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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MAY 31 1988

MEMORANDUM

SUBJECT: Rat and Rabbit Teratology Studies on Tilt

TO: Ms. Lois Rossi, PM 21
Registration Division (TS-767C)

FROM: Byron T. Backus, Toxicologist Byron T. Backus 5/27/88
Toxicology Branch (TS-769C)

THROUGH: Marcia van Gemert, Ph.D.
Section Head, Review Section III M. van Gemert 5/21/88
Toxicology Branch (TS-769C)

and

Theodore M. Farber, Ph.D., D.A.B.T.
Branch Chief
Toxicology Branch (TS-769C)

EPA Record No. 210009

Project No. 8-0456

Tox. Chem. 323EE

Action Requested:

Review and comment on 1) two rat teratology studies submitted by Ciba-Geigy on Tilt, especially relating to the increased incidence of cleft palate in fetuses from high-dosed (300 mg/kg/day) rat dams, and 2) additional statistical analyses and a correction relating to a previously submitted and reviewed rabbit teratology study on this chemical.

Comments and Recommendations:

1. The first rat teratology study (report 86004) has been classified as core minimum data. The test material was administered at 0 (vehicle control), 30 and 90 mg/kg/day. Additionally, there was a high-dose group which initially received 360 mg/kg/day, subsequently reduced to 300 mg/kg/day. The NOEL for maternal and fetal toxicity was 30 mg/kg/day, and the LEL was 90 mg/kg/day.

It is concluded that the occurrence of cleft palate at a low incidence in the fetuses of dams which received 90 and 360/300 mg/kg/day is a compound-related (but not necessarily teratogenic) effect. This is based on the lack of any occurrence of cleft palate among 5431 control fetuses in 19 studies conducted at this laboratory.

It is also noted that both occurrences of cleft palate at the 360/300 mg/kg/day level in this study were detected on visceral examination (the one reported occurrence at 90 mg/kg/day was detected on external examination, but it was one of a number of malformations existing in one fetus). It is possible that there might have been additional (undetected) cases of cleft palate since only 148/285 fetuses were examined viscerally (the remaining 137 were examined skeletally). However, in one of the two high-dose fetuses with cleft palate, anasarca (generalized edema) was noted on external examination, leaving just one high-dose fetus in which cleft palate was the only malformation. Overall, the extremely low incidence of this finding by itself, as well as the indications of delayed development in high-dose fetuses (cleft palate might result from delayed development) are strong evidence that what was observed in this study was not a result of teratogenic activity. The teratogenic NOEL = 300 mg/kg/day (HDT).

2. The second rat teratology study (Report 86189) has been classified as supplementary. However, it is recognized that the purpose of this study was to confirm the finding of an increased incidence of cleft palate in the first study. In this second study, 0/2122 controls and 2/2064 fetuses from dosed (300 mg/kg/day, days 6-15) dams showed cleft palate. While this incidence is considerably lower than that (2/285) reported in the first study, there is no indication that fetuses in this second study were examined other than externally. The two affected fetuses in the high-dose group of the previous study had been found to have cleft palate on visceral (rather than external) examination.

   It is recommended that a clarification be obtained as to whether or not the fetuses in this second study were examined only externally. If the examination was only external, then they should be more completely examined for cleft palate.

   The findings of this second study do not change the previous conclusion that the test material is not teratogenic, expecially if the incidence is 2/2064.

3. The additional data and corrections relating to the previously submitted and reviewed rabbit teratology study do not affect the conclusions of the original DER (Phang, 3/10/87) relating to NOEL and LEL for maternal or developmental toxicity, nor do they affect the re-classification of the study to Core Minimum (Taylor, 11/16/87).

CASWELL FILE

Reviewed by: Byron T. Backus Byron T. Backus 5/23/88
Section 3, Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert Marcia van Gemert 5/24/88
Section 3, Tox. Branch (TS-769C)

DATA EVALUATION REPORT I

STUDY TYPE: Teratology - Rat

TOX. CHEM. NO: 323EE

ACCESSION NUMBER: 404250-01

MRID NO: not given

TEST MATERIAL: CGA-64250

SYNONYMS: Propicanozole, TILT

STUDY NUMBER(S): Toxicology/Pathology Report 86004 (MIN 852148)

SPONSOR: Ciba-Geigy Corporation

TESTING FACILITY: Pharmaceuticals Division
Ciba-Geigy Corporation

TITLE OF REPORT: CGA 64250 Technical - A Teratology (Segment II) Study in Rats

AUTHOR(S): Marcsisin, J. F., Wimbert, K. V., Giknis, M. L. A., Arthur, A. T., and Yau, E. T.

REPORT ISSUED: 1/28/87

Classification: Core Minimum Data

Special Review Criteria (40 CFR 154.7)

CONCLUSIONS:

1. The test material was orally administered from day 6 through day 15 of gestation at 0 (vehicle only), 30 and 90 mg/kg/day. High-dose animals initially received 360 mg/kg/day, but because of severe symptoms this was reduced to 300 mg/kg/day. Some of the high-dose dams received as many as 5 doses at 360 mg/kg/day, while some received as few as 2.

2. The NOEL for maternal toxicity was 30 mg/kg/day, with a LEL of 90 mg/kg/day, based on reduced dam body weight gains and the occurrence of rales in 1/24 dams.

