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DATA EVALUATION REPORT

STUDY TYPE: Teratology - rabbit (83-3)

TOX. CHEM. NO.: 628C

ACCESSION NUMBER/MRID NO.: 407343-02

TEST MATERIAL: Paclobutrazol

SYNONYMS: Clipper 2SC formulation, PP333

STUDY NUMBER(S): RB0334

REPORT NUMBER: CTL/P/1460

SPONSOR: ICI Americas Inc., Agricultural Products, Wilmington, Del. 19897

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

TITLE OF REPORT: Paclobutrazol: Second Teratogenicity Study in the Rabbit Including Individual Animal Data

AUTHOR(S): M.E. Killick, G.H. Pigott, I. Pate, P.B. Banham

REPORT ISSUED: October 28, 1986

CONCLUSION: Paclobutrazol was tested in a teratology study in New Zealand White rabbits at the following dose levels: 0, 25, 75 or 125 mg/kg/day. The maternal toxicity NOEL is 75 mg/kg/day and the maternal LEL is 125 mg/kg/day based on decreases in body weights and body weight gain and food consumption during dosing. The developmental toxicity NOEL is 75 mg/kg/day and the LEL is 125 mg/kg/day based on the increased incidence of extra ribs in pups.

Classification: Guideline

Testing Guideline Satisfied: Teratology - Rabbits (83-3)

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-(1,2,4-triazol-1-yl)pentan-3-ol

Description: Buff colored powder
Batch #(s), Other #(s): Batch P29, CTL Ref. Nos. Y00001/001/017, Y00001/001/035
Purity: 92.4% w/w
Source: ICI PLC, Plant Protection Division, England
Vehicle (if applicable): corn oil

2. Test Animals:

Species and Strain (sexes): Proven male and virgin female New Zealand White rabbits
Age: 13-22 weeks (females), male ages unreported
Weight(s): 2.8-4.0 g (females), male weights unreported
Source(s): Interfauna, UK, Limited, Huntingdon, Cambridgeshire, UK

3. Procedure:

a. Dosage Preparation:

Frequency of preparation: Not explicitly stated, assumed only one bulk preparation was prepared for each dose level which was divided into aliquots.

Storage conditions: In the dark in the animal room.

Stability Analyses: Stability in corn oil was established for another study conducted on this chemical and was not part of this report.

Homogeneity Analyses: Samples from the lowest and highest concentrations were taken at the beginning, at the mid-point and at the end of the process of subdividing one of the bulk preparations into aliquots. The samples were analyzed to confirm that the subdivision had been achieved homogeneously.

Concentration Analyses: A sample of each bulk preparation was analyzed prior to the start of dosing to verify the achieved concentration of Paclobutrazol in corn oil.

b. Basis For Selection of Dose Levels: Based on results of preliminary studies.

c. Animal Assignment and Dose Levels:

Test Group	Dose Administered mg/kg bodyweight/day
Controls	0
1	25
2	75
3	125

d. Insemination and Dosing Protocol: Eighteen female rabbits were tested per dose level. Approximately 3 weeks prior to insemination, each female was given an intravenous injection of 25IU chorionic gonadotrophin in order to promote ovulation. On the day of insemination, semen was collected from each male using an artificial vagina. The volume of each semen sample was measured prior to dilution to 5 ml with physiological saline. Each female was inseminated with 1 ml of the diluted semen. Each replicate of females was inseminated with semen from one male. After insemination, each female was intravenously dosed with 25IU chorionic gonadotrophin to promote ovulation.

All inseminated females were dosed once daily by gavage with 1 ml dosing formulation from days 7-19 inclusive of gestation. The volume given was adjusted daily according to bodyweight.

e. Clinical Observations and Mortality: All animals were checked daily for clinical signs of toxicity and mortality.

f. Body Weight Determinations: The bodyweight of each animal was recorded on days 1 and 4, 7-19 (inclusive) and on days 22, 26, and 30 of gestation.

g. Food Consumption: Food consumption was measured by giving a weighed quantity of food on days 1, 4, 7, 10, 13, 16, 19, 22, 26, and 30 respectively.

h. Gross Necropsy:

Animals (groups) which died or were sacrificed in moribund condition and were subjected to complete gross pathological examinations: all animals.

