8-14-92

DOC 930131

FINAL

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DATA EVALUATION REPORT

CIMECTARB

Study Type: Developmental Toxicity

Prepared for:

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Office of Pesticide Programs
U.S. Environmental Protection Agency
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Date: 8/20/92

DATA EVALUATION REPORT

STUDY TYPE: Developmental toxicity

EPA IDENTIFICATION NUMBERS

PC Code: 112602

MRID No.: 418695-24

TEST MATERIAL: CGA 163935

SYNONYM: Cimectarb

SPONSOR: Agricultural Division, Ciba-Geigy Corporation, P.O. Box 18300, Greensboro, NC

STUDY NUMBER: CBG 484/891971

TESTING FACILITY: Huntingdon Research Center, Ltd., Cambridgeshire, England

TITLE OF REPORT: Developmental Toxicity (Teratogenicity) Study with CGA 163935 Technical in Rabbits

AUTHOR: E. Hughes

REPORT ISSUED: June 26, 1990

CONCLUSIONS: A developmental toxicity study was conducted in which New Zealand White rabbits were administered CGA 163935 via gavage at 0, 10, 60, or 360 mg/kg/day on gestational days (GD) 7-19, inclusive. Maternal toxicity was not observed in chis study. Therefore, the maternal NOEL was >360 mg/kg/day.

Developmental toxicity was observed at 360 mg/kg/day and manifested as a decreased number of live fetuses per litter. Consequently, the NOEL and LOEL for developmental toxicity were 60 and 360 mg/kg/day, respectively.

CLASSIFICATION: Core Minimum Data. This study meets the minimum requirements set forth under EPA Guideline Series 83-3 for a developmental toxicity study in rabbits.

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A. MATERIALS

1. Test Compound

Purity:	96.6%
Description:	Dark brown liquid
Batch number:	P.705002
Receipt date:	November 30, 1988
Contaminants:	Not reported

2. Vehicle:

2% Aqueous methylcellulose (source not reported)

3. Test Animals

Species:	Rabbit
Strain:	New Zealand White
Source:	Interfauna UK, Ltd., Huntingdon, Cambridgeshire, England
Age:	16-24 weeks
Weight:	3.4-4.0 kg

B. STUDY DESIGN

This study was designed to assess the potential of CGA-163935 to cause developmental toxicity in rabbits when administered daily via gavage from GDs 7-19, inclusive.

Insemination: Females were mated with untreated male rabbits of proven fertility used specifically for breeding. Following coitus, the does were injected with 25 I.U. of luteinizing hormone (Chorulon®) to ensure ovulation. The day of mating was designated day 0 of gestation.

Animal husbandry: Food (SDS Standard Rabbit Diet SQC) and tap water were available ad libitum throughout the study. A 14/10-hour light/dark cycle was maintained. Temperature and humidity ranges were 17-23°C and 37%-75%, respectively.

Group arrangement: Animals were assigned to dose groups using a sequential numbering system as follows:

Test Group	Dose Level (mg/kg/day)	Number Assigned per Group
Control	0	16
Low dose	10	16
Mid dose	60	17
High dose	360	17

Dose administered: Doses were administered daily via gavage from GD 7 through 19 in a volume of 5 ml/kg. The GD 7, 9, 11, and 15 recorded body weights were used to calculate the concentration of the doses. Dosing suspensions were prepared daily and stirred continuously prior to and during dosing. Homogeneity, stability, and concentration of the dosing suspensions were analyzed on samples taken from day 1 of dosing from all dose groups.

Dose rationale: Concentrations of the doses were selected based upon a preliminary study (CBG/493 tox number 891161) in which pregnant does were administered CGA-163935 via gavage at dose levels of 0, 40, 400, or 800 mg/kg/day. Maternal toxicity was reported at 40 mg/kg/day and above and characterized as follows. At 800 mg/kg/day, 4/6 does died. At 400 mg/kg/day, one doe died; there was a slight increase in the incidence of cold ears postdosing; there was a transient depression in food intake on GDs 7-9; and there was a transient marked weight loss to GD 9. At 40 mg/kg/day, there were a slight increase in cold ears postdosing and transient weight loss to GD 9 (3/5 animals). (Reviewer's note: Due to the lack of maternal effects noted in the main study, this reviewer believes that a dose of ≥400 mg/kg/day, instead of 360 mg/kg/day, should have been used in the main study.)