3. The NOEL for fetotoxicity was 30 mg/kg/day, and the LEL (significantly increased incidence of unossified sternebrae, and increased - not significantly so, but part of dose-related trends - incidences of rudimentary ribs, and shortened or absent renal papillae) was 90 mg/kg/day. At 360/300 mg/kg/day there was a high incidence of rudimentary ribs, a highly significant ($p \leq 0.01$) incidence of unossified sternebrae, as well as statistically significant increased incidences of shortened and absent renal papillae and dilated ureter.

4. It is concluded that the occurrence of cleft palate (reported for 1/302 fetuses at 90 mg/kg/day, and in 2/285 at 360/300 mg/kg/day) is probably a compound-related (but not necessarily teratogenic) effect. This is based on the the lack of any occurrences of cleft palate among controls in 19 teratology studies conducted at this laboratory with (presumably) this rat strain, or an incidence of 0/5431. By Fisher's Exact Test the probability of a distribution of 0/5431 (control) and 2/285 is 0.00248.

   Both occurrences of cleft palate at the high dose level in this study were detected on visceral examination (the one reported occurrence at 90 mg/kg/day was detected on external examination, but was one of a number of malformations in one fetus). It is possible that there might have been additional (undetected) cases of cleft palate in this group since only 148/285 fetuses were examined viscerally (137 were examined skeletally). However, in one of the two high-dose fetuses with cleft palate, anasarca (generalized edema) had been identified on external examination, leaving just one high-dose fetus in which cleft palate was the only malformation.

   Overall, the extremely low incidence of this finding by itself, as well as the indications of delayed development in high-dose fetuses (cleft palate might result from delayed development) are strong evidence that what was observed in this study was not a result of teratogenic activity. The teratogenic NOEL = 300 mg/kg/day (HDT).

A. MATERIALS:

1. Test compound: CGA 64250 Technical. Batch no. FL 850083. According to the text in Acc. 404250-03 the material was a gold-colored mixture of thick liquid and crystals. The mean composition of 6 samples was >92.1% (range: 91.7 to 92.3%). The test material was administered as a 0.3%, 0.9% or 3.6% suspension in 3% aqueous cornstarch containing 0.5% Tween 80.

2. Test animals: Species: rat; Strain: CrL:COBS CD (SD)BR VAF/PLUS; Age: "sexually mature" virgins. Weight: 210-300 grams. These were mated with sexually mature males of the same strain.

B. STUDY DESIGN:

1. Mating

"Following a period of approximately 3 weeks for acclimation... a total of 120 females were mated with 60 sexually mature males of the same strain."

2. Animal assignment

"A maximum of 24 sperm positive animals were placed on study within each dose group." Assignment was random. The following were the dose groups:

Group	Dose Level (mg/kg/day)	Number of Females/group
1 (vehicle controls)	0	24
2 (low)	30	24
3 (intermediate)	90	24
4 (high)	360/300*	23

*4 animals received 5 doses of 360 mg/kg, 9 received 4 doses of 360 mg/kg, 9 received 3 doses of 360 mg/kg, and 2 received 2 doses of 360 mg/kg. Subsequent doses were 300 mg/kg/day in this group.

3. Dosing:

"CGA 64250 Technical...was administered to Groups 2 (30 mg.), 3 (90 mg.) and 4 (360 mg.)... The high dose group was reduced to 300 mg/kg/day on May 4, 1985 due to severe maternal toxicity. This decrease was accomplished by changing the dose volume... from 10.0 ml/kg/day to 8.3 ml/kg/day... The animals in Group 1 served as the vehicle controls... Dams were treated from day 6 through 15 of gestation, the period of organogenesis in the rat."

4. All animals were given access to their diet (Purina #5002 Certified Chow) and to tap water ad libitum.

5. Statistics - From p. 15-16: A number of types of statistical analyses were performed on body weight, body weight gain, feed consumption, fetal weight, number of corpora lutea, implantations, resorption sites, viable fetuses, % post-implantation loss, and fetal sex ratios.

6. There is a signed and dated "Certification of Good Laboratory Practices" on p. 3, and a signed and dated Quality Assurance Unit Statement on p. 417.

C. METHODS AND RESULTS:

1. Observations: From p. 14: "The dams were observed daily for changes in appearance or behavior."

Results:

Mortality: From p. 20: "One dam from the control group (No. 22) was found dead on day 20 of gestation in this study. The cause of death was thought to be complications resulting during early labor and delivery... All other females survived to scheduled necropsy."

Symptoms: From p. 20: "There were no treatment-related clinical observations in dams of the low dose group and only a single observation of rales in the intermediate dose group. However, severe signs of compound-related maternal toxicity were observed in dams of the high dose group during the first week of dosing. The compound-related signs of maternal toxicity observed in the high dose group included a statistically significant increase in the incidence of lethargy, ataxia, and salivation when compared to the control group and biologically significant signs of rales, prostration, hypothermia and bradypnea. Due to the severity of these toxic signs, the high dose level was lowered from 360 mg/kg/day to 300 mg/kg/day on the sixth day of dosing." From table 6.1 (p. 33) 9/23 high dose animals showed lethargy (compared to 0/24 controls), 4/23 showed salivation (0/24 controls) and 3/23 showed ataxia (0/24 controls).

