Primary Review by: Deborah L. McCall, Review Section, Toxicology Branch II, (H7509C) /HED DMcCall 11-4-90

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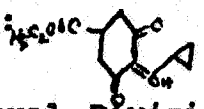
DATA EVALUATION RECORD

Study Type: Teratology study in rats (§83-3)

EPA Identification No.s: EPA MRID (Accession) No.: 415639-23
EPA ID No.:
EPA Record No.:
Caswell No.: 271N
HED Project No.: 0-1874

Test Material: CGA 163935 Technical
Synonyms: 4-(cyclopropyl-α-hydroxy-methylene)-3,5-dioxo-cyclohexane carboxylic acid ethylester.

Chemical Structure:  mw 252.27

Sponsor: Agricultural Division, CIBA-GEIGY Corporation, P.O. Box 18300, Greensboro, North Carolina

Study Number(s): 861128

Testing Facility: CIBA-GEIGY LTD., Experimental Toxicology GU 5, Facility Sisseln, CH-4332 Stein, Switzerland

Title of Report: Developmental Toxicity (Teratogenicity) Study with CGA 163935 Tech. in Rats

Author(s): Dr. M. Schoch

Report Issued: December 20, 1988

Conclusions: CGA 163935 was orally dosed to pregnant rats at dose levels of 20, 200, and 1000 mg/kg (24 rats/group) during gestation days 6-15. At a dose of 1000 mg/kg, CGA 163935 appeared to have effects on developmental toxicity by an increase in asymmetrically shaped sternebra. No other treatment-related effects were noted.

Developmental Toxicity NOEL ≥ 200 mg/kg;

Maternal Toxicity NOEL ≥ 1000 mg/kg, the highest dose tested.

Core Classification: Core minimum

This study satisfies the Guideline requirements (83-3), Developmental Toxicity (Teratology) Study in rats.

1. MATERIALS AND METHODS

A. Test Compound: Purity: 96.6%
Description: solid
Lot No.: P.705002
Stability: within acceptable ranges

1. Vehicle(s): Oleum arachidis (peanut oil)

[Reviewers note: No other information was provided on the vehicle.]

B. Test Animal(s): Species: Rat, female, nulliparous
Strain: Tif: RAIf(SPF), hybrids of RII/1 x RII/2
Source: Animal Production, WST 455, CIBA-GEIGY LTD, 4332 Stein, Switzerland
Age: Approximately 2 months
Weight: 196-200 grams

C. Study Design: This study was designed to assess the developmental toxicity potential of CGA 163935 Technical when administered by the oral route to rats on gestation days 6 through 15, inclusive.

1. Mating: Nulliparous female rats were mated overnight with males of the same stock and of proven fertility at a ratio of 3 females to 1 male in racks containing modified mating cages. Presence of the copulation plug in the vagina or presence of spermatozoa on a vaginal smear was considered as confirmation of copulation and designated as gestation day '0'.

2. Group Arrangement: The mated females were randomized to experimental and control groups by weight stratification. The animals were identified by color code on the tail and placed in groups of 4 per cage.

Test Group	Dose Concentration (mg/mL)	Dose Level (mg/kg)	No. of Rats Assigned
Control	- -	0	24
Low	4	20	24
Mid	40	200	24
High	200	1000	24

3. Dosing: All doses were in a volume of 5 mL/kg of body weight/day. The dosing suspensions were analyzed for concentration

and stability. Dosing was based on daily body weights.

4. Observations: The animals were checked for mortality, signs of abortion, and abnormal conditions daily. Dams were sacrificed on day 21 of gestation. Examinations at sacrifice consisted of: macroscopic pathology, number of corpora lutea, weight of uterus, number and location fetuses (live/dead) in each horn, and number of intrauterine resorptions.

The fetuses were examined by: gross inspection, sex-determination, and individual fetal body weights. Historical control data were provided to allow comparison with concurrent controls.

D. Statistical analysis: A copy of the statistical methods used for the data analysis is attached (Appendix A).

E. Compliance: A Quality Assurance Statement and a Statement of Compliance with FIFkA Good Laboratory Practice Standards were signed and dated December 22, 1988.

