C11061

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DATA EVALUATION RECORD 6/21/1994

STUDY TYPE: Multigeneration Reproduction - Rat (Guideline 83-4)

MRID NUMBER: 407343-03

TEST MATERIAL: Technical grade paclobutrazol (stated purity of 92.4%) described as an off white powder (Batch No. P29).

SYNONYMS: Paclobutrazol, [(2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H,1,2,4-triazol-1-yl)pentan-3-ol]

STUDY NUMBER(S): CTL/P/1496 and CTL/P/1496S

SPONSOR: ICI Americas, Inc., Agricultural Products, Wilmington, DE.

TESTING FACILITY: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK

TITLE OF REPORT: Paclobutrazol: Two Generation Reproduction Study in Rats Including Individual Animal Data.

AUTHOR(S): Wickramaratne, G. A.

DATE REPORT ISSUED: February 27, 1987.

CONCLUSIONS:

Paclobutrazol (technical, 92.4% pure) was tested in a two-generation reproduction study in Alpk:AP (Wistar derived) rats at the following dose levels: 0, 50, 250 and 1250 ppm (0, 2.5, 12.5 or 62.5 mg/kg/day) in the diet. Each generation began with 17 males and 34 females for each control and high dose group and 15 males and 30 females for each low and mid-dose group. One male was mated with 2 females from the same test group until sperm plugs were observed in vaginal smears. The F_0 parental animals were given test diets for 12 weeks before they were mated, and the F_1 parental animals wee not mated until 11 weeks after they were selected from the F_{1A} litters. Selection of parents for the F_1 generation was made when the pups were 36 days of age, and the mated animals in the study were between 16 and 20 weeks of age at mating.

At 250 ppm and above, an increased incidence of chromodacryorrhea and thickened eyelids was observed in both generations. This was observed in both the parental animals and in the pups. In the

parental animals, the incidences were within the historical control range. At 1250 ppm, increases in liver weights were observed in females in both generations (23% absolute, 26% relative F_0; 7% absolute, 14% relative F_1) and fatty change in the liver was observed in F_0 females (23/30 versus 0/30 for controls). Increased liver weights, mottling or accentuation of the lobular structure, liver enlargement and pallor and discoloration were observed in male and female pups from both generations. In addition to the changes in the liver, dental malocclusion was observed in both generations (parental animals and pups, incidence in parental animals within historical control range) and thickening of the urinary bladder wall was observed in 1 F_0 and in 3 F_1 high dose females. **The reproductive/systemic LOEL is 250 ppm (12.5 mg/kg/day) based on increases in liver weights and on fatty change in the liver of parental females; and on an increased incidence of chromodacryorrhea and thickened eyelids, dental malocclusion, increased liver weights, mottling or accentuation of the lobular structure, liver enlargement, pallor and discoloration in male and female pups. The reproductive/systemic NOEL is 50 ppm (2.5 mg/kg/day).**

The study is classified as **Core Guideline Data** and **satisfies the regulatory requirement for a multigeneration study in rats.**

I. PROTOCOL

A. Materials

1. Test species: Twenty-one-day old male and female Alpk:AP (Wistar derived) rats were obtained for the first parental generation of the study from the Specific Pathogen Free (SPF) colony at the Alderley Park Breeding Unit of the testing facility. The rats were acclimated for a period of 15 or 16 days before they were placed into the study.

2. Diet preparation: The composition of the basal diet (CT1 diet from Special Diets Services, Ltd., Stepfield, Witham, Essex, UK) is shown in Addendum A below. Addendum A describes the methods used in preparing the test diets used in the study.

   Test diets were analyzed for homogeneity of mixtures and chemical stability in dietary mixtures at nominal levels of 100 and 1500 ppm in a previous study. The results of that report were not included.

B. Procedures and Study Design

1. Mating: One male was caged with two females from the same test group until sperm cells were observed in vaginal smears taken daily during the mating period. If sperm were not found after 10 days' observation, the first male was removed and three days later was replaced by another male from a group of males with proven fertility in the same test group. In the first parental generation only (F_0 parental animals),

there were two matings to produce F_{1A} and F_{1B} litters. If two attempts at mating were unsuccessful the report stated that no further matings were tried. Brother-sister matings were also avoided.

After successful mating, each pregnant female was individually placed into a cage with a solid bottom and autoclaved paper bedding where they were kept through gestation and lactation.

2. Mating schedule: The F_0 parental animals were given test diets for 12 weeks before they were mated, and the F_1 parental animals were not mated until 11 weeks after they were selected from the F_{1A} litters. Selection of parents for the F_1 generation was made when the pups were 36 days of age, and the mated animals in the study were between 16 and 20 weeks of age at mating.

