5-6-94

Microfiche

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

010943

MAY ' 6 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

Subject: PIRATE® Insecticide-Miticide (AC 303,630): Review of subchronic and developmental studies in the rat

P.C.#: 129093
Submission #s: S442669
Project No. D196061
G pet-temp toler
EPA ID#: 3G04223

From: Guruva B. Reddy, D.V.M., Ph. D. 5/3/94
Section 4
Toxicology Branch I
Health Effects Division (7509C)

To: Dennis Edwards/Meredith Johnson
Project Manager 19
Registration Division (7505C)

Thru: Marion P. Copley, D.V.M., D.A.B.T. Marion Copley 5/3/94
Section Head
Section 4, Toxicology Branch I
Health Effects Division (7509C)

I. **CONCLUSIONS:**

The rat developmental and rat subchronic studies have been reviewed and are acceptable. The results of these studies did not alter the temporary tolerance established through memo dated November 3, 1993. This action completes review of all studies submitted under D192279.

A copy of the DERs are attached.

1536

cc: CCB, OREB (Dorsey)

Recycled/Recyclable
Printed with Soy/Canola Ink on paper that contains at least 50% recycled fiber

II. ACTION REQUESTED:

American Cyanamid Company, has submitted an application for an Experimental Use Permit and a petition for Temporary Tolerance for PIRATE® Insecticide-Miticide. All the studies included in this package were reviewed, except for two studies listed below (D196061).

Guideline #	Study Type	MRID #
82-1(a)	Subchronic Oral (rodent)	427702-19
83-3(a)	Teratology (rodent)	427702-21[1]/428842-02

1. The study (MRID # 427702-21) was reanalyzed and resubmitted under MRID # 428842-02

III. STUDIES REVIEWED:

STUDY/CLASSIFICATION	TB-I COMMENTS
82.1 (a) 90-Day feeding-rodent American Cyanamid Co., USA Study # T-0316; 4/93 MRID # 427702-19 core-Guideline	At 600 ppm, males had an decreased body weight gain (14%) and increased relative liver weights (19%), while females exhibited decreased hemoglobin (14.9%) and increased absolute/relative liver weights (16.8%/21.6%). At 900 ppm, body weight gain (25%/21%) and feed consumption in males/females, RBC numbers, %HCT and %HGB in females were decreased. At the same dose level, platelets, ALK in males, absolute/relative liver weights (18.3%/33.1%) in females, relative liver weights (15%) in males and absolute/relative spleen weights in males and females increased. At 1200 ppm, male rats exhibited decreased activity, ataxia, anorexia, chromodacryorrhea and dark brown material around nose. Additionally, in males/females, body weight gains (37%/24%), feed consumption, RBC numbers, %HCT and %HGB decreased and platelet counts, BUN in males, ALK levels in males/females, absolute/relative liver (25.9%/44.8%) and splenic weights in females and absolute/relative splenic weights and relative liver (47%) weights in males were increased. The LEL of 600 ppm is based on decreased body weight gain and increased relative liver weight in males and decreased HGB and increased absolute/relative liver weights in females. The NOEL is 300 ppm.

83-3 (a) Developmental Toxicity - rat Argus Res. labs., Study # American Cyanamid- 371-90-177; 7/93 MRID #427702-21/428842-02 Core - Guideline	Maternal toxicity was noted in the form of a dose-related decrease in body weight gain in the mid (21.2%; 6-12 days) and high (23.4%; 6 - 16 days) dose groups, a dose-related decrease in relative feed consumption in the mid (6.3%) and high (12.2%) dose groups and a decrease in water intake in the high (12.9%) dose group; the body weight gain, relative feed intake and water consumption rebounded to control levels in both groups during the post-dosing (16 - 20 days) period. Therefore, the **Maternal Toxicity LEL = 75 mg/kg/day, and the Maternal Toxicity NOEL = 25 mg/kg/day, based on reduced body weight gain, reduced relative feed intake and reduced water consumption.** Developmental toxicity was not observed either in the form of maternal cesarean section observations or fetal external, visceral or skeletal malformations and variations. Therefore, the **Developmental Toxicity LEL is greater than 225 mg/kg/day and the NOEL is greater than or equal to 225 mg/kg/day.**

010949

Reviewed by: Guruva B. Reddy, D.V.M., Ph.D. 4/14/94
Section 4, Tox. Branch I (7509C)
Secondary reviewer: Marion P. Copley, D.V.M., D.A.B.T.
Section 4, Tox. Branch I (7509C) Marion Copley 4/15/94

DATA EVALUATION REPORT

STUDY TYPE: 90-Day Oral Toxicity Study - Rats

GUIDELINE NO: 82-1

P. C. NO.: 129093

MRID NO.: 427702-19

TEST MATERIAL: AC 303,630

SYNONYMS: Pyrrole -3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-1-(ethoxymethyl)-5-(trifluromethyl)

STUDY/PROJECT NUMBERS: Study No. T-0316
Study No. T-0221

SPONSOR/TESTING FACILITY: American Cyanamid Company
Princeton, NJ 08543-0400

TITLE OF REPORT: AC 303,630: 13-Week and 28-Day Dietary Toxicity Studies in the Albino Rat

AUTHOR(S): Joel E. Fischer

REPORT ISSUED: April 8, 1993

EXECUTIVE SUMMARY:

In a sub-chronic oral toxicity study, technical AC 303,630 (Lot. # AC7171-141A; 93.6% a.i.) was administered in feed to 20/sex/dose Crl:CD® (SD) rats at dose levels of 0, 150, 300, 600, 900 or 1200 ppm (measured intake of 0, 11.7, 24.1, 48.4, 72.5 or 97.5 mg/kg/day, respectively) for 90 days (MRID # 427702-19; Study # T-0316).

At 600 ppm, males had an decreased body weight gain (14%) and increased relative liver weights (19%), while females exhibited decreased hemoglobin (14.9%) and increased absolute/relative liver weights (16.8%/21.6%). At 900 ppm, body weight gain (25%/21%) and feed consumption in males/females; RBC numbers, %HCT and %HGB in females were decreased. At the same dose level, platelets, ALK in males, absolute/relative liver weights (18.3%/33.1%) in females, relative liver weights (15%) in males and absolute/relative spleen weights in males and females increased. At 1200 ppm, male rats exhibited decreased activity, ataxia, anorexia, chromodacryorrhea and dark brown material around nose. Additionally, in

4

males/females, body weight gains (37%/24%), feed consumption, RBC numbers, %HCT and %HGB decreased and platelet counts, BUN in males, ALK levels in males/females, absolute/relative liver (25.9%/44.8%) and splenic weights in females and absolute/relative splenic weights and relative liver (47%) weights in males were increased. **The LEL of 600 ppm is based on decreased body weight gain and increased relative liver weight in males and decreased HGB and increased absolute/relative liver weights in females. The NOEL is 300 ppm.**
This study is **core-guideline** and **satisfies** guideline requirement for a 82-1(a) study in the rat.

A. MATERIALS:

1. Test compound: CL No. 303,630, Description - white solid; insoluble in water and soluble in acetone; Lot # AC7171-141A; Purity - 93.6 %.

2. Test animals: Species: rats, Strain: CD® [Crl:CD®(SD)], Age: 3 weeks, Weight: Males - 86 to 105 g; Females - 70 to 88 g, Source: Charles River Breeding Labs., Inc., Wilmington, Massachusetts.

