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WASHINGTON, D.C. 20460

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MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: 125601. Paclobutrazol. Re-evaluation of Rat Developmental Toxicity Study

PC Code 125601
Tox. Chem. No. 628C
Accession No. 251747

TO: Cynthia Giles-Parker, PM Team # 22
Registration Division (7505C)

FROM: Pamela M. Hurley, Toxicologist Pamela M. Hurley 11/30/93
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THRU: Roger L. Gardner, Section Head Roger Gardner 3/23/94
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In the process of preparing the Paclobutrazol data package for review by the HED RfD Committee, the first rat developmental toxicity study was reassessed. In the original Data Evaluation Report (DER), the study was classified as Core Supplementary due to the fact that a NOEL could not be established because of an increase in delayed ossification at all dose levels. For the analysis of this study, both the Registrant and the Agency conducted statistical analyses only on the fetal incidences and not on the litter incidences. For the reassessment, the litter incidences were statistically analyzed at the lowest dose level where the fetal incidences were statistically significant. The litter incidences were not statistically significant. Therefore, the NOEL can be redefined at the lowest dose level. The LEL is very close to 40 mg/kg/day, but is redefined to 100 mg/kg/day. The study is reclassified as Core Minimum. The following paragraph summarizes the results of the study.

Teratogenicity Study in the Rat No. 1. Report No. CTL/P/842. July 13, 1983.

Conclusions: Paclobutrazol was tested in a developmental toxicity study in Wistar derived Alderley Park rats at the following dose levels: 0, 40, 100 or 250 mg/kg/day on days 6 - 15 of gestation. The NOEL for maternal toxicity is 40 mg/kg/day and the LEL is 100 mg/kg/day. The effects were decrease in body weight gain (100 mg/kg/day and above), pallor and enlargement of

the liver, decreased food consumption and food utilization, staining of the genital and ventral areas and mortality (250 mg/kg/day). The NOEL for developmental toxicity is 40 mg/kg/day (LDT) and the LEL is 100 mg/kg/day based on a dose-related increase in the incidence of delayed ossification at various sites and partial ossification of the odontal bone in the mid- and high dose groups and partial ossification of the occipital bone in the high dose group. The LEL is very close to 40 mg/kg/day because the increased incidence of delayed ossification was statistically significant for fetuses but not for litters. Cleft palate was also observed at dose levels that were maternally toxic. The study is reclassified as Core Minimum.

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Teratogenicity Study in the Rat No. 1. Report No. CTL/P/842. July 13, 1983.

Conclusions: Paclobutrazol was tested in a developmental toxicity study in Wistar derived Alderley Park rats at the following dose levels: 0, 40, 100 or 250 mg/kg/day on days 6 - 15 of gestation. The NOEL for maternal toxicity is 40 mg/kg/day and the LEL is 100 mg/kg/day. The effects were decrease in body weight gain (100 mg/kg/day and above), pallor and enlargement of the liver, decreased food consumption and food utilization, staining of the genital and ventral areas and mortality (250 mg/kg/day). The NOEL for developmental toxicity is 40 mg/kg/day (LDT) and the LEL is 100 mg/kg/day based on a dose-related increase in the incidence of delayed ossification at various sites and partial ossification of the odontal bone in the mid- and high dose groups and partial ossification of the occipital bone in the high dose group. The LEL is very close to 40 mg/kg/day because the increased incidence of delayed ossification was statistically significant for fetuses but not for litters. Cleft palate was also observed at dose levels that were maternally toxic. The study is classified as Core Minimum.

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DATA EVALUATION RECORD

Citation: Killick, M. E., G. H. Pigott, P. B. Banham, and M. R. Thomas. July 13, 1983. Paclobutrazol: Teratogenicity study in the rat. Unpublished report no. CTL/P/842 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 251747.

Materials and Methods

Test substance: The test substance contained 92.4% (w/w) (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-(1,2,4-triazol-1-yl) pentan-3-ol.