3. Animal assignment: F_0 animals were randomly assigned to test groups as follows:

Test groups		Dose	Animals per group **	
No.	Designation	(ppm) *	Males	Females
1	Control	0	17 ***	34 ***
2	Low (LDT)	50	15	30
3	Mid	250	15	30
4	High (HDT)	1250	17 ***	34 ***

* Diets were administered from the beginning of the study until the animals were sacrificed.

** The same number of animals were picked from the F_{1A} litters as parents for the F_2 generation.

*** These groups had 2 males and 4 females added as microbiological sentinels to monitor the health status of the animals during the course of the study.

C. Observation Schedule

1. Parental animals: Observations and the schedule for those observations is summarized from the report as follows:

Type of observation	Number of animals per sex per group	Frequency
Mortality and signs of toxicity	All	Twice a day during premating and growth periods.
Detailed clinical observations	All	Once a week during growth and breeding periods.
Body weight	All	At beginning of study and weekly through growth and mating periods.
	Maternal animals	Days 1, 8, 15, and 22 of gestation; days 1, 5, 11, 22, and 29 post partum; and weekly until sacrifice.
	Paternal animals	Weekly post-mating until sacrifice.
Food consumption	All	Weekly during premating period.

2. Reproductive performance: Parental reproductive performance was assessed from breeding and parturition records of animals in the study. A mating was considered successful if it resulted in the production of a litter that contained at least one live pup on the day of birth. The length of gestation was noted in days (calculated from the day a positive vaginal smear was found to the day of birth). The length of the precoital interval (number of days between the date of mating and the date a positive vaginal smear was found) was noted for each pair.

The following indexes were calculated:

$$\text{Male fertility index} = \frac{\text{No. females pregnant}}{\text{No. males mated}} \times 100$$

$$\text{Female fertility index} = \frac{\text{No. females pregnant}}{\text{Total no. females mated}} \times 100$$

$$\text{Gestation index} = \frac{\text{No. live litters born}}{\text{No. pregnancies}} \times 100$$

3. Litter observations: According to the report, the following litter observations were made:

Observation	Time of observation (lactation day) Birth	Day 5	Day 11	Day 22	Day 29
Number of live pups	Yes	Yes	Yes	Yes	Yes
Pup weight*	Yes	Yes	Yes	Yes	Yes
External alterations	Yes	Yes	Yes	Yes	Yes
Number of dead pups	Yes	Yes	Yes	Yes	Yes
Sex of each pup	Yes	Yes	Yes	Yes	Yes

* Pups were not individually identified, and their weights were recorded by sex and litter until individuals were selected as parents for the next generation.

Dead pups were examined grossly for external and internal abnormalities, and a possible cause of death was determined for pups born or found dead.

The following indexes were calculated:

$$\text{Gestation index} = \frac{\text{No. live pups born}}{\text{No. live + dead pups born}} \times 100$$

$$\text{Viability index} = \frac{\text{No. live pups at day 22}}{\text{No. pups born alive}} \times 100$$

4. Necropsy

a. Parental animals: All surviving parental males were sacrificed as soon as possible after the last litters in each generation were produced. Maternal animals were sacrificed after the last litter of each generation was weaned. These animals were subjected to post mortum examinations as follows:

Animals examined	Macroscopic	Microscopic
Found dead	Yes	Yes
Unscheduled sacrifice *	Yes	Yes
Scheduled sacrifice	Yes	Yes

* Includes apparently pregnant females failing to produce a litter on the expected date of parturition and mated non-pregnant females.

b. Offspring: The F1A, F1B, and F2 offspring were sacrificed at 35 days of age. These animals were subjected to post mortum examinations as follows:

Animals examined	Macroscopic	Microscopic
Found dead **	Yes	Yes
Scheduled sacrifice	Yes ***	Yes ***

** Only those 18 days of age or older.

*** Included 5 per sex from each group in the F1A and F1B litters and 10/sex/group in the F2 litter. The report also stated, "All additional clinically abnormal pups and further normal pups received a gross necropsy so that a minimum of 2 pups of each sex were examined from each litter where possible."

c. Necropsy observations: Gross necropsy consisted of external and internal examinations including the cervical, thoracic, and abdominal viscera.

The liver from each fully necropsied animal was weighed.

The following tissues were prepared for microscopic examination:

X	Ovaries	X	Epididymides
X	Uterus	X	Prostate
X	Unusual lesions	X	Seminal vessicles
X	Vagina/cervix	X	Testes

Additional tissues prepared for microscopic examination included the eyes, Harderian gland, liver and mammary gland (parental females only). The eyes, skull and lower jaw from the F_2 offspring were also prepared for microscopic examination.