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned randomly to the following test groups:

Table 1

Test Group	Dose in diet (ppm)	male	female
I	0	20	20
II	150	20	20
III	300	20	20
IV	600	20	20
V	900	20	20
VI	1200	20	20

Animals were housed individually in stainless-steel, suspended, screen-bottomed cages held on racks, with absorbent paper to collect urine and feces. The rats were maintained in an environment with a room temperature of 22 ± 2°C, relative humidity of 50 ± 20 % and a light/dark cycles of 12 hours. The air was changes 10 - 20 times/hour. The basal diet (Purina Certified Rodent Chow #5002,

5

Ralston Purina, St. Louis, MO) or the test diets as appropriate and water were provided ad libitum.

The above doses were selected based on the **4-week rat study T-0221** (levels: 0, 600, 900, 1,200, 1,600 and 2,000 ppm; 5/sex). Table 2 presents those clinical parameters of males and females which were statistically significantly different from the controls.

TABLE 2. 28-DAY DOSE-RANGE FINDING STUDY[1]

OBSERVATIONS	DOSE (PPM)					
	0	600	900	1200	1600	2000
Mortality:						
♂	0/5	0/5	0/5	0/5	1/5	2/5
♀	0/5	0/5	0/5	0/5	0/5	0/5
Food Consumption (%):						
♂	0	-8.0	-8.6	-15.3*	-24.0*	-21.3*
♀	0	-5.6	-7.0	-13.7*	-16.9*	-21.1*
Body Wt. Gain (%):						
♂	0	-3.8	-15.4	-16.6	-30.2*	-45.3*
♀	0	-7.5	-9.6	-16.0*	-26.6*	-36.8*
Liver Wts. (g): Absolute/Relative(%):						
♂	10.2/3.6	10.6/3.9	11.1/4.4*	11.6/4.6*	12.5*/5.7*	11.8/6.1*
♀	7.5/3.6	8.7/4.4*	9.1*/4.6*	9.4*/5.0*	9.9*/5.7*	10.2*/6.5*
Spleen Wts. (g): Absolute/Relative(%):						
♂	0.67/0.24	0.59/0.22	0.56/0.22	0.82/0.33*	0.95*/0.43*	0.68/0.35*
♀	0.59/0.28	0.52/0.26	0.61/0.31	0.62/0.33	0.64/0.37*	0.66/0.43*
BUN (mg/dl):						
♂	15.0	17.0	17.6	19.2	22.3*	25.7*
♀	14.2	18.0	17.0	19.2*	20.2*	22.2*
Total Protein (g/dl):						
♂	7.3	7.1	7.0	7.2	7.6	7.0
♀	6.7	7.1	7.1	7.3*	7.3*	7.4*
Albumin (mg/dl):						
♂	4.1	3.8	3.6	3.6*	3.5*	3.4*
♀	3.9	3.7	3.6	3.7	3.5*	3.5*
GGPT (U/l):						
♂	0.0	0.0	0.2	0.6	2.8*	4.7*
♀	0.6	0.8	1.0	1.4	2.0	5.4*
SGPT (IU/l):						
♂	62.2	55.8	66.0	78.2	89.3*	106.3*
♀	37.0	45.2	61.8	50.4	66.2*	73.2*

[1] Data extracted from study Tables 5.2.1, 5.3.2, 5.5.1, 5.5.2, 5.6.1 and 5.6.2.

* $P \leq 0.05$

Based on dose-related increase in liver weights and hepatocellular hypertrophy at the 1600 and 2000 ppm dosages and statistically significant differences in the relative liver weights in females, the author concluded that the NOEL in the rat was less than 600 ppm, however, **TB-I considers 600 ppm as NOEL for AC 303,630 in the rat.** This is based on absolute and relative liver weight increases and decreased body weight gain (9.6%) in females, at the 900 ppm dose level.

Necropsy and histopathology of the deceased were not pathognomonic of treatment-related changes.

2. Diet preparation

Diet was prepared every 2 weeks. The diets were prepared by adding the proper amount of test substance to a small portion of the basal diet (premix) which was then mixed with the appropriate amount of the basal diet to obtain desired dietary concentrations. The test diets were sealed in polythene bags and stored at room temperature until use. Samples from each batch were collected and frozen for analysis of AC 303,630 content. Homogeneity and stability were determined on low- and high-dose levels prior to commencement of the study.

Results - At 150, 300, 600, 900 and 1200 ppm, the nominal concentration (%) found at 4 sampling times ranged from 97.6% to 98.3%. The concentration of the test material in the 150 and 1200 ppm diets, during 28 days storage at room temp., ranged from 89.8% to 97.5% (mean = 92.5%) and 95.1% to 103.3% (mean = 100%) of the nominal concentration, respectively. The homogeneity of the compound in two test diets ranged from 92.2% to 97.4 % of the nominal concentration.

3. Animals received food and water ad libitum.

4. Statistics - Standard one-way ANOVA was performed for body weight, body weight gains, food consumption, hematology, clinical chemistry, urinalysis, organ weights and organ-body weight percentages. If the differences were significant then a Dunnet's t-test was used for pairwise comparisons between treated groups and the control.

5. A signed quality assurance statement was enclosed.

7

C. METHODS AND RESULTS:

1. Observations:

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

Results: At 1200 ppm, male rats exhibited decreased activity (15%), anorexia (25%), ataxia (15%), chromodacryorrhea (40%) and dark brown material around nose (45%). The above signs are considered treatment-related. At the lower doses, no other signs of toxicity or mortality were observed, except for 2 female rats, one each from the control and 600 ppm died accidently during the 43-44 day bleeding.

2. Body weight

Animals were weighed 8 days prior to study initiation, on day 0 and weekly thereafter. The report included both group mean weekly body weights and body weights gains, but only body weight gains are presented in Table 3.

TABLE 3. MEAN BODY WEIGHT GAINS AND PERCENT GAIN OR LOSS RELATIVE TO CONTROLS (%)[a]

INTERVAL (WEEKS)	DOSE (PPM)					
	0	150	300	600	900	1200
1 - 5						
♂	225	221 (-1.8)	214 (-4.9)	201 (-10.7)	177 (-21.3)*	159 (-29.3)*
♀	122	120 (-1.6)	112 (-8.2)	111 (9.0)	99 (-18.9)	97 (-20.5)
5 - 10						
♂	112	94 (-16)	97 (-13.4)	93 (-17.0)	77 (-31.3)	62 (-44.6)
♀	51	55 (7.8)	51 (0)	48 (-5.9)	37 (-27.5)	37 (-27.5)
10 - 13						
♂	40	32 (-20.0)	37 (-7.5)	29 (-27.5)	31 (-22.5)	16 (-60.0)
♀	16	18 (12.5)	21 (31.0)	16 (0)	13 (-18.8)	11 (-31.3)
1 - 13						
♂	376	347 (-7.7)	348 (-7.4)	324 (-13.8)*	284 (-24.5)*	237 (-37.0)*
♀	189	192 (1.6)	184 (-7.4)	175 (-7.4)	149 (-21.1)*	144 (-23.8)*

a Data extracted from study Tables 5.3.1, 5.3.2, 5.3.3 and 5.3.4.
* Statistically significant at $P \leq 0.05$.

