9-10-93

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

Subject: Cacodylic Acid: Review of Subchronic Oral Toxicity in Rats

FROM: Steven L. Malish, Ph.D., Toxicologist S. L. Malish 9/8/93
Tox. Branch II, Review Section IV
HED (H7509C)

TO: Virginia Dietrich, Product Manager (51)
Ron Kendall - PM team Reviewer
Registration Division (H7508W)

THRU: Jess Rowland, M.S., Acting Section Head Jess Rowland 9/10/93
Tox. Branch II, Review Section IV
HED (H7509C)

and

Marcia van Gemert, Ph.D., Branch Chief
Tox Branch II; HED (H7509C)

Task Identification: Submission: S442517 DP Barcode: D192215
P.C. Code: 012501 Caswell No.: 133

ACTION REQUESTED: Review of Subchronic Oral Toxicity: 90 Day Study/Rat [MRID 427677-01] to upgrade the chronic toxicity/ carcinogenicity study in rats [MRID 418621-01].

Response:

A Data Evaluation Report for the above referenced study is attached. A summary is provided.

Cacodylic acid (99.5%) was incorporated into the diet of 6 groups of 60 specific pyrogen free Fischer F344 rats per sex at concentrations, respectively, of 0 (Control), 5, 50, 500, 2000 and 5000 ppm for at least 13 weeks.

All rats treated with 2,000 or 5,000 ppm died or were sacrificed during the first 5 weeks of treatment. Two (2) males and 2 females died at 500 ppm during weeks 4 and 13, respectively. At 50 and 500 ppm, treatment related signs and observations included increased water intake and urine output.

Body weight gains in both sexes were depressed at 500 ppm.

Slightly decreased hematological parameters (hematocrit, hemoglobin, red blood cell count and MCHC and MCV (females) occurred in both sexes at 500 ppm. Females at 50 ppm showed a slight decrease in hemoglobin and red blood cell values versus the controls. A compensatory increase in the reticulocyte count occurred at 500 ppm in both sexes.

The thyroid gland in the female showed a dose related decrease in the absolute and relative weights. The adrenal gland showed a dose related decrease in the absolute and relative weights of the male and a decrease in the relative weight of the female versus the control. At 500 ppm, the absolute weight of the testes was decreased.

At 50 and 500 ppm the thyroid presented hypertrophic follicular epithelium. At 500 ppm, a reduced volume of smooth muscle of the uterus occurred; hyperplasia of the epithelium lining the renal papilla associated with papillary necrosis was seen.

Pathological changes were also seen in the aorta, bone marrow, heart and testes.

Based on these results, the following NOEL and LOEL was established. The LOEL is based on mortality, decrease in body weight gain, alterations in the hematology, changes in the absolute and relative organ weights and histopathology lesions in the thyroid, kidney, aorta, bone marrow, heart, testes and uterus.

NOEL: 5 ppm (0.4 mg/kg/day).
LOEL: 50 ppm (males: 4 mg/kg/day; females: 4.5 mg/kg/day).

CORE CLASSIFICATION: Minimum

Reviewed by Steven L. Malish, Ph.D. Steven L. Malish 9/8/93
Tox. Branch II, Section IV (H7509C)
Secondary Reviewer: Jess Rowland, M.S. Jess Rowland 9/10/93.
Tox. Branch II, Section IV (H7509C)

Data Evaluation Report

STUDY TYPE:	82-1 Subchronic Oral Toxicity: 90 Day Study/Rat
IDENTIFICATIONS:	Submission: S442517 DP Barcode: D192215
MRID NO.:	427677-01 Caswell No.: 133
P.C. Code:	012501
TEST MATERIAL:	Cacodylic Acid
SYNONYMS:	Dimethylarsinic acid
SPONSOR:	Luxembourg Industries (Pamol) Ltd. 27 Hamered St. P.O. 13 Tel-Aviv 61000, Israel
STUDY NO.:	PAL/009/CAC
TESTING FACILITY:	Life Science Research Israel, Ltd. PO Box 139, Ness Ziona, 70 451 Israel
TITLE OF REPORT:	Cacodylic Acid Toxicity in Dietary Administration to Rats for 13 weeks: A Preliminary Study
AUTHORS:	S. Crown, G. Kenan, A. Nyska, T. Waner
REPORT ISSUED:	May 1987

SUMMARY:

Cacodylic acid (99.5%) was incorporated into the diet of 6 groups of 60 specific pyrogen free Fischer F344 rats per sex at concentrations, respectively, of 0 (Control), 5, 50, 500, 2000 and 5000 ppm for at least 13 weeks.

