynamics.

In 14 studies submitted, the range of astrocytomas was 0 to 3.3 percent. The individual studies provided percentages of 1.7, 0.08, 0.86, 3.3, 0.08, 0, 0, 0, 0, 0.8, 0, 1.8, 0, and 1.7.

Other gliomas besides astrocytomas were recorded in rat brains in these 14 studies.

Additionally, it should be noted that the duration of the historical control studies generally exceeded 24 months.

The full significance of the oncogenic potential of Arsenal to male rat brains must await full statistical analysis and possibly Peer Review.

There was no compound-related effect in female rat brain tumors.

The Bio/dynamics historical control data are appended to this report.

b. Thyroid C-cell Tumors

The incidence of C-cell thyroid neoplasms, showed an increase at the mid- and high-dose. More specifically, however, the C-cell carcinoma incidence was increased at the high-dose only.

The following data summarizes the findings.

Male Rats

Group	1	2	3	4
No. examined	65	65	65	65
C-cell carcinoma	1	1	1	5
C-cell adenoma	2	3	3	4

The high-dose male rats showed a higher incidence of C-cell carcinoma (5/65, 7.69%), when compared to the control (1/65, 1.53%), low-dose (1/65, 1.53%), and mid-dose (1/63, 1.58%) male rats. Additionally, the high-dose incidence (7.69%) was within the range of 0 to 13.7 percent from historical control data collected at Bio/dynamics. Also, the high-dose was reported to be without statistical significance.

The fate of the individual male rats with C-cell carcinoma is presented below as tabulated in the report.

Male Rats with C-cell Carcinoma

Group	Sex	Animal No.	Death Code	Day of Study of Death	Week of Study of Death
I	M	1024	D	614	88
II	M	2027	D	691	99
III	M	3059	T	739	106
IV	M	4017	D	654	99

Male Rats with C-cell Carcinoma (cont'd)

Group	Sex	Animal No.	Death Code	Day of Study of Death	Week of Study of Death
IV	M	4023	S	639	92
IV	M	4040	S	669	96
IV	M	4055	T	739	106
IV	M	4064	S	665	95

Key: T = Terminal Sacrifice; D = Spontaneous Death;
S = Sacrificed Moribund.

It can be seen from this table there is no apparent decrease in latency of the C-cell tumor.

Proliferative lesions of the male thyroid in this study are summarized below as presented the report:

Table 1

Thyroid Gland

Summary-Incidence of Proliferative Lesions

Sex	Males			
Group	I	II	III	IV
Thyroid gland # Examined	65	65	63	65
C-cell hyperplasia	15	8	13	6
%	23.10	12.31	20.63	9.23
C-cell adenoma	2	3	9	4
%	3.10	4.62	14.29	6.15
C-cell carcinoma	1	1	1	5
%	1.54	1.54	1.59	7.69
C-cell adenoma and carcinoma	3	4	10	9
%	4.62	6.15	15.87	13.85

Summary-Incidence of Proliferative Lesions (cont'd)

Sex		Males			
Group		I	II	III	IV
C-cell hyperplasia, adenoma and carcinoma combined		17	12	21	15
	%	26.15	18.46	33.33	23.08

The differences between the incidences of all groups are not statistically significant. The following tables, taken from the report, are of historical control data from Bio/dynamics.

Table 2

Thyroid Gland - Selected Findings
Historical Control Data - Male Charles River Albino CD® Rats
(Compiled from 14 Studies Conducted at Bio/dynamics, Inc.)

	Range of Incidence of Historical Data		Mean Incidence of Historical Data
	Low	High	
# Examined	73	69	1413
C-cell hyperplasia	0	10	60
Percentage	0	14.59	4.25
# Examined	73	69	1413
C-cell hyperplasia	0	10	60
Percentage	0	14.59	4.25
# Examined	131	70	1413
C-cell ademoma	0	8	72
Percentage	0	11.43	5.10
# Examined	129	131	1413
C-cell carcinoma	0	18	58
Percentage	0	13.74	4.10
# Examined	54	70	1413
C-cell adenoma and carcinoma combined	0	12	129
Percentage	0	17.14	9.13

Thyroid Gland - Selected Findings (cont'd)

	Range of Incidence of Historical Data		Mean Incidence of Historical Data
	Low	High	
# Examined	54	70	1413
C-cell hyperplasia, adenoma and carcinoma combined	0	18	183
Percentage	0	25.71	12.95

For the purposes of a combined finding, those animals having more than one finding were counted only once.