Results: The body weights of males and females in the 900 and 1200 ppm groups were significantly ($P \leq 0.05$) lower during most of the study, when compared to the controls. In males, body weight differences

were initially observed during the first week in the 1200 ppm and during the second week in the 900 ppm groups, whereas in females the differences were initially observed during the third week in both groups and appear dose-related. In addition, 600 ppm group males exhibited significant weight differences during weeks 8 - 13. The body weight differences of males and females in the 150 and 300 ppm groups were not statistically significant (data not presented in table).

The body weight gain data presented in Table 3 were abstracted from the sponsor's data, however, statistical significance for study periods 5 - 10 and 10 - 13 weeks were not given. Total body weight gains of males/females in the 1200 and 900 ppm and males in the 600 ppm group decreased significantly (37%/24%, 25%/21% and 14%, respectively), when compared to the controls and the decrease was dose-related. The total body weight gain of males in the 1200, 900 and 600 ppm groups and females in the 1200 and 900 ppm groups are considered treatment-related. Total body weight gain of the 600 ppm females was lower (7.4%) than controls and was not statistically significant, therefore, considered to be of no biological significance. The total body weight gains of males and females in the 300 and 150 ppm groups were slightly decreased but were not statistically significant.

3. Food consumption and compound intake

Consumption was determined weekly during the exposure period. Group mean compound intake was calculated from the consumption and dietary concentration. Following is the compound intake during the study:

Dietary Concentration (ppm)	Achieved Intake (mg/kg/day) Mean
150	11.7
300	24.1
600	48.4
900	72.5
1200	97.5

Water consumption was not measured.

Food consumption was reported decreased significantly ($P \leq 0.05$) in the males and in the

females throughout the treatment period, except for week 8 in males and weeks 3 and 10 in females, in the 1200 ppm group, when compared to the controls. At this dose, the mean percent reduction in food consumption of males/females during the weeks of 1 - 5, 5 - 10, 10 - 13 and 1 - 13 was 12.8/7.2, 14.1/12.6, 18.9/6.4 and 15.2/9, respectively, when compared to the controls. At 900 ppm, the mean food consumption of males/females, for the respective treatment periods was 7.3%/8.6%, 9%/15.9%, 6.6%/6.4% and 7.3%/10.4%, when compared to the controls; the decrease was significant ($P \leq 0.0\%$) during most of the study. The decreased mean feed consumption of 1200 and 900 ppm males/females was considered treatment-related (Table 4). Food consumption of males decreased sporadically ($P \leq 0.05$) in the 600, 300 and 150 ppm groups and was considered to be of no biological significance. At this dose female food consumption was not statistically different (data not presented in table).

TABLE 4. MEAN FOOD CONSUMPTION (G/WEEK) AND PERCENT GAIN OR LOSS RELATIVE TO CONTROLS (%)[a]

INTERVAL (WEEKS)	DOSE (PPM)					
	0	150	300	600	900	1200
1 - 5						
♂	179	173 (-3.4)	170 (-5.0)	170 (-5.0)	166 (-7.3)	156 (-12.8)
♀	139	138 (-0.7)	140 (0.7)	136 (-2.2)	127 (-8.6)	129 (-7.2)
5 - 10						
♂	199	189 (-5.0)	185 (-7.0)	184 (-7.5)	181 (-9.0)	171 (-14.1)
♀	151	147 (-2.6)	143 (-5.3)	140 (-7.3)	127 (-15.9)	132 (-12.6)
10 - 13						
♂	196	188 (-4.1)	195 (-0.5)	192 (-2.0)	183 (-6.6)	159 (-18.9)
♀	141	149 (5.6)	150 (6.4)	147 (4.4)	132 (-6.4)	132 (-6.4)
1 - 13						
♂	191	183 (-4.2)	183 (-6.4)	182 (-4.7)	177 (-7.3)	162 (-15.2)
♀	144	145 (0.7)	144 (0)	141 (-2.1)	129 (-10.4)	131 (-9.0)

a Data extracted from study Tables 5.2.1 and 5.2.2; the statistical significance was not determined.

4. Ophthalmological examination

Performed on all animals 6 days prior to exposure to the chemical and at the termination of experiment. No compound related effects were observed. At termination, unilateral focal retinopathies were observed in 2 males and 1 female at the 300 ppm, 1 male at the 600 ppm, 1 female at

the 900 ppm and 1 male at the 1200 ppm levels. In addition, one female each with iris stroma (300 ppm) unilateral blepharoptosis (600 ppm) and unilateral phthisis (900 ppm) were observed. The above findings were not dose-related and were commonly found in rats of this strain, therefore, were considered not related to treatment.

5. Blood was collected from 10 rats/sex/group (fasted overnight) on study days 43 and 44 and on the day of sacrifice for clinical chemistry determinations. Hematology was done only at termination. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)		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
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

Results - In males/females, at the 1200 ppm level, the mean number of RBCs (7.4M/7.4M vs 8.3M/8.1M), % hematocrit (43.2/43.4 vs 46.7/47.7) and % hemoglobin (14.7/14.6 vs 15.8/16.0) decreased significantly ($P \leq 0.05$), when compared to the controls. At the 900 ppm level, in females, the mean number of RBC, % hemoglobin and % hematocrit values were 7.4M, 43.8% and 14.5%, respectively, when compared to the controls and the differences were significantly ($P \leq 0.05$) different. Further, in the 600 ppm females, the % hemoglobin (14.9) decreased significantly. In addition, platelet counts of 1200 ppm and 900 ppm males increased by approximately 20% (1331 vs 1109; $P \leq 0.05$), respectively, when comparison to the controls. **The above hematological changes were expected due to body weight losses and were associated with increased splenic weights, and are considered to be treatment-related.** The platelet counts in the females receiving 900 and 1200 ppm doses increased slightly (1212 and 1209 vs 1062, respectively), but not significantly, when compared to the controls; therefore, considered to be of no biological significance. None of the above hematological parameters in males receiving 600 ppm levels or

either sexes receiving 300 ppm or 150 ppm doses in the diet were affected.

b. Clinical Chemistry

X	Electrolytes:	X	Other:
	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorous		Cholesterol
X	Potassium		Globulins
X	Sodium		Glucose
	Enzymes	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum Protein (TP)
	Cholinesterase (ChE)		Triglycerides
	Creatinine phosphokinase		Serum protein electrophores
	Lactic acid dehydrogenase (LAD)		
X	Serum alanine aminotransferase (also SGPT)		
X	Serum aspartate aminotransferase (also SGOT)		
X	Gamma glutamyl transferase (GGTP)		
	Glutamate dehydrogenase		

Table 5 presents those clinical chemistry parameters which were affected due to treatment with AC 303, 630.

Results - During the interim sampling, BUN (58%), GGTP (217%), SGPT (36%) in males and BUN (37%) and GGTP (71%) levels in females increased significantly ($P \leq 0.05$) in the 1200 ppm dose, when compared to the controls and are considered treatment-related. At the same dose, albumin levels in males decreased 5.6% ($P \leq 0.05$), which was expected due to reduced body weight gain and reduced feed consumption, however, the mean was within the range (3.2 - 5.2) established for this strain and age of rat, therefore, considered to be of no biological significance. Alkaline phosphatase levels in the 900 ppm group males increased 44% ($P \leq 0.05$)) compared to the controls and was considered treatment-related. The ALK levels in males and females in the 1200 ppm group increased, however, the increases were not significant, therefore, considered to be of no biological significance. Phosphorus levels in 900 ppm females increased 22% ($P \leq 0.05$), but the increase lacked dose-response and considered to be of equivocal significance (data not presented). At the 600 ppm, 300 ppm and 150 ppm, none of the clinical chemistry parameters of either sex were affected.

