

US EPA ARCHIVE DOCUMENT

9/13/93

Tricloram



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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Picloram: review of a chronic feeding toxicity study in the dog.

Tox.Chem No.: 039
MRID No.: 408343-01
DP Barcode: D194559
Submission No.: S446937
PC Code: 005101

From: John C. Redden, Toxicologist
Section 3
Toxicology Branch 1
Health Effects Division (H7509C)

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To: Venus M. Eagle
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Thru: Karen L. Hamernik, Ph.D.
Section Head Section 3
Toxicology Branch 1
Health Effects Division (H7509C)

K.L.H. 9/17/93 *KB* 9/14/93

ACTION:

Review of a chronic feeding toxicity study in the dog.

CONCLUSIONS:

Doses tested in diet: 0, 7, 35 or 175 mg/kg/day in Beagle dogs. The NOEL or LOEL \geq 175 mg/kg/day. The study is classified as **core Minimum**. The information presented for this Chronic Feeding Toxicity Study, satisfies the criteria set forth in Subdivision, Σ Series 83-1(b).

Urine volume and appearance were not recorded. While this information should have been included in the report, the deficiency is not considered significant enough to invalidate the conclusions of the study since there were ^{no} signs of nephrotoxicity or histopathology to suggest toxicity.

The authors concluded that the NOEL = 35 mg/kg/day and LOEL = 175 mg/kg/day, based on increased absolute liver weights in males and

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relative liver weights in males and females receiving 175 mg/kg/day picloram in the diet. TB-1 disagrees with the authors' conclusion and consider the NOEL to be ≥ 175 mg/kg/day rather than 35 mg/kg/day as the NCEL for this study. However, the HDT is supported by the range-finding studies even though there were no signs of toxicity in this study.

MRID 408343-01

HED Doc 010553

Reviewed by: Guruva B. Reddy, D.V.M., Ph.D. *ESR/MSY*
Section IV, Tox. Branch I (H7509C) *7/19/93*
Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T.
Section IV, Tox. Branch I (H7509C) *Marion P. Copley*

DATA EVALUATION REPORT

STUDY TYPE: Chronic Feeding Toxicity - Dog

GUIDELINE NUMBER: 83-1(b)

TOX. CHEM NO: 039

P. C. NO: 005101

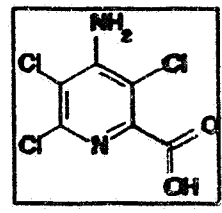
MRID NO.: 408343-01

TEST MATERIAL: Picloram - technical

SYNONYMS: 4-amino-3,5,6-trichloropicolinic acid

STUDY NUMBER: TXT:K-038323-040

SPONSOR: Dow Chemical Co.



TESTING FACILITY: Health & Environmental Sciences-Texas
The Dow Chemical Co.
Lake Jackson Research Center
Freeport, TX 77541

TITLE OF REPORT: Picloram: 12-Month Dog Chronic Dietary Toxicity Study

AUTHOR(S): T. Barna-Lloyd, J. R. Szabo and N. L. Davis

REPORT ISSUED: June 8, 1988

CONCLUSION: Doses tested in diet: 0, 7, 35 or 175 mg/kg BW/day in Beagle dogs. The NOEL or LOEL \geq 175 mg/kg/day
The HDT is supported by the range finding studies even though there were no signs of toxicity in this study. *MPC*
Classification: core-Minimum

The information presented for this Chronic Feeding Toxicity Study, satisfies the criteria set forth in Subdivision, F Series 83-1(b).

A. MATERIALS:

- Test compound:** Picloram - technical. Description - tan powder, Identity - AGR-219562, Purity - \approx 94 %.
- Test animals:** Species: Canine, Strain: Beagle, Age: 17 -

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18 weeks. Weight: Males - 8.6 to 8.8 kg, Females - 7.2 to 7.6 kg, Source: Marshall Research Farms, North Rose, NY. Acclimated for 35 days during which time the animals were screened for health status and any abnormalities. A step-by-step procedure is described for selecting healthy animals for the study. The dogs were housed 2/pen, except for mid dose male penmates, 86T2391 and 86T2392, which were penned individually starting Study Day 218 because of feed wasting problems. The animals were maintained in a temperature of 72°F, a 12-hour light/dark cycles and 13 air changes/hour.

One female control dog (86T2405) was diagnosed infected with demodectic mites. Therefore, all dogs were treated with Mitaban® for control/prophylaxis of demodectic mange on study Days 28 and 101.