Test species: Female Wistar derived Alderley Park strain rats were used. Each female was mated overnight with a male and the following morning vaginal smears were examined for the presence of spermatozoa. The day spermatozoa were found was designated Day 0 of gestation. Test animals weighed between 222 and 280 g and were 12 weeks old when selected for the study.

Experimental procedures: The test substance was suspended in corn oil and administered by gavage on days 6 through 15 of gestation. Doses of 0, 40, 100, or 250 mg test substance per kg body weight were given to groups of 24 mated dams.

Each dam was observed daily for occurrence of toxic signs and mortality. Bodyweight determinations were made on days 0, 6-15, and day 21 of gestation. Food consumption was estimated for three day periods throughout gestation according to the report.

The rats were sacrificed on day 21 of gestation and subjected to a gross necropsy. Gravid uteri and individual fetuses from each dam were weighed, and the numbers of corpora lutea, implantation sites, live and dead fetuses, and embryonic deaths were noted. Live fetuses were grossly examined and two-thirds of them were prepared for skeletal examination. The remainder were prepared for soft tissue examination, and abnormalities were noted.

Early embryonic deaths were described as implantation sites with decidual or placental tissue only, while late deaths showed embryonal or fetal tissue with placenta at implantation sites according to the report.

The degree of ossification in the manus and pes was assessed according to the following scale:

1 = good---metacarpals/metatarsals and first, second, and third phalanges fully ossified.

2 = metacarpals/metatarsals and first and third phalanges fully ossified, some of second row not ossified.

3 = metacarpals/metatarsals fully ossified; all first and third row present, the majority fully ossified; most of second row not ossified, occassionally phalanx may be partially ossified.

4 = one metacarpal or metatarsal may be partially ossified, while the remainder of these bones may be fully ossified; second row of phalanges not ossified, most of first and third rows ossified.

5 = poor---one metacarpal or metatarsal partially ossified or not ossified at all, the remainder of these bones may be fully ossified; second row of phalanges not ossified, occassionally phalanges of the first and third rows partially ossified, and the rest are not ossified.

Major abnormalities were characterized as rare or possibly lethal, and minor abnormalities were defined as those commonly observed. The report stated that variations in the degree of ossification were considered as minor defects when observed to occur more frequently than similar observations in control or background data. Extra thoracic ribs were considered to be minor variants.

Statistical procedures are discussed below as apropriate. The report noted that animals that died during gestation, aborted, or were not pregnant were not included in the analysis of results.

Reported Results

The report stated that one rat died and four others were sacrificed in extremis. All of these animals were from the high dose group, and they died after 2 to 5 doses. The only clinical sign which was related to treatment according to the authors was staining of the genital and ventral areas. There were 4, 3, or 6 of 24 with the staining in the control, low, and mid dose groups, respectively, while 10 of the 19 survivors in the high dose group exhibited the effect.

Maternal bodyweight gain during the treatment period (days 6-15 of gestation) showed a dose-related decrease (not statistically significant). During that period the control, low, mid and high dose groups gained an average of 54.5, 53.2, 50.6, and 49.2 g, respectively. The only statistically significant difference between treated and control group means was reported for the high dose group dams during days 6-9 of gestation (3.9 g compared with 11.3 g for the control group; $p<0.01$, Student's t test). The authors also noted a slight decrease in bodyweight gain (8.8 g) during the same period for the mid dose group, but they noted no statistical significance. See page 7.

Group mean food consumption for the high-dose group was also statistically significantly less than the control group. The control group animals consumed an average of 23.4 g of food per observation period during dosing compared with 20.7 g for the high dose group ($p<0.01$, Student's t test). During days 6-9 and 9-12 of gestation the mean food consumption values for the high dose group were 15.2 and 20.6 g, respectively. The respective control group values for the two times were reported to be 20.4 and 23.4 g. See page 7.

The ratio between bodyweight gain and food consumption (g bodyweight gain per 100 g food consumed) was significantly decreased in the high dose group below that reported for the control group dams during days 6-9 of gestation. The reported group means were 17.8 and 5.3 ($p<0.01$, Student's t test). See page 8

At necropsy the investigators noted pallor, lobulation, and enlargement of the livers in 10 of the 19 survivors in the high-dose group dams as well as the 5 which died during the study. Pallor of the kidney was also noted in the high dose group animals. No other group was reported to have dose related gross pathology.

