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

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JUL 11 1984

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Rereview of Rat Teratology and Rat Range Finding Study of CGA-72662 Technical. CASWELL# ~~168B~~ 168B

TO: Herb Harrison, Branch Chief
Insecticide and Rodenticide Branch
Registration Division (TS-767)

FROM: Stephen C. Dapson, Ph.D.
Pharmacologist, Section V
Toxicology Branch/HED (TS-769C)

Stephen C. Dapson 7/6/84

THRU: Laurence D. Chitlik, DABT
Section Head, Section V
Toxicology Branch/HED (TS-769C)

LDC 7/10/84

Bdd 7/11/84

Action Requested: As requested the following is the rereview of the rat teratology study and rat range finding study for CGA-72662 Technical.

Recommendation: The pilot teratology study (IRDC#382-069) appeared adequate to establish dosing levels in the primary study although some essential data for complete assessment was not submitted for review (see page 4). This study is classified as Core Supplementary Data.

The primary rat teratology study (IRDC#382-070) is also classified as Core Supplementary Data. Additional information (listed on page 12 of this review) is requested. Available data, however, supports a tentative NOEL of 100 mg/kg for maternal toxicity. Fetotoxicity was demonstrated at all dose levels (NOEL for fetotoxicity < 100 mg/kg). No teratogenic potential was evident at dose levels tested (100, 300 and 600 mg/kg) however additional data is requested in order to complete this assessment.

Due to some unusual findings reflected in the submitted data (see Table 3, page 8, high dose and control values), a lab audit was requested. Summary results of the lab audit now available as per the memo of L. Chitlik, July 5, 1984, indicated that these identical findings should be considered due to a chance occurrence.

As noted in the conclusion section of the study review, additional data should be requested. As per personal communication with L. Chitlik and the July 5, 1984 memo, I have been informed that the requested data are available in the raw data at IRDC and should be forewarded to the Agency.

Study Identification:

Study I

Study Title: Pilot Teratology Study in Rats

Sponsor: Ciba-Geigy Corporation

Testing Laboratory: International Research and Development Corporation (IRDC)
Mattawan, Michigan 49071

Study Number: 382-069

Date: August 7, 1979

Data Review:

Materials and Procedures: A copy of the procedures section from the original report is appended.

There was no mention of analysis of the dosage dilutions to verify if the actual concentration of the test compound was present. Also no mention of the timing of the dosing (time of day) and whether it was kept constant.

No data collection for food consumption was mentioned.

Results:

Maternal observations: All rats in the 2500 mg/kg/day dosage group died by gestation day 8. The cause of death was not determined. In the 1500 mg/kg/day dosage group, 2 rats died, one each on day 8 and 20 respectively. The cause of death was also not determined in these cases. Although the investigators stated that necropsy was conducted on the rats that had died, no gross pathological findings were provided.

An increase in "activity" was occasionally seen soon after test article administration in the 1500 and 2500 mg/kg/day dosage groups.

The 1000 and 1500 mg/kg/day dosage group presented with an increase in oral discharge, matting of the haircoat around the mouth and matting and staining of the abdominal and anogenital haircoat. The 600 mg/kg/day dosage group presented with an increase in oral discharge and matting of the haircoat around the mouth. No apparent appearance or behavioral changes occurred in the 300 mg/kg/day dosage group. No individual data was supplied for any of the above observations and the above information was extracted from the text of the report. Also the oral discharge was not further described nor was the "increase in activity" further characterized.

The dosages used in this pilot study affected the maternal weight gain in a dose related manner (Table 1) with the 1000 and 1500 mg/kg/day dosage groups showing the greatest reduction in weight gain. All animals in the 2500 mg/kg/day dosage group died 2 days after the start of the dosing period.

There was no mention of post-mortem findings in the mothers, other than uterine observations.

TABLE 1

Pilot Teratology Study in Rats
Group Mean Maternal Body Weights (grams) and Body Weight Change
CGA-72662 Technical (mg/kg/day)

Days of Gestation	0	300	600	1000	1500
0	242	246	238	245	244
6	274	280	264	274	267
9	285	280	269	277	269
12	299	297	279	283	278
16	326	325	300	298	288
20	390	380	356	305	277
Days of Gestation					
0-6	32	34	26	29	23
6-9	11	0	5	3	2
9-12	14	17	10	6	9
12-16	27	28	21	15	10
16-20	64	55	56	7	-11
0-20	148	134	118	60	33
6-16	52	45	36	24	21

Data extracted from IRDC Study #382-069 Table 1.