Animals (groups) sacrificed at the end of the treatment/observation period which were subjected to complete gross pathological examinations: all animals.

i. Uterine Examinations: The intact gravid uterus (minus ovaries and trimmed free of connective tissue) was removed from each female and weighed. The ovaries and uteri were then examined and the following data were recorded:

1) Number of corpora lutea in each ovary.

2) Number and position of implantations subdivided into:

a) live fetuses
b) early intra-uterine deaths (showed decidual or placental tissue only)
c) late intra-uterine deaths (showed embryonic or fetal tissue in addition to placental tissue)

3) Individual fetal weights.

j. Fetal Examinations: Each fetus was examined for external and visceral abnormalities. They were then sexed, eviscerated and fixed in methanol. The head of each fetus was cut along the fronto-parietal suture line and the brain was examined for macroscopic abnormalities. The carcasses were processed and stained using the method of Staples and Schnell and then examined for skeletal abnormalities and degree of ossification. The individual bones of the manus and pes were assessed and the result converted to a 5 point scale. Abnormalities were classified as major (rare or possibly lethal or both) or minor (deviations from normal that are not uncommon) defects. Variations were also recorded and classified as minor defects or variants depending on the frequency of occurrence in relation to background data.

l. Statistical Analyses: Only those females with live fetuses in utero at termination on day 30 of gestation were included in the statistical analyses. Data were analyzed using analysis variance, the Student's t-test and Fisher's Exact Test.

B. RESULTS:

1. Dosage Preparation: All of the samples of the dosing solutions taken for analysis contained a greater amount of the test chemical than the nominal concentrations. The achieved concentrations were up to 7.2% above the nominal concentrations. The homogeneity analyses showed that the dosing solutions were homogeneously mixed. For the lowest dose level, the mean concentrations ranged from 25.1 (100.4%) to 25.8 (103.2%) mg/ml and for the highest dose level, the mean concentrations ranged from 128 (102.4%) to 129 (103.2%) mg/ml.

2. Maternal Toxicity:

   a. Clinical Observations and Mortality: One animal in the mid-dose group was sacrificed *in extremis* due to a mechanical error in the dosing procedure. This animal was not pregnant. No treatment-related clinical signs of toxicity were observed in any does at any dose level.

   b. Body Weight Determinations: There were no differences in maternal body weight gain in any of the treated groups during the predosing period when compared to controls. In the highest dose group, weight loss was observed during the first 3 days of the dosing period (days 7-10 of the gestation period). For the remainder of the dosing period, body weight gain was greater than the controls for this dose group. No effects were noted for the 2 lower dose levels. For the highest dose group, weight gain was generally lower than the controls during the post-dosing period, however, overall weight gain was similar to controls and the authors stated that there was no evidence to suggest that this was due to an adverse effect of exposure to paclobutrazol.

The investigators supplied the following data:

Table I: Body Weight Gains (grams)[a]

Group:	Prior to Dosing Period	Dosing Days 7-10	Dosing Days 10-13	Entire Dosing Period	Post Dosing Period	Entire Gestation Period
Control	211.0	33.7	60.2	216.7	303.7	731.4
LDT	266.0	12.5	105.4	270.1	292.4	828.6
MDT	233.9	21.7	65.5	230.7	289.1	753.6
HDT	256.4	-58.6**	74.6	224.9	260.3	741.7

[a] Data extracted from report number CTL/P/1460

c. Food Consumption: Maternal food consumption during the pre-dosing period was similar in all groups. In the high dose group, food consumption was 25% less than controls during the first 3 days of dosing. As a result, overall food consumption for the entire dosing period was slightly less than controls for this dose group. Food consumption was also slightly less (but not significantly so) for the mid-dose group during the dosing period. No changes in food consumption for the low dose group were observed during the dosing period and no changes in any dose group were observed during the post-dosing period.