Tissue samples from the liver and reproduction organs of parental animals in the control and high dose group were examined microscopically. The reproductive organs of parental animals in the low and mid dose groups suspected of infertility were also examined microscopically. Tissues showing abnormalities macroscopically (urinary bladders of F_1 parental animals according to the report) were also examined under the microscope.

Tissue samples from the liver and reproduction organs of pups in the control and high dose group and livers observed grossly to be abnormal in the low and mid dose groups were examined microscopically. Tissues showing abnormalities macroscopically (eyes and Harderian glands from the F_2 pups according to the report) were also examined under the microscope.

D. Data Analyses

1. General considerations: The report noted that all analyses were done for each generation separately, and in the first generation analyses considered each litter separately for all parameters except those noted during premating and liver weight results. Pre-mating observations, parental and pup liver weights, fertility, pup weights, and pup weight gain were analyzed separately for each sex.

The percentage pups born live and pup survival were calculated by litter in each group before the group's mean values were determined. The report stated that three dams (one from the F_{1B} mating in the mid dose group and one each from the control and high dose group in the F_2 mating) were excluded from the analyses of the reproductive performance, pregnancy weights, lactation weights, viable litter size, total litter weights, and pup weights because all pups in those litters were born dead.

2. Statistical analyses: The report stated that all analyses were two-sided except for fertility, live born index, and survival index which were one-sided.

Analysis of variance was performed on the following observations: parental body weight gain to each week, weekly food consumption and food utilization during pre-mating; initial and final body weights and body weight gain during gestation and lactation with a covariate to represent the effect of re-mating (remated females tend to weigh more according to the report); litter size and total litter weight during lactation; mean pup weight and weight gain during lactation including final pup weight on Day 29; and parental liver weights and mean pup weights per litter, with and

without the relavant body weight as a covariate. The report stated that the proportion of pups surviving to day 22 was calculated for each litter individually and transformed using the double arcsine transformation and weighted by the denominator in the proportion.

The Student's t test was used to compare treatment group means to control group means to determine significance of differences.

The Fisher's Exact Test was used to assess proportions of fertile animals in each group and the proportions of litters with all pups born alive on day 1 as well as the proportion of litters with all the pups alive on day 1 that had all pups survive to day 22 of lactation.

II. REPORTED RESULTS

A. Analysis of test diets: According to the report, analysis of the diets showed that concentrations were within 10% of nominal. Four exceptions were noted in the mid and low dose groups. Two of those values were just above and just below nominal (+10.4%) at the mid dose level, and the third was 16% below the nominal mid dose concentration. One analysis of the low dose diet showed a mean concentration that was 19.4% below that expected.

According to the report, there were 9 samples analyzed for accuracy of each dietary concentration. The mean concentrations determined for the 50, 250, and 1250 ppm diets were 48.9, 245, and 1229 ppm, respectively. The respective ranges for the low, mid and high dose levels were 40.3 to 54.2, 210 to 276, and 1157 to 1287 ppm.

B. Parental animals

1. Mortality and clinical signs: The investigators noted that chromodacryorrhea, thickened eyelids, and dental malocclusion (twisted snout) occurred frequently in F_1 males and females. Both the 250 and 1250 ppm dose groups were affected, however, the report stated that the number of occurrences were within the historical control range. These results are summarized from the report as follows:

Observation	Dose group			
	Control	Low	Mid	High
F_0 Generation - Males				
Chromodacryorrhea	0	0	0	1
Thickened eyelids	0	0	0	0
Dental Malocclusion	0	0	0	1
F_1 Generation - Males				
Chromodacryorrhea	0	0	2	3
Thickened eyelids	0	0	2	1
Dental Malocclusion	0	0	1	0
F_0 Generation - Females				
Chromodacryorrhea	1	1	2	1
Thickened eyelids	1	1	2	1
Dental Malocclusion	1	0	0	0
F_1 Generation - Females				
Chromodacryorrhea	0	0	3	7
Thickened eyelids	2	0	2	8
Dental Malocclusion	0	0	1	1

Several animals were either found dead or were sacrificed because of their moribund condition. These deaths were not associated with treatment.

2. Body weight and food consumption: The report noted that the rats given the 1250 ppm diet had reduced body weight gains throughout the premating period for both generations. There were no effects on body weight observed in rats given the 50 ppm diet, and only males in the 250 ppm dose group showed a marginal reduction in body weight gain according to the investigators.