All rats treated with 2,000 or 5,000 ppm died or were sacrificed during the first 5 weeks of treatment. Two (2) males and 2 females died at 500 ppm during weeks 4 and 13, respectively. At 50 and 500 ppm, treatment related signs and observations included increased water intake and urine output.

Body weight gains in both sexes were depressed at 500 ppm.

Slightly decreased hematological parameters (hematocrit, hemoglobin, red blood cell count and MCHC and MCV (females) occurred in both sexes at 500 ppm. Females at 50 ppm showed a slight decrease in hemoglobin and red blood cell values versus the controls. A compensatory increase in the reticulocyte count occurred at 500 ppm in both sexes.

The thyroid gland in the female showed a dose related decrease in the absolute and relative weights. The adrenal gland showed a dose related decrease in the absolute and relative weights of the male and a decrease in the relative weight of the female versus the control. At 500 ppm, the absolute weight of the testes was decreased.

At 50 and 500 ppm the thyroid presented hypertrophic follicular epithelium. At 500 ppm, a reduced volume of smooth muscle of the uterus occurred; hyperplasia of the epithelium lining the renal papilla associated with papillary necrosis was seen.

Pathological changes were also seen in the aorta, bone marrow, heart and testes.

Based on these results, the following NOEL and LOEL was established. The LOEL is based on mortality, decrease in body weight gain, alterations in the hematology, changes in the absolute and relative organ weights and histopathology lesions in the thyroid, kidney, aorta, bone marrow, heart, testes and uterus.

NOEL: 5 ppm (0.4 mg/kg/day).
LOEL: 50 ppm (males: 4 mg/kg/day; females: 4.5 mg/kg/day).

CORE CLASSIFICATION: Minimum

This study satisfies the data requirement for 82-1 for a Subacute Oral Toxicity: 90 days Study in rodents and is acceptable for regulatory purposes.

I. INTRODUCTION:

The study was designed to test the subchronic toxic effects associated with dietary feeding of the test compound for a least 13 weeks.

II. MATERIALS:

A. Test Compound

Chemical name: Dimethyl hydroxyarsine oxide
Trade Name: Dimethylarsenic acid, cacodylic acid
Common name: Cacodylic acid
Batch No: 1007
Trade Name: Dimethylarsenic acid, cacodylic acid
Purity: 99.5% a.i.
Description: White crystalline solid
Storage: Room temperature

B. Test Animals:

Species: Rat
Strain: Charles River CDF (Fischer F344)
Age: 4 to 5 weeks of age on arrival
Weight: Females: mean-112; Males: mean- 141 gm at initiation
Source: Charles River Breeding Laboratories, USA
Acclimatization: 7 days
Housing: 5 animals per cage
Feed: powdered rodent diet Altromin 1321N (Atromin Specialfutterwerke GmbH, Lage FRG) ad libitum
Tap water: Sterilized tap-water ad libitum. Supplied by polyethylene bottles.
Environment (target): 21°C.; humidity 55%; Air changes 15/hr.; light cycle: 12 hours light/12 hours dark

III. STUDY DESIGN:

A. Treatment

Sixty (60) rats per sex were assigned randomly by weight to six (6) test groups and administered 0, 5, 50, 500, 2000 and 5,000 ad-mixed in the feed for at least 13 weeks (Table 1).

Table 1

Animal Test Group Assignments

Group	Treatment	Dietary Conc. (ppm)	Animals on Test (M/F)
1	Control	0	10/10
2	Cacodylic acid	5	10/10
3	Cacodylic acid	50	10/10
4	Cacodylic acid	500	10/10
5	Cacodylic acid	2000	10/10
6	Cacodylic acid	5000	10/10

B. Test Article Formulation:

A measured amount of the test material was incorporated with mixing into about 1 Kg of the powdered basal diet. The diet was diluted again to 5,000 ppm with basal diet and mixed. Serial dilutions were then used to make the lower concentrations. All test diets were prepared weekly.

C. Dosing:

Dietary concentrations of the test material were feed for up to 13-14 weeks. During the week of necropsy animals continued to receive their respective test diets.

D. Analysis of Test Article Formulations:

Stability and Homogeneity

Homogeneity and stability of cacodylic acid dispersal in the rodent diet was determined from trial samples prior to commencement of the study. Homogeneity was determined from 6 spaced samples from each concentration. Stability was determined by analysis of the 5,000 ppm sample homogeneity dispersal sample on days 7, 14, and 28 after preparation. Samples were stored at room temperature prior to analysis.

Concentration Analysis

The test material was assayed to verify the content of the active ingredient and checks made to verify the test material content at the end of Weeks 1 (0, 5, 50 and 500 ppm) and week 3 (2,000 ppm).