B. STUDY DESIGN:

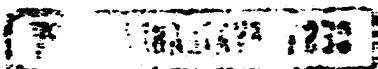
1. Animal assignment

Animals were assigned randomly to the following test groups:

Test Group	Dose in diet (mg/kg)	Main Study months	
		male	female
1 Cont	0	4	4
2 Low (LDT)	7	4	4
3 Mid (MDT)	35	4	4
4 High (HDT)	175	4	4

a. **Dose Selection:** Dose levels were selected based on results of 1-month (HED Doc. # 003954) and 6-month (MRID # 00110534 and Doc. #s 003954 and 007069) previously submitted dietary toxicity studies in dogs. One-liners, the DERs and the sponsor's summary of 6-month study indicated a LOEL = 35 mg/kg, based on increased liver weights in males only. At 175 mg/kg, both males and females exhibited decreased food consumption, weight gain and alanine aminotransferase and increased liver weights and alkaline phosphatase; however, the aforementioned alterations were not accompanied by histopathological changes due to toxicity. Under the current toxicity evaluation criteria the significance of lesions at 35 mg/kg is questionable.

b. **Additional Information:** In a range-finding study (MRID Acc. #247156 and Doc. #001889) with 1 dog/group receiving 1000 mg/kg for 7 days or 2000 mg/day for 4 days, there was emesis, reduced feed consumption and decreased body weight. In a follow-up canine palatability study (MRID Acc.



#247156 and Doc. #001889), at doses levels of 250, 500 or 1000 mg/kg/day for 7 days or 500 mg/kg for 14 days, no overt signs of toxicity was observed.

In a 3 month study (MRID #s091152^(sec) and 00069969 and Doc. #003954 and 007069), rats fed 3000 ppm resulted in liver necrosis and bile duct proliferation. It is likely that the aforementioned liver histopathological changes seen in rats could be induced in dogs if the doses are high enough.

2. Diet preparation

Diets and premixes were prepared weekly. Diets were made by serial dilution of the premixes. Mid-dose samples taken on Day 1 were analyzed for homogeneity. The test diets (all doses including control) were assayed to determine the achieved concentration of test material at study Days 1, 85, 176, 274 and 365. Stability of the test material was based on an earlier study (MRID #00110534 and Doc. #s 003954 and 007069).

Results - The concentration of test material in the test diets ranged from 98.6 to 100.5% of the target. The compound was stable for 28 days. The concentration of the compound in the mid-dose sample was 97.6% of the nominal concentration.

3. Animals received food (Purina Certified Canine Diet #5007) and water ad libitum.
4. **Statistics** - A detailed description of the statistical methodology, taken from the study report, is attached (Appendix 1). The results of all statistical analysis are in Table 5 of Study Report. Methodology was difficult to follow.
5. **Quality Assurance**

A statement of compliance with Good Laboratory Practices and a signed statement of Quality Assurance were included in the submission.

C. METHODS AND RESULTS:

1. Observations:

Animals were inspected once daily for signs of toxicity and mortality.

Results - No treatment-related Toxicity/Mortality was observed during the study except for one mid-dose female (86T2414) which developed anemia and died on Day 67 with an

immune-mediated hemolytic disorder. In addition, one high-dose male (86T2388) on Day 147 was depressed, had stiff gait and developed bronchopneumonia. Treatment with penicillin improved the condition. This dog appeared healthy through 6-month clinical examination and Day 346 and was lethargic remainder of the study. Pre-terminal clinical chemistry showed elevated WBC and ALP activity. Necropsy revealed enlarged liver consistent with increased liver size seen in high-dose level dogs, however, histopathology revealed no morphological changes associated with toxicity or clinical signs. The death of mid-dose female and clinical course of high-dose male are considered unrelated to treatment.

2. Body weight

Animals were weighed on Day -1, then weekly thereafter and before necropsy.