There were also body weight gain reductions observed during pregnancy for the F_{1A} and F_{1B} litters in 1250 ppm dose group females. The F_0 and F_1 dams did not show adverse effects on body weight during lactation according to the report. In examining the body weight data, it is noted that the reductions were never below 90% of the control values. Although many of the values were statistically significantly below the control values, it does not appear that they were biologically significant.

Food consumption was marginally reduced for the 1250 ppm males in the F_0 generation, and food consumption was reduced in both generations for the high dose group females. The report noted that there was no consistent effect on food utilization (g body weight increase/g food consumbed).

Reported body weight and selected food consumption results are summarized as follows:

Observation and study week	Dose group Control	Low	Mid	High
F_0 Generation Males - Pre-mating				
Mean body weight (g)				
0	73.4	74.9	71.7	72.7
12	496.5	504.1	485.8	475.1 *
Mean weight gain (g)				
0 - 12	423.1	429.2	414.1	402.4 *
Mean food consumption (g/rat/day)				
1	19.1	19.1	18.7	17.2 **
2	25.1	26.6 *	25.2	23.6 *
12	32.6	33.6	32.1	32.0
F_0 Generation Females - Pre-mating				
Mean body weight (g)				
0	69.3	68.4	68.5	69.8
12	262.0	264.8	261.5	255.2
Mean weight gain (g)				
0 - 12	192.7	196.4	193.0	185.4 *
Mean food consumption (g/rat/day)				
1	16.2	16.1	16.6	15.3 **
5	20.7	21.0	20.8	19.8 *
6	21.3	21.5	21.4	20.2 *
7	21.0	21.3	21.5	20.0 *
8	21.5	21.6	21.6	20.7 *
12	20.8	20.8	20.7	19.9 *

* Statistically significantly different from control, $p<0.05$.
** Statistically significantly different from control, $p<0.01$.

Observation and study week	Dose group Control	Low	Mid	High
F_1 Generation Males - Pre-mating				
Mean body weight (g)				
0	117.8	127.5	130.5	125.2
10	460.9	478.9	458.3	453.3
Mean weight gain (g)				
0 - 10	343.1	351.5	327.9	328.1
Mean food consumption (g/rat/day)				
1	23.6	23.8	23.4	23.2
6	34.1	34.2	33.0	30.0 *
11	33.1	35.0	33.1	33.0
F_1 Generation Females - Pre-mating				
Mean body weight (g)				
0	110.9	112.6	120.2	110.9
10	263.7	264.9	270.4	250.3 *
Mean weight gain (g)				
0 - 10	152.8	152.3	150.2	139.4 *
Mean food consumption (g/rat/day)				
1	20.1	19.8	20.5	19.0 *
4	22.7	22.5	22.2	21.1 *
8	23.7	23.1	23.3	21.1 **
11	22.3	22.5	21.9	20.5 **

* Statistically significantly different from control, $p<0.05$.
** Statistically significantly different from control, $p<0.01$.

Selected group mean body weight values for pregnant or nursing dams were summarized in the report as follows:

Observation and study time	Dose group: Control	Low	Mid	High
F_0 Generation - Litter A				
Mean body weight (g)				
Day 1 of gestation	273.8	278.7	273.3	265.8
Day 22 of gestation	402.3	404.5	396.4	386.7 **
Day 1 of lactation	304.3	312.2	311.6	294.7
Day 29 of lactation	322.4	333.3 *	324.8	318.9
Mean body weight gain (g)				
Days 1-22 of gestation	128.7	126.7	123.4	118.6 **
Day 1-29 of lactation	18.1	21.1	13.2	24.1
F_0 Generation - Litter B				
Mean body weight (g)				
Day 1 of gestation	309.6	321.8 *	313.7	307.2
Day 22 of gestation	426.1	446.3 *	425.8	420.6
Day 1 of lactation	341.5	345.0	343.4	325.2 *
Day 29 of lactation	356.4	362.8	355.8	353.4
Mean body weight gain (g)				
Days 1-22 of gestation	116.4	124.3	111.6	113.4
Day 1-29 of lactation	15.0	17.8	12.4	28.2 *
F_1 Generation				
Mean body weight (g)				
Day 1 of gestation	287.9	285.4	293.4	266.9 **
Day 22 of gestation	412.5	412.5	415.9	381.3 **
Day 1 of lactation	315.6	323.6	320.2	290.2 **
Day 29 of lactation	337.3	336.1	334.9	314.7 **
Mean body weight gain (g)				
Days 1-22 of gestation	120.3	126.7	121.8	112.2
Day 1-29 of lactation	21.8	12.6	13.6	24.7

* Statistically significantly different from control, $p<0.05$.
** Statistically significantly different from control, $p<0.01$.