IV. METHODS:

A. Observations:

Rats were inspected at least twice a day (once daily on weekends and public holidays) during treatment for pharmacologic and toxicologic effects. In addition, all animals were handled once weekly.

B. Mortality:

Severely debilitated animals were sacrificed or isolated to prevent cannibalism. Animals judged in extremis were sacrificed to preclude autolysis.

Animals found dead outside normal working hours were preserved at 4°C. and necropsied as soon as possible the following day. A complete necropsy was performed in all cases.

C. Body Weights:

Each animal was weighed on the first day of treatment and at weekly intervals.

D. Food Consumption:

The quantity of food eaten by each cage was calculated weekly by the measurement of food given minus the food consumed.

E. Food Efficiency:

Efficiency of food conversion (ratio between body weight change to the weight of food consumed) was calculated each week for the first 13 weeks of the study.

F. Compound Consumption:

Compound consumption expressed as mg/kg/day was calculated for each group/sex from the concentration of the test compound in the food, food consumption and body weight data.

G. Water Consumption:

The amount of water imbibed by each cage of rats was recorded weekly for 13 weeks. Group means were calculated at each time period.

H. **Clinical Pathology**:

Blood samples from all surviving animals in each group were collected for hematology and blood chemistry during weeks 11 and 12. The animals were fasted overnight prior to the drawing of blood samples. Blood samples were taken from the retro-orbital sinus with each rat under ether anesthesia. EDTA (for hematology) or lithium heparin (for blood chemistry) were used as anticoagulants.

The following parameters were examined.

Hematology

x Hematocrit (HCT)*	x Leukocyte count (WBC)*
x Hemoglobin (HGB)*	x Platelet count*
x Erythrocyte count (RBC)*	x Leukocyte differential*
Mean corpuscular HGB (MCH)	x Mean corpuscular HGB Concentration (MCHC)
x Mean corpuscular volume (MCV)	Blood clotting measurements
x Reticulocyte count (Retic)	Erythrocyte morphology

*Required for subchronic studies
x Parameters examined.

Clinical Chemistry

Electrolytes:	Other:
x Calcium[a]	x Albumin (Album)[a]
x Chloride[a]	Blood creatinine (Creat)[a]
Magnesium	x Blood urea nitrogen (BUN)[a]
x Phosphorus[a]	x Total cholesterol(Chol)[a]
x Potassium[a]	x Globulins
x Sodium	x Glucose[a]
Enzymes:	x Total bilirubin[a]
x Alkaline phosphatase	x Direct bilirubin
x Alanine aminotransferase (ALT)[a]	x Total protein[a]
x Aspartate aminotransferase (AST)[a]	Triglycerides
Cholinesterase[b]	Serum protein electrophoresis
x Creatinine phosphatase (CPK)[a]	Triiodothyronine (T_3)
Lactic acid dehydrogenase	Thyroxine (T_4)
x τ-Glutamyl transpeptidase [GGPT]	A/G Ratio
	x Uric Acid

[a] Required for subchronic and chronic studies.
x Parameters examined.

I. Urinalysis:

Urine was collected during week 11 of the study. The rats were deprived of drinking water for 4 hours on the day of collection and were placed into individual metabolism cages with out food and water for ≈16 hours.

The following parameters were examined.

Urinalysis

Parameter Examined	Sediment Examination[a]
Appearance[a]	x Epithelial Cells
x Volume[a]	x Polymorphonuclear leukocytes
x Specific Gravity	x Red blood cells
x pH	x Casts
x Urobilinogen	x Crystals
x Glucose[a]	x Other abnormal components
x Ketones[a]	
x Bilirubin	
x Blood (occult)[a]	
x Protein[a]	

[a] Required for subchronic and chronic studies.
x Parameter examined.

J. Organ Weights

The following organ weights were evaluated: adrenals (L/R), brain (3 levels), kidney (3 levels), liver (2 lobes), spleen, testes (L/R) and thyroids (L/R) including the parathyroids.

K. Histopathology

The checked (X) tissues from all 0 and 500 ppm rats were examined. In addition, the liver, kidneys, lungs, and gross lesions were examined for all low and mid-dose animals. Tissues from the 2,000 and 5,000 ppm animals were not examined.