Results - Table 1 presents the total feed consumption and total body weight gain during the study. No statistically significant differences in the body weight or body weight gains of the treated animals were observed when compared to the controls. The body weight gains in the males and females

DOSE (MG/KG/DAY)	MALES			FEMALES		
	FC	BW	FE	FC	BW	FE
Control (0)	17620	3274	18.6	16526	3444	20.8
7	17706 (100) ^a	3619 (120)	22.1	15336 (92.8)	4613 (134)	30.1
35	18310 (104)	4415 (135)	24.1	15624 (94.5)	2578 (74.8)	16.5
175	18190 (103)	3054 (93)	16.8	12272 (74.3)	2290 (66.5)	18.7

* Data were extracted from Report Tables 6 - 9

^a (%)

FC = Feed Consumption (g)

BW = Body Weight Gain (g)

FE = Feed Efficiency (%)

of all test groups ranged from 93% to 135% and 66.5% to 134% of the control values, respectively. The authors explained that decreased weight gains in high dose females were due to reduced feed consumption because of palatability of the test compound not to the compound intake. We concur with the authors explanation.

3. Food consumption, compound intake and feed efficiency

Consumption was determined and mean daily diet consumption

was calculated. Compound intake was calculated from the actual consumption. Feed efficiency was not reported.

Results - Table 1 presents total feed consumption and feed efficiency. Feed consumption was not affected in males; however, the high dose females consumed less than the controls due to unpalatability of the test substance. The feed consumption of males/females in the 7, 35 and 175 mg/kg/day groups was 100/92.8, 104/94.5 and 103%/74.3%, respectively, of the controls. In males and females, the compound intake was 7, 35 and 175 mg/kg/day. The TB-I calculated feed efficiency of males/females in the control, 7, 35 and 175 mg/kg/day groups was 18.6/20.8, 22.1/30.1, 24.1/16.5 and 16.8%/18.7%, respectively (Table 1). The reduced food consumption in females corresponds to reduced weight gains.

4. Ophthalmological examination

Performed on all animals prior to start of the study and at the termination of experiment. No compound related effects were reported.

5. Blood was collected before treatment (-14 days) and at 100, 178 and 358 days for hematology and clinical chemistry from all animals. In addition, blood samples were taken from male dogs on study days -8 for evaluation of alkaline phosphatase activity. The pre-study alanine aminotransferase (ALT) values in the report are the average of -8 and -14 days values. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)		Mean corpusc. HGB conc. (MCHC)
X	Erythrocyte count (RBC)		Mean corpusc. volume (MCV)
X	Platelet count		Reticulocyte count
X	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

Results - Hematological parameters that showed time-dose interactions in both sexes are presented in Table 2. There was a significant time-dose interaction for platelets and WBC counts in the high dose males and females when compared to the controls. However, the increased platelet and WBC numbers are within the range established for adult dogs and the differences are considered as normal biological variation.

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TABLE 2. MEAN HEMATOLOGY VALUES THAT DIFFERED SIGNIFICANTLY FROM CONTROLS ¹								
DOSE (MG/KG/DAY)	MALES (INTERVAL - MONTHS)				FEMALES (INTERVAL - MONTHS)			
	Pre-study	3	6	12	Pre-study	3	6	12
	<i>% of Control</i> PLATELETS ²							
0	382	311	306	282	366	382	362	363
7	98-373	95-297	92-283	98-275	359	331	305	255
35	112-426	120-374	119-384	111-312	484	438	442	490
175	116-442	150-485*	156-476*	173-657*	339	422*	410*	462*
	WBC ³							
0	11.7	11.1	11.0	12.2	12.4	13.8	12.7	11.2
7	11.5	13.1	11.0	12.3	11.8	12.8	11.7	10.4
35	12.3	13.3	13.1	10.3	16.2	15.5	14.6	13.1
175	11.4	18.1*	16.3*	17.9*	11.6	19.0*	14.0*	14.3*

¹ Data were extracted from study Tables 10 - 25

² 10³/mm³

* = P ≤ 0.05, based on One Way ANOVA

b. Clinical Chemistry

X

Electrolytes:

- X Calcium
- X Chloride
- Magnesium
- X Phosphorous
- X Potassium
- X Sodium

Enzymes

- X Alkaline phosphatase (ALK)
- Cholinesterase (ChE)
- Creatinine phosphokinase
- Lactic acid dehydrogenase (LAD)
- X Serum alanine aminotransferase (also SGPT)
- X Serum aspartate aminotransferase (also SGOT)
- Gamma glutamyl transferase (GGT)
- Glutamate dehydrogenase

X

Other:

- X Albumin
- Blood creatinine
- X Blood urea nitrogen
- X Cholesterol
- X Globulins
- X Glucose
- X Total bilirubin
- X Total serum Protein (TP)
- Triglycerides
- Serum protein electrophoresis

Results - Clinical chemistry parameters which showed time-dose or time-dose-sex interactions are presented in Table 3. Although, statistical significance was observed for the clinical chemistry parameters - mean alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, cholesterol, total protein and albumin, none of the significant differences can be attributed to the treatment since the values either lacked dose-response or were within the normal values published for this strain and age of dogs. In addition, the glucose, globulin, total bilirubin, urea

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nitrogen, sodium, potassium, calcium or phosphorus levels were not affected due to treatment with picloram.