3. Test Substance Intake: Based on food consumption, body weight, and dietary analyses results, the doses expressed as mg test substance/kg body weight were as follows during the pre-mating period:

	Dose levels (ppm)					
	Males			Females		
Week	50	250	1250	50	250	1250
			F_0 Generation			
1	7.55	38.60	183.76	7.27	37.60	177.58
4	5.54	27.77	138.94	5.45	27.48	134.55
8	3.93	19.54	100.04	4.53	22.95	111.96
12	3.33	16.52	84.19	3.93	19.79	97.47
			F_1 Generation			
1	6.52	31.95	160.66	6.58	32.56	163.68
4	4.99	24.09	124.81	5.25	25.41	128.03
8	3.89	19.27	97.28	4.57	22.50	110.03
10	3.61	18.06	90.10	4.34	20.25	103.38

4. Reproductive performance: There were no consistent effects on reproductive performance noted by the investigators.

Results for the parental animals are summarized from the report as follows:

Observation	Dose group Control	Low	Mid	High
F_0 Generation - Litter A				
Median precoital interval (days)	3	3	4	3
Males				
Mated	15	15	15	15
Fertile	12	12	15	14
Fertility not determined	1	0	0	0
Intercurrent deaths	0	0	0	0
Females				
Number mated	30	30	30	30
Number fertile	25	27	28	28
Fertility not determined	0	0	0	0
Intercurrent deaths	0	0	0	0
Median gestation interval (days)	22	22	23	22
Number of litters	25	27	28	28
Total litter losses	0	0	0	0
Mean litter size (Day 1)	12.0	11.9	9.8**	10.9
Mean litter size (Day 29)	11.8	11.2	9.0	9.8
Number of pups (Day 1)	303	320	279	305
Number of pups (Day 29)	296	302	258	277
Pup deaths (Days 1-29)	7	18	21	28
Mean pup weight (g) (Day 1)	5.8	5.7	6.2	5.9
Mean pup weight (g) (Day 29)	75.9	77.3	84.2	76.0

* Statistically significantly different from control, $p<0.05$.
** Statistically significantly different from control, $p<0.01$.

Observation	Dose group Control	Low	Mid	High
F_0 Generation - Litter B				
Median precoital interval (days)	2	3	2	2
Males				
Mated	15	15	14	15
Fertile	14	14	13	14
Fertility not determined	0	0	1	0
Intercurrent deaths	0	0	1	0
Females				
Number mated	25	27	28	28
Number fertile	24	26	27	26
Fertility not determined	0	0	1	0
Intercurrent deaths	5	3	2	2
Median gestation interval (days)	22	22	22	22
Number of litters (Day 1)	24	26	27	26
Total litter losses	3	2	4	2
Mean litter size (Day 1)	9.3	11.2	8.4	10.2
Mean litter size (Day 29)	8.7	10.2	7.7	9.4
Number of pups (Day 1)	237	262	223	250
Number of pups (Day 29)	188	245	182	222
Pup deaths (Days 1-29)	49	26	41	28
Mean pup weight (g) (Day 1)	6.4	6.0	6.5	6.0
Mean pup weight (g) (Day 29)	79.2	73.6	80.4	75.0

* Statistically significantly different from control, $p<0.05$.
** Statistically significantly different from control, $p<0.01$.

Observation	Dose group Control	Low	Mid	High
	F_1 Generation			
Median precoital interval (days)	2	3	3	3
Males				
Mated	15	15	14	15
Fertile	14	14	15	14
Fertility not determined	0	0	0	0
Intercurrent deaths	0	0	0	0
Females				
Number mated	30	30	30	30
Number fertile	27	27	30	27
Fertility not determined	0	0	0	0
Intercurrent deaths	0	0	0	0
Median gestation interval (days)	22	22	22	22
Number of litters (Day 1)	27	27	30	27
Total litter losses	0	0	0	1
Mean litter size (Day 1)	10.4	10.9	11.1	10.1
Mean litter size (Day 29)	9.8	10.6	10.5	9.7
Number of pups (Day 1)	282	295	334	266
Number of pups (Day 29)	264	285	305	256
Pup deaths (Days 1-29)	18	10	17	10
Mean pup weight (g) (Day 1)	6.1	6.1	6.0	6.0
Mean pup weight (g) (Day 29)	79.5	77.8	78.1	73.2

* Statistically significantly different from control, $p<0.05$.
** Statistically significantly different from control, $p<0.01$.