Observations: Animals were observed daily for mortality and signs of toxicity. Body weight and food consumption data were recorded on GDs 1, 5, 7, 9, 11, 15, 20, 24, and 29. On GD 29, does were sacrificed by cervical dislocation and litters were delivered by cesarean section. Examination of the does at sacrifice included the following:

- Gross pathology observations
- Number of corpora lutea
- Number of implantation sites
- Numbers of resorptions (early and late) and live and dead fetuses

Examination of all live fetuses included the following:

- Individual fetal weight
- External anomalies
- Visceral anomalies and sex determination
- Brain anomalies (After sacrifice and fixation in 740P industrial methylated spirits, the head was sliced through the line of the frontoparietal suture)
- Skeletal anomalies using a modification of Dawson's technique (1926)
- Suspected abnormalities were examined by alternative procedures (e.g., microdissection and histopathology).

Statistical analysis: The following methods were used:

- Litter size, pre- and post-implantation loss, litter weight, mean fetal weight, and incidences of anomalies--Kruskall-Wallis and Jonckheere test for an ordered series of treatments.

Compliance

- A signed Statement of No Data Confidentiality Claim, dated January 11, 1991, was provided.
- A signed Statement of Compliance with US EPA, OECD, United Kingdom, and Japanese MAFF GLPs, dated June 26 and July 3, 1990, was provided.
- A signed Quality Assurance Statement, dated October 5, 1990, was provided.

C. RESULTS

Test Material Analysis

Analyses conducted on dosing suspensions for concentration revealed values from 88% to 96% of target. Stability of the test compound in the vehicle after 1 hour in the dark at room temperature revealed values from 99% to 101% of target. Stability analyses after 4 and 24 hours rendered values that were outside the acceptable range of ±10% of target. Homogeneity of the dosing suspensions revealed values from 95% to 100% of target. However, homogeneity was not achieved following 4- and 24- hours storage of the dose suspensions and therefore dosing was conducted within 1 hour of dose preparations.

Maternal Toxicity

Mortality: At 60 mg/kg/day, one doe was sacrificed following a gavage error on GD 8. At 360 mg/kg/day, one pregnant doe (#412) was found dead on GD 13; necropsy revealed convulsion prior to death. A second pregnant doe (#403) in this dose group was sacrificed on GD 24 following abortion on GD 22 and severe weight loss; necropsy revealed hemorrhagic depressions in the stomach. Clinical signs and weight loss and subsequent death in this doe are suspected to be related to treatment. In the preliminary study, one pregnant rabbit died at 400 mg/kg/day, with a few hemorrhagic depressions in the stomach and the 800 mg/kg/day does that died were noted with this same clinical finding.

Abortion: No compound-related abortions were observed. Three abortions were noted, one in the control group on GD 27; the second at 60 mg/kg/day (day of abortion not reported); and the third (#403) at 360 mg/kg/day on GD 22 (killed on GD 24 due to severe weight loss).

Clinical observations: No compound-related clinical signs were observed in the does that survived the study. The following signs were noted at similar incidences in all dose groups: alopecia; cold ears post-dose; few/no soft feces; nervousness postdosing; and off-feed.

Body weight: No compound-related effect was observed in body weight or body weight gain in the does that survived the study. A summary of maternal body weight gain data for selected intervals is presented in Table 1. The reviewers calculated and reanalyzed (ANOVA on untransformed data for significant dose group effect; Dunnett's test for group comparisons) the body weight data to present summary data for the pre-dosing, dosing, post-dosing and gestational periods. Body weights on GD 1, 7, 20, and 29 did not differ significantly from controls (data not shown).

Corrected body weight gain was not reported and could not be calculated since gravid uterine weight data either was never recorded or provided in the study report.

Food consumption: No compound-related effect was observed in food consumption in the does that survived the study. A summary of food consumption data (g/animal/day) is presented in Table 2. The reviewers calculated and reanalyzed (ANOVA on untransformed data for significant dose group effect; Dunnett's test for group comparisons) the food consumption data to present summary data for the pre-dosing, dosing, postdosing, and gestational periods.