TABLE 3. CLINICAL CHEMISTRY VALUES THAT DIFFERED SIGNIFICANTLY FROM THE CONTROLS*								
DOSE (MG/KG/DAY)	MALES (MONTHS)				FEMALES (MONTHS)			
	Pre-study	3	6	12	Pre-study	3	6	12
ALKALINE PHOSPHATASE (ALP: U/l)								
0	119.3	70.0	43.3	41.5	128.5	66.8	43.1	38.7
7	124.0	68.3	39.0	30.7	150.1	79.0	59.3	39.5
35	110.3	70.0*	50.1*	43.5*	117.3	52.8*	45.8*	43.8*
175	116.2	67.2	55.4	63.1	103.2	62.3	42.9	42.5
ALANINE AMINOTRANSFERASE (ALT: U/l)								
0	23.8	31.0	31.7	37.5	29.8	24.6	26.7	30.3
7	25.4	25.6	27.7	31.7	23.2	24.4	24.6	32.2
35	29.6	24.2**	25.5**	31.7**	24.8	25.6**	31.8**	31.9**
175	27.9	20.7	26.9	47.9	20.9	18.1	20.1	33.4
ASPARTATE AMINOTRANSFERASE (AST: U/l)								
0	27.2	34.3	39.3	41.8	31.6	29.2	23.6	25.2
7	26.4	30.7	24.8	26.6	24.7	30.2	22.3	26.4
35	31.9	30.8*	25.5*	31.1*	23.4	25.7*	26.8*	32.8*
175	33.2	28.1*	21.2*	30.3*	22.7	24.6*	21.6*	20.9*
CHOLESTEROL (mg/dl)								
0	194.7	154.6	154.0	129.7	202.3	153.7	173.9	154.0
7	234.5	206.9	181.4	150.2	205.8	162.6	237.3	180.6
35	197.5	214.5*	208.6*	167.5*	211.2	201.5*	241.9*	198.9*
175	212.1	240.4*	207.7*	190.8*	198.6	230.8*	214.1*	198.8*
TOTAL PROTEIN (g/dl)								
0	6.1	6.4	5.9	6.1	6.2	6.5	6.2	6.5
7	6.3	6.1	5.8	6.3	5.9	6.3	5.9	6.3
35	5.9	6.3	6.0	6.2	6.1	6.3	6.2	6.5
175	6.2	6.4*	5.9*	6.5*	6.0	6.0*	5.8*	5.9*
ALBUMIN (g/dl)								
0	3.2	2.9	3.4	3.1	3.2	3.0	3.7	3.4
7	3.2	3.0	3.5	3.4	3.1	3.1	3.5	3.2
35	3.0	3.0*	3.7*	3.4*	3.0	3.2*	3.9*	3.4*
175	3.2	3.1*	3.3*	3.3*	3.1	2.9*	3.3*	3.4*

* Data were extracted from study Tables 26 to 33

* = $P \leq 0.05$

** = $P \leq 0.001$

Alkaline phosphatase - At 35 mg/kg, in males and females, the mean alkaline phosphatase (ALP) levels were significantly different from the controls for time-dose interaction. In males, the high-dose ALP values for 6 (55.4 ± 13.9) and 12 (63.1 ± 21.0) month evaluation appear higher than the controls (41.5 ± 16.9), however, the mean differences were

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not statistically significant because large variations in individual values as evidenced by large standard deviations. As anticipated the ALP levels decreased with age. Since the ALP levels lacked dose-response and were within the range established for this strain and age of dogs, the differences are considered to be of no toxicological significance.

Alanine aminotransferase (ALT) - mid-dose males and females exhibited a statistically significant time-sex-dose interaction. In the dog, persistent ALT elevations suggest liver necrosis. These statistical differences lacked dose-response, were within the range established for this strain and age of dog and lacked liver lesions associated with toxicity. The differences are considered to be of no biological significance.