Pathology

Digestive System	Respiratory System
x Salivary glands[a]	x Trachea[a]
x Esophagus[a]	x Lung[a]
x Stomach	Pharynx[c]
x Duodenum[a]	Larynx[c]
x Jejunum[a]	x Head with nasal turbinate
x Cecum[a]	Nose[c]
x Colon[a]	
x Ileum[a]	Cardiovascular/Hemo.System
x Rectum[a]	
x Liver[a,c]	x Aorta (thoracic)[a]
x Pancreas[a]	x Heart[a]
Gall bladder[a,b]	x Bone marrow[a]
	x Lymph nodes[a]
Neurological System	x Spleen[a]
	x Thymus[a]
x Brain[a,c]	
x Pituitary[a]	Urinogenital System
x Peripheral nerve[a,b]	
x Spinal cord (3 levels)[a,b]	x Kidneys[a,c]
x Eyes (optic nerve)[a,b]	x Urinary bladder[a]
	x Testes[a,c]
Glandular System	x Epididymides
	x Prostate
x Adrenals[a]	x Seminal vesicles
Lacrimal glands[b]	x Uterus[a]
x Parathyroids[a]	x Ovaries[a,c]
x Thyroids[a]	
	Others
	x Skin
	x Mammary glands
	x All gross lesions and masses
	x Skeletal muscle[a]

[a]Required for subchronic studies.
[b]In subchronic studies, examined only if indicated by toxicity or target organ involvement.
[c]Organ weights required in subchronic studies.
x Tissues and Organs examined.

L. Statistics:

Presumptive differences between control and treated groups were tested for significance using the Student's t test using pooled intragroup error variance for the following parameters: body weight, food consumption, hematology, blood chemistry, urinalysis, organ weights and ratios of organ weight to body weight.

M. Regulatory Compliance:

A statement of compliance with Good Laboratory Practice Standards and a statement of no data confidentiality claims was signed and dated.

V. RESULTS:

A. Analytical:

Homogeneity and Stability

All analysis for homogeneity and stability performed prior to initiation of the study (day 0) at 50, 500, 2000 and 5,000 ppm and on days 7, 14 and 28 at 5,000 ppm were within found to be within 8% of the stated concentration (Table 2).

Table 2

Homogeneity and Stability of the Test Compound in the Diet Mix[1]

Concentration Stated (ppm)	Number of Replicates	Concentration Present (ppm) at Day 0
0	2	0
50	6	54
500	6	504
2000	6	2015
5000	6	5207[a]

[1]Adapted from original report, p. 98.
[a]Day 7, 5130 ppm; Day 14, 5137 ppm; Day 28, 4833 ppm; 2 replicates at each point.

Concentration Analysis:

Concentration analysis of cacodylic acid in the diet on week 1 at 5, 50, 500, 2000 and 5,000 ppm and week 3 at 2,000 ppm was tabulated below (Table 2A).

Table 2A

Concentration Analysis of Cacodylic Acid in Feed on Weeks and 1 and 3

Group No.	Concentration Stated (ppm)	Concentration Present (ppm)
1	0	0.0
2	5	4.9
3	50	47.7
4	500	508
5	2000	2,006[2]
6	5000	5,032

[1]Adapted from original report, p. 99.
[2]Week 3, 1957 ppm.

B. Clinical Signs:

Treatment related signs most prominently seen at 2,000 and 5,000 ppm consisted of hunching, thinness, emaciation, decreased motor activity, urogenital wetting, diarrhea, emaciation, snout staining and failure to groom.

C. Mortality:

Mortality was seen in both sexes at doses of 500, 2000 and 5,000 ppm. At 5,000 ppm, all females died or were killed *in extremis* during the first week; 1 male died during week 1 while 7 males were found dead and 2 were killed *in extremis* during week 2. At 2,000 ppm, all males died from week 2 to 5 and all females except 1 (which was killed *in extremis* during week 3) died during weeks 1 to 3. At 500 ppm, 2 males died during week 4 while 2 females died during week 13. The authors note that the 1 female died shortly after blood collection at 500 ppm (Table 3).

Table 3

Mortality at Various Time Periods During the Study[1]

	Concentration (ppm)					
Week	0	5	50	500	2,000	5,000
1	-	-	-	-	0M, 1F	1M, 10F[d]
2	-	-	-	-	2M, 6F	9M[c], 0F
3	-	-	-	-	4M, 3F	-
4	-	-	-	2M[a], 0F	3M, 0F	-
5	-	-	-	-	1M, 0F	-
6-12	-	-	-	-	-	-
13	-	-	-	0M, 2F[a]	-	-
Total	-	-	-	2M, 2F	10M[a], 10F[b]	10M, 10F

[1]Adapted from original report, p. 43 to 44.
- = no deaths
[a]found dead
[b]all found dead except for 1 female at week 3 which was killed in extremis
[c]7 animals found dead, 2 killed in extremis
[d]5 animals found dead, 5 killed in extremis

D. Body Weight:

Body weight parameters were not evaluated at the 2,000 and 5,000 ppm dose levels because of the high mortality during the early part of the study.