The investigators supplied the following data:

Table II: Food Consumption Data (g/day)[a]

Group:	Prior to Dosing Period	Dosing Days 7-10	Dosing Period	Post-Dosing Period	Entire Gestation Period
Control	205.4	173.0	167.3	192.9	565.6
LDT	213.5	180.2	170.5	188.5	572.5
MDT	198.4	166.4	152.4	191.9	542.7
HDT	213.2	130.7**	149.5	190.2	552.9

[a] Data extracted from report number CTL/P/1460.

d. Gross Pathology: No treatment-related macroscopic findings were observed in any of the groups.

e. Cesarean section observations: The report stated that "there was no evidence of a treatment-related effect on pre- or post-implantation loss, early or late intra-uterine death, the proportion of male fetuses or gravid uterine weight". Total litter

weight for the mid- and high dose groups and mean fetal weight for the high dose group were less than the controls but not significantly so. The authors stated that they were within the range expected for rabbits of this strain in this laboratory.

Table III: Cesarean Section observations[a]

	Control	LDT	MDT	HDT
Dose (mg/kg/day)	0	25	75	125
# Animals Assigned	18	18	18	18
# Animals Mated/Inseminated	18	18	18	18
Pregnancy Rate (%)	83%	94%	89%	83%
Maternal Wastage				
# Died	0	0	1	0
# Died/pregnant	0	0	0	0
# Non pregnant	3	1	2	3
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Total Corpora Lutea	162	194	162	160
Corpora Lutea/dam	10.8	11.4	10.1	10.7
Total Implantation	134	152	130	135
Implantations/Dam	8.9	8.9	8.1	9.0
Total Live Fetuses	109	129	103	106
Live Fetuses/Dam	7.3	7.6	6.4	7.1
Total Resorptions	25	23	27	29
Early	12	10	10	10
Late	13	13	17	19
Resorptions/Dam	1.7	1.4	1.7	1.9
Mean Fetal Weight (gm)	42.0	41.9	42.9	39.7
Preimplantation Loss(%)	17.3	21.6	19.8	15.6
Postimplantation Loss(%)	18.7	15.1	20.8	21.5
Sex Ratio (% Male)	45.9	48.8	47.6	51.9

[a] = Data extracted from report number CTL/P/1460.

3. Developmental Toxicity: There were no treatment-related effects for major defects. The percentage of fetuses with minor visceral defects was slightly increased in the high dose group when compared to controls because of a statistically significant

increase in the number of fetuses with pale spleens. The authors stated that this finding alone was considered to be of no toxicological significance.

There was a significant increase in partial ossification of the transverse processes of the 7th cervical vertebra in the mid- and high dose groups. However, the dose-response was weak and the number of litters affected was higher in the mid-dose group than in the high-dose group.

The proportion of fetuses with partially ossified 5th sternebrae was statistically significantly increased in all the treated groups when compared to the controls. However, the numbers affected diminished with increasing dosage and the control value was at the low end of the historical range. In addition, none of the litters were statistically significantly elevated.

The proportion of fetuses with extra ribs was increased in the high dose group when compared to controls. Both normal length ribs and short length floating ribs were increased with a corresponding decrease in short length articulating ribs (not statistically significant due to the one-sided test). Again, however, for the normal length ribs, none of the litters were statistically significantly elevated. However, there was a statistically significant increase in the number of litters with short length floating ribs. The authors stated that, "in the case of extra (13th) ribs, the total incidence of short length ribs is comparable to control and the variation between articulating and floating ribs is considered of no consequence. Normal length 13th ribs were also seen at increased frequency at this top dose. It should be noted that the incidence at 75 mg/kg/day paclobutrazol was below the control value by about the same proportion that the incidence at 125 mg/kg/day exceeded it indicating the degree of variability seen with this parameter. However, an effect of treatment at this top dose group cannot be discounted as the incidence of extra ribs, at least in the rat, correlates with maternal stress (Wickramaratne 1986) and in this instance could therefore be a further reflection of maternal toxicity at this dose. The following tables summarize some of the more significant findings. External and visceral findings are summarized together in one table.