Aspartate aminotransferase (AST) - the AST levels of mid and high-dose males and females were significantly different for time-dose interaction. The AST levels in dogs are not liver specific; were generally lower than the controls, lacked dose-response, therefore, considered to be of no toxicological significance.

Cholesterol - the mid and high-dose cholesterol levels in both sexes were statistically higher than the controls. Although the cholesterol levels were higher than the concurrent control values, the levels were within the range for this strain and age of dogs, therefore considered to be of no biological significance.

Total protein and albumin - in both sexes, the total protein in high dose group and albumin in the mid and high dose groups were statistically different than the controls. These differences lacked dose-response and were generally within the published range for adult dogs and therefore, considered to be of no biological significance.

6. Urinalysis

Urine was taken from the bladder at necropsy on fasted animals. The CHECKED (X) parameters were examined.

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 - Compound related effects were not observed in any treated groups. Urine volume and appearance were not

recorded. While this information should have been included in the report, the deficiency is not considered significant enough to invalidate the conclusions of the study since there were no signs of nephrotoxicity or histopathology to suggest toxicity.

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		Cardiovasc./Hemat.		Neurologic
X	Tongue	X	Aorta	XX	Brain
X	Salivary glands	X	Heart	X	Periph. nerve
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	X	Spleen	X	Eyes (optic n.)
X	Jejunum	X	Thymus		Glandular
X	Ileum		Urogenital	XX	Adrenal gland
X	Cecum	XX	Kidneys		Lacrimal gland
X	Colon	X	Urinary bladder	X	Mammary gland
	Rectum	XX	Testes	XX	Parathyroids
XX	Liver	X	Epididymides	XX	Thyroids
X	Gall bladder	X	Prostate		Other
X	Pancreas		Seminal vesicle	X	Bone
	Respiratory	XX	Ovaries	X	Skeletal muscle
X	Trachea	X	Uterus	X	Skin
X	Lung	X	Cervix	X	All gross lesions and masses
	Nose	X	Oviducts		
	Pharynx	X	Vagina	X	Mediastinal tissue
	Larynx			X	Mesenteric lymph node
X	Tonsil			X	Mesenteric tissue

- a. Organ weight - No differences in absolute or relative weights of adrenals, brain, kidneys, testes, ovaries and thyroid gland were attributed to the administration of Picloram[®]. There was a non-statistical increase in absolute (101.7%) and relative (121.4%) ovarian weights of high-dose animals was observed, however, the increase was not associated with histological changes suggestive of toxicity. The authors concluded that the ovarian weight increases was probably due to the cyclic changes. We concur with their conclusions. In addition, at 175 mg/kg/day, the absolute liver weights in males and females increased non-significantly by 54.8% and 6.7 %, respectively, when compared to the controls. The relative weights of high-dose males and females increased statistically ($P \leq 0.01$) by 54.8% and 24.9%, respectively, when compared to the controls. The authors attributed the

increased absolute liver weights in males and relative liver weights in males and females to the administration of test compound. TB-I does not agree with the study authors' conclusions that these organ weight changes were probably treatment-related, since the changes were not associated with any overt signs of toxicity and/or histopathological changes suggestive of toxicity. These changes are considered adaptive changes and considered to be of questionable toxicological significance. Based on this information TB-I considers 175 mg/kg/day instead of 35 mg/kg/day the NOEL for this study.

- b. **Gross pathology** - No treatment-related gross morphological changes were noted at necropsy, except enlarged livers in 3/4 males and 2/4 females receiving 175 mg/kg/day test substance in the diet. The enlarged livers are considered compensatory changes and considered to be of equivocal significance, since no compound related histopathology was observed.
- c. **Microscopic pathology** - No histopathological lesions attributable to administration of Picloram[®] were found in any tissues.

D. DISCUSSION:

Study Deficiencies - Urine volume and appearance were not recorded. While this information should have been included in the report, the deficiency is not considered significant enough to invalidate the conclusions of the study since there were no signs of nephrotoxicity or histopathology to suggest toxicity.

The data reporting was adequate. The authors concluded that the NOEL = 35 mg/kg/day and LOEL = 175 mg/kg/day, based on increased absolute liver weights in males and relative liver weights in males and females receiving 175 mg/kg/day picloram in the diet. TB-I disagrees with the authors' conclusions and consider NOEL to be \geq 175 mg/kg/day rather than 35 mg/kg/day as NOEL for this study.