5. Necropsy results

a. Organ weights: The report noted a significant increase in liver weights above control group values for 1250 ppm female rats in both generations with the increase being greater in the first generation than in the second. There were no dose-related effects on liver weight noted for males of either generation. Liver weight results for female rats are summarized from the report as follows:

Observation	Dose group			
	Control	Low	Mid	High
F_0 Generation				
Organ weight (g)	15.8	15.9	15.1	19.4 **
Adjusted for body weight (g)	15.6	15.5	15.4	19.7 **
F_1 Generation				
Organ weight (g)	11.9	12.0	12.2	12.7 *
Adjusted for body weight (g)	11.7	11.8	11.9	13.3 **

* Statistically significantly different from control, $p<0.05$.
** Statistically significantly different from control, $p<0.01$.

b. Pathology

i. Macroscopic examination: The report noted the following observations to be related to the administration of the test substance:

Liver - mottling or accentuation of the lobular structure in 11/30 F_0 females from the 1250 ppm dose group. Liver enlargement, pallor, and discoloration were observed in females from both generations.

Urinary bladder - thickening of the bladder wall in 3 F_1 and one F_0 female from the 1250 ppm dose group. Soft deposits in the bladders of 4 F_1 males receiving the 1250 ppm diet were characterized as toxicologically insignificant and normal for male rats.

ii. Microscopic examination: Changes in the liver were described in the report as follows:

> In sections of liver stained with hematoxylin and eosin, fatty change was visible as vacuolation of hepatocytes in the centrilobular zone. In mildly affected lobules, the change appeared to commence

in the outer part of the centrilobular zone and the innermost cell layers were not vacuolated. Even in severely affected lobules, a few hepatocytes immediately adjacent to the centrilobular vein remained unvacuolated. These "spared" cells often showed cytoplasmic eosinophilia,...individual hepatocytes were swollen, up to five times normal size and the cytoplasm was distended with multiple small to medium sized vacuoles to give a "ground glass" appearance. In some cells, vacuoles coalesced to form a single large clear vacuole. Examination of Oil Red O stained sections...con-firmed that vacuolation was due to...fatty change.

No other microscopically examined tissues from adult rats in the study indicated a dose or treatment related effect of the test substance.

Observation	Dose Group			
	Males		Females	
	Control	High	Control	High
	F_0 Generation			
Number Examined	15	15	30	30
Centrilobular fatty change	0	0	0	23
Increased cytoplasmic eosinophilia of centri-lobular hepatocytes	0	0	0	14
Inflammatory cell infiltration	3	0	1	11
	F_1 Generation			
Number Examined	15	15	30	30
Centrilobular fatty change	0	1	1	1
Increased cytoplasmic eosinophilia of centri-lobular hepatocytes	0	0	0	0
Inflammatory cell infiltration	2	0	1	1

* Statistically significantly different from control, $p<0.05$.
** Statistically significantly different from control, $p<0.01$.

C. Offspring

1. Viability and clinical signs: The authors noted that there were 3, 2, 4, and 2 whole litter losses in the F_{1B} generation. No effects on the total number of pups born alive were observed in the A or B litters of the first generation or in the F_2 generation; however, the mean litter size was lower than controls for the F_{1A} litters from both the 250 ppm and 1250 dose groups. Reductions in litter size in the B litters were neither statistically significant nor dose-related. There were no apparent reductions in litter size in the F_2 litters. There appeared to be a reduction in pup survival in the F_{1A} mid- and high dose litters. This reduction in survival was not apparent in the F_{1B} litters nor in the F_2 generation. In addition, the authors stated that the values were within recent historical control values (data not provided). Reductions in body weights and body weight gains were neither dose-related nor consistent between generations.

Viability results from pups during lactation are summarized from the report as follows:

Observation and study time	Dose group: Control	Low	Mid	High
F1 Generation				
Litter A				
Mean percentage surviving in each litter	98.3	95.1	94.1	91.8
Mean transformed value	1.389	1.323	1.302 *	1.279 **
No. litters with all pups surviving to Day 22/total no. litters	20/25	18/27	19/28	18/28
Litter B				
Mean percentage	81.7	84.3	78.4	86.6
Transformed value	1.177	1.255	1.130	1.246
No. litters with all pups surviving to Day 22/total no. litters	15/24	14/25	15/27	14/26
F_2 Generation				
Mean percentage	94.5	97.5	94.7	92.1
Transformed value	1.301	1.371	1.321	1.356
No. litters with all pups surviving to Day 22/total no. litters	17/27	21/27	18/29	19/27

* Statistically significantly different from control, $p<0.05$.
** Statistically significantly different from control, $p<0.01$.