Fetal Observations: No statistically significant differences were seen in mean values for viable fetuses, late and early resorptions, postimplantation loss, total implantations or corpora lutea in the 300, 600 and 1000 mg/kg/day dosage groups. The 1500 mg/kg/day dosage group presented with a decreased mean of viable fetuses and total implantations and increased means of late resorptions and postimplantation loss. However, it should be emphasized that data for only 3 dams were available at 1500 mg/kg/day. Also a decrease in mean implantation/dam would suggest early dosing prior to the completion of implantation. In this dose group, the mean values were primarily affected by 1 dam (#21048) which had only 4 implantations and 4 late resorptions.

Conclusions: Severe toxicity was observed in the 2500 mg/kg/day dose group. At the 1500 mg/kg/day dosage level significant reduction in maternal weight gains and other observed clinical signs of toxicity (including 2 mortalities) may preclude use of this level in a definitive teratology study as well. Reported results at the 300, 600 and 1000 mg/kg/day dosage levels would potentially support use of these levels and below in primary teratology studies.

It is recommended that this study be considered adequate as a range finding study for rat teratology studies.

Core Classification: Core Supplementary Data since it is only a range finding study. The report lacks: necropsy data on the animals that died; post-mortem findings on dams other than uterine observations; individual clinical observation data on dams.

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Study II

Study Title: Teratology Study in Rats

Sponsor: Ciba-Geigy Corporation

Testing Laboratory: International Research and Development Corporation (IRDC)
Mattawan, Michigan 49071

Study Number: 382-070

Date: December 21, 1979

Data Review:

Materials and Procedures: A copy of the procedures section from original report is appended.

In general, the procedures which were followed approximated the 1978 Proposed Guidelines and therefore are acceptable. The following additional comments should be noted:

It was not stated whether or not an analysis of the dosage solutions was carried out to verify the actual concentration of the test compound present. The animals were dosed on days 6 through 19 of gestation rather than days 6 through 15, the period of major organogenesis. The timing (time of day) of the dosing was not mentioned.

Apparently, food consumption was not determined in the study. These data would have been helpful is assessing maternal toxic effects.

Fetal length determinations were not mentioned nor internal confirmation of the sex of the fetuses.

Results:

Maternal observations: The investigators stated that "biologically meaningful differences in behavior and/or appearance were noted in all treatment groups when compared to the control group."

The high dose group presented with a red nasal discharge and an increase in activity during the first portion of the treatment period in all animals 1 1/2 hours after dosing. Midway through the treatment period, on several days, a clear oral discharge and inactivity were observed. The oral discharge was noted in 11 rats for several days during subsequent dosing. An increase in matting and staining of the anogenital haircoat was noted.

The mid-dose group had a red nasal discharge during the first portion of the treatment period in all animals 1 1/2 hour after dosing. Near the end of the treatment period a clear oral discharge was present in 5 rats prior to dosing on several days. An increase in matting and staining of the anogenital haircoat was noted.

The low dose group had a clear oral discharge in 2 rats on 2 separate days.

Other observations included "occasional hair loss, anal discharge, soft stool and dry red matter in the region of the mouth, forelimbs and/or nose, were noted in similar frequency in all groups."

All of the dams survived. "At sacrifice, hydronephrosis was noted in two dams, one each in the control and 600 mg/kg/day dosage groups (Dam #26962 and #27027, respectively)." Other than these stated findings and the uterine findings, no other post-mortem data on the mothers was presented.

No individual data on the clinical appearance and behavioral observations in the dams was furnished and the above information was derived from the text of the report. The investigators did not determine what the red nasal discharge or the clear oral discharge was, nor did they further characterize the "increased activity" or "inactivity" which they observed.

As can be seen in Table 2, there is no significant difference in maternal weight gain in the low dose group as compared to the control group. The mid dose group showed a slight reduction in overall weight gain (days 0 to 20) whereas the high dose group showed a reduction in both overall weight gain and weight gain during the dosing periods (days 6 to 19, see 6-20 on Table) and period of organogenesis (days 6 to 15, see 6-16 on Table). The first 3 days of treatment (day 6 to 9) showed the greatest reduction in weight gain.

The investigators did not provide any food consumption data with which to correlate these weight changes.

TABLE 2

Teratology Study in Rats
Summary of Group Mean Maternal Body Weights and
Body Weight Change (grams) CGA-72662 Technical (mg/kg/day)

DAY	CONTROL	100	300	600
0	269	270	266	271
6	302	299	298	300
9	306	306	294	293
12	321	322	307	302
16	345	346	335	322
20	416	415	392	379
0-20	147	145	126	108
6-16	43	47	37	22
6-20	114	116	94	79
0-6	33	29	32	29
6-9	4	7	-4	-7
9-12	15	16	13	9
12-16	24	24	28	20
16-20	71	69	57	57

Data extracted from IRDC Report #382-070 Table 1.