The reported group mean corpora lutea per dam ranged from 13.5 in the mid dose group to 14.7 in the control group. Group mean implantations per dam ranged from 12.8 in the low and mid dose groups to 13.7 in the control group (high-dose group averaged 13.0), and the group mean number of live fetuses per litter ranged from 11.8 in the mid-dose group to 12.7 in the control group (the mean for the high dose group was 12.4). None of these three parameters exhibited a relationship to dose. See page 9.

Group mean gravid uterine weights for the control, low, mid and high-dose groups were reported to be 86.5, 86.0, 83.1,

and 87.2 g, respectively. The respective mean fetal weights were 5.1, 5.2, 5.3, and 5.1 g for the control, low, mid, and high dose groups.

The overall incidence of fetuses with defects in each group was reported as follows:

	Dose groups			
Observation	Control	Low	Mid	High
	External/visceral			
No. examined*	305	297	283	234
With external defects (%)	15 (5)	16 (5)	12 (4)	12 (5)
	Skeletal			
No. examined**	204	198	190	153
With defects (%)	84 (41)	110 (56)	117 (61)	111 (73)

*All fetuses were examined externally. Also includes those examined for visceral abnormalities (one-third of the fetuses.

**Two-thirds of the fetuses were examined for skeletal defects.

The authors noted that there were 3, 2, 1, and 3 fetuses in the control, low, mid, and high dose groups with major defects. One fetus from the low dose group was reported to have cleft palate along with three from the high dose group. Two of the latter group were litter mates, and the third exhibited exencephaly according to the report. The other major defects noted included hydrocephaly and multiple defects of the vertebrae, sternebrae and ribs in effected fetuses.

The report stated that a dose-related increase in the incidence of skeletal defects was observed in fetuses from treated dams. The defect which contributed most to the increase was classified as a minor defect and involved partial ossification of the 7th cervical vertebra's transverse processes. Incidence data for this and other skeletal observations which were reported to be dose-related are summerized as follows:

Observation	Dose groups			
	Control	Low	Mid	High
No. examined	204	198	190	153
Cervical defect (%)	13 (6)	32 (16)	49 (26)	47 (31)
Extra rib (unilateral) (%)	22 (11)	36 (18)	101 (53)	104 (68)
Extra rib (bi- and unilateral) (%)	54 (26)	54 (27)	135 (71)	126 (82)

Partial ossification was also noted in the mid and high dose group fetuses in the odontal bone as well as in the occipital bone of high dose group fetuses. Control, mid, and high dose groups had 9.3, 18.9, and 23.5% of the fetuses with the first effect, while the high dose group and controls had respective incidences of 11.1 and 2.5% for the latter effect. See page 10 for details.

Discussion and Conclusions

The data presented in the report are adequate to support the conclusions of the investigators. They concluded that the no-observed-effect level (NOEL) for maternal toxicity with respect to decreased bodyweight gain during dosing (days 6-9 of gestation) is 40 mg/kg/day (lowest dose tested). The lowest-effect dose (LEL) is 100 mg/kg/day. The highest dose caused mortality (5/24 animals in the group) as well as grossly observable liver effects (pallor and enlargement).

Fetuses exhibited a dose-related increase in the incidence of delayed ossification at all doses, and the authors concluded that a NOEL for these effects was not established. They also presented a discussion of the incidence of cleft palate observed in the study. They stated:

> Cleft palate is rare as a spontaneous abnormality in the Alderley Park rat with a historic incidence of 1 in approximately 1500 fetuses in recent studies...in this Laboratory...The observed incidence of cleft palate in this study at 250 mg/kg/day paclobutrazol may be of biological significance...When the results of the preliminary study are taken into account (Dosages of 80 mg/kg/day caused cleft palate in 1 of 110 fetuses.) the possibility of a treatment related effect cannot be ignored.