Table 3 shows the individual studies for the data from the Bio/dynamic files.

Table 3

Thyroid Gland - Selected Findings
Historical Control Data - Male Charles River Albino CD® Rats

Study	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Date Initiated	1978	1977	1977	1978	1978	1977	1977	1978	1977	1977	1978	1978	1977	1978
Date Terminated	1980	1980	1980	1981	1980	1980	1980	1980	1980	1979	1981	1980	1980	1981
# Examined	68	139	139	70	69	131	123	54	139	139	70	73	129	70
C-cell adenoma	0	14	6	8	4	0	0	0	5	8	8	2	13	4
%	0	10.1	4.3	11.4	5.8	0	0	0	3.6	5.8	11.4	2.7	10.1	5.7
C-cell carcinoma	2	4	4	4	2	18	12	0	3	1	0	0	0	8
%	2.9	2.9	2.9	5.7	2.9	13.7	9.8	0	2.2	0.7	0	0	0	11.4
C-cell adenoma and carcinoma	2	18	10	12	6	18	12	0	8	9	8	2	13	11
%	2.9	12.9	7.2	17.1	8.7	13.7	9.8	0	5.8	6.5	11.4	2.7	10.1	15.7
C-cell hyperplasia	4	3	9	6	10	9	2	0	11	2	2	0	1	1
%	5.9	2.2	6.6	8.6	14.5	6.9	1.6	0	7.9	1.4	2.9	0	0.8	1.4
C-cell adenoma and carcinoma and hyperplasia	6	20	19	18	16	23	14	0	19	11	9	2	14	12
%	8.8	14.4	13.7	25.7	23.2	17.6	11.4	0	13.7	7.9	12.9	2.7	10.9	17.1

Evaluation of Tables 1, 2, and 3 show that the incidences of C-cell proliferative lesions in the AC 243,997 study (C-cell hyperplasia, C-cell adenoma, C-cell carcinoma), individually or in combination, were within the range of Bio/dynamic historical control data. In addition, the incidences do not exhibit a stepwise dose-response progression from hyperplasia to adenoma to carcinoma (see Table 1).

As can be seen by comparing the incidences of proliferative lesions from Table 1 (the data from the current study) to the Bio/dynamic historical control data in Table 2, the incidences of the proliferative lesions at the high-dose are all within the range of the historical control data as shown below.

Table 4

Thyroid Findings

C-cell	High-Dose Incidence of Current Arsenal Study	Range of Hist. Controls from Bio/dynamics
Hyperplasia	9.23%	0 - 14.59%
Adenoma	6.15%	0 - 11.43%
Carcinoma	7.69%	0 - 13.74%
Adenoma and Carcinoma	13.85%	0 - 17.14%
Hyperplasia and Adenoma and Carcinoma	23.08%	0 - 25.71%

The report states that Suzuki et al. (1979) reported the incidence of medullary carcinoma in the thyroid gland of Sprague-Dawley rats to be 79 percent (33/42) in males and 49 percent (19/39) in females.

The registrant employed an outside consultant, W. Roy Brown, D.V.M., Ph.D., to examine the thyroid gland of male rats and render an opinion.

Dr. Brown's analysis is presented below in Table 5.

Table 5
Dr. Brown's Analysis

Summary Incidence of Proliferative Lesions of C-cell Origin in the Thyroid Gland of Male Rats

Dose Group	I	II	III	IV
Number of Thyroid Glands Examined	65	65	65	65
C-cell hyperplasia (all degrees)				
Incidence	8	13	14	7
Percent	12.3	20.0	21.5	10.8
C-cell adenoma				
Incidence	2	1	7	2
Percent	3.1	1.5	10.8	3.1
C-cell carcinoma				
Incidence	1	1	1	4
Percent	1.5	1.5	1.5	6.2
C-cell adenoma and carcinoma				
Combined Incidence	3	2	8	6
Percent	4.6	3.1	12.3	9.2
C-cell hyperplasia adenoma and carcinoma				
Combined Incidence	11	15	22	13
Percent	16.9	23.1	33.8	20.0