2. Food consumption:

Individual food consumption for days 0-6 and daily thereafter was determined. Group means were calculated. The following (from table 6.3, p. 36-37) includes all intervals for which one or more of the dosed groups showed a significantly lower mean food consumption than controls:

Interval	Mean Daily Food consumption (gms) ± S.D. during interval			
	Group 1	Group 2	Group 3	Group 4
0-6	19.45 ± 2.18	20.57 ± 2.67	20.75 ± 1.49	19.96 ± 3.15
6-7	21.00 ± 3.88	21.90 ± 3.52	18.73 ± 3.51	18.82 ± 5.39
7-8	21.00 ± 2.93	21.43 ± 3.99	20.32 ± 2.80	16.82 ± 4.84*
8-9	22.18 ± 2.30	22.24 ± 3.55	19.68 ± 2.75*	18.58 ± 4.38*
9-10	21.77 ± 2.83	23.19 ± 2.84	20.82 ± 3.59	16.68 ± 4.10*
10-11	22.77 ± 2.78	22.38 ± 3.46	19.82 ± 5.78*	20.36 ± 3.55

*Different from control with $p < 0.05$.

3. Dam body weights and gains:

Individual dam body weights were measured on gestation days 0, 6, 8, 12, 16 and 20.

Results:

From p. 22: "There were no significant differences in body weight...between the control animals and any treatment group during the course of the study." From table 6.4 (p. 38):

Group mean body weights:

Day	Group 1	Group 2	Group 3	Group 4
0	255.68 ± 16.62	251.29 ± 14.92	259.05 ± 14.88	257.55 ± 19.65
16	331.55 ± 23.01	327.67 ± 23.59	332.95 ± 19.47	325.82 ± 27.06
20	380.82 ± 35.17	379.00 ± 32.52	392.21 ± 18.98	385.73 ± 32.13

From p. 22: "Statistically significant decreases in maternal weight gain were detected in the intermediate and high dose groups as compared to the control group for the interval of gestational days 6-8." However, there were no significant differences between groups over the entire 20 days of the study. From table 6.5 (p. 39):

Interval (Days)	Group 1	Group 2	Group 3	Group 4
0-6	29.05 ± 7.74	32.00 ± 7.50	32.41 ± 6.98	28.09 ± 9.29
6-8	7.14 ± 4.23	3.76 ± 5.22	3.14 ± 4.70*	2.68 ± 5.44*
8-12	16.59 ± 4.59	18.43 ± 6.83	16.71 ± 4.72	14.23 ± 4.99
6-16	46.82 ± 7.83	44.38 ± 10.32	45.62 ± 6.83	40.18 ± 11.24
0-20	125.14 ± 25.14	127.71 ± 26.43	133.05 ± 18.75	128.18 ± 19.90

*Different from control with $p < 0.05$.

4. Reproductive parameters:

From p. 14: "The dams were necropsied on day 20 of presumed gestation. The uteri including their contents were weighed and corpora lutea, live fetuses, dead fetuses and intrauterine resorption sites were counted."

Results:

From p. 23: "There were no significant treatment-related effects on any of the reproductive parameters examined."

5. Mean fetal body weights:

Individual fetuses were weighed at necropsy.

Results:

There were no significant differences (or even indications of possible dose-related differences) between groups with respect to fetal weights. The mean fetal weight (both males and females) is reported (p. 41) as 3.5 grams for each dosage group.

6. Variations and malformations:

From p. 14: "The fetuses were sexed and numbered in order of their positions in the uterus from the ovarian end of the left horn to the ovarian end of the right horn... The fetuses were examined for gross abnormalities. Approximately one half of the fetuses from each litter were placed into Bouin's fixative for subsequent visceral examination. The remaining half of the litter was placed in 95% ethanol for skeletal examination."

Visceral examination - From p. 14: "Approximately 1/2 of the fetuses were fixed in Bouin's solution for at least one week and then examined viscerally for abnormalities..." The following were examined:

Central nervous system:	brain, eyes and spinal cord
Cardiovascular system:	heart and major blood vessels
Respiratory system:	nasal passages, trachea, lungs, diaphragm
Gastrointestinal sytem:	oral cavity, tongue, esophagus, stomach, intestines, liver, pancreas
Lymphoid structures:	thymus, spleen
Urinary system:	kidneys, ureters, bladder
Endocrine system:	adrenals
Reproductive system:	ovaries, uterus or testicles

Skeletal examination - Approximately 1/2 of the fetuses from each litter were stained with Alizarin Red S and cleared according to the method of Staples and Schnell and examined for skeletal abnormalities.

Results:

There were 1141 apparently viable fetuses in this study. The following is the summary of fetal malformations as reported in table 6.8 (p. 42):

	Dose Group 1	2	3	4
External:				
Anasarca	0	0	0	1
Cleft/Hare Lip	0	0	1[b]	0
Cleft Palate	0	0	1[b]	0
Club Foot	0	0	1[b]	0
Micromelia	0	0	1[b]	0
No. fetuses examined	270	284	302	285
Visceral:				
Cleft/Hare Lip**	0	0	1	0
Cleft Palate**	0	0	0	2[a]
Hydromelia	0	0	0	1[a]
Protruding Tongue	0	0	0	1[a]
No. fetuses examined	141	148	156	148
Skeletal:				
Lacrimal Bone - Agenesis	0	0	1[b]	0
Rudimentary Ribs - Thoracic 13th	3	1	0	1
No. fetuses examined	129	136	146	137

**Malformations were identified on the visceral examination
[a] Same fetus (Female 108 - #5) one fetus (Female 108 - #5) anal cleft palate, hydromelia of protruding tongue
[b] Same fetus (Female 69 - #16)

The following is a summary of visceral fetal variations (from table 6.9, p. 43):

	Dose Group 1	2	3	4
Renal papilla(e): short	32	27	40	57*
absent	4	4	8	16*
Dilated ureter(s)	38	21	38	63*
No. fetuses with variations	44	29	58	80
No. fetuses examined	141	148	156	148