There were no statistically significant differences in mean numbers of viable fetuses, late or early resorptions, postimplantation loss, total implantations, corpora lutea or fetal sex distribution in any treatment group compared to control. The mean fetal weight showed a slight lowering in the mid dose group. The high dose group, however, was statistically significantly lower as compared to the control group (Table 3). Some of the data on this table were recalculated to check reported mean values per dam, and specifically for the value in the control group for mean corpora lutea per dam which was originally reported as 17.1 $\pm$ 2.33.

TABLE 3

Teratology Study in Rats
Summary of Group Mean Maternal and Fetal Observation at Cesarean Section CGA-72662 Technical (mg/kg/day)

	0	100	300	600	Histori
Dams - Gravid	24	22	25	24	383
Total Corpora Lutea	393	388	425	418	-
Corpora Lutea/Dam	16.38±4.17*	17.64±2.82	17.00±2.06	17.42±4.18	15.5
Total Implantations	364	346	391	364	-
Implantations/Dam	15.17±3.28	15.72±1.98	15.64±1.89	15.17±2.26	13.8
Total Postimplantation Loss	23	15	30	23	-
Postimplantation Loss/Dam	0.96±0.86	0.68±0.72	1.20±1.29	0.96±1.20	0.8
Total Early Resorptions	22	15	29	22	-
Early Resorptions/Dam	0.92±0.78	0.68±0.72	1.16±1.28	0.92±1.18	-
Total Late Resorptions	1	-	1	1	-
Late Resorptions/Dam	0.04±0.20	-	0.04±0.20	0.04±0.20	-
Total Viable Fetuses	341	331	361	341	-
Viable Fetus/Dam	14.21±3.48	15.05±1.91	14.44±2.65	14.21±2.34	13.0
Sex Ratio - Male	175	165	190	173	2506
Female	166	166	171	168	2502
Mean Fetal Body Weight (gm)	3.50±0.26 (based on 23 litters)	3.46±0.20	3.36±0.28	3.27±0.23 $p < 0.01$	3.6

Data extracted from IRDC Study #382-070 Tables 3 & 4 and Appendix III.

All values have been recalculated from individual cesarean section observation data to verify reported means and/or incidences per dam.

*Value different from original IRDC table.

In both the treated and control groups in this study, the postimplantation loss per dam is slightly increased and the mean fetal weight is lower than that of available combined historical controls data. The individual fetal weight data was not presented in the submitted report. It should be noted that the historical data is not presented in the most appropriate format. In order to properly compare historical data to the test groups in this study, the data should be presented by individual study and dated. Data should be made available for at least a 2 year period prior to this study and 2 years after it's completion for all assayed parameters.

The fetal morphological observations consisted of one malformation in the control group, one fetus with a thread-like tail and small anus and 2 malformations in the high dose group, one fetus with an omphalocele and 3 fetuses in one litter with a cartilage anomaly (Table 4). The cartilage anomaly consisted of increased girth in the thoracic region predominantly due to increased length of the costal cartilage. The space between both the ribs and vertebrae was reduced and kyphosis was observed in all three fetuses. The investigators believe that the cartilage anomaly is genetic in origin, based on findings from some of their other studies, but they presented no direct evidence for this. In terms of what is considered developmental variations, there is a dose related increase in unossified sternebrae #5 and/or #6 (Table 4), with all three treatment group observations statistically significant from control based on statistical assessment performed by B. Litt, statistician in the Toxicology Branch using Peto's test for dose weighted trend of response. If the responses were adjusted for litter effect the level of significance would be greater. For the variation called "other" sternebrae unossified, using the same statistical method, the noted differences between the high and low doses and control are statistically significant (both on a litter basis and when adjusted for litter effect). The treated groups also presented with reduced ossification of the skull, but this was not statistically different from control. The investigators did not provide any statistical assessment of variations in the report.

Summary of the Incidence of Fetal Malformation
and Developmental and Genetic Variation
CGA-72662 Technical (mg/kg/day)

	0		100		300	
No. of litters examined	23		22		25	
No. of fetuses examined externally	341		331		361	
No. of fetuses examined viscerally	117		114		123	
No. of fetuses examined skeletally	224		217		238	
Malformation	Fetuses	Litters	Fetuses	Litters	Fetuses	Litters
Thread Like Tail, Small Anus	1(0.3%)	1(4.3%)	-	-	-	-
Omphalocele	-	-	-	-	-	-
Cartilage Anomaly	-	-	-	-	-	-
Rib Anomalies	-	-	-	-	-	-
Variations						
Sternebrae #5 and/or #6 unossified	48(21.4%)	17(73.9%)	63(29%) $p \cong 0.03$	19(86.4%)	79(33.2%) $p < 0.004$	22(88.0%)
"Other" sternebrae unossified	2(0.9%)	2(8.7%)	5(2.3%) $p = 0.12$	4(18.2%) $p < 0.05$	5(2.1%) $p = 0.21$	4(16.0%) $p \cong 0.17$
Skull reduced in ossification	-	-	2(0.9%)	1(4.5%)	1(0.4%)	1(4.0%)

Data extracted from IRDC Study #382-070 Table 5 and Appendix III.