12

TABLE 5. CLINICAL CHEMISTRY VALUES[a]

PARAMETERS	DOSE (PPM)					
	0	150	300	600	900	1200
43-44 Days						
BUN (mg/dl) ♂	14.2	15.0	17.0	16.8	17.7	22.4*
♀	13.9	14.4	15.0	17.3	16.3	19.1*
GGTP (IU/L) ♂	0.6	0.8	0.8	1.0	1.0	1.9*
♀	1.7	1.4	2.1	1.8	2.6	2.9*
SGPT (IU/L) ♂	54.0	52.4	51.4	46.6	63.5	73.6*
♀	52.9	48.4	55.5	43.8	55.7	63.4
ALK (IU/L) ♂	199.8	163.6	190.7	209.1	288.0*	242.1
♀	139.6	131.4	174.3	165.4	159.8	209.1
Terminal						
BUN (mg/dl) ♂	17.1	16.0	17.5	17.9	18.6	22.3*
♀	15.7	15.0	14.2	15.2	14.9	16.6
GGTP (IU/L) ♂	0.0	0.0	0.0	0.0	0.0	0.3
♀	0.0	0.0	0.0	0.0	0.0	0.0
SGPT (IU/L) ♂	50.2	47.7	47.6	52.7	57.0	62.7
♀	48.3	50.7	56.6	39.7	47.7	46.4
ALK (IU/L) ♂	110.4	107.1	125.8	143.4	232.9*	225.0*
♀	58.6	54.2	79.4	72.3	101.1*	118.5*

a Data abstracted from report Tables 5.5.1, 5.5.2, 5.5.3 and 5.5.4

* $P \leq 0.05$

At the terminal sacrifice, BUN (30%) in 1200 ppm males and ALK levels in 900 and 1200 ppm males/females (111%/73% and 104%/102%, respectively) were increased significantly, when compared to the controls and were considered treatment-related (Table 5). In males, at the 1200 ppm, chloride (100.7 vs 95.8) and albumin levels were significantly higher , but were still within the control ranges established for this strain and age of rat and were not considered to be biologically significant. In addition, the serum phosphorus levels of males in the 600 and 900 ppm groups, increased 15%, respectively, however, the increase lacked dose-response and were considered

not related to treatment. In females, at the 900 and 1200 ppm dose levels, the total proteins increased 7.8%, respectively, compared to the controls; the results are inconsistent and difficult to explain in the light of reduced body weight gain. At the 300 and 150 ppm doses, the above clinical parameters were not affected.

6. Urinalysis

Urine was collected from 10 rats/sex/dose group at termination (13 weeks). The CHECKED (X) parameters were examined.

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

Results - No significant changes in the urinalysis parameters were observed, except for decrease in urine pH (5.5 vs 6.2) at the 1200 ppm level, in male rats. In the absence of changes in the other urine parameters, the decrease in urine pH of males rats was considered to be of no biological significance.

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, and only tissues from the controls and 1200 ppm groups were subjected to histological examination. In addition, histologic examination was restricted to all gross lesions and lungs, liver, kidneys, heart, spleen, thyroids, adrenals, brain and gonads from all 150, 300, 600, 900 and 1200 ppm groups. The (XX) organs, in addition, were weighed.

X	Digestive system
	Tongue
X	Salivary glands
X	Esophagus
X	Stomach
X	Duodenum
X	Jejunum
X	Ileum
X	Cecum
X	Colon
X	Rectum
XX	Liver
	Gall bladder
X	Pancreas
	Respiratory
X	Trachea
X	Lung
	Nose
	Pharynx
	Larynx

X	Cardiovasc./Hemat.
X	Aorta
XX	Heart
X	Bone marrow
X	Lymph nodes
XX	Spleen
X	Thymus
	Urogenital
XX	Kidneys
X	Urinary bladder
XX	Testes
X	Epididymides
X	Prostate
X	Seminal vesicle
XX	Ovaries
XX	Uterus
X	Vagina

X	Neurologic
XX	Brain
X	Periph. nerve
X	Spinal cord (3 levels)
X	Pituitary
X	Eyes (optic n.)
	Glandular
XX	Adrenal gland
	Lacrimal gland
X	Mammary gland
XX	Parathyroids
XX	Thyroids
	Other
X	Bone
X	Skeletal muscle
X	Skin
X	All gross lesions

a. Organ weight

Results: At the termination, several statistically significant increases/decreases in organ weights or relative organ weights were exhibited (see Table 6). The only significant finding related to treatment were the liver and splenic weight changes. In females, the absolute/relative liver weights in the 600 ppm, 900 ppm and 1200 ppm groups, increased 16.8%/21.6%, 18.3%/33.1% and 25.9%/44.8%, respectively, when compared to the controls and the response was dose-related. In males, at the 900 ppm dose, the absolute liver weight increased 15.2%, when compared to the controls and the increase lacked a dose-response. The relative liver weights of males rats in the 600 ppm, 900 ppm and 1200 ppm groups increased 19%, 15% and 47%, respectively, when compared to the controls. These differences are considered related to treatment with AC 303,630. TB-I disagrees with the study author's conclusion that increased relative liver weights (13%) of the 300 ppm group males were treatment-related, since the increase was not reflected either in the decreased body weight gain or in the increased liver enzymes. The absolute liver weights in both sexes and relative liver weights in female rats have increased slightly, at the 300 ppm. Liver weights of 150 ppm males and females were

15

comparable to those of the control rats.

TABLE 6 ABSOLUTE AND RELATIVE (% BW) ORGAN WEIGHTS[a]

TISSUES: Wt. (G)/% BW	DOSE (PPM)					
	0	150	300	600	900	1200
Liver						
♂	13.89/2.768	14.24/2.961	14.93/3.127*	15.10/3.303*	16.00*/3.184*	14.99/4.081*
♀	8.20/2.813	8.51/2.821	8.74/3.021	9.58*/3.422*	9.70*/3.745*	10.32*/4.073*
Kidney						
♂	3.31/0.661	3.47/0.724	3.34/0.703	3.22/0.705	2.99*/0.715	2.69*/0.736
♀	2.26/0.777	2.28/0.760	2.24/0.776	2.25/0.804	2.03*/0.782	2.00*/0.789
Heart						
♂	1.[illegible]9/0.303	1.524/0.319	1.528/0.320	1.511/0.332	1.421/0.339*	1.370/0.373*
♀	1.[illegible]7/0.359	1.130/0.375	1.088/0.376	1.096/0.392*	0.991/0.384	1.024/0.405*
Spleen						
♂	0.81/0.161	0.81/0.168	0.84/0.175	0.83/0.183	0.98*/0.236*	0.96*/0.263*
♀	0.59/0.202	0.59/0.197	0.61/0.211	0.62/0.222	0.69*/0.265*	0.72*/0.282*
Brain						
♂	2.07/0.415	2.11/0.442	2.09/0.440	2.03/0.448	2.00/0.485	2.04/0.588*
♀	1.91/0.661	1.89/0.630	1.96/0.682	1.91/0.690	1.89/0.740*	1.91/0.760*
Adrenals						
♂	0.064/0.013	0.063/0.013	0.061/0.013	0.059/0.013	0.059/0.014	0.062/017*
♀	0.078/0.027	0.085/0.028	0.080/0.028	0.082/0.030	0.073/0.028	0.071/0.028
Thyroids						
♂	0.032/0.006	0.031/0.006	0.033/0.007	0.028/0.006	0.033/0.008	0.034/0.009*
♀	0.030/0.010	0.028/0.009	0.030/0.010	0.082/0.011	0.028/0.011	0.028/0.011
Testes/Ovaries						
♂	3.315/0.664	3.366/0.704	3.245/0.683	3.163/0.691	3.053/0.710	3.173/0.851*
♀	0.702/0.242	0.741/0.248	0.779/0.270	0.829/0.296*	0.805/0.311*	0.778/0.310*