Changes in mean litter sizes were summarized in the report as follows:

Observation and study time	Dose group Control	Low	Mid	High
	F1 Generation			
Litter A				
Day 1	12.0	11.9	9.8 **	10.9
Day 5	11.9	11.3	9.4 **	9.9 **
Day 11	11.9	11.3	9.2 **	9.9 **
Day 22	11.9	11.2	9.1 **	9.9 **
Litter B				
Day 1	9.3	11.2	8.4	10.2
Day 5	9.0	10.3	7.7	9.7
Day 11	8.9	10.3	7.7	9.6
Day 22	8.8	10.3	7.7	9.5
	F_2 Generation			
Day 1	10.4	10.9	11.1	10.1
Day 5	9.8	10.7	10.5	9.7
Day 11	9.8	10.6	10.5	9.7
Day 22	9.8	10.6	10.5	9.7

* Statistically significantly different from control, $p<0.05$.
** Statistically significantly different from control, $p<0.01$.

The report stated that the majority of clinical observations made on F_1 and F_2 pups had a low incidence and were unrelated to treatment. However, the investigators noted that chromodacryorrhea, thickened eyelids, and dental malocclusion had an incidence that was apparently dose related and occurred most frequently in the pups from the 250 and 1250 ppm dose groups. The results of these observations are summarized from the report as follows:

Observation	Dose group Control	Low	Mid	High
(No. of litters with one or more affected pups)				
F_1 Generation - Litter A				
Litters weaned (Day 29)	25	27	28	28
No. of litters affected	4	0	5	19
Chromodacryorrhea	4	0	5	17
Thickened eyelids	4	0	4	18
Dental Malocclusion	0	0	0	9
F_1 Generation - Litter B				
Litters weaned (Day 29)	21	24	23	24
No. of litters affected	1	1	4	8
Chromodacryorrhea	1	1	4	5
Thickened eyelids	1	1	4	8
Dental Malocclusion	0	0	0	3
F_2 Generation				
Litters weaned (Day 29)	27	27	30	26
No. of litters affected	0	2	7	13
Chromodacryorrhea	0	2	7	8
Thickened eyelids	0	0	4	8
Dental Malocclusion	0	0	0	5

2. Body weight: Selected group mean body weights are summarized from the report as follows:

Observation and study time	Dose group Control	Low	Mid	High
F1 Generation				
Litter A				
Males				
Body weight (g) - Day 1	6.0	5.9	6.4 *	6.1
Weight gain (g) - Day 22	36.2	36.3	41.1 *	35.9
Females				
Body weight (g) - Day 1	5.6	5.5	6.0 *	5.6
Weight gain (g) - Day 22	34.9	34.7	39.9 *	34.2
Litter B				
Males				
Body weight (g) - Day 1	6.6	6.2 *	6.6	6.2
Weight gain (g) - Day 22	37.4	34.3	39.1	35.8
Females				
Body weight (g) - Day 1	6.2	5.8 *	6.3	5.7 *
Weight gain (g) - Day 22	36.2	32.9	37.1	33.5
F_2 Generation				
Males				
Body weight (g) - Day 1	6.2	6.3	6.2	6.2
Weight gain (g) - Day 22	38.8	36.8	37.9	35.0 *
Females				
Body weight (g) - Day 1	6.0	5.9	5.8	5.8
Weight gain (g) - Day 22	37.4	35.3	35.8	32.8 *

* Statistically significantly different from control, $p<0.05$.
** Statistically significantly different from control, $p<0.01$.

3. Necropsy results

a. Organ weights: Increased liver weights were observed in the 1250 ppm dose group pups from both generations. Slight increases were observed in the 250 ppm dose group also. These results are summarized from the report as follows:

Observation	Dose group: Control	Low	Mid	High
F_1 Generation				
Litter A				
Males				
Organ weight (g)	5.82	5.86	6.65 **	6.99 **
Body weight (g)	124	127	137 *	126
Adjusted for body weight (g)	6.08	5.97	6.19	7.18 **
Females				
Organ weight (g)	5.14	5.05	5.51	6.10 **
Body weight (g)	112	110	117	108
Adjusted for body weight (g)	5.15	5.14	5.25	6.29 **
Litter B				
Males				
Organ weight (g)	6.13	5.82	6.51	7.13 **
Body weight (g)	131	122	135	124
Adjusted for body weight (g)	5.98	6.15	6.15	7.36 **
Females				
Organ weight (g)	5.34	5.05	5.57	6.09 **
Body weight (g)	114	107	117	108
Adjusted for body weight (g)	5.21	5.31	5.30	6.27 **
F_2 Generation				
Males				
Organ weight (g)	6.19	6.23	6.42	6.93 **
Body weight (g)	131	132	133	121 *
Adjusted for body weight (g)	6.08	6.11	6.23	7.38 **
Females				
Organ weight (g)	5.38	5.20	5.25	5.99 **
Body weight (g)	116	116	113	107 *
Adjusted for body weight (g)	5.23	5.07	5.24	6.30 **