Body weight gains were depressed in both sexes receiving 500 ppm or more of the test compound versus the control. In the males and females at 13 weeks, the absolute weight was decreased by 7.5% and 6%, respectively. Statistical significance weight decreases were seen in males and females at the majority of the weekly intervals. Decreased body weight gain of 13% in males and 17% in females occurred by the end of the study. No changes in absolute or body weight gains were seen at the lower dose levels versus the controls (Table 4, 5).

Table 4

Mean Body Weight and Body Weight Change in Males[1]

	Dose(ppm)			
Weeks	0	5	50	500
0	141	140	141	137
1	173	172	174	165[b]
4	220	218	215	198[b]
8	257	255	255	234[a]
12	269	269	270	250[a]
13	267	265	268	247[a]
Abs. Wgt.[2]	--	2.0	1.0	-20.0
%[3]	--	-0.7	0.3	-7.5
Change[4]	126	125	127	110
%[3]	--	<1	<1	-13

---

[1]Adapted from original report, p. 47.
[2]Absolute weight (gm) compared to the control at 13 weeks.
[3]Percentage
[4]Change in weight gain (gm) compared to the control from 0 thru 13 weeks.
[a]$p<0.05$
[b]$p<0.01$

Table 5

Mean Body Weight and Body Weight Change in Female Animals[1]

	Dose (ppm)			
Weeks	0	5	50	500
0	113	114	112	112
1	128	128	127	123[a]
4	151	150	148	141[b]
8	164	165	164	155[b]
12	167	170	167	155
13	167	169	168	157[a]
Abs. Wgt.[2]	--	2	1	-10
%[3]	--	1	0.6	- 6
Change[4]	54	55	56	45
%[3]	--	2	4	-17

---

[1]Adapted from original report, p. 47.
[2]Absolute weight (gm) compared to the control at 13 weeks.
[3]Percentage
[4]Change in weight gain (gm) compared to the control from 0 thru 13 weeks.
[a]$p<0.05$
[b]$p<0.01$

E. Food Consumption:

Food consumption was similar between the control and treated animals throughout the 13 week period. A decrease in food consumption occurred during week 3 at the 500 ppm dose level in both sexes. A compensatory increase was seen during week 4 in the females.

F. Food Conversion Ratio:

Food conversion ratio was similar between the control and treated animals throughout the study.

G. Compound Consumption:

Mean compound consumption over the 13 week period is shown below in Table 6.

Table 6

Mean Compound Consumption During the 13 Week Study[1]

Dose (ppm)	Males (mg/kg/day)	Females (mg/kg/day)
0	0	0
5	0.4	0.4
50	4.0	4.5
500	43.2	45.7

[1]Adapted from original report, p. 50.

H. Water Consumption:

Males showed statistically significant increases at the 50 (160 ml/animal/day) and 500 ppm (169 ml/animal/day) dose levels versus the control (117 ml/animal/day). Females showed statistically significant increases at the 50 (129 ml/animal/day) and 500 ppm (141 ml/animal/day) dose levels versus the control (105 ml/animal /day). The increase in water consumption occurred in both sexes during weeks 3 thru 13.

I. Hematology:

Results are tabulated in Table 7.

Male rats showed a dose related decrease in the hematocrit, hemoglobin and red cells versus the respective controls which was statistical significance at 500 ppm. At the same dose level, MCHC showed a statistical significant decrease while MCV showed a decrease versus the respective controls. All hematology parameter decreases were <10% versus the control. The reticulocyte count showed a statistically significant increase of 67% raising from 1.5 in the control to 2.1 at 500 ppm (Table 7).

Female rats showed a dose related decrease in hematocrit, hemoglobin, red cells and MCHC values compared to the respective control. Statistical significance was achieved at 50 and 500 ppm for the hemoglobin and red blood cells and at 500 ppm for the hematocrit and MCHC. The MCV values showed increases at 5, 50 and 500 ppm compared to the control which were statistically significant at 5 and 500 ppm dose levels. All hematology parameter decreases were <10% versus the control. The reticulocyte count showed a statistically significant increase of 26% raising from 1.5 in the control to 1.9 at 500 ppm (Table 7).