Dr. Brown states "It is my opinion that the difference between the control and high dosage group male rats with respect to the C-cell carcinomas is of no biological significance. The incidence in the high-dose rats is consistent with that which can occur spontaneously and those that have been reported in control rats in studies of similar type at Bio/dynamics, the site of the study. An incidence of as high as 79% (33/42) of C-cell carcinoma have been reported in male Sprague-Dawley rats (Suzuki, et al.). Other studies indicate an increase of 16-40% of C-cell carcinomas in other strains of rats, including Long-Evans, Sprague-Dawley, Wistar and wild rats (Rattus norvegicus). The highest group incidence of C-cell carcinomas in this study was 6.2%." [End of quotation].

c. Adrenal Medullary Tumors

An additional tumor type of possible concern occurred in the adrenal gland.

In female rats, there was an increased incidence of adrenal medullary tumors at the high-dose. The incidence was as follows for the number of female rats examined by effective proportions:

Female Adrenal Medulla

Group	1	2	3	4
No. examined	27	34	22	31
Carcinoma	0	0	0	1
Adenoma	1	2	0	6
Carcinoma and Adenoma (Combined)	1	2	0	7
Percentanges	4.0	5.8	0	22.5%

Animal Number	Dose	Week Death	Tumor
1540	0	106	Adenoma
2503	1000	104	Adenoma
2561	1000	106	Adenoma
4507	10,000	106 (unscheduled)	Adenoma
4521	10,000	106	Carcinoma
4524	10,000	106	Adenoma
4528	10,000	104	Adenoma
4534	10,000	107	Adenoma
4537	10,000	106	Adenoma
4551	10,000	107	Adenoma

It can be seen from the week of death for the Arsenal female tumor-bearing animals that the earliest pheochromocytoma occurred at week 104. Therefore, when the number of animals examined is adjusted for effective proportions the high-dose percentage is 22.5 percent.

The historical control data from Bio/dynamics for 14 studies showed pheochromocytomas ranging from 0 to 15.5 percent. The individual percentages were 6.7, 4.2, 0, 6.7, 1.9, 3.5, 11.0, 0, 4.5, 2.6, 8.7, 3.5, 5.3, and 15.5 percent.

Additionally, it should be noted that the historical control data is unculled for mortality (although no pheochromocytomas were found prior to 12 months).

The historical control data is appended to the report.

Reviewer's Conclusion - The incidences of thyroid proliferative lesions and neoplasms in this study are considered unrelated to the administration of AC 243,997.

Additional statistical analyses of the incidences of astrocytomas in male rats need to be performed. The increase in these tumors at the high-dose may be compound-related.

With respect to the adrenal medullary gland in females, it appears that the increased incidence at the high-dose may be compound-related depending on interpretation by statistics. However, survival disparity analysis and statistical analysis are needed.

If the number of female adrenal medullary gland tumors is not adjusted for effective proportions and the essentially unculled proportions are examined, the following incidences are observed.

Female Adrenal Medullary Tumors

Group	1	2	3	4
No. examined	55	55	55	55
Adenomas and carcinomas	1	2	0	7
Percentages	1.8%	3.6%		12.7%

In this situation, the unculled data are within the range of Bio/dynamics historical controls from 0 to 15.5 percent which are also unculled.

Additionally, in male rats in the Arsenal study, pheochromocytomas were more frequent and occurred at earlier periods than in females.

What may be being observed in females is a compound-related geriatric increase of pheochromocytoma.

For males in the Arsenal study, the weeks at which pheochromocytomas were found were as follows:

Control		Low		Mid		High	
A.N.	Week	A.N.	Week	A.N.	Week	A.N.	Week
1001	106	2005	87	3003	91	4002	81
1008	106	2007	106	3012	106	4013	106
1020	93	2012	106	3018	105	4020	102
1023	92	2018	106	3039	98	4027	106
1031	106	2034	100	3040	96	4044	106
1043	106	2039	106	3059	106	4048	106
1052	97	2045	106			4051	106
1055	106	2052	106			4054	103
		2054	106			4059	104
		2055	88			4062	89
		2064	106				

The incidences, distributions, and time-to-tumor for other benign and malignant neoplasms were considered unrelated to treatment.