These effects occurred at maternally toxic doses.

The dose related increase in the number of fetuses with skeletal abnormalities is associated with the increases in delayed ossification as shown in the tabulated summaries of incidence data above. However, the uncertainty with regard to the occurrence of cleft palate in fetuses from treated dams in this and a preliminary study suggest that paclobutrazol may have a teratogenic potential at maternally toxic doses.

Core classification: Supplementary since there is no NOEL for fetal effects.

Mean Body Weight Gains (grams)[a]

Group:	Prior to Dosing Period (0-6)	Dosing Period (6-15)	Days 6-9	Post Dosing Period (15-21)	Entire Gestation Period (0-21)	Corrected Body Weight Gain[b]
0	29.6	54.5	11.3	82.0	166	79.5
40[c]	28.9	53.4	10.9	77.7	160	74.0
100	31.3	50.6	8.8	81.6	164	80.9
250	28.7	49.2	3.9**	80.1	158	70.8

♀ in each group: 24, 24, 24, 19 in control, low dose, mid-dose and high dose, respectively.

[a]Data extracted from (report number CTL/P/842 and tables 4 and 7, adjusted for replicate structure of study design)

[b]corrected body weight gain for entire gestation period = body weight gain for entire gestation period minus gravid uterus weight (calculated by reviewer - no statistical analysis)

[c]mg/kg/day

**Statistically significant at 1% level.

Food Consumption Data (g)[a]

Group:	Prior to Dosing Period	Dosing Period	Post-Dosing Period	Total Food Consumption g/rat
Control	24.2	23.4	32.3	549
LDT	24.2	22.9	32.2	544
MDT	23.8	22.3	31.7	534
HDT	24.2	20.7**	31.8	522

[a]Data extracted from (report number CTL/P/842 and table 5)

**Statistically significant at 1% level (Student's t-test, two-sided)

Food Utilization (g Bodyweight Gain/100 g Food Consumed)

Period (days)	Dose Level of Paclobutrazol (mg/kg) 0	40	100	250
0-6	20.3	19.8	21.8	19.6 (19)
6-9	17.8	17.8	14.2	5.3** (19)
9-12	28.3	28.9	29.5	32.2* (19)
12-15	29.2	29.2	29.1	32.4 (19)
15-21	42.4 (23)	40.4	43.2	42.1 (19)
Overall	30.2 (23)	29.4	30.6	30.3 (19)

Data extracted from (report number CTL/P/842 and table 6)
Means based on 24 observations/group unless otherwise indicated by a number in parentheses.

* $p < 0.05$, ** $p < 0.01$

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Cesarean Section Observations[a]

	Control	LDT	MDT	HDT
Dose (mg/kg/day)	0	40	100	250
# Animals Assigned	24	24	24	24
# Animals Mated/Inseminated	24	24	24	23
Pregnancy Rate (%)	100	100	100	96
Maternal Wastage				
# Died	0	0	0	5
# Died/pregnant	0	0	0	4
# Non pregnant	0	0	0	1
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Total Corpora Lutea	353	328	324	263
Corpora Lutea/dam	14.7	13.7	13.5	13.8
Total Implantations	328	308	305	248
Implantations/Dam	13.7	12.8	12.8	13.0
Total Live Fetuses	305	297	283	234
Live Fetuses/Dam	12.7	12.4	11.8	12.4
Total Resorptions	23	11	23	14
Early	22	11	23	12
Late	1	0	0	2
Resorptions/Dam	0.96	0.46	0.96	0.74
Mean Fetal Weight (gm)	5.1	5.2	5.3	5.1
Preimplantation Loss(%)	7.1	6.1	5.8	5.7
Postimplantation Loss(%)	7.0	3.6	7.5	5.6
Sex Ratio (% Male)	54.8	53.2	47.0	50.0
Total Litter Weight (g)	64.9	64.2	61.9	63.4

[a]Data extracted from report number CTL/P/842, table 7 and Appendices A and D.