2. RESULTS

A. Analyses of Suspensions: Methods and results of analyses were provided. The nominal concentrations for the samples ranged from 91.2 - 98.7% for low (20 mg/kg), 89.1 - 96.4% for mid (200 mg/kg), and 95.2 - 97.5% for the high dose (1000 mg/kg). These concentrations were within acceptable ranges.

B. Maternal Toxicity

1. Mortality: No animals were reported to have died during the study.

2. Clinical Observations: Cage-side observations were 'not remarkable' for the entire study period.

3. Body Weight: The animals were weighed daily. The investigators supplied the following data: group mean and individual animal data.

During study days 11-16; a statistically significant increase in body weights was seen in the 20 mg/kg dose group when compared to the controls (see Table 1). Body weight gain in the 1000 mg/kg dose group was reduced during study periods 0-6 and 0-21, but no effects were noted during the dosing period (6-15). These effects are not compound-related and should be considered of little toxicological significance. The corrected mean body weights are in Table 2.

Table 1: Mean Body Weight Changes (g)

Dose (mg/kg)	Number of Animals[1]	Days of Gestation 0-6	6-11	11-16	16-21	0-21
Control	22	37 ±6.1	32 ±6.0	47 ±4.6	79 ±8.5	195 ±16.5
20	20[2]	35 ±5.5	30 ±5.2	43 ±6.1*	81 ±8.1	189 ±17.8
200	24	34 ±7.5	31 ±6.4	44 ±7.3	77 ±15.6[3]	187 ±28.2
1000	24	34 ±5.9*	30 ±4.0	46 ±5.7	75 ±12.4	184 ±20.4*

[1] = Only females with litters were included;
[2] = Females (45,46,47, & 48) were excluded from the statistics due to defective water bottle on days 3-4;
[3] = Body weights were not measured for animals #'s 69, 70, 71, & 72 due to technical error on Day 19.
* = Significant at $P < 0.05$ (student's t-test).
(Data was extracted from Table 3, pg 37.)

Table 2: Mean Maternal Corrected Body Weight (g)

Dose (mg/kg)	Number of Animals[1]	Body Weight Day 6	Day 21	Gravid Uterus	Corrected Body Wgt[3]	Corrected B. Wgt Gain[4]
Control	22	236	394	122	272 ±14.2	36 ± 8.6
20	20[2]	234	387	120	268 ±14.1	34 ±11.1
200	24	233	385	117	269 ±24.2	36 ±16.0
1000	24	234	384	115	269 ±14.8	35 ±10.9

[1] = Only females with litters were included;
[2] = Females (45,46,47, & 48) were excluded from the statistics due to defective water bottle on days 3-4;
[3] = Body weight on Day 21 minus Gravid Uterus;
[4] = Corrected Body Weight minus Body Weight on Day 6.
± = Standard deviation.

(Data was extracted from Table 4, pg 39 and Appendix 9.1.1, pg 76).

4. Food Consumption: A pelleted, certified standard diet and tap water (plastic bottles) was provided ad libitum. Food consumption was recorded on days 6, 11, 16 and 21 (see Table 3).

A decrease in mean food consumption (statistically significant) was noted in the 20 mg/kg dose group for study days 11-16. This decrease corresponds to the reduced body weight gain seen in the same dose group during the same test days. No compound-related effects were seen and this finding should be considered of little toxicological significance.

Table 3: Food Consumption Data (grams)

Dose (mg/kg)	Gestation Days 0-6	6-11	11-16	16-21	0-21
Control	22.1 ±1.8	22.2 ±1.3	25.0 ±1.6	25.8 ±2.8	23.7 ±1.6
20	21.3 ±0.8	21.2 ±0.9	23.0 ±1.3*	25.7 ±1.1	22.8 ±0.8
200	21.2 ±1.6	22.2 ±1.5	23.4 ±1.3	25.6 ±2.0	23.0 ±1.2
1000	21.6 ±0.7	21.8 ±0.9	24.7 ±1.1	25.9 ±1.1	23.4 ±0.8

(Food consumption was measured by cages, 6/group, with each cage containing 4 rats.)

* = Significant at $P < 0.05$ (student's t-test).

(Data extracted from Table 5, pg 41.)

C. Gross Pathological Observations: No gross pathological differences were seen in the control, 20 or 200 mg/kg dose groups. A small spot was noted on the liver of one rat in the 1000 mg/kg. The spot was attributed to an injury prior to the beginning of the study. This finding does not appear to be compound-related.