Chart continues on next page

Chart continues

	600		Historical	
No. of litters examined	24		383	
No. of fetuses examined externally	341		5008	
No. of fetuses examined viscerally	117		3072	
No. of fetuses examined skeletally	224		1936	
Malformations	Fetuses	Litters	Fetuses	Litters
Thread Like Tail, Small Anus	-	-	-	-
Omphalocele	1(0.3%)	1(4.2%)	-	-
Cartilage Anomaly	3(0.9%)	1(4.2%)	-	-
Rib Anomalies	-	-	11(0.6%)	10(2.6%)
Variations				
Sternebrae #5 and/or #6 unossified	171(76.3%) $p < 0.0001$	23(95.8%)	384(19.8%)	175(45.7%)
"Other" sternebrae unossified	24(10.7%) $p < 0.0001$	11(45.8%) $p < 0.0001$	23(1.2%)	19(5.0%)
Skull reduced in ossification	2(0.9%)	2(8.3%)	52(2.7%)	31(8.1%)

Data extracted from IRDC Study #382-070 Table 5 and Appendix III.

No rib anomalies were noted, although according to the historical data 0.6% of the fetuses and 2.6% of the litters of controls have presented with incidences of this malformation. None of the tables provided contained individual fetal expression of the variations for comparison. The reporting of "reduced ossification of the skull" or "other sternebrae unossified" should have been reported more precisely; the exact bones should be identified.

Conclusions: The dosages of CGA-72662 Technical, based on the data presented, produced dose-related signs of maternal and fetal toxicity. The data suggest that the maternal no effect level is 100 mg/kg based on maternal weight gain depression and clinical observations (red nasal discharge and clear oral discharge) at 300 mg/kg. The data also demonstrate that the NOEL for fetotoxicity has not been determined. Statistically significant increases in unossified sternebrae were noted. At the dosage levels

tested (100, 300 and 600 mg/kg/day) CGA-72662 Technical was not found to be teratogenic. It should be noted that since fetotoxicity is demonstrated in this study at dose levels far above those observed in the rabbit study (10 mg/kg/day) and since this study clearly demonstrates no teratogenic potential in the rat, that little would be gained by requiring a repeat of this study solely to determine a NOEL for fetotoxicity; the rabbit is clearly more sensitive.

Due to some unusual findings reflected in the data (see high dose and control cesarean section data in Table 3, page 8), a lab audit was requested. A summary of the audit findings, memo of L. Chitlik, July 5, 1984 indicates: the raw data for the cesarean section observations were examined, one animal though mislabelled was found to be properly recorded elsewhere. Overall the data had been reported correctly. The daily observation data reporting was somewhat inconsistent between "room" daily observation records and individual observation data. One soft tissue observation of a fetus was excluded from the final report. There was no retention of Bouin's preserved specimens. No stability information or storage instructions for the test compound were included in the raw data. There was no statistical assessment of fetal variation data in the raw data or final report. The raw data usually contained adequate information on the bones of the skull that were unossified that were left out in the final report and also the raw data contained the individual fetal body weights.

It was concluded according to the memo of L. Chitlik, July 5, 1984, that "the primary concern of the similarities between high dose and control cesarean section data are apparently a chance happening. The final report, within the limitations of the "for cause" audit, was found to be adequately supported by the raw data".

Core Classification: Core Supplementary Data with possible upgrade to Core Minimum if reporting deficiencies are corrected. The following data should be requested from the registrant:

1. Individual clinical observation data on the mothers including criteria for what was considered as observed changes in "activity". Clarification of what was found at post-mortem in the dams in terms of internal organ changes (other than uterine findings and the 2 dams with hydronephrosis).

2. Individual weight data on fetuses, individual fetal data on expression of variations seen. Description of the exact bones involved in "other" sternebrae reduced in ossification and the bones of the skull which are reduced in ossification.

3. The historical data should be presented by individual study and dated. The data should be made available for at least a period comprising 2 years prior to and 2 years following completion of the study for all assayed parameters. This will allow a proper comparison of test groups in this study. Also necessary is historical data relating to the cartilage defect that was observed and reported.

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Superseded Tox Review

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