Skeletal Examinations

Observations[a]	Control	Low Dose	Mid Dose	High Dose
#pups (litters) examined	204 (24)[b]	198 (24)	190 (24)	153 (19)
Skeletal defects # with minor or major defects	84 (24)	110[d] (23)	117[d] (23)	111[d] (19)
Skull occipital partially ossified	5 (5)	9 (6)	2 (2)	17[d] (7)
Skull odontoid not ossified	19 (10)	16 (9)	36[d] (13)	36[d] (13)
Transverse processes on 7th cervical vertebrae partially oss. (unilat.)	12 (7)	20 (9)	33[d] (16)	29[d] (15)
Transverse processes on 7th cervical vertebrae partially oss. (bilat.)	1 (1)	12[d] (4)	16[d] (8)	18[d] (12)
Transverse processes on 7th cervical vertebrae partially oss. (unilat. or bilateral)	13 (8)	32[d] (10)	49[d] (17)	47[d] (15)
Extra (14) rib (bilateral)	22 (13)	36[c] (14)	101[d] (23)	104[d] (19)
Extra (14) rib (bi- and unilateral)	54 (11)	54 (8)	135[d] (19)	125[d] (14)
2nd Sternebra partially oss.	12 (8)	13 (8)	16 (10)	24[d] (12)

[a]fetal (litter) incidence

[b]litters counts conducted by EPA reviewer. The statistical significance of litters at the lowest dose level where the fetal incidences were statistically significant were checked and found not to be statistically significant when compared to controls.

[c]$p < 0.05$

[d]$p < 0.01$

Fore and Hindlimb Assessment

	Dose Level (mg/kg/day)			
	0	40	100	250
# Litters examined	24	24	24	19
Mean manus score/fetus	2.15	2.15	2.21	2.52**
Mean pes score/fetus	2.72	2.66	2.64	3.01*

* $p < 0.05$; ** $p < 0.01$

Summary of Incidence of Cleft Palate in Two Studies

Preliminary Study	**Dose Level (mg/kg/day)**			
	0	80	160	240
# Fetuses examined	130	110	118	85
# With cleft palate	1	1	0	6*
Present Study	**Dose Level (mg/kg/day)**			
	0	40	100	250
# Fetuses examined	305	297	283	234
# With cleft palate	0	1	0	3

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DATA EVALUATION RECORD

1. CHEMICAL: Paclobutrazol
(+)-(R*,R*)-beta-[(4-chlorophenyl)methyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol or (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazole-1-yl)-pentan-3-ol

2. TEST MATERIAL: Paclobutrazol (92.4%) was used (see Item 1.)

3. STUDY/ACTION TYPE: Teratogenicity - rats; (EUP for new chemical)

4. STUDY IDENTIFICATION: Killick, M. E., G. H. Pigott, P. B. Banham, and M. R. Thomas. June 1, 1984. Paclobutrazol: Second teratogenicity study in the rat. Unpublished report no. CTL/P/997 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 254864.

5. REVIEWED BY:

Name: Roger Gardner
Title: Toxicologist
Organization: Review Section 6
Toxicology Branch
Signature: Roger Gardner
Date: 3/13/85

6. APPROVED BY:

Name: Jane Harris, Ph. D.
Title: Section Head
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Signature: Jane E Harris
Date: 3/13/85

7. CONCLUSIONS:

A no-observed-effect level (NOEL) for maternal toxicity in this experiment is greater than 100 mg/kg/day (highest dose tested). Dose-related fetal effects (renal dilatation, hydroureter, and minor skeletal defects or variations) were observed at 40 and 100 mg/kg/day dose levels, and a NOEL of 10 mg/kg/day was established for fetal effects.

Core classification: Minimum

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8. MATERIALS AND METHODS

Test species: Female Wistar derived Alderley Park strain rats were used. Each female was mated overnight with a male and the following morning vaginal smears were examined for the presence of spermatozoa. The day spermatozoa were found was designated Day 1 of gestation. Test animals weighed between 262 and 300 g and were 12 weeks old when selected for the study.