D. Cesarean Section Observations: A statistically significant decrease in the mean number of corpora lutea/Dam was noted in the 1000 mg/kg dose group (see Table 4). Also, a nonsignificant decrease was seen in the percent of preimplantation losses in the 1000 mg/kg group. These findings should be considered of little toxicological significance. The number of early resorptions was comparable in all the dose groups, and no late resorptions or aborted fetuses were noted.

The total number of corpora lutea of the 1000 mg/kg dose group was lower (statistically significant) than the control group. However, it is within the range of the historical controls.

E. Developmental Toxicity:

1. External Observations: No external observations were noted in the 200 mg/kg dose group (see Table 5). One fetus in the control group was missing the lower jaw. Four fetuses in the 20 mg/kg dose group had generalized edema (same litter); and the authors considered this finding as incidental. One fetus in the 1000 mg/kg dose group showed fibrous adhesion of the right fore-limb with the tail and position anomaly of the right fore-limb. The skeleton examination of this fetus revealed no abnormality. Therefore, this reviewer agrees with the study author that the adhesion should be attributed to an accidental injury, with the position anomaly being noted as a malformation.

Table 4: Cesarean Section Observations

Dose (mg/kg)	Control	20	200	1000
# Animals Assigned	24	24	24	24
# Nonpregnant	2	0	0	0
Pregnancy Rate (%)	92	100	100	100
Maternal Wastage				
# Died	0	0	0	0
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Total # of Litters	22	24	24	24
Total Corpora Lutea	420	448	448	408
Corpora Lutea/Dam	19.1 ±2.5	18.7 ±3.1	18.7 ±2.8	17.0 ±2.5**
Total Implantations	365	382	386	376
Implantations/Dam	16.6 ±1.5	15.9 ±1.8	16.1 ±1.8	15.7 ±2.0
Total Live Fetuses	351	372	367	359
Live Fetuses/Dam	16.0 ±1.9	15.5 ±2.1	15.3 ±1.8	15.0 ±2.4
Total Resorptions	14	10	19	17
Early	14	10	19	17
Late	0	0	0	0
Resorptions/Dam	0.6 ±0.7	0.4 ±0.7	0.8 ±1.5	0.7 ±0.8
Total Dead Fetuses	0	0	0	0
Fetal Body Weight				
Males/litter	5.7	5.7	5.7	5.7
Females/litter	5.4	5.4	5.3	5.4
Mean Litter Fetal Wgt (g)	5.5	5.5	5.5	5.5
Preimplantation Loss (%)[a]	12.4	13.7	12.9	8.3
Postimplantation Loss (%)[a]	4.0	2.8	4.6	4.9
(%) Male/Litter	49.0 ± 11.3	47.7 ± 14.4	48.5 ± 8.2	46.9 ±13.1

[a] = Calculated on a per litter basis.
** = Significant at P < 0.01 (student's t-test).

(Data extracted from Table 7, 8 & 9 pg 48-51).

Table 5: External Examinations

Dose (mg/kg)	0	20	200	1000
Litters examined	22	24	24	24
Fetuses examined	351	372	367	359
Malformations				
Missing mandibula				
affected fetuses	1[a]	0	0	0
(%)	0.3	-	-	-
affected litters	1	0	0	0
(%)	4.5	-	-	-
Generalized edema				
affected fetuses	0	4[b]	0	0
(%)	-	1.1	-	-
affected litters	0	1	0	0
(%)	-	4.2	-	-
Adhesion & position anomaly of right fore-limb				
affected fetuses	0	0	0	1[c]
(%)	-	-	-	0.3
affected litters	0	0	0	1
(%)	-	-	-	4.2

[a] = fetus also showed skeletal malformation;
[b] = 2/4 fetuses also showed visceral anomalies and 1/4 showed skeletal malformation;
[c] = fetus also showed skeletal malformation.

(Chi-square test (1% confidence level) was performed on the above data and it was not significant. Data was extracted from Table 11, pg 53.)