*Significantly different from controls with $p \leq 0.01$

The following is a summary of skeletal fetal variations which with noticeably elevated incidences in the intermediate and/or high dose groups (from table 6.11, p. 37-38):

	Dose Group 1	2	3	4
Ribs:				
rudimentary	0	1	4	53†
Sternebrae				
bipartite	0	1	0	4
not ossified	49	54	83*	99**
No. fetuses examined	129	136	146	137

†Not reported as significantly different (see p. 45)
*Significantly different from controls with $p < 0.05$
**Significantly different from controls with $p < 0.01$

5. Historical control data - cleft palate:

According to information in Appendix 7.22.1 the incidence of cleft palate in controls for all rat teratology studies (including this one) conducted at this laboratory 1983-1985 was 0/5431. Examining the total incidences of cleft palate at low, intermediate and high-dose levels the following values can be derived:

	Dose Group 1	2	3	4
Incidence of cleft palate: all studies	0/5431	0/5453	1/5811	3/4425
Incidence of cleft palate in other studies	0/5161	0/5169	0/5509	1/4140
Incidence of cleft palate in this study	0/270	0/284	1/302	2/285

In short, out of 19,979 fetuses in 18 other studies, one (in a high-dose group) had cleft palate. In this one study, out of 871 fetuses exposed to the test material, 3 showed cleft palate.

D. DISCUSSION:

The NOEL for maternal toxicity was 30 mg/kg/day, with a LEL of 90 mg/kg/day, based on reduced dam body weight gains and the occurrence of rales in 1/24 dams. These effects were more pronounced at 360/300 mg/kg/day, along with lethargy and ataxia.

The NOEL for fetotoxicity was 30 mg/kg/day, and the LEL (significantly increased incidence of unossified sternebrae, and increased - not significantly so, but part of dose-related trends - incidences of rudimentary ribs, and shortened or absent renal papillae) was 90 mg/kg/day. At 360/300 mg/kg/day there was a a high incidence of rudimentary ribs, a highly significant ($p \leq 0.01$) incidence of unossified sternebrae, as well as statistically significant increased incidences of shortened and absent renal papillae and dilated ureter.

It is concluded that the occurrence of cleft palate (reported for 1/302 fetuses at 90 mg/kg/day, and in 2/285 at 360/300 mg/kg/day) is probably a compound-related (but not necessarily a teratogenic) effect. This is based on the the lack of any occurrences of cleft palate among controls in 19 teratology studies conducted at this laboratory with (presumably) this rat strain, or an incidence of 0/5431. By Fisher's Exact Test, the probability of a distribution of 0/5431 (control) and 2/285 is 0.00248.

It is also noted that both occurrences of cleft palate at the high dose level in this study were detected on visceral examination (the one reported occurrence at 90 mg/kg/day was detected on external examination, but was one of a number of malformations existing in one fetus). It is possible that there might have been additional (undetected) cases of cleft palate in this group since only 148/285 fetuses were examined viscerally (137 were examined skeletally). It is possible that there might have been additional (undetected) cases of cleft palate in this group since only 148/285 fetuses were examined viscerally (137 were examined skeletally). However, in one of the two high-dose fetuses with cleft palate, anasarca (generalized edema) had been identified on external examination, so there was just one high-dose fetus in which cleft palate was the only malformation.

Overall, the extremely low incidence of this finding by itself, as well as the indications of delayed development in high-dose fetuses (cleft palate might result from delayed development) are strong evidence that what was observed in this study was not a result of teratogenic activity.

This study is classified as core minimum.

CASWELL FILE

Reviewed by: Byron T. Backus Byron T. Backus
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Secondary reviewer: Marcia van Gemert
Section 3, Tox. Branch (TS-769C) Marcia van Gemert 5/24/88

DATA EVALUATION REPORT II

STUDY TYPE: Teratology (modified) - rat

TOX. CHEM. NO: 323EE

ACCESSION NUMBER: 404250-02

MRID NO: not given

TEST MATERIAL: CGA-64250

SYNONYMS: Propicanozole, TILT

STUDY NUMBER(S): Toxicology/Pathology Report 86189 (MIN 862244)

SPONSOR: Ciba-Geigy Corporation

TESTING FACILITY: Pharmaceuticals Division
Ciba-Geigy Corporation

TITLE OF REPORT: A Modified Teratology Study in Albino Rats with CGA-64250

AUTHOR(S): Mallows, S., Levy, E., Giknis, M. L. A., and Yau, E. T.

REPORT ISSUED: 2/16/87

Classification: Core Supplementary Data

Special Review Criteria (40 CFR 154.7)

CONCLUSIONS:

1. The purpose of this study was not to satisfy or meet a data requirement, but was to determine whether the finding of a previous study (DER I in this review set) indicating that the test material might cause cleft palate in rat fetuses was correct. In the previous study, 1/302 fetuses at 90 mg/kg/day and 2/285 at 360/300 mg/kg/day had cleft palate, but the incidence in control fetuses was 0/5431 for 19 teratology studies at this laboratory.

2. In this study 0/2122 controls and 2/2064 fetuses from dosed (300 mg/kg/day, days 6-15) dams showed cleft palate. While this incidence is considerably lower than that (2/285) observed previously there is no indication that fetuses in this study were examined other than externally. The two affected fetuses in the previous study were found to have cleft palate on visceral (rather than external) observation.

3. It is recommended that a clarification be obtained as to whether or not the fetuses of this study were examined only externally. If so, then they should be more completely examined for cleft palate.