Experimental procedures: The test substance was suspended in corn oil and administered by gavage on days 7 through 16 of gestation. Doses of 0, 2.5, 10, 40, or 100 mg test substance per kg body weight were given to groups of 24 mated dams.

Each dam was observed daily for occurrence of toxic signs and mortality. Body weight determinations were made on days 1, 4, 7-16, 19 and 21 of gestation. Food consumption was estimated for three day periods throughout gestation according to the report.

The rats were sacrificed on day 21 of gestation and subjected to a gross necropsy. Gravid uteri and individual fetuses from each dam were weighed, and the numbers of corpora lutea, implantation sites, live and dead fetuses, and embryonic deaths were noted. Live fetuses were grossly examined and two-thirds of them were prepared for skeletal examination. The remainder were prepared for soft tissue examination, and abnormalities were noted.

Early embryonic deaths were described as implantation sites with decidual or placental tissue only, while late deaths showed embryonal or fetal tissue with placenta at implantation sites according to the report.

The degree of ossification in the manus and pes was assessed according to the following scale:

1 = good---metacarpals/metatarsals and first, second, and third phalanges fully ossified.

2 = metacarpals/metatarsals and first and third phalanges fully ossified, some of second row not ossified.

3 = metacarpals/metatarsals fully ossified; all first and third row present, the majority fully ossified; most of second row not ossified, occassionally phalanx may be partially ossified.

8. MATERIALS AND METHODS (continued)

4 = one metacarpal or metatarsal may be partially ossified, while the remainder of these bones may be fully ossified; second row of phalanges not ossified, most of first and third rows ossified.

5 = poor---one metacarpal or metatarsal partially ossified or not ossified at all, the remainder of these bones may be fully ossified; second row of phalanges not ossified, occassionally phalanges of the first and third rows partially ossified, and the rest are not ossified.

Major abnormalities were characterized as rare or possibly lethal, and minor abnormalities were defined as those commonly observed. The report stated that variations in the degree of ossification were considered as minor defects when observed to occur more frequently than similar observations in control or background data. Extra thoracic ribs were considered to be minor variants.

Statistical procedures are discussed below as apropriate. The report noted that animals that died during gestation, aborted, or were not pregnant were not included in the analysis of results.

9. REPORTED RESULTS

The report stated that there were no treatment-related effects on dams with respect to the occurrence of toxic signs, mortality, body weight, or macroscopic observations at necropsy. See Appendix I for body weights and food consumption.

The reported group mean corpora lutea per dam ranged from 14.4 in the 10, 40, and 100 mg/kg/day groups to 14.8 in the 2.5 mg/kg/day dose group (the control group mean was 14.6). Group mean implantations per dam ranged from 12.9 in the control group to 14.0 in the 2.5 mg/kg/day dosed group, and the group mean number of live fetuses per litter ranged from 12.0 in the control group to 13.3 in the lowest-dosed group. None of these three parameters exhibited a relationship to dose. See Appendix I for tables.

Group mean gravid uterine weights for the 0, 2.5, 10, 40, and 100 mg/kg/day dosed groups were reported to be 76.4, 85.3, 82.3, 87.9, 85.4, respectively. The respective mean

9. REPORTED RESULTS (continued)

fetal weights were 4.7, 4.9, 4.9, 4.8, and 4.9 g.

The overall incidence of fetuses with defects in each group was reported as follows:

	Dose (mg/kg/day)				
Observation	Control	2.5	10	40	100
	External/visceral				
No. examined*	264	318	301	302	305
With external defects (%)	60 (22.7)	35 (23.6)	73 (24.3)	138 (45.7)	147 (48.2)
	Skeletal				
No. examined**	176	213	199	200	202
With defects (%)	61 (34.7)	80 (37.6)	75 (37.7)	89 (44.1)	106 (52.5)

*All fetuses were examined externally. Also includes those examined for visceral abnormalities (one-third of the fetuses.