* Statistically significantly different from control, $p<0.05$.
** Statistically significantly different from control, $p<0.01$.

b. Pathology

i. Macroscopic examination: The report noted the following observations to be related to the administration of the test substance:

Liver - mottling or accentuation of the lobular structure in F_{1A}, F_{1B}, and F_2 pups from the 1250 ppm dose group. Liver enlargement, pallor, and discoloration were observed in pups of both sexes from both generations at the high dose level.

The incidence of selected liver lesions is summarized from the report as follows:

Observation	Dose group * Control	Low	Mid	High
	F_1 Generation			
Litter A				
Males (number examined)	50	58	58	61
Pale	0	0	0	5
Speckled/mottled/prominant reticular pattern	0	0	0	4
Litter B				
Males (number examined)	45	51	56	52
Prominant reticular pattern	0	0	0	10
Females (number examined)	41	50	48	53
Prominant reticular pattern	0	0	0	8
	F_2 Generation			
Males (number examined)	66	63	73	66
Discoloration (focal or generalized)	0	0	0	6
Pale (focal/generalized)	0	0	0	4
Prominant/accentuated lobular/ reticular markings	0	0	0	36
Females (number examined)	62	60	69	58
Prominant/accentuated lobular/ reticular markings	0	0	0	18

* No statistical significance was indicated in the report.

III. DISCUSSION

A. Investigators' conclusions: The conclusions of the investigators were described in the report as follows:

"Evidence of slight toxicity was seen in the parent rats receiving 1250 ppm Paclobutrazol as a reduction in body weight gain in both generations. This reduced weight gain was statistically significant in comparison to controls. A slight reduction in weight gain was apparent at 250 ppm, but this was not consistent since it was only seen in the F_1 males, and considered to be too small to be of any toxicological significance...There were no treatment-related effects on the number of pups born alive, their survival or on the incidence of total litter losses in either generation." In the F_{1a} litters, the total litter weights of all treated groups were lower than controls. However, since only the total litter weight of the 250 ppm dose group in the F_{1b} mating was less than controls, this was not considered to be of toxicological signficance. In both parents and offspring, there was an increased incidence of chromodacryorrhea, thickened eyelids and dental malocclusion at both the 250 and 1250 ppm dose levels. In addition, in the 1250 ppm dose group, increases in liver weight were observed in both the parents (females, especially the F_0 generation) and the offspring (both sexes). In general, these increases were associated with macroscopic changes of accentuation of the lobular pattern and microscopic changes consistent with centrilobular fatty change. The histologic changes were restricted to those females which had recently completed lactation and they were not observed in the F_1 females. The authors stated that this may have been due to the fact that the F_0 females, while raising two litters each, were exposed to the test chemical for a longer period than the F_1 females (which only raised one litter each) and would have ingested larger amounts, particularly during pregnancy and lactation.

B. Reviewer's discussion:

The design and conduct of this study deviate slightly from the EPA Guidelines (mainly, age of animals on commencement of dosing, age at weaning and number of males used for mating). None of these deviations are considered to be crucial. The study is acceptable for

regulatory purposes and is classified as Core Guideline.

Systemic/Reproductive Toxicity: In the 1250 ppm females, an increase in liver weights was observed in both generations; the increase was more evident in the first generation than in the second generation. Macroscopic examinations showed mottling or accentuation of the lobular structure in the F_0 high dose females and enlargement, pallor and discoloration in high dose females from both generations. Microscopic examinations of these livers showed evidence of fatty change. In addition to the changes in the liver, thickening of the urinary bladder wall was observed during the gross examination of 3 F_1 and 1 F_0 high dose females.

In the offspring, there was an increased incidence of chromodacryorrhea, thickened eyelids and dental malocclusion in both the 250 ppm and the 1250 ppm dose groups for both generations and sexes. There were increased liver weights in both sexes in all generations at 1250 ppm. In support of the increased liver weights, there was mottling or accentuation of the lobular structure in both generations of litters from the 1250 ppm dose group. Liver enlargement, pallor and discoloration were also observed. However, no statistical significance was indicated in the report. The NOEL is 50 ppm and the LOEL is 250 ppm.