2. Non-Neoplastic

There were increased incidences of three non-neoplastic lesions in female rats. One lesion was extramedullary hematopoiesis of the spleen. The second lesion was peliosis hepatis of the liver. The third lesion was B-squamous cysts of the thyroid.

With respect to the spleen, the overall incidence of the lesion was as follows:

Spleen Females

Group	1	2	3	4
No. examined	65	65	65	65
Extramedullary hematopoiesis	12	17	17	20
Grades of the lesion	2,3,2,4, 4,3,2,4, 2,2,2,2	3,2,2,2, 4,3,3,4, 2,4,4,5, 2,5,2,2, 2	3,2,2,2, 4,4,5,4, 2,2,2,2, 5,4,4,2, 2	2,2,3,3, 4,4,2,3, 2,2,4,4, 2,3,5,5, 3,5,2,2

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As can be seen from the incidence and grades of the lesion, the high-dose group is the LEL and the mid-dose group is the NOEL. The grade of 5 is associated with 0, 2, 2, and 3 lesions in the control, low-, mid-, and high-dose groups, respectively. Additionally, the grade of 4 is associated with 3/12 (25%), 4/17 (23%), 5/17 (29%), and 4/20 (20%) of the lesions in the control, low-, mid-, and high-dose groups, respectively.

Additionally, there did not appear to be any association of the the increased incidence of this splenic lesion with earlier deaths based on analysis of the data. Also, associative anemia was not observed in high-dose females.

Although the full toxicological significance of this lesion is uncertain, it does not appear to be compensatory. In any case, due to the incidences and grades of the lesion, it appears to be a compound-related effect.

The distribution of the lesion in the spleen of female rats is shown below:

Interim Kill

Group	1	2	3	4
No. Examined	10	10	10	10
Lesion	0	0	0	0

Died on Study

Group	1	2	3	4
No. Examined	30	24	34	28
Lesion	8	7	10	11

Terminal Kill

Group	1	2	3	4
No. Examined	25	31	21	27
Lesion	4	10	6	9

In female rats, there was an increased incidence of peliosis hepatis of the liver in the mid- and high-dose groups. This lesion is the presence in hepatic lobules of multiple microscopic pools of blood which may become lined by endothelium. It is a rare condition that may result from the congestion of the liver with necrosis. However, there was no compound-related occurrence of hepatic necrosis (either by incidence or grade of lesion) in female rats in this study.

The incidence of the peliosis hepatis in the liver was as follows:

Female Liver

Group	1	2	3	4
No. Examined	65	65	65	65
Peliosis hepatis	4	3	6	8

The grades of the lesion were comparable between control and high-dose rats and there was no indication of a decrease in latency of the lesion at the high-dose. Based on these considerations, the slight increase in this lesion in the mid- and high-dose groups is not considered compound-related.

The third lesion in female rats, which was ungraded (only P present in individual animal data), which occurred at an increased incidence was B-squamous cysts of the thyroid in female rats.

The occurrence was as follows:

Female Thyroid

Group	1	2	3	4
No. Examined	65	65	65	65
B-squamous cysts	5	7	5	12

The NOEL for this finding is the mid-dose of 5000 ppm and the LEL is the high-dose of 10,000 ppm.

Other non-neoplastic lesions occurred at similar frequency and grade between control and treated male and female rats.

Reviewer's Conclusion - The NOEL for non-neoplastic lesions is considered to be the mid-dose of 5000 ppm. The LEL is the high-dose of 10,000 ppm and the effects are increased incidences of extramedullary hematopoiesis of the spleen and B-squamous cysts of the thyroid in female rats. These compound-related lesions do not appear to be life-threatening and may not be used to establish an MTD for female rats.

R:55662:Dykstra:C.Disk:KENCO:01/22/90:CT:VO:SW:CT:ka
R:55665:Dykstra:C.Disk:KENCO:01/25/90:CT:VO:CT