The study satisfies the requirements set forth in Subdivision F Guideline, 83-1(b) for Chronic Feeding Toxicity Study in the Dog.

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Final: 7/19/93

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**Chronic Feeding Toxicity - Dog
Guideline 83-1(b)**

APPENDIX I

STATISTICAL METHODOLOGY

Statistical Evaluation

All parameters examined statistically were first tested using Bartlett's test for equality of variance. If the results from Bartlett's test did not reject the equality of variances, the parameter was subjected to further parametric analysis as described below. If Bartlett's test demonstrated inequality of variances, the data were transformed using log, inverse, and square root transformations, in the order given, to obtain equality of variances. If none of the transformations satisfied Bartlett's test for equality of variances, the transformation or raw data with the lowest Bartlett's statistic was used. The transformed data was then subjected to the appropriate parametric analysis as described below.

In-life body weight, hematologic, and clinical chemistry data were evaluated using a three-way repeated measures analysis of variance (ANOVA) for time (the repeated factor), sex, and dose. In the three-way ANOVA, differences between groups were primarily detected by the time-dose interaction.

Final fasted body weight, absolute and relative organ weights (excepting testes and ovaries), and specific gravity of urine were evaluated using a two-way ANOVA for the factor of sex and dose.

Results for absolute and relative testicular and ovarian weight were analyzed using a one-way ANOVA. Upon determination of significant dose effects among groups, separate dose levels were compared to control using separate one-way ANOVAs with Bonferroni's correction.

For those parameters examined by two-way ANOVA, examination was made first for a significant sex-dose interaction. If this was found, a one-way ANOVA was done separately for each sex. If no sex-dose interaction was identified, and a dose effect was identified, or if, in the subsequent one-way ANOVA, a dose effect was identified, then the appropriate type of ANOVA was repeated separately for each dose level vs control.

Parameters analyzed by the three-way repeated measures analysis required a few additional examinations. First, examination was made for a time-sex-dose interaction; if present, the analysis was repeated separately for each sex. The next examination was for a sex-dose interaction. When identified, the data were reexamined with separation of the sexes. After accounting for the sex factor, by eliminating concern or controlling for sex, the time-dose interaction was examined. When a time-dose interaction was identified, the analysis was repeated for each dose against controls. In a few instances, a sex-dose interaction was identified for a particular dose, leading to separation by sex and reanalysis.

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**Chronic Feeding Toxicity - Dog
Guideline 83-1(b)**

APPENDIX I (Continued)

STATISTICAL METHODOLOGY

The nominal alpha levels used and the test references were:

One-Way ANOVA	
Bartlett's test (Winer, 1971)	alpha=0.01
1st ANOVA (Steel & Torrie, 1960)	alpha=0.10
2nd ANOVA (Steel & Torrie, 1960)	alpha=0.05
Two-Way ANOVA	
Bartlett's test (Winer, 1971)	alpha=0.01
First ANOVA (Winer, 1971)	
Sex-dose interaction	alpha=0.05
Dose factor	alpha=0.10
Second ANOVA (Winer, 1971)	
Sex-dose interaction	alpha=0.05
Dose factor	alpha=0.05
Three-Way Repeated Measures ANOVA	
Bartlett's test (Winer)	alpha=0.01
First ANOVA (Winer, 1971)	
Time-sex-dose interaction	alpha=0.01
Sex-dose interaction	alpha=0.05
Time-dose interaction	alpha=0.10
Second ANOVA	
Time-sex-dose interaction	alpha=0.01
Sex-dose interaction	alpha=0.05
Time-dose interaction	alpha=0.05

The following data were recorded, made part of the study file, and reported, but not analyzed for differences of statistical significance: body weight gains from baseline, WBC differential count, urinalysis readings aside from specific gravity, and feed consumption figures. The p-values calculated for individual dose comparisons to control values were

corrected for the multiple comparisons with Bonferroni's correction (Miller, 1966). Because repeated measures analysis cannot be done when some data are missing, the prestudy values for female 86T2414, which died prior to the three-month evaluation, were excluded from statistical analysis. However, all data from 86T2414 (i.e., prestudy and at the time of death) are included in the study file and shown in this report.

Because numerous measurements were statistically compared in the same group of animals, the overall false-positive rate (Type I errors) could be much greater than the above-cited alpha levels might suggest. As a consequence, the final interpretation of numerical data considered statistical outcomes together with other factors, such as dose-response relationships and the plausibility of results in light of biologic and pathologic findings.

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