Gross pathology observations: No compound-related gross pathology findings were observed in the does that survived the study. Incidental findings included short tail; cortical scarring of the kidney; and serosanguineous fluid present around vagina.

Cesarean section observations: Compound-related effects were observed at 360 mg/kg/day. A summary of cesarean section data is presented in Table 3. Significant trends for decreased number of live fetuses/litter and increased percent of pre- and post-implantation losses were observed at 360 mg/kg/day. Group comparisons with controls for these parameters did not reach significant levels. Pre-implantation was completed before the start of treatment and consequently, the increase in this parameter was not caused by the test compound. The decreased number of live fetuses, however, was considered to be treatment-related. The historical control incidences for postimplantation loss were 5.7%-15.5% and the number of live fetuses/litter were 6.1%-9.5%; both of these effects in this study were outside of these ranges.

Developmental Toxicity

No compound-related effects were observed at any dose level. Incidences of external, visceral, and skeletal malformations are presented in Table 4.

External examinations: At 0, 10, 60, and 360 mg/kg/day, 3 (3 litters), 2 (2 litters), 5 (4 litters), and 1 fetus(es), respectively, exhibited a single external malformation or multiple external malformations (Table 4). No variations were noted.

Visceral examinations: At 0, 10, 60, and 360 mg/kg/day, 1, 2 (2 litters), 3 (2 litters), and 1 fetus(es), respectively, exhibited a single visceral malformation or multiple visceral malformations (Table 4). Variations

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TABLE 1. Mean Body Weight Gain (g ± S.D.)[a,b,c]

Dose Group (mg/kg/day)	Prior to Dosing Period (GD 1-7)	Dosing Period (GD 7-20)	Post-Dosing Period (GD 20-29)	Gestation Period (GD 1-29)
0	56.9 ± 82.3	122.3 ± 120.8	207.7 ± 115.6	386.9 ± 234.7
10	52.5 ± 130.9	130.6 ± 54.5	233.8 ± 101.9	416.9 ± 108.2
60	120.0 ± 80.8	146.4 ± 106.5	200.0 ± 80.9	466.4 ± 138.6
360	105.7 ± 114.6	125.7 ± 165.0	167.9 ± 104.5	399.3 ± 233.4

[a]Data were extracted from Study No. CBG 484/891971, Appendix 4.

[b]Calculated and analyzed by the reviewers.

[c]Animals which were killed, aborted, or were not pregnant were excluded from the analysis.

TABLE 2. Mean Food Consumption (g/animal/day ± S.D.)[a,b,c] 009711

Dose Group (mg/kg/day)	Prior to Dosing Period (GD 1-6)	Dosing Period (GD 7-19)	Post-Dosing Period (GD 20-28)	Gestation Period (GD 1-28)
0	153.2 ± 22.2	146.7 ± 22.8	136.8 ± 28.2	144.9 ± 19.6
10	149.5 ± 33.6	141.0 ± 23.8	139.1 ± 25.4	142.2 ± 18.1
60	155.7 ± 33.9	159.5 ± 25.2	150.3 ± 31.5	155.7 ± 24.9
360	148.8 ± 30.3	145.1 ± 36.6	143.0 ± 39.7	145.2 ± 32.3

[a]Data were extracted from Study No. CBG 484/891971, Appendix 3.

[b]Calculated and analyzed by the reviewers.

[c]Animals which were killed, aborted, or were not pregnant were excluded from the analysis.

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TABLE 3. Cesarean Section Observations[a]