a Data abstracted from study Tables 5.7.1, 5.7.2, 5.7.3 and 5.7.4

* Significant at $P \leq 0.05$

The absolute and relative spleen weights of males/females in the 1200 and 900 ppm groups increased significantly. The response of relative splenic weights in males and absolute and relative splenic weights in females were dose-dependant. In males, the absolute/relative spleen weights, at the 1200 ppm and 900 ppm, increased 18.5%/63.4% and 21%/46.5%, respectively, when compared to the controls. In females, at the same dosages, the absolute/relative spleen weights increased 22%/39.6% and 16.9%/31.2%, respectively, when compared to the controls. The above changes were correlated with decreased %HGB, %HCT and RBC

16

numbers observed in these animals and are considered treatment-related.

At the 1200 and 900 ppm, the relative kidney weights of males/females decreased 18.7%/11.5% and 9.7%/10.2%, respectively, when compared to the controls. The decrease lacked the dose-response. These changes are due to reduction in body weights as the relative organ weights remained constant and the effects are considered not treatment related. Moreover, there was no evidence of histopathological changes which could be considered due to treatment. The absolute and relative kidney weights in the 600 ppm, 300 ppm and 150 ppm levels were generally comparable to those of the controls.

In males, at the 1200 ppm and 900 ppm levels, the relative heart weights increased 23% and 11.9%, respectively, when compared to the controls. In females, the relative heart weights in the 1200 ppm and 600 ppm dose levels increased 12.8% and 9.2%, respectively. The changes are considered due to decreased body weight gain rather than compound effect since no histopathological changes associated with treatment were observed. Heart weights of animals in the 300 ppm and 150 ppm levels were comparable to the controls.

At the 1200 ppm, in males the relative weights of brain (41.7%), adrenal (30.8%), thyroid (50%) and testes (28.2%) increased significantly, when compared to the controls. At the same dose, in females, the relative brain (15%) and ovarian weights (28.1%) increased. In addition, in females, the relative brain weight (12%) in the 600 ppm group and relative ovarian weights in the 900 ppm and 600 ppm groups (28.5% and 22.3%, respectively), increased significantly, when compared to the controls. These obsolete/relative organ weight changes, noticed above were not supported by histopathology, were probably due to the reduction in body weights in both sexes and considered to be of no biological significance. None of the above organ weights in the 300 ppm and 150 ppm dose levels were affected due to AC 303,630 administration.

b. Gross pathology - There were no gross pathological changes in the organs which were attributed to treatment with AC 303,630 were observed.

c. Microscopic pathology - Hepatocellular hypertrophy in 1 of 20 male rats each at the 1200 ppm and 900 ppm groups and 1/20 females at the 600 ppm dose level were observed. There was no other evidence of severe toxicity such as necrosis was observed in any of liver tissues. Spongyform myelopathy in brain and spinal cord of male rats in the 1200 ppm (2/20), 900 ppm (2/20) and 600 ppm (1/20) were observed. The severity of cellular changes was described as moderate, however, these changes did not affect the clinical signs such as ataxia or locomotor activity. In the absence of clinical signs the study pathologist considered the aforementioned cellular changes may be due to abnormal fixing. We concur with their conclusions. In addition, one of affected males in the 1200 ppm group exhibited lesions in the sciatic nerve and the other affected 1200 ppm male exhibited the lesion in the optic nerve. Further, male rats at the 1200 ppm (2/20), 900 ppm (3/20) and 600 ppm (2/20) levels exhibited unilateral/bilateral atrophy of seminiferous tubules. These changes, with the exception of 1 animal at the 600 ppm level and 1 animal at the 900 ppm, occurred in the same animals which exhibited spongyform myelopathy. The study authors concluded that the above changes were probably due to reduced feed intake and reduced weight gain. We concur with their conclusions.

There were no microscopic findings in either sex at the 300 ppm or 150 ppm levels that could be attributed to AC 303,630.

D. DISCUSSION:

The data reporting was thorough and the summary means were supported by individual animal data.

E. CONCLUSIONS:

Doses administered: 0, 150, 300, 600, 900 or 1200 ppm (**measured** intake of 0, 11.7, 24.1, 48.4, 72.5 or 97.5 mg/kg/day, respectively) of technical AC 303,630 (Lot. # AC7171-141A; 93.6% a.i.) to 20/sex/dose Crl:CD® (SD) rats for 90 days in feed (MRID # 427702-19; Study # T-0316).

At 600 ppm, males had an decreased body weight gain (14%) and increased relative liver weights (19%), while females exhibited decreased hemoglobin (14.9%) and increased absolute/relative liver weights (16.8%/21.6%). At 900 ppm, body weight gain (25%/21%) and feed consumption in

18

males/females, RBC numbers, %HCT and %HGB in females were decreased and platelets, ALK in males, absolute/relative liver weights (18.3%/33.1%) in females, relative liver weights (15%) in males and absolute/relative spleen weights in males and females increased. At 1200 ppm, male rats exhibited decreased activity, ataxia, anorexia, chromodacryorrhea and dark brown material around nose. Additionally, in males/females, body weight gains (37%/24%), feed consumption, RBC numbers, %HCT and %HGB decreased and platelet counts, BUN in males, ALK levels in males/females, absolute/relative liver (25.9%/44.8%) and splenic weights in females and absolute/relative splenic weights and relative liver (47%) weights in males were increased. **The LEL of 600 ppm is based on decreased body weight gain and increased relative liver weight in males and decreased HGB and increased absolute/relative liver weights in females. The NOEL is 300 ppm. This is contrary to the study author's conclusion of 300 ppm as LEL, since it was based on relative liver weights which alone is not adequate to determine whether MTD has been reached to evaluate potential oncogenicity of the chemical.**

This study is **core-guideline** and **satisfies** guideline requirement for a 82-1(a) study in the rat.

Reddy/AC 303,630 90-day rat.der/4-4-94
Final: 4-14-94
DP Barcode: D196061

010949

GUIDELINE: 83-3(a)

Review by: Guruva B. Reddy, DVM, PHD 4/14/94
Review Section IV, Toxicology Branch I (7509C)
Secondary Reviewer: Marion P. Copley, DVM, DABT
Section Head, Review Section IV, Toxicology Branch I (7509C)
Marion Copley 4/15/94

DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity - Rat

TOX. CHEM. NO.: N.A.