Table 7

Mean Hematology Values at Termination[1]

Dose /Sex	HCT %	Hb gm%	RBC 10^6/ul	MCHC gm%	MCV u^3	Retic. %
0 M	46.0	15.9	8.46	34.5	54.4	1.5
5 M	45.8	15.8	8.41	34.5	54.5	---
50 M	45.5	15.7	8.30	34.6	54.8	---
500 M	42.5[c]	14.4[c]	7.90[c]	34.0[a]	53.9	2.1[b]
0 F	45.7	16.1	8.17	35.3	55.9	1.5
5 F	45.6	15.9	7.97	34.9	57.2[a]	---
50 F	44.8	15.6[a]	7.89[a]	34.8	56.8	---
500 F	42.4[c]	14.7[c]	7.36[c]	34.6[a]	57.7[b]	1.9[b]

[1] Adapted from original report, p. 52.
[a] $p<0.05$
[b] $p<0.01$
[c] $p<0.01$

J. Clinical Chemistry:

All parameters are listed in Table 8 below.

At 500 ppm, males showed statistically significant albeit slight increases in ALT, AST, GGTP, creatinine, cholesterol and blood urea nitrogen. At 50 ppm in the male, ALT and blood urea nitrogen showed statistically significant slight increases. At 500 ppm, females showed a similar increase which was not statistically significant except for the cholesterol value. In both sexes at 500 ppm, albumin showed statistically significant albeit slight decreases versus the respective controls (Table 8).

Table 8

Mean Clinical Chemistry Values at Termination[1]

Group /Sex	ALT IU/l	AST IU/l	GGTP IU/l	CPK IU/l	Creat umol/l	Chol mmol/l	BUN mmol/l	Album g/l
0 M	30	49	1.4	168	54.9	1.3	6.8	38.3
5 M	32	46	3.2[a]	72[c]	59.0	1.4	6.5	37.9
50 M	33[a]	52	1.9	157	55.5	1.4	7.7[a]	38.4
500 M	35[b]	55[a]	3.5[a]	124[a]	61.4[b]	1.8[c]	8.5[c]	36.8[a]
0 F	40	61	4.5	186	54.5	1.8	7.2	36.7
5 F	37	55	6.0	160	52.8	1.7	7.6	37.2
50 F	34	52	6.4	141	57.3	1.9	7.9[a]	36.9
500 F	44	69	10.4	157	54.4	2.2[c]	7.8	35.3[a]

[1]Adapted from original report, p. 54-55.
[a]p<0.05
[b]P<0.01
[c]P<0.01

K. Urinalysis

Animals at 50 and 500 ppm excreted a more dilute and voluminous urine. These values were statistically significant. In males, a dose related increase in pH occurred with statistical significance seen at 500 ppm. In females, the pH decreased versus the control with statistical significance seen at 5 and 50 ppm (Table 9).

Two (2) of 9 females at 500 ppm showed a red blood cells (+2/+2) in the urine sediment versus a grade of 0 in the 10 control animals.

Table 9

Mean Urinalysis Values At Termination[1]

Observa -tions	Dose Level (ppm)							
	0	5 M	50 M	500 M	0 F	5 F	50 F	500 F
Volume	2.7	3.0	4.4[b]	4.6[c]	1.6	1.7	2.6[b]	3.1[c]
pH	6.8	6.9	6.9	7.2[a]	6.7	6.5[a]	6.4[a]	6.5
Sp. Gr.	1.06	1.05	1.04[c]	1.04[c]	1.07	1.06	1.05[c]	1.04[c]

[1]Adapted from original report, p. 56.
[a]$p<0.05$
[b]$p<0.01$
[c]$p<0.01$

L. Organ Weight:

Adrenal glands of both sexes showed a dose related decrease in absolute weight. Statistical significance was seen at 500 ppm in the male and 50 and 500 ppm in the female. The relative adrenal weight showed a dose related decrease at all dose levels with the decrease reaching statistical significance in the males at 500 ppm (Table 10, 11).

The thyroid gland in the female showed a dose related decrease in the absolute weight and was correlated with a decrease in the relative body weight ratio. Statistical significant was not seen in either parameter. Males showed a sporadic increase in the absolute thyroid weight at 50 ppm which was correlated with a statistical significant increase in the relative body weight ratio (Table 10, 11).

Increased kidney relative body weight ratios occurred at 500 ppm in both sexes and was statistically significant in the females (Table 10).

The testes showed a dose related decrease in the absolute weight (Table 10); however, the relative body weight ratio was not statistically significant.

Other statistically significant changes in the absolute organ weights occurred: liver weight decreases in the female animals at 500 ppm and sporadic weight increase in the spleen in the males at 5 ppm.