2. Visceral Examination: One third of the fetuses/litter were examined by the Wilson technique (see Table 6). No malformations were noted in the control, 200 or 1000 mg/kg dose groups. Two of the fetuses with generalized edema in the 20 mg/kg dose group were processed for the visceral examination. The exam revealed the edema was localized in the subcutaneous area. No visceral anomalies were noted in the control, 20 or 200 mg/kg dose groups. In the 1000 mg/kg group one fetus had hypoplasia of the testicle (left). Also, blood was found in the orbital area of one fetus in the 200 mg/kg group. No visceral findings were reported for concurrent historical controls.

Table 6: Visceral Examinations

Dose (mg/kg)	0	20	200	1000
Litters examined	22	24	24	24
Fetuses examined	117	124	122	120
Malformations				
Subcut. edema				
affected fetuses	0	2	0	0
(%)	-	1.6	-	-
affected litters	0	1	0	0
(%)	-	4.2	-	-
Anomalies				
Hypoplasia of testicle				
affected fetuses	0	0	0	1
(%)	-	-	-	0.8
affected litters	0	0	0	1
(%)	-	-	-	4.2

(Data was extracted from Table 13, pg 58.)

(Chi-square test (1% confidence level) was performed on the above data and it was not significant.)

3. Skeletal examination: Skeletal assessment was performed on two thirds of the fetuses/litter using the Dawson staining technique (see Table 7). No malformations were noted in the 200 or 1000 mg/kg dose groups. In tne control group one fetus was missing the mandibula and had a bipartite occipital bone. One fetus in the 20 mg/kg group had an accumulation of skeletal anomalies and another had reduced ischium. The authors stated 'no significant differences' in the incidences or type of skeletal anomalies were noted for the test groups when compared with controls. The asymmetrically shaped sternebra in the 1000 mg/kg group are outside of the historical controls; i.e. 29.2% (litter) vs 15.08%, historical controls (see Appendix B).

F. Discussion/Conclusions:

1. Maternal Toxicity: No evidence of maternal toxicity was seen at the limit dose (1000 mg/kg).

Maternal Toxicity NOEL ≥ 1000 mg/kg, the highest dose tested.

2. Developmental Toxicity: At a dose of 1000 mg/kg, CGA 163935 appeared to have effects on developmental toxicity by an increase in the asymmetrically shaped sternebra. No other treatment-related effects were noted.

Table 7: Skeletal Examinations

Dose (mg/kg)	0	20	200	1000
Litters examined	22	24	24	24
Fetuses examined	234	248	245	239
Missing Mandibula & bipartite occipital bone				
affected fetuses	1	0	0	0
affected litters	1	0	0	0
Reduced ischium				
affected[a]	0	1/1	0	0
(%)	-	4.2	-	-
Accumulation of thoracic anomalies				
affected	0	1/1	0	0
(%)	-	4.2	-	-
Sternebra:				
Fused				
affected	4/3	6/4	6/5[b]	1/1
(%)	13.6	16.7	20.8	4.2
Asymmetrically shaped				
affected	2/2	4/4	5/4	8/7
(%)	9.1	16.7	16.7	29.2
Fragmentary				
affected	-	-	-	1/1
(%)	-	-	-	4.2
Bipartite				
affected	-	-	2/2	-
(%)	-	-	8.3	-
Bipartite thoracic vertebral centers				
affected	1/1	1/1	-	-
(%)	4.5	4.2	-	-
Wide fontanel				
affected	-	-	1/1	-
(%)	-	-	4.2	-

[a] = Fetuses per litter;
[b] = Some observations may be grouped together;
% = Percent per litter;
(Data was extracted from Table 15, pg 63-64.)

(Chi-square test (1% confidence level) was performed on these data.)

a. Deaths/Resorptions: No treatment-related effects were noted.

b. Altered Growth: No treatment-related effects were noted.

c. Developmental Anomalies: An increase in the asymmetrically shaped sternebra appeared to be a treatment-related effect in the 1000 mg/kg group. The litter and fetal incidence of this finding was outside of historical control values for this laboratory.

d. Malformations: No treatment-related effects were noted.

Developmental Toxicity NOEL ≥ 200 mg/kg.

G. Study Deficiencies: The footnotes are missing or mislabeled for Table 7, pg 48.

H. Core Classification: Core Minimum

Page ___ is not included in this copy.

Pages 11 through 16 are not included in this copy.

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