4. If the incidence of cleft palate in fetuses from dosed dams was 2/2064, then this low incidence (90% lower than was seen in the previous study) provides some reinforcement for the previous conclusion (see DER I) that the test material is not teratogenic.

5. It is noted that, in contrast to the previous rat teratology study, the fetuses of the dosed dams (300 mg/kg/day) had significantly lower weights than did those of controls.

A. MATERIALS:

1. Test compound: CGA 64250 Technical. Batch no. FL 850083. According to information in Acc. 404250-03 the material was a gold-colored mixture of thick liquid and crystals. The mean composition of 6 samples was >92.1% (range: 91.7 to 92.3%). The test material was administered as a 3.0% suspension in 3% aqueous cornstarch containing 0.5% Tween 80.

2. Test animals: Species: rat; Strain: CrL:COBS CD (SD)BR; Age: "sexually mature" virgins. Weight: 206-305 grams. These were mated with sexually mature males from the same strain.

B. STUDY DESIGN:

1. Mating

Four hundred females were used. "Mating began by initially placing the first 120 females into a cage of one of the 199 male animals in a ratio of 1:1. On subsequent days no more than 120 females were placed with males, and if 2 females were inseminated by the same male during the course of the study, one was assigned to the control group and one was assigned to the treated group when possible."

2. Animal assignment

"A total of 178 sperm positive animals were placed on study in the control group and 189 sperm positive animals were placed on study in the treated group. Twenty-three...of the control animals and twenty-eight of the treated animals were no pregnant..." The data from these animals were not included in the report. The control animals were designated as Group 1 and the dosed animals were designated as Group 2.

3. Dosing:

"CGA 64250 Technical...was administered to Group 2 (300 mg/kg/day) once daily by gastric intubation as a 3.0% suspension in 3% aqueous cornstarch containing 0.5% Tween 80. The animals in Group 1 served as the vehicle controls and received 10 ml/kg/day of 3% cornstarch with 0.5% Tween 80 which was a volume equivalent to that received by treated rats... Dams were treated from day 6 through 15 of gestation, the period of organogenesis in the rat."

4. All animals were given access to their diet and to tap water ad libitum. On p. 19 it is reported that "due to a technical error" 6 control females were without feed for 1-2 days.

5. Statistics - From p. 13-14: A number of types of statistical analyses were performed on body weight, body weight gain, feed consumption, fetal weight, number of corpora lutea, implantations, resorption sites, viable fetuses, % post-implantation loss, and fetal sex ratios.

6. There is a signed and dated Good Laboratory Practice statement on p. 407, and a signed and dated Quality Assurance Unit Statement on p. 408.

C. METHODS AND RESULTS:

1. Observations: From p. 13: "The dams were observed twice daily for mortality and daily for changes in appearance or behavior."

   Results:

   Mortality: From p. 17: "Four dams from the treated group died or were sacrificed during the course of the study. Two dams were found dead...and these deaths were attributed to compound-toxicity. In addition, one dam died due to a dosing accident... and one dam delivered early and was subsequently sacrificed. All other females survived to scheduled necropsy."

   Symptoms: From p. 17: "Severe signs of compound-related maternal toxicity were observed in dams of the CGA 64250-treated group during the treatment period. The compound-related signs of maternal toxicity observed...included a statistically significant increase in the incidence of ataxia, coma, lethargy, prostration, audible respiration, labored respiration, and salivation when compared to the control group and a biologically significant incidence of ptosis, lacrimation, pale color and death..." From table 6.1 (p. 25) 83/189 dosed animals showed lethargy (compared to 0/178 controls), and 79/189 dosed animals showed ataxia (0/178 controls).

2. Food consumption: Mean food consumption was essentially the same for controls and group II (300 mg/kg/day Propiconazole days 6-15) in the pre-dose period. Group II mean daily food consumption values were significantly lower than controls during the dosage period, and then tended to be higher. The following is from table 6.3, p. 27-28:

Interval (gestation days)	Mean food consumption (gms) ± S.D. during interval Group I (controls)	Group II (dosed)
0-6	145.28 ± 16.50	146.16 ± 15.88
6-7	25.87 ± 7.75	*19.66 ± 7.80
7-8	25.24 ± 4.68	*15.91 ± 6.12
8-9	25.35 ± 4.07	*15.13 ± 6.16
9-10	25.66 ± 4.56	*18.41 ± 6.09
10-11	26.32 ± 4.27	*21.39 ± 6.09
11-12	28.19 ± 5.29	*23.36 ± 6.39
12-13	28.08 ± 4.86	*25.52 ± 5.59
13-14	27.12 ± 5.27	*25.45 ± 5.14
14-15	28.01 ± 4.73	*25.64 ± 5.33
15-16	29.19 ± 4.75	*25.92 ± 5.67
16-17	30.01 ± 4.97	29.15 ± 5.91
17-18	29.45 ± 4.99	30.12 ± 4.29
18-19	28.60 ± 5.36	*31.64 ± 4.72
19-20	28.06 ± 5.11	29.13 ± 5.03

* Reported as significantly ($p<0.05$) different from control value.

3. Dam body weights and gains:

Individual dam body weights were measured on gestation days 0, 6, 8, 12, 16 and 20. Additionally, individual body weights for day 20 less uterus, placentas and fetuses are also reported.