P. C. NO.: 129093

MRID NO.: 423842-02/427702-21

TEST MATERIAL: AC 303,630

SYNONYMS: Pyrrole -3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-1-(ethoxymethyl)-5-(trifluromethyl)

STUDY NUMBER or LAB. PROJECT ID: Argus Project No. 101-015
American Cyanamid No. 971-90-177

SPONSOR: American Cyanamid Company
Princeton, NJ 08543-0400

TESTING FACILITY: Argus Research Labs., Inc.
Horsham, PA 19044

TITLE OF REPORT: An Oral Developmental Toxicity (Embryo-Fetal Toxicity/Teratogenicity) Definitive Study with AC 303,630 in Rats

AUTHOR(S): Terry Martin, DVM, MS, ABVT

REPORT ISSUED: July 22, 1993

EXECUTIVE SUMMARY: In a developmental toxicity (teratology) study, 25 timed-pregnant rats per dose group of Crl:CD®BR VAF/Plus® (SD), received either 0, 25, 75 or 225 mg/kg/day by oral gavage from gestation day 6 through 16, inclusive. The test compound (Lot # AC 7504-59A, Purity 94.5%) in 0.5% carboxymethylcellulose was administered in 10 mL/kg body weight (MRID #428842-02; Study # American Cyanamid- 971-90-177).

Maternal toxicity was noted in the form of a dose-related decrease in body weight gain in the mid (21.2%; 6-12 days) and high (23.4%; 6 - 16 days) dose groups, a dose-related decrease in relative feed consumption in the mid (6.3%) and high (12.2%) dose groups and a decrease in water intake in the high (12.9%) dose group; the body weight gain, relative feed intake and water consumption rebounded to control levels in both groups during the

20

post-dosing (16 - 20 days) period. Therefore, the **Maternal Toxicity LEL = 75 mg/kg/day, and the Maternal Toxicity NOEL = 25 mg/kg/day, based on reduced body weight gain, reduced relative feed intake and reduced water consumption.**

Developmental toxicity was not observed either in the form of maternal cesarean section observations or fetal external, visceral or skeletal malformations and variations. Therefore, the **Developmental Toxicity LEL is greater than 225 mg/kg/day and the NOEL is greater than or equal to 225 mg/kg/day.**

The study is classified as Core - Guideline Data and satisfies the requirement (§ 83-3 a) for a developmental toxicity (teratology) study in rats.

A. **MATERIALS**

1. Test Compound: AC 303,630; Description - Tan solid; Lot # - AC 7504-59A; Purity -94.5%

   Vehicle(s): Carboxymethylcellulose from Sigma Chemical Co.; Lot # - 38F-0529

2. Test Animal(s): Species: Rats; Strain: Charles River Crl:CD®BR VAF/Plus®; Age: M - 30 days, F - 66 days old at receipt; Weight: M - 420 to 1011g and F - 174 to 225g at initiation of cohabitation; Source: Charles River Labs. Inc., Portage, MI.. Acclimated for ≈ a month.

B. **STUDY DESIGN**

This study was designed to assess the developmental toxicity potential of AC 303,630 when administered by gavage to timed-pregnant Crl:CD®BR VAF/Plus® Sprauge Dawley rats on gestation days 6 through 15, inclusive. The pregnant rats were housed individually in wire-bottomed stainless steel cages suspended above absorbent paper and was offered feed (Certified Rat Chow® #5002, Ralston Purina Co., St. Louis, MO) and water was provided ad libitum. Animals were maintained at a temperature of 68°F to 80°F, relative humidity of 35% to 70% and a 12 hour light and dark cycle. Air was changed 10/hour. On Day 0, a total of 25 naturally mated females each were randomly assigned to the treatment groups as presented in Table 1.

Group Arrangement:

Table 1

Test Group	Dose Level (mg/kg)	Number Assigned
I (Control)	0	25
II (Low)	25	25
III (Mid)	75	25
IV (High)	225	25

C. METHODS

1. Mating

Acclimated, untreated female (140) rats were mated 1:1 with untreated fertile males of the same breed. Animals were cohabitated overnight and successful matings identified by examining vaginal smears for sperm or by the presence of a copulatory plug. These females were considered to be fertilized and that day was designated as Day 0 of presumed gestation.

2. Dosing

Range Finding Studies: Dose levels were selected based on results of a range finding study in this report (Argus Research Labs., Inc., Study Protocol #101-015P). Dose levels were tested in pregnant (0, 20, 40, 80 and 160 mg/kg/day) and non-pregnant rats (180, 200, 270 and 350 mg/kg/day). No pregnant rats died in the study. The reduced feed consumption/weight gain during the treatment (6 - 16 days) was 5.3%/6.5%. Neither the reduced feed consumption nor the reduced body weight gain were statistically different to indicate that maternal toxic dose has been reached. There were no adverse effects on embryo-fetal survival, sex ratios, body weights or morphology.

Individual data or summary tables for the non-pregnant rat section of the study were provided. It was reported that in the 180 and 200 mg/kg/day dosage levels a dose-dependent difference in body weight gains were observed after seven days of dosing; it was not explicit whether the difference was positive or negative. In addition, at the 270 and 350 mg/kg/day, there were slight reduction in feed intake and weight gain and increased liver weights were reported. There were no deaths, no other clinical signs or necropsy findings suggestive of compound administration were seen, except for emaciation and decreased motor

27

activity in one 350 mg/kg/day group rat. The TB-I is not convinced that MTD has been achieved.

In the main study, the test substance, diluted in 0.5% carboxymethylcellulose to a constant volume of 10 ml/kg, was administered by gavage. Controls received 0.5% carboxymethylcellulose at a dose equivalent to that used in high dose group. Daily, dosage adjustments were based on the recent body weights.

Test substance analysis: Determination of concentration and homogeneity of AC 303,630 in 0.5% carboxymethylcellulose suspensions was performed using HPLC-UV by the sponsor. Dosage suspensions were prepared weekly during the study and were stored refrigerated. Homogeneity of AC 303,630 in 0.5% carboxymethylcellulose aqueous suspension was determined in the pilot study. Concentration was determined in the pilot and definitive studies on the first and last day of dosing period.

Results: The purity of undiluted test compound was reported as 94.5%. No impurities were listed. Homogeneity of the samples (2 and 16 mg/ml) ranged from 93% to 98% of target concentration. The mean concentrations for the pilot study (2, 4, 8 and 16 mg/mL) ranged from 91% to 97% of target concentration. The mean concentration in the main study (2.5, 7.5 and 22.5 mg/mL) ranged from 84% to 100% of target concentration.

3. **Observations**

The animals were checked once or twice daily for mortality or abnormal conditions during the course of the study. Dams were sacrificed by carbon dioxide asphyxiation on day 20 of gestation and thoracic, abdominal and pelvic cavities and viscera were examined for abnormalities. Uteri and ovaries were removed and live and dead fetuses, and early and late resorption sites were noted in each uterus. Corpora lutea were counted and recorded for each ovary. Uteri weighed and which appeared non-pregnant was stained with 10% ammonium sulfide to confirm pregnancy. All fetuses were counted, weighed, sexed and examined for external and visceral anomalies. Approximately one-half of the fetuses in each litter were fixed in Bouin's solution and examined for soft tissue alterations according to Wilson's sectioning technique. The remaining fetuses were eviscerated, cleared and stained with alizarin red S for skeletal alterations. All abnormalities, malformations and alterations were photographed.