**Two-thirds of the fetuses were examined for skeletal defects.

The authors noted that there were 3, 2, 1, 0, and 9 fetuses in the control, 2.5, 10, 40, and 100 mg/kg/day dose groups with major defects, respectively. Eight of the 9 fetuses in the highest dosed group were reported to have hydroureter. Two litters contained one fetus each with the defect, and two additional litters contained 3 each with the defect. The authors stated that all cases were associated with some renal pelvic dilatation, and 3 litter mates from dam number 116 were reported to have distended bladders also. The reported incidence of urogenital defects is presented in Appendix 1 below.

The reported minor skeletal defects were characteristic of delayed ossification, and the authors stated:

> The only individual defect to show a substantial treatment-related increase was the incidence of

9. REPORTED RESULTS (continued)

> partial ossification of the transverse processes of the seventh cervical verterbra...There were other minor indications of increased or decreased ossification seen in these dose groups (40 and 100 mg/kg/day groups) but none attained statistical significance.

The only skeletal variation reported to be significantly increased by the two highest doses was the incidence of 14th rib. The incidence of these defects is presented in Appendix 2.

The number of litters with one or more fetuses with external/visceral or skeletal defects is summarized as follows:

	Dose (mg/kg/day)				
Observation	Control	2.5	10	40	100
	External/visceral				
No. examined	22	24	24	24	24
With external defects	17	20	21	23	23
	Skeletal				
With defects	22	21	22	24	24

No other observations in the study showed compound related effects.

10. DISCUSSION

The authors noted that a no-observed-effect level (NOEL) for maternal toxicity in this experiment is greater than 100 mg/kg/day (highest dose tested). Dose-related fetal effects (renal dilatation, hydroureter, and minor skeletal defects or variations) were observed at 40 and 100 mg/kg/day dose levels, and a NOEL of 10 mg/kg/day was established on the basis of the results described above and historical control data (see Appendix 3).

Adequate data were presented in the report to support the authors' conclusions. The investigators also provided a discussion of published literature to which substantiates their interpretation of the results (see Appendix 4).

APPENDIX 1

Incidence of Urogenital Defects

Body Weight Gains

Food Consumption

Cesarian Section Observations

Fetal Defects

Mean Body Weight Gains (grams)[a]

Group:	Prior to Dosing Period (1-7)	Dosing Period (7-16)	Post Dosing Period (16-22)	Entire Gestation Period (1-22)	Corrected Body Weight Gain[b]
0	27.6	44.7	50.3	122.6	46.2
2.5[c]	29.2	47.3	50.4	126.8	41.5
10	27.1	47.9	55.1	130.2	47.9
40	29.7	43.2	52.0	124.9	43.0
100	29.8	47.8	52.9	130.5	45.1

♀ in each group: control: 21 to day 16, 22 thereafter; low dose: 24; mid-dose 1: 24; mid-dose 2: 24; high dose: 24 to day 7, 22 thereafter.

[a]Data extracted from (report number CTL/P/997 and tables 3 and 5, adjusted for replicate structure of study design)

[b]corrected body weight gain for entire gestation period = body weight gain for entire gestation period minus gravid uterus weight (calculated by reviewer - no statistical analysis)

[c]mg/kg/day

Food Consumption Data (g)[a]

Group:	Prior to Dosing Period	Dosing Period	Post-Dosing Period	Total Food Consumption g/rat
Control	23.5	19.9	23.5	461
LDT	23.5	20.6	23.0	465
MDT1	22.9	20.5	24.1	466
MDT2	23.9	19.8	22.8	459
HDT	24.0	20.1	23.3	465

[a]Data extracted from (report number CTL/P/997 and table 4)

Cesarean Section Observations[a]