Results:

Body weights and body weight gains during the dosing period were significantly lower in dosed animals than in controls:

From table 6.4 (p. 29):

Day of gestation	Mean body weight (gm) Controls	Dosed
0	249.88 ± 13.88	252.08 ± 16.54
6	278.12 ± 15.28	282.85 ± 18.52*
16	339.02 ± 21.16	324.24 ± 22.64*
20	397.29 ± 27.77	387.10 ± 31.10*
20†	319.71 ± 20.14	309.93 ± 22.28*

† Body weight less uterus, placentas and fetuses
* Reported as significantly ($p < 0.05$) different from control value.

From table 6.5 (p. 31):

Interval in terms of days of gestation	Mean maternal body wt. gain (gm) Controls	Dosed
0-6	28.25 ± 8.16	30.77 ± 8.89*
6-16	60.90 ± 12.93	41.06 ± 13.30*
16-20	58.27 ± 12.92	62.99 ± 16.33*
0-20	147.41 ± 22.12	134.97 ± 25.66*
0-20†	69.82 ± 14.28	57.75 ± 15.73*

† Body weight gains without uterus, placentas and fetuses.
* Reported as significantly ($p < 0.05$) different from control value.

4. Reproductive parameters:

From p. 13: "Dams surviving to term were necropsied on day 20 of presumed gestation. The uteri including their contents were weighed, and corpora lutea, live fetuses, dead fetuses and intrauterine resorption sites were counted. The fetuses were sexed and numbered in order of their positions in the uterus from the ovarian end of the left horn to the ovarian horn of the right horn." Group means for corpora lutea, implantation sites, early resorptions, late resorptions, total resorptions, live fetuses and dead fetuses were calculated, along with % post-implantation losses and fetal sex ratios.

Results:

There were no significant differences between dosed animals and their controls with respect to fetal sex ratio or mean numbers of corpora lutea, implantation sites and dead fetuses. The mean number of live fetuses was significantly ($p < 0.05$) lower in dosed animals, due to somewhat lower means for implantation sites and higher means for total resorptions in the dosed animals, although these values were not significantly different from the controls. From table 6.6, p. 32:

Parameter	Controls	Dosed
Mean no. corpora lutea	16.93 ± 2.73	17.01 ± 3.02
Mean no. implantation sites	14.50 ± 3.17	14.21 ± 3.36
Mean no. early resorptions	0.8 ± 0.95	1.0 ± 1.56
Mean no. late resorptions	0.1 ± 0.64	0.1 ± 0.40
Mean no. total resorptions	0.81 ± 1.11	1.15 ± 1.63
% Post-implantation loss	5.78 ± 8.34	8.58 ± 13.43
Mean no. live fetuses	13.69 ± 3.24	13.06 ± 3.41*

* Reported as significantly ($p < 0.05$) different from control value.

5. Mean fetal body weights: Fetal body weights were collected at necropsy.

Results:

Mean fetal weights for both males and females were significantly lower in dosed animals. The following values were calculated using a weighted analysis (see p. 355-356) to take into account variations in litter sizes. From table 6.7, p. 33:

Parameter	Controls ± S.E.	Dosed ± S.E.
Fetal weight - male (gm)	3.569 ± 0.0258	3.403 ± 0.0257**
Fetal weight - female (gm)	3.387 ± 0.0228	3.232 ± 0.0228**

** Reported as significantly ($p < 0.001$) different from control value.

6. Variations and malformations:

The fetuses were examined for external abnormalities. From p. 13: "All of the fetuses from each litter were injected intraperitoneally with T-61 euthanasia solution, examined for cleft palate, tagged, placed into Bouin's fixative, and subsequently saved in 95% ethyl alcohol." There is no indication that the fetuses were examined other than externally.

Results:

From p. 20: "There were no statistically significant treatment-related, external, gross observations among fetuses in this study.

The following malformation incidences are reported (table 6.8, p. 34):

	Controls	Treated
Agnathia	1/2122	0/2064
Filament tail	2/2122	0/2064
Spina bifida with astroschisis, exencephaly, protruding tongue	1/2122	0/2064
Cleft palate	0/2122	2/2064
No. of litters examined	155	158

The fetuses with cleft palate are identified (p. 34) as fetus #8 of dam 1337 and fetus #5 of dam 1352. From information on p. 334 fetus 8 was a female, one of a litter of 12 (with an additional 2 resorptions), and a fetal weight of 2.44 grams (there was one smaller fetus in this litter, with a weight of 1.75 grams). From p. 338 fetus 5 was a female, one of a litter of 16 (no resorptions), with a fetal weight of 2.90 grams (there were two smaller fetuses in this litter, weighing 2.75 and 2.54 grams).

7. Historical control data - cleft palate:

According to information in Appendix 7.20.1 the incidence of cleft palate in controls for all rat teratology studies (not including this one) conducted at this laboratory 1983-1985 was 0/5431.

D. DISCUSSION:

The purpose of this study was not to satisfy or meet a data requirement, but was to determine whether the finding of a previous study (DER I in this review set) indicating that the test material might cause cleft palate in rat fetuses was correct. This was the reason why there were only two groups of dams, controls and those dosed at 300 mg/kg/day. In the previous study, 1/302 fetuses at 90 mg/kg/day and 2/285 at 360/300 mg/kg/day had cleft palate, but the incidence in control fetuses was 0/5431 for 19 teratology studies at this laboratory.

In this study 0/2122 controls and 2/2064 fetuses from dosed (300 mg/kg/day, days 6-15) dams showed cleft palate. While this incidence is considerably lower than that (2/285) observed previously there is no indication that fetuses in this study were examined other than externally. The two affected fetuses in the previous study were found to have cleft palate on visceral (rather than external) observation. A clarification should be obtained as to whether or not the fetuses were examined only externally for the presence of cleft palate. If so, then they should be more completely examined for this defect.