Historical control data were provided from 90 studies on reproductive parameters and maternal necropsy observations, 76 studies for fetal external alterations and 40 studies for fetal skeletal variations and malformations to allow comparisons with concurrent controls. The studies covered from 1987 - 1989.

4. **Statistical analysis**

Fetal and maternal body weights, maternal body weight gains, food consumption, gravid uterine weights, percent male fetuses, % resorbed conceptus, % fetal implantations, fetal alterations and fetal ossification sites were analyzed using Bartlett's Test of Homogeneity of Variances and one-way analysis of variance (ANOVA), followed by Dunnett's test if significant. Non-homogenous data was analyzed using Kruskal-Wallis, Fisher's Exact or Dunn's Method of Multiple Comparisons Test, as appropriate. All other caesarean sectioning data were analyzed using Kruskal-Wallis Test.

5. **Compliance**

A signed Statement of Confidentiality Claim was provided.

A signed Statement of compliance with EPA GLP's was provided.

A signed Quality Assurance Statement was provided.

D. **RESULTS**

1. **Maternal Toxicity**

a. **Mortality** - Animals were observed twice daily for mortality.

Results - No treatment-related deaths, abortions or premature deliveries occurred during the study.

b. **Clinical Observations** - observed for general appearance several times during the acclimation and Day 0 of pregnancy. Rats were examined for clinical signs associated with test substance administration, premature deliveries and/or abortions and deaths, immediately before intubation, one hour after intubation and once daily during the post-dosage period.

Results - No treatment-related clinical signs,

34

premature deliveries and/or abortions and deaths were noticed during the study. Localized alopecia and chromodacryorrhea were observed, which were neither dose-related nor statistically significant.

c. **Body Weight** - Maternal body weights were measured one week before mating and on gestation Days 0 and 6 through 20 of presumed gestation. Table 2 summarizes body weight gains for the specified intervals.

Results - Mean body weight gains during the treatment period (6 - 16 days) in the high-dose dams decreased by 23.4% when compared to the controls and was significant ($P \leq 0.01$). Within the treatment period, the mean maternal body weight gains during the 6 - 9 and 6 - 12 days treatment periods were -72.3% and -57.7% ($P \leq 0.01$), respectively, when compared to the controls (Table 2). The reduced body weight gains in this group is considered treatment-related and was adequate to test the potential developmental toxicity of the chemical. At the 75 mg/kg/day, the mean body weight gain was significantly ($P \leq 0.05$) lower (21.1%) during the initial 6 - 12 days of treatment, however, the weight gains during the entire treatment period (6 - 16 days), post-treatment (16 - 20 days) and the gestation periods (0 - 20 days) were not statistically significant; slightly less than the controls but were comparable. **Although reduced weight gain did not last through the entire treatment period, the reduction during the 6 - 12 days treatment was sharp and considered as toxic manifestation of the chemical and will be used to establish LEL for the chemical.** The mean body weight gains of 25 mg/kg/day group during the study was slightly lower than the controls but comparable.

85

TABLE 2. BODY WEIGHT GAIN (GRAMS)[1]

	AC 303,630 MG/KG/DAY			
INTERVAL	0	25	75	225
PRETREATMENT:				
0 - 6 DAYS	23.5	23.8	23.2	24.4
TREATMENT:				
6 - 9 DAYS	8.1	7.3	5.2	0.6**
9 - 12 DAYS	12.6	11.7	11.2	8.2
12 - 16 DAYS	26.1	23.8	25.6	27.1
6 - 12 DAYS	20.8	19.1	16.4*	8.8**
6 - 16 DAYS	46.9	42.8	42.0	35.9**
POST-TREATMENT:				
16 - 20 DAYS	61.4	58.3	62.2	63.2
GESTATION:				
0 - 20 DAYS	131.8	123.9	127.4	123.6
0 - 20* DAYS	88.7	81.2	83.4	80.2
6 - 20* DAYS	65.2	58.5	60.2	55.7**

[1] Data taken from summary Table 4 of study.
* Corrected maternal body weight (Body wt. - Litter wt.)
* $P \leq 0.05$, ** $P \leq 0.01$

d. **Food Consumption** - Food consumption was recorded on days 0 and daily days 6 through 29 of presumed gestation. The data in the study report is given in both g/animal/day and g/kg/day and only relative feed consumption are presented in Table 3.

Results - Average food consumption, calculated as g/animal/day and g/kg/day was significantly reduced ($P \leq 0.01$) for the entire treatment period (days 6 - 16 of gestation), at the 75 and 225 mg/kg/day (Table 3). The absolute/relative feed consumption in the mid- and high-dose groups during the entire treatment period decreased 8.0%/6.3% and 15.1%/12.2%, respectively, when compared to the controls. Within this dosing period, significant reductions ($P \leq 0.05$ to $P \leq 0.01$) in absolute/relative feed consumption values occurred in the 75 and 225 mg/kg/day groups on days 8 to 9 (12.3%/11.5% and 23.2%/22.2%, respectively), 6 to 9 (9.4%/8.4% and 18.9%/17.7%, respectively), 9 to 12 (9.1%/7.3% and 19.7%/16.4%, respectively) and 6 to 12 (8.3%/7.9% and 19.1%/17.0%, respectively) days of gestation. Althcugh the reduced feed consumption in the mid-dose dams did not result in the significant body weight gain reduction during the treatment days 6 - 16 (Table 2), the reduced feed consumption during this period was highly significant, therefore, was

considered treatment-related and will be used as the toxicity end point for LEL. Feed consumption was not affected due to treatment in the 25 mg/kg/day group in contrast to the controls.

TABLE 3. RELATIVE FEED CONSUMPTION (G/KG/DAY)[1]

	AC 303,630 MG/KG/DAY			
INTERVAL	0	25	75	225
PRETREATMENT: 0 - 6 DAYS	76.7	76.0	75.6	75.1
TREATMENT:				
6 - 9 DAYS	71.2	69.1	65.2**	58.6**
9 - 12 DAYS	70.8	69.6	65.6**	59.2**
12 - 16 DAYS	71.4	70.7	68.7	67.7
6 - 12 DAYS	71.0	69.4	65.4**	58.9**
6 - 16 DAYS	71.2	69.9	66.7**	62.5**
POST-TREATMENT: 16 - 20 DAYS	68.8	67.6	68.9	71.2
GESTATION: 0 - 20 DAYS	69.4	68.5	67.2	65.9*

[1] Data taken from summary Table 6 of study.
* = $P \leq 0.05$, ** = $P \leq 0.01$

e. **Water consumption** - Water consumption was recorded daily throughout the study.