Table IV: External/Visceral Examinations

Observations†	Control	Low Dose	Mid Dose	High Dose
#pups(litters) examined	109(15)	129(17)	103(16)	106(15)
#pups (litters) showing major defects	2(2)	4(3)	3(3)	4(4)
#pups showing minor defects only	23	25	23	27
Encephalocoele, cleft palate, gross malformation of skull	0	0	1	0
Cardiac defects*	1	4	0	0
Umbilical hernia	0	0	1	0
Limb defects*	1	0	2	4
Scoliosis	0	0	0	1
Pale spleen (%)	5(5)	3(2)	7(7)	13(12)**
# Litters (%)	2(13)	3(18)	2(13)	6(40)

*Major defects only
** Statistically significant

Table V: Skeletal Examinations

Observations[+]	Control	Low Dose	Mid Dose	High Dose
#pups(litters) examined	109(15)	129(17)	103(16)	106(15)
#pups showing major defects	0	0	1	1
#pups showing minor defects only	39	38	38	47
#pups showing variants	109	129	103	106
Skull - gross malformation[1]	0	0	1	0
Hyoid - partially ossified[2]	3	0	1	5
Odontoid - part. ossified[3]	5	4	4	4
Cerv. Vertebrae (%) - transverse process partially ossified, 7th[2]	2(2)	6(5)	8(8)*	8(7.5)*
# litters (%)	2(13)	3(18)	5(51)	3(20)
Tranverse processes				
of 2nd lumbar part. oss.[3]	42	56	48	44
of 3rd lumbar fully oss.[3]	40	60	61*	38
of 3rd lumbar part. oss.[3]	65	71	44	60
of 4th lumbar part. oss.[3]	13	20	16	20
Sternebrae (%)				
Partially ossified 5th[2]	30(28)	55(43)*	47(46)**	45(43)*
# Litters	9(60)	15(88)	13(81)	13(87)
Extra Ribs[3] (%)				
13th normal length	49(45)	42(33)	38(37)	62(59)*
# Litters	14(93)	12(71)	13(81)	14(93)
13th short length and floating	9(8)	15(12)	7(7)	21(20)*
# Litters	6(40)	9(53)	4(25)	12(80)*

[1] Major defect
[2] Minor defect
[3] Variation
* Statistically significant

Manus and Pes Assessment: The authors stated that "there was no evidence for a compound-related effect on the manus or pes scores.

C. DISCUSSION:

1. Maternal Toxicity: On the first three days of treatment, there was a significant loss in bodyweight at the high dose level. There was also a significant decrease in food consumption at the same level for these same three days. In addition, there was a greater incidence of pups at the highest dose level with extra ribs. The authors stated that this was indicative of maternal stress. Although the authors

stated that the decrease in bodyweight was probably not due to maternal toxicity, these data in combination with the pup and food consumption data indicate that the NOEL is probably very close to the highest dose tested. Therefore, to be on the conservative side, the NOEL for maternal toxicity is 75 mg/kg/day and the LOEL is 125 mg/kg/day.

2. Developmental Toxicity:

   a. Deaths/Resorptions: No significant differences were observed between treated and control animals.

   b. Altered Growth: No significant differences were observed between treated and control animals.

   c. Developmental Anomalies: There were no treatment-related developmental anomalies.

   d. Malformations: No significant differences were observed between treated and control animals.

D. Study Deficiencies: No major deficiencies.

E. Core Classification: Core Guideline Data.

Maternal NOEL = 75 mg/kg
Maternal LOEL = 125 mg/kg/day (HDT)
Developmental Toxicity NOEL = 75 mg/kg/day
Developmental Toxicity LOEL = 125 mg/kg/day (HDT)