In contrast to the previous rat teratology study, the fetuses from dams dosed at 300 mg/kg/day had significantly lower weights than did those of controls.

Reviewed by: Byron T. Backus Byron T. Backus 5/20/88 CASWELL FILE
Section 3, Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert Marcia van Gemert 5/24/88
Section 3, Tox. Branch (TS-769C)

DATA EVALUATION REPORT III

STUDY TYPE: Teratology - rabbit

TOX. CHEM. NO: 323EE

ACCESSION NUMBER: 404250-04

MRID NO: not given

TEST MATERIAL: CGA-64250

SYNONYMS: Propicanozole, TILT

STUDY NUMBER(S): Toxicology/Pathology Report 86043 (MIN 852172)
(Supplement to EPA Acc. No. 265796)

SPONSOR: Ciba-Geigy Corporation

TESTING FACILITY: Pharmaceuticals Division
Ciba-Geigy Corporation

TITLE OF REPORT: CGA 64250 Technical - A Teratology Study in New Zealand White Rabbits (Amended)

AUTHOR(S): Raab, D. M., Yourneff, M. A., Giknis, M. L. A., and Yau, E. T.

REPORT ISSUED: 6/16/87

Classification: Core Minimum Data (note: this is the previous review classification of this study; this submission contains additional material relating to the original study).

Special Review Criteria (40 CFR 154.7)

COMMENTS AND CONCLUSIONS:

1. This study was previously reviewed (Phang, 3/10/87 and Taylor, 11/16/87). In the initial review by Phang it was classified as supplementary; subsequently, it was upgraded to minimum by Taylor on the basis of additional material relating to animals which were sacrificed before the study termination.

2. The material in Acc. 404250-04 consists of statistical analyses which incorporate data (previously excluded) from the dams which were sacrificed before study termination, as well as a correction relating to statistically significant increases in body weight in the intermediate and high-dose groups on gestational days 20-24.

3. The material in Acc. 404250-04 do not affect the conclusions of the original DER (Phang, 3/10/87) that: "The NOEL for maternal toxicity was estimated to be 100 mg/kg; LOEL, 250 mg/kg. The NOEL for developmental toxicity was estimated to be 400 mg/kg (HDT)," nor do they affect the re-classification of the study to Core Minimum (Taylor, 11/16/87).

DATA EVALUATION REPORT

STUDY TYPE: Teratology Study in Rabbits.

ACCESSION NUMBER: 265796 Project No. 7-0227

CASWELL NO.: 323EE RECORD NO.: 185584

TEST MATERIAL: CGA 64250 Technical; 92.1% purity

SYNONYMS: Propiconazole; Tilt

SPONSOR: Agricultural Division, CIBA-GEIGY Corp.

TESTING FACILITY: Research Department, Pharmaceuticals Division, CIBA-GEIGY Corp., Summit, NJ.

CITATION: Raab, D.M., Youreneff, M.A., Giknis, M.L.A., et al. (1986). CGA-64250 Technical: A Teratology Study in New Zealand White Rabbits. Report No. 86043 (MIN 852172); CIBA-GEIGY Corp., NJ. (Aug 1, 1986).

REVIEWER: Whang Phang, Ph.D. 3/10/87
Pharmacologist
Toxicology Branch/HED (TS-769C)

SECONDARY REVIEWER: Marcia van Gemert, Ph.D.
Section Head M. van Gemert 3/10/87
and
William Burnam, Deputy Branch Chief
Toxicology Branch/HED (TS-769c)

CONCLUSIONS: Groups (19/group) of pregnant rabbits were administered CGA-62450 at doses of 100, 250, and 400 mg/kg from gestation days 7 through 19. At 250 and 400 mg/kg, the treated animals showed decreased food consumption and body weight gain during the treatment period. At 400 mg/kg, treated rabbits also showed increased incidence of abortion. There was, however, no evidence of developmental toxicity.

Based upon the data, the NOEL for maternal toxicity was estimated to to be 100 mg/kg; LOEL, 250 mg/kg. The NOEL for developmental toxicity was estimated to be 400 mg/kg (HDT).

Classification: Core Supplementary. The report must contain the data on the animals which were sacrificed before the termination of the study, specifically those rabbits which had aborted or delivered early in the study.

A. MATERIALS:

1. Test compound: CGA-64250, Description: not specified. Batch #: FL 850083, Purity: 92.1%.

2. Test animals: Species: rabbits; Strain: New Zealand White; Age: "Sexually" mature; Weight: 6-7 lbs; Source: HARE-MARLAND, Hewitt, NJ.

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned randomly to the following test groups:

Test Group	Dose (mg/kg/day)	No. of Animals female
1	0	19
2 low dose	100	19
3 mid dose	250	19
4 high dose	400	19

2. Dosage Preparation

Appropriate amounts of CGA-64250 were mixed in 0.5% Tween 80 and 3% aqueous corn starch. The chemical analysis of the stability of the test solution was not submitted.

The animals were dosed by gavage.