Table 10

Mean Absolute Organ Weight[1]

Dose (ppm)	Testes (gm)		Adrenals (mg)		Thyroid (mg)	
	Male	Female	Male	Female	Male	Female
0	2.9	-	39.8	43.2	14.2	17.4
5	2.8	-	35.6	38.5	13.5	15.6
50	2.8	-	34.1	37.3[a]	17.2[a]	14.9
500	2.4	-	30.0[b]	35.5[b]	15.1	14.7

[1]Adapted from original report, p. 57.
[a]$p<0.05$
[b]$p<0.01$

Table 11

Group Mean Organ/Body Weight Ratios[1]

Dose (ppm)	Kidney (%)		Adrenals x 1000		Thyroid x 1000	
	Male	Female	Male	Female	Male	Female
0	0.76	0.81	14.4	25.3	5.2	10.2
5	0.75	0.81	13.3	22.5	5.0	9.1
50	0.77	0.82	12.6	22.3	6.3[a]	8.9
500	0.84[b]	0.87[a]	11.8[b]	22.6	5.9	9.1

[1]Adapted from original report, p. 58.
[a]$p<0.05$
[b]$p<0.01$

M. Pathology:

Animals Dying or Sacrificed During the Study

(1) Gross Pathology:

The gross pathology mirrored the histopathological findings, except for the gastrointestinal findings.

Many of the animals at 2,000 and 5,000 ppm were autolyzed and cannibalized. Most of these animals were emaciated and showed depletion of the abdominal fat pads. Congestion and hemorrhagic contents were seen in the gastrointestinal tract.

At 500 ppm, 1/2 male animals that died during week 4 showed congestion and hemorrhagic contents in the gastrointestinal tract, namely the stomach, duodenum, ileum, jejunum, caecum and colon. These changes, however, were not confirmed by histopathological examination. Autolysis precluded histopathological examination of the gastrointestinal tract of the other animal.

(2) Histopathology:

Treatment related histopathological lesions were summarized below and the incidences presented in Table 12 and 13 for males and females, respectively (except for the stomach and prostate lesions).

Kidney

Two (2) males at 500 ppm exhibited papillary necrosis involving the tip/mid of the papilla with 1 animal having subchronic pyelonephritis. Hyperplasia of the epithelium lining the renal papilla was seen in 4M and 1F at 500 ppm. One (1) male in both the 50 and 500 ppm showed focal medullary cystic dilation.

Slight focal basophilic tubules were seen in 2 males and 2 females at 500 ppm, 1 male at 50 ppm and 1 female at 5 ppm.

Heart

Three (3) males at 500 ppm showed multiple foci of subchronic myocarditis. The lesion was characterized by multiple foci and areas of myocardial degeneration and mononuclear cell infiltration.

Aorta:

Focal medial mineralization was observed in 3 males at 500 ppm.

Thyroid

Hypertrophied follicular epithelium exhibited by the cells being lined with cuboidal to columnar epithelium were seen in most animals at 500 ppm and many animals at 50 ppm. In the control and 5 ppm levels follicles were lined with flatten to cuboidal epithelium and only a few follicles were lined with cuboidal to columnar epithelium. Colloidal changes were not seen.

Bone Marrow

Slight to reduced cellularity was seen 5 males and 2 females at

500 ppm.

Testes

Two (2) males at 500 ppm showed reduced spermatozoa in the lumen while 2 decadent males showed degeneration of the germinal epithelium.

Uterus

Seven (7) incidences of a denser myometrium (reduced volume of smooth muscle cytoplasm) occurred at 500 ppm versus 3 cases in the control group.

Stomach:

Two (2) decadent males at 500 ppm showed diffuse mineralization of the glandular mucosa while 1 male showed erosion of the glandular mucosa.

Prostate:

Subchronic prostitis was seen in 1 male at 500 ppm.

Note: The reviewer notes that no explanation was given for the lack of histopathological examinations at the 2 higher doses (2,000 and 5,000 ppm) as required by the Subdivision F Guidelines, although the raw data indicated that the some of these animals were autolytic and/or cannibalized.

Table 12

Histopathological Lesions in Male Rats Fed Cacodylic Acid for 13 Weeks

Tissue (Number Examined: 10/sex/dose)	0 ppm	5 ppm	50 ppm	500 ppm
Thyroid:				
1. Most follicles lined by cuboidal to columnar epithelium	0	0	1	7
2. Many follicles lined by cuboidal to columnar epithelium	0	0	5	2
3. Few follicles lined by cuboidal to columnar epithelium	10	10	4	0
Kidneys:				
1. Basophilic tubules - slight	0	0	1	2
2. Pelvic transitional cell hyperplasia - slight/diffuse	0	0	1	1
3. Pyelonephritis - subchronic	0	0	0	1
4. Papillary necrosis involving tip/mid portion of papilla	0	0	0	2
5. Epithelial lining renal papilla:				
a. hyperplasia, occasional/focal/ slight/diffuse	0	0	0	4
6. Tubular cystic dilation - focal, occasional	0	0	1	1
Aorta: Focal medial mineralization	0	0	0	3
Bone Marrow: Reduced cellularity-slight	0	0	0	5
Heart: Myocarditis, subchronic, multiple, focal	0	0	0	3
Testes:				
1. Reduced sperm in lumen	0	0	0	2
2. Degeneration of germinal epithelium	0	0	0	2