Dose (mg/kg/day)	0	2.5	10	40	100
# Animals Assigned	24	24	24	24	24
# Animals Mated/Inseminated	22	24	24	24	24
Pregnancy Rate (%)	92	100	100	100	100
Maternal Wastage					
# Died	0	0	0	0	0
# Died/pregnant	0	0	0	0	0
# Non pregnant	2	0	0	0	0
# Aborted	0	0	0	0	0
# Premature Delivery	0	0	0	0	0
Total Corpora Lutea	322	356	345	346	346
Corpora Lutea/dam	14.6	14.8	14.4	14.4	14.4
Total Implantations	284	335	321	317	324
Implantations/Dam	12.9	14.0	13.4	13.2	13.5
Total Live Fetuses	264	318	301	302	305
Live Fetuses/Dam	12.0	13.3	12.5	12.6	12.7
Total Resorptions	20	17	20	15	19
Early	19	17	15	14	18
Late	1	0	5	1	1
Resorptions/Dam	0.91	0.71	0.83	0.63	0.79
Mean Fetal Weight (gm)	4.7	4.9	4.9	4.8	4.9
Preimplantation Loss(%)	11.8	5.9	7.0	8.4	6.4
Postimplantation Loss(%)	7.0	5.1	6.2	4.7	5.9
Sex Ratio (% Male)	56.8	55.3	53.2	52.6	50.2
Total Litter Weight (g)	56.1	64.7	61.4	60.2	62.3

[a]Data extracted from report number CTL/P/997, table 5 and Appendix D.

Intergroup Comparison of Fetal Defects

	Dose Level				
External/Visceral Defects	0	2.5	10	40	100
# Fetuses Examined	264	318	301	302	305
# Litters Examined	22	24	24	24	24
# Fetuses Showing Any Defect	60	75	73	138	147
% of Fetuses Affected	22.7	23.6	24.3	45.7[b]	48.2[b]
# Litters Affected	17	20	21	23	23
# Fetuses Showing Major Defects	2	1	1	0	9
% of Fetuses Affected	0.8	0.3	0.3	0	3.0
# Litters Affected	1	1	1	0	4
Skeletal Defects					
# Fetuses Examined	176	213	199	202	202
# Fetuses Showing Any Defect	61	80	75	89	106
% of Fetuses Affected	34.7	37.6	37.7	44.1[a]	52.5[b]
# of Litters Affected	22	21	22	24	24
# Fetuses Showing Major Defects	1	1	0	0	0
% of Fetuses Affected	0.6	0.5	0	0	0
# of Litters Affected	1	1	0	0	0
Skeletal Variants					
# Fetuses Examined	176	213	199	202	202
# of Fetuses Showing Variants	172	203	188	196	195
% of Fetuses Affected	97.7	95.3	94.5	97.0	96.5
# of Litters Affected	22	24	24	24	24

[a]$p < 0.05$; [b]$p < 0.01$

Intergroup Comparison of Fetal Defect Incidence
Incidence by Fetus (Litter)

Observation	Dose Level (mg/kg/day) 0	2.5	10	40	100
External/Visceral Defects					
Kidney - unilateral pelvic dilatation					
Extreme	0 (0)	1 (1)	0 (0)	0 (0)	1 (1)
Moderate	0 (0)	3 (2)	1 (1)	2 (2)	6[a] (3)
Slight	24 (12)	34 (14)	27 (15)	63[b] (18)	49[b] (19)
Ureter:					
Bilateral dilated:					
Moderate	0 (0)	1 (1)	0 (0)	1 (1)	2 (1)
Slight	3 (3)	5 (3)	3 (3)	10 (8)	21[b] (8)
Unilateral dilated:					
Moderate	1 (1)	1 (1)	1 (1)	2 (2)	9[a] (3)
Slight	18 (9)	17 (10)	18 (8)	39[a] (22[b])	49[b] (17[a])
Unilateral hydroureter	0 (0)	0 (0)	0 (0)	0 (0)	8[b] (4)
Unilateral kinked	19 (11)	22 (13)	22 (15)	57[b] (19[a])	50[b] (19[a])
Skeletal Defects					
Vertebrae:					
10th Thoracic Centrum partially ossified	0 (0)	3 (3)	1 (1)	1 (1)	5[a] (4)
7th Cervical transverse process partially ossified:					
Bilateral	3 (3)	4 (3)	1 (1)	7 (5)	14[a] (7)
Unilateral	7 (6)	16 (10)	18[a] (11)	35[b] (15[a])	40[b] (20[b])

[a]$p < 0.05$; [b]$p < 0.01$

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