3. Animals received food and water ad libitum.

4. Mating: Females were artificially inseminated. The day of insemination was designated as day 0 of gestation.

5. All animals were observed daily for clinical signs. They were also weighed on day 0, 7, 10, 14, 20, 24, and 29.

6. Food consumption of the pregnant females was measured daily for gestational days 5 to 29.

7. On day 29 of gestation, females were sacrificed and the fetuses were removed. In addition, the following observations and measurements were made:
   a). Macroscopic abnormalities of the reproductive tract of the dam.
   b). Number of corpora lutea in each ovary.
   c). Weight of gravid uterus.
   d). Numbers of live fetuses and number and distributions of resorption sites in each uterine horn.
   e). Placental weights.
   f). Fetal weights.

g). External abnormalities and sex of each fetus were determined.
h). Each fetus was sacrificed; soft tissue and skeletal abnormalities were examined.

8. Statistics - The following procedures were utilized in analyzing the numerical data: Bartlett's test, Dunnett's method of multiple comparision, and Healy Analysis.

9. Quality assurance: A quality assurance statement was submitted.

C. RESULTS:

Maternal Toxicity

1). Survival Rates: The survival rates were good for all treated and control animals. There were two deaths, one doe from the control and the other from mid dose animals. The death of one of the animals was due to a dosing accident.

In high-dose animals, 5/19 does were sacrificed early due to abortion or early delivery. In the mid dose group, one doe aborted early. One control animal delivered early.

2). Necropsy and Clinical Observations:

Among animals of the high dose group, there was an increased incidence of stool alterations, which could be compound related because similar results were not observed in the corresponding controls. There was also an increased incidence of abortion (Table I). Other parameters were comparable between the control and treated animals.

3). Food Consumption:

During the dosing period (days 7-19), the high and mid dose groups consistently consumed less food. The decrease in food intake was significantly different from that of the controls. Subsequent to the termination of dosing, the food consumption of these animal was increased (Table II).

4). Body Weight:

The body weight gain of the mid and high dose animals was supressed between the gestation days 7-20 (Table III). Similar to food consumption, the body weight gain of the affected groups of animals rebounded to normal after withdrawal of the test compound.

Developmental effects:

The developmental parameters were relatively similar between the controls and treated animals (Table IV). Although there appeared to be an increase in the value of Mean No. Resorption, the apparant increase was heavily influenced by a completely resorbed litter.

Fetotoxicity:

The Mean No. of Live Fetuses and the Mean Fetal Weights were comparable between control and treated groups (Table IV and V).

There were no significant differences in visceral and skeletal malformations between the treated and control fetuses (Table VI, VII, and VIII). Although increased incidence of the formation of 13th rib was observed, this incidence was shown to be associated with the maternal toxicity in other studies (Kavlock et al., 1985).

Discussion:

Based upon the reported data, CGA-62450 Technical at 250 and 400 mg/kg caused decreases in food consumption and body weight in treated does. At 400 mg/kg, the test compound also induced early delivery and abortion in pregnant rabbits. The report has a major deficiency; the data on food consumption, body weight gain, and other parameters of the animals which delivered or aborted early in the study were not presented in the report. These data are important in evaluating the possible maternal toxicity of the test agent, and they should be submitted for review.

The experimental results did not shown any increase in the incidence of cleft lip or palate in treated animals. Based upon the available data, the LOEL for maternal toxicity was estimated to be 250 mg/kg; NOEL, 100 mg/kg. The NOEL for developmental toxicity was estimated to be 400 mg/kg (HDT).

Reference

Kavlock, R.J., Chernoff, N., and Rogers, E.H. (1985). The effect of Acute Maternal Toxicity on Fetal Development in the Mouse. Teratogenisis, Carcinogenesis , and Mutagenesis 5: 3-25 (1985).

MAY 27 1988

NOTE TO: Byron Backus
Review Section III

FROM: Quang Bui

THRU: Marcia van Gemert

Re: Developmental toxicity studies with TILT
Issue on Cleft Palate in rats

Ciba Geigy submitted two developmental toxicity studies with Tilt in rats. In the first study, doses of 0, 30, 90, and 300 mg/kg/day were used whereas in the second study a single dose level of 300 mg/kg/day was used.

L?

Cleft palate was found at a very low incidence (1/302 fetuses at 90 mg/kg/day; 2/285 fetuses at 300 mg/kg/day; 2/2064 fetuses at 300 mg/kg/day in the second study) as compared to 0 incidence in both the concurrent and historical control data.

Both developmental and maternal toxicity NOELs were established at 30 mg/kg/day with both LELs at 90 mg/kg/day.

Issue: Should Tilt be classified as a teratogen based upon the findings of cleft palate.

Cleft palate was found at a very low incidence at maternally toxic doses (90 and 300 mg/kg/day) along with other adverse developmental effects (delayed ossification and altered growth). Is it necessary to classfy this chemical as a teratogen?

1. From a regulatory standpoint, developmental toxicity assessment is based upon all manifestations of developmental toxicity and not solely on malformations since
   a. All types of manifestations are of concern
   b. An adverse effect on development can be manifested differently depending on the species being tested
   c. Distinction between malformations and variations varies among testing facilities and investigators
   d. Current study protocol is designed to investigate developmental toxicity and is not intended to maximize the findings of malformations

2. Developmental toxicity risk assessment takes into consideration the developmental toxicity NOEL (30 mg/kg/day) and not a "teratogenic" NOEL since once an adverse effect is recognized, it is the dose and not the type of adverse effect that is critical.

3. Findings of cleft palate are not truly dose-related

From the data submitted, I concur with your maternal and developmental toxicity NOELs and LELs. Under the conditions of both studies, there is no evidence to indicate that Tilt is a teratogen in rats. Developmental toxicity occurs at 90 and 300 mg/kg/day but in conjunction with maternal toxicity.