Table 13

Histopathological Lesions in Female Rats Fed Cacodylic Acid for 13 Weeks

Tissue (Number Examined: 10/sex/dose)	0 ppm	5 ppm	50 ppm	500 ppm
Thyroid				
1. Most follicles lined by cuboidal to columnar epithelium	0	0	0	10
2. Many follicles lined by cuboidal to columnar epithelium	0	0	7	0
3. Few follicles lined by cuboidal to columnar epithelium	10	1	3	0
Kidneys:				
1. Basophilic tubules - slight	0	1	0	2
2. Epithelial lining renal papilla:				
a. hyperplasia, occasional, focal, slight	0	0	0	1
Uterus: Myometrium: Denser	2	2	0	7
Bone Marrow: Reduced cellularity -Slight	0	0	0	2

VI. DISCUSSION:

At 5,000 ppm, all males died during week 1 and 2 while all females died during week 1. At 2,000 ppm females died during weeks 1 to 3 while males died during weeks 3 to 5. No data were reported for the 2,000 and 5,000 ppm animals (except for some minor gross necropsy), presumably because at least some of the animals were autolytic and/or cannibalized. The highest dose level in the study with a full data set was 500 ppm.

Throughout the study at 500 ppm, body weight gains were depressed 13% in the male and 17% in the female although no change in food consumption occurred.

Increased water intake at 50 and 500 ppm in both sexes correlated with the increased urine output and the decrease specific gravity. The increased hemodilution may have been responsible for the slight decrease in the albumin noted at 500 ppm in both sexes.

The mild anemia noted in both sexes probably was due to the well known effect of arsenic binding with the red blood cell membrane resulting in increased red blood cell destruction. A compensatory increase in the formation of red blood cell precursor cells (reticulocytes) occurred.

The damaged red blood cells were excreted in the urine and may have caused the blood cells found in the urinary sediment in females at 500 ppm.

Slight to mild effects on various enzymes and other clinical chemistry parameters were noted at 500 ppm in both sexes. No effect in the liver was seen at necropsy to account for these changes.

The relative weight increase in the kidney of the males was probably due to a decrease in the body weight at 500 ppm. The dose related relative weight decrease in the adrenals of the males can not be explained.

The dose related decrease in the adrenals in both sexes versus the respective controls was not correlated with any histopathological changes.

The authors note that in the 1 male at 500 ppm that died during week 4, gross pathology revealed changes indicative of irritation in the ileum and jejunum; these changes were not confirmed histologically. Gross necropsy in the other male correlated with the necropsy data.

At 50 and 500 ppm effects were noted in the thyroid (hypertrophic follicular epithelium). These changes, at least in the female, could be correlated with a dose related decrease in the absolute weight and decrease in the relative thyroid weight versus the controls.

The uterus showed a reduced volume of smooth muscle versus the control. The authors note that this effect may have been a consequence of the reduced body weight.

Both 500 ppm animals that died on week 4 showed degeneration of the testes germinal epithelium while 2 animals that were sacrificed at termination showed reduced sperm in the testes tubules. These results could be correlated with the reduced weight of the testes.

Changes in the aorta and heart also occurred and were considered to be due to compound administration.

Changes in the prostate and focal basophilic tubules of the kidney appear to be within the normal background for this type of rat at the laboratory. At 500 ppm, 2 decedent males showed stomach lesions which may have been due to the debilitated condition of these animals.

The astrocytoma seen in one 500 ppm female appeared to be a spurious effect not related to the administration Cacodylic Acid.

VII. CONCLUSION:

Cacodylic acid (99.5%) was incorporated into the diet of 6 groups of 60 specific pyrogen free Fischer F344 rats per sex at concentrations, respectively, of 0 (Control), 5, 50, 500, 2000 and 5000 ppm for at least 13 weeks.

Based on the results of this study, the following NOEL and LOEL are established. The LOEL is based on mortality, decrease in body weight gain, alterations in the hematology, changes in absolute and relative organ weights and histopathology lesions in the thyroid, kidney, aorta, bone marrow, heart, testes and uterus.

NOEL: 5 ppm (0.4 mg/kg/day).
LOEL: 50 ppm (males: 4 mg/kg/day; females: 4.5 mg/kg/day).

VIII. CORE CLASSIFICATION: Minimum

This study satisfies the data requirement for a subacute oral toxicity in rodents [82-1a] and is acceptable for regulatory purposes.