Parameter	Dose Level (mg/kg/day) 0	10	60	360
No. animals mated	16	16	17	17
No. animals pregnant	14	16	16	16
Pregnancy rate (%)	88	100	94	94
Maternal wastage				
No. died/nonpregnant	0	0	0	0
No. died, sacrificed/pregnant	0	0	1	2
No. nonpregnant	2	0	1	1
No. aborted	1	0	1	1[b]
No. premature delivery	0	0	0	0
No. live litters on GD 29	12[c]	16	14	14
Total corpora lutea[d]	134	174	152	143
Corpora lutea/doe	10.3 ± 2.7[e,f]	10.9 ± 1.8	10.9 ± 1.9	10.2 ± 1.3
Total implantations[d]	114	147	127	107
Implantations/doe	8.8 ± 2.7[f]	9.2 ± 2.8	9.1 ± 2.0	7.6 ± 2.4
Total live fetuses[d]	92	134	98	80
Live fetuses/doe	7.7 ± 2.8[f]	8.4 ± 2.6	7.0 ± 2.3	5.7 ± 2.7*
Total resorptions[d]	22	13	29	27
Early resorptions	7	3	19	14
Late resorptions	15	10	10	13
Resorptions/doe	1.7 ± 2.3	0.8 ± 1.0	2.1 ± 2.3	1.9 ± 1.9
Total dead fetuses	0	0	0	0
Dead fetuses/doe	0	0	0	0
Fetal weight/litter (g)	44.4 ± 5.6	43.8 ± 3.7	47.0 ± 6.1	45.2 ± 6.6
Preimplantation loss (%)	14.3	16.5	16.2	24.3*
Postimplantation loss (%)	13.2	8.1	21.4	24.8*
Sex ratio (% male)	40.9	56.9	53.5	56.7

[a]Data were extracted from Study No. CBG 484/891971, Tables 1 and 4 and Appendix 5.

[b]Animal aborted and was then killed due to severe weight loss.

[c]One doe had 100% resorptions.

[d]Calculated by the reviewers

[e]Mean ± S.D. (S.D. calculated by the reviewers)

[f]Includes one doe with 100% resorptions

*Significant decreasing or increasing trend ($p<0.05$)

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TABLE 4. Incidences of Fetal Malformations[a] 009711

Findings[b]	Dose Level (mg/kg/day) 0	10	60	360
No. fetuses (litters) examined	92 (12)	134 (16)	98 (14)	80 (14)
External Malformations				
Scoliosis	1	1	2	0
Hydrocephaly	1	0	1	0
Left forelimb flexure	1	0	0	1
Left fore limb brachydactyly	0	0	1	0
Spina bifida	0	1	1	0
Irregular white area at back of eye	0	1	0	0
Total No. fetuses (litters) with any external malformation(s)	3 (3)	2 (2)	5 (4)	1
Visceral Malformations				
Retro-oesophageal right subclavian artery	1	1	1	1
Dilated ascending aorta/aortic arch	0	1	0	0
Narrow pulmonary trunk	0	1	0	0
Interventricular septal defect	0	2	2	0
Diaphragmatic hernia	0	1	0	0
Subcutaneous edema	0	1	0	0
Atelectasis	0	1	0	0
Transposition of ascending aorta	0	0	1	0
Displaced pulmonary trunk	0	0	1	0
Cardiovascular irregularities	0	0	1	0
Total No. fetuses (litters) with any visceral malformation(s)	1	2 (2)	3 (2)	1
Skeletal Malformations				
Branched/absent/misaligned ribs	1	0	0	0
Fusion/reductions of ribs	0	1	0	0
Fused vertebral arches	0	0	1	0
Fused/flattened sternebrae	0	1	0	0
Flattened cranium	0	1	1	0
Total No. fetuses (litters) with any skeletal malformation(s)	2 (2)	1	3 (3)	1
Total No. fetuses (litters) with any malformation(s)	4 (4)	3 (3)	7 (5)	2 (2)

[a]Data were extracted from Study No. CBG 484/891971, Tables 5a, 5b, and 5c and Appendix 6b.

[b]More than one type of anomaly may be found in one fetus.

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included iridial hemorrhage, cardiovascular irregularities, dilated pulmonary trunk, liver cysts, and absent/bilobed/bifurcated gallbladder; these were noted in all dose groups (data not shown).

Skeletal examinations: At 0, 10, 60, and 360 mg/kg/day, 2 (2 litters), 1, 3 (3 litters), and 1 fetus(es), respectively, exhibited multiple skeletal malformations (Table 4). Variations were noted in the cranial region (jugular, maxilla, and sutural bones); cervical region (arches, ribs, and centra); and thoracic regions (ribs, sternebrae, arches, and centra). These variations were noted in all dose groups (data not shown). Incomplete ossification was noted at similar incidences in all dose groups (data not shown)

D. DISCUSSION/CONCLUSIONS

Acceptance Criteria

The reviewers have completed an Acceptance Criteria check list (Attachment I) to be included with the evaluation of the study. Criterion number 3 (maternal toxicity) was not fulfilled. All other criteria were satisfied.