Results - Water consumption was significantly reduced ($P \leq 0.05$ to $P \leq 0.01$) in the mid and high-dose groups on days 6 - 7 (22.9% and 38.9%, respectively) and in the 225 mg/kg/day group on days 6 - 12 (18.9%) of presumed gestation. In the 225 mg/kg/day group water consumption tend to be lower (12.9%) during entire gestation period, when compared to the controls and was considered treatment-related. Although reduced feed intake of the 75 mg/kg/day dams was considered treatment-related, the mean water consumption in the 75 mg/kg/day group was -8.2% during the entire gestation period and was considered to be of no biological significance, since it lacked dose-response relationship. There was no difference in the water consumption in low-dose group.

f. **Gross Pathological Observations** - No treatment-related gross pathological observations were noticed in dams at necropsy among any treatment groups except for marked and slight dilation of the right kidney pelvis of one control and one high-dose group rat and considered to

37

be of no biological significance. **Organ weights were not recorded.**

g. **Cesarean Section Observations**

Cesarian section was performed on a total of 22, 25, 24 and 25 rats in the control, 25, 75 and 225 mg/kg/day groups, respectively. No statistically significant differences for the number of live fetuses, corpora lutea, implantatiors, litter size, early resorptions and late resorptions, number of dams with resorptions, fetal weights, sex ratios and percent resorbed conceptuses were observed in dams at necropsy (Table 4). The percent conception in the control, 25, 75 or 225 mg/kg/day groups was 88.1, 100, 96 and 100%, respectively. There were no dead fetuses and none of the dams had only resorbed conceptus.

TABLE 4. CESARIAN SECTION OBSERVATIONS*

PARAMETER	DOSE (MG/KG/DAY)			
	0	25	75	225
# Animal Mated	25	25	25	25
# Animal Pregnant (% of total)	22 (88.1)	25 (100)	24 (96.0)	25 (100)
Maternal Wastage				
# Died	0	0	0	0
# Pregnant	22	25	24	25
# Non pregnant	3	0	1	0
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Total Corpora Lutea	351	396	389	392
Corpora Lutes/Dam	16.0	15.8	16.2	15.7
Total Implantations	314	346	352	354
Implantations/Dam	14.3	13.8	14.7	14.2
Total Live Fetuses	292	323	319	337
Live Fetuses/Dam	13.3	12.9	13.3	13.5
Total Resorptions (Early/Late)	22/0	23/0	31/2	16/1
Resorptions/Dam (Early/Late)	1/0	0.9/0	1.3/0.1	0.6/0.04
Resorbed Conceptus/Litter (%)	7.5	6.8	9.1	4.3
# of Dams with Resorptions (%)	14 (63.6)	13 (52.0)	18 (75.0)	10 (40.0)
Total Dead Fetuses	0	0	0	0
Dead Fetuses/Dam	0	0	0	0
Mean Fetal Weight (gm)	3.25	3.31	3.32	3.21
Preimplantation Loss (%)	10.5	12.6	9.5	9.7
Postimplantation Loss (%)	0	0	3.4	4.8
Sex Ratio (% Male)	52.9	50.3	52.6	50.1

* Data extracted from Report Tables 8, 9 and 20

2. Developmental Toxicity

A total of 292/22, 323/25, 319/24 and 337/25 fetuses/litter from the control, 25, 75 and 225 mg/kg/day, respectively, were examined for external alterations. Of these respective fetuses, 140, 156, 153 and 163 fetuses were examined for soft tissue alterations, and 152, 167, 166 and 174 fetuses were examined for skeletal alterations and fetal ossification site averages.

The number of litters with fetal alterations in the control (0), 25, 75 and 225 mg/kg/day groups were 8

(36.4%), 8 (32.0%), 7 (29.2%) and 11 (44.0%), respectively. The number of fetuses with any alterations were 18 (6.2%), 10 (3.1%), 10 (3.1%) and 22 (6.5%) and the mean percentage of fetuses with any alteration/litter were 6.41, 3.05, 4.24 and 6.52, in these respective groups. None of these differences were statistically significant.

a. **External Examinations** - No treatment-related external malformations/variations were observed in fetuses at necropsy in any treated groups. External malformations were observed in three low dose, one middle dose and two high dose fetuses. In the low dose, the incidence included anasarca in one fetus (litter/fetal - 4.0/0.3), and thread like tail in two fetuses (litter/fetal - 8.0/0.6). Umbilical hernia (litter/fetal - 4.2/0.3) was observed in one mid-dose fetus. In the high dose, one fetus was a conjoined twin (litter/fetal - 4.0/0.3) and one fetus had thread like tail (litter/fetal - 4.0/0.3).

b. **Visceral Examinations** - Treatment with AC 303,630 had no effect on the visceral malformations/variations. Two low dose fetuses exhibited soft tissue malformations. One externally malformed fetus (17168-10) had a diaphragmatic hernia and a small kidney (litter/fetal - 4.0%/0.6%) and other fetus (17173-2) had slight/moderate dilation of the left/right renal pelvis (litter/fetal - 4.0%/0.6%).

One fetus (17199-17) from the mid dose presented slight dilation of the renal pelvis (litter/fetal - 4.2%/0.6%) which was classified as fetal variation (reversible developmental delay).

The above incidences were sporadic and lacked dose-response and were within the historical control range established for this strain and age of rats. The incidences are considered spontaneous and therefore of no biological significance.

c. **Skeletal Examinations:**

No treatment-related skeletal malformations and/or variations were noted. Appendix I presents fetal skeletal alterations and Appendix II presents ossification site endpoints that significantly differed from the control group.

At the 225 mg/kg/day group, the mean litter incidence of supernumery ribs increased (13.04 vs 13.0; $P \leq 0.05$) and was accompanied by increased litter averages for

ossified thoracic vertebrae (13.05 vs 13.0; $P \leq 0.05$) and decreased litter averages for lumbar vertebral ossification sites (5.94 vs 6.0; $P \leq 0.05$, Appendix II). The respective historical control ranges were 0 - 20, 0 - 11.8 and 0 - 4.3, based on 40 studies which included 7003 fetuses from 818 litters. The above litter means in the study are at or near the concurrent/historical controls, and therefore, considered to be unrelated to treatment.

In the high-dose group the fetal incidence of absent sternal ossification increased by 5.2% ($P \leq 0.01$) compared to the controls (Appendix I). This incidence was considered unrelated to treatment since the litter incidence was not affected and was within the historical control range of 0 - 6.5 established for this strain and age of rats. In addition, when the incidence of incomplete and absent ossification were combined, the statistical significance at the 225 mg/kg/day group vanished, when compared to the controls. Furthermore, when other endpoints of delayed sternal ossification were considered (incomplete ossification), the fetal incidences were significantly reduced ($P \leq 0.01$) in the 25, 75 and 225 mg/kg/day dosage groups. This further supports that increased incidence of absent sternebral ossification is unrelated treatment.

The fetal incidence of incompletely ossified ischia was 0.6, 0.6 and 1.7% in the 25, 75 and 225 mg/kg/day, respectively, when compared to the 4.6% of the controls and were statistically significant ($P \leq 0.01$). The incidence of incompletely ossified ischia was considered not related to treatment since the effect was opposite of developmental toxicity and the litter incidence was not affected.

There were no other fetal alterations that occurred at significant litter or fetal incidences.

E. **DISCUSSIONS**

The data reporting was thorough and the summary means were supported by the individual animal data.

F. **CONCLUSIONS**

a. **Maternal NOEL: 25 mg/kg/day. LEL: 75 mg/kg/day,** based upon, reduced body weight gain (6 - 12 days) and reduced relative feed consumption (6 - 16 days) during treatment.

b. **Developmental NOEL > 225 mg/kg/day.**

As presented, the study satisfies the requirements set forth in Subdivision F Guideline, 83-3 (a) for Developmental Toxicity Study in Rats.

Reddy/AC 303,630/dev-rat/3-14-94
Final: 4-14-94
Project #: D196061

CHLORFENAPYR

Page _____ is not included in this copy.

Pages 33 through 36 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.