Test Material Analyses

Analyses of concentration, stability, and homogeneity of the test material in the vehicle were within ±10% of target with one exception; the concentration of the low-dose group was 88% of the nominal value. This did not impact negatively on the outcome of the study.

Maternal Toxicity

No compound-related maternal toxicity was observed in this study. One of the two mortalities in the high-dose group revealed hemorrhagic depressions in the stomach at necropsy. A similar finding was observed in the animals that died at 800 mg/kg/day (4/6 animals) and at 400 mg/kg/day (1/6 animals) in a range-finding study and was considered to be due to the test compound. In the present study in the absence of other affected end-points, the reviewers do not consider the death of one animal at the high-dose level to be sufficient evidence for designation of a LOEL and NOEL. The range-finding study demonstrated that 800 mg/kg/day exceeded the maximum tolerated dose (MTD); while 400 mg/kg/day did not reach a clear MTD with significant body weight changes, increased clinical signs, and/or necropsy findings. The present study also demonstrated that exposure to 360 mg/kg/day of Cimectarb does not produce maternal toxicity. This reviewer believes that a higher dose could have been tolerated by the rabbits.

Based on a lack of maternal toxicity, the maternal NOEL was >360 mg/kg/day.

Developmental Toxicity

Deaths/resorptions: A significant trend towards decreasing number of fetuses/litter and increasing percent of post-implantation loss with increasing dose were observed. At 360 mg/kg/day, these endpoints were 14% decreased and 88% increased, respectively, above/below controls. Although

neither parameter reached a significant level upon group comparisons, they were considered to be effects of the test compound. In the range-finding study at 400 mg/kg/day, no such effects were reported which may be explained by a small number of litters evaluated in that study. In comparison with historical control data, these endpoints were outside of the laboratory's historical control range.

Altered growth: No compound-related effects were observed.

Developmental anomalies: No compound-related effects were observed.

Based on the decreased number of live fetuses/litter, the NOEL and LOEL for developmental toxicity were 60 and 360 mg/kg/day, respectively.

Study/Reporting Deficiencies

No protocol was submitted.

The uteri of apparently nonpregnant animals were not stained for detection of early embryonic death, and consequently, the reported pregnancy rate may or may not be correct.

Gravid uterine weight data was not recorded/reported.

Sufficient analyses of the data were not presented (i.e., standard deviation were often missing and body weight and food consumption data were not summarized for appropriate intervals). The reviewers calculated standard deviations and created summary tables for body weight gain and food consumption data.

E. CORE CLASSIFICATION: Core Minimum Data.

Maternal NOEL = >360 mg/kg/day
Maternal LOEL = Not determined

Developmental Toxicity NOEL = 60 mg/kg/day
Developmental Toxicity LOEL = 360 mg/kg/day (decreased number of live fetuses per litter)

ATTACHMENT I

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83-3 Teratology Studies

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1.	YES	Technical form of the active ingredient tested.
2.	YES	At least 20 pregnant animals/dose group for mice, rats, or hamsters are available. At least 12 pregnant animals/dose group for rabbits are available (three test groups and control group).
3.	NO	At the high dose, overt maternal effects such as slight weight loss are reported (or a limit dose is given, 1,000 mg/kg).
4.*	YES	At the low dose, no developmental toxicity is reported.
5.	YES	Dosing duration is at least during the period of major organogenesis, but may extend up to one day prior to term.
6.*	YES	Analysis for test material stability, homogeneity, and concentration in dosing medium.
7.	YES	Individual daily observations.
8.	YES	Individual body weights.
9.	YES	Individual food consumption.
10.	YES	Necropsy on all animals.
11.	YES	Individual uterine examination, including numbers of fetal deaths, early and late resorptions, and viable fetuses per sex.
12.	YES	All ovaries examined to determine number of corpora lutea.
13.	YES	Individual litter weights and/or individual fetal weights/sex/litter.
14.	YES	Individual fetal external examination.
15.	YES	Individual fetal skeletal examination for 1/3 to 1/2 of each litter for rodents and all for rabbits.
16.	YES	Individual fetal soft tissue examination.

Criteria marked with a * are supplemental, may not be required for every study