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TR-2247

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

002247

10/26/82

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: M. Mautz (16)
Registration Division (TS-767)

SUBJECT: Review of Curacron Teratology Studies; 100-598, 599;
PP#8F2057, 8H5177
CASWELL#266AA Acc. Nos.: 247784-7, 247919, 070884

Petitioner: Ciba-Geigy Corp.
Agricultural Division
Greensboro, N.C. 27409

Recommendation:

It is recommended that the submitted studies be classified as follows:

1. Teratology, Rats (conducted at Science Applications). Core Guidelines, NOEL for maternal toxicity is 90 mg/kg and the NOEL for fetotoxicity is 120 mg/kg. A teratogenic effect is not indicated at the highest dose tested.

2. Teratology, Rats (conducted by Ciba-Geigy Corporation). Invalid.

3. Teratology, Rabbits (conducted by Ciba-Geigy Corporation). Supplementary Data. Maternal toxicity was not observed at the highest dose tested and questions remain regarding study execution.

A Core-Minimum teratology in a second species (other than rats) remains as a data gap.

Review of Data:

1. Teratology, Rats. Conducted at Science Applications, Inc., San Diego, California, (Study No. 282009, July 22, 1982) and submitted by Ciba-Geigy Corporation on July 28, 1982.

CGA-15324 Technical was administered by gavage to (SD) FBR Sprague-Dawley Derived rats, approximately 76 days of age, on days 6-15 of gestation. Females had been previously inseminated with evidence of mating confirmed by the presence of vaginal plugs or sperm in the vagina. All females were administered a

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volume of 10 ml/kg of body weight containing either 0, 10, 30, 60, 90 or 120 mg/kg of test material in corn oil. Animals were observed at least twice daily from the time of receipt from the supplier to their sacrifice. Animals were weighed on days 0, 6-15 and 20 of gestation. Food consumption was measured on days 6, 13 and 20 of gestation. On day 20, females were sacrificed by CO_2 asphyxiation and the uteri removed. The ovaries were examined in situ and the number of corpora lutea counted. Number of live and dead fetuses and resorption sites were counted. Each fetus was examined externally and viscerally. One half of each litter were decapitated, the heads fixed in Bouin's solution, and examined using the technique of Wilson. Each fetus was stained with Alizarin Red and examined for skeletal anomalies.

Results:

Maternal toxicity was evident only at 120 mg/kg. Three high dose females died and one female was sacrificed in a moribund state. During the period of dosing, ataxia was observed in one animal, diuresia in three, labored breathing in one, and ocular porphyrin discharge, tremors, hyperactivity and hypothermia in four. Although a single 60 mg/kg animal died during the dosing period, characteristic symptoms of toxicity were not observed in lower dose groups. Body weight appeared to be reduced only in the high dose group. No characteristic gross lesions were observed at any dose level.

No effects of treatment were observed on the following parameters: percentage of live fetuses, number of resorbed fetuses, number of dead fetuses, or mean sex ratio.

In addition, the incidence of each type of variation and malformation was similar for controls and treated groups. Major malformations were observed only in a single fetus of the high dose group.

Core Classification: Core Guidelines. The NOEL for maternal toxicity is 90 mg/kg and the NOEL for fetotoxicity is 120 mg/kg. Not teratogenic at 120 mg/kg (HDT).

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2. Teratology - Rats. Conducted and submitted by Ciba-Geigy Corporation (Project No. 2274099).

(Background Information:

In response to a request from L. Chitlik of Toxicology Branch on June 2, 1982, supporting data for a teratology study conducted in the rat has been submitted. The study was conducted by Ciba-Geigy and the Final Report was dated May 29, 1974.)

The submitted data consists of handwritten summary tables for maternal food consumption and body weights, parturition data, fetal weights and visceral findings. The data is not considered "raw data" by this reviewer as it does not appear to be the original raw data collected at the time of the experiment.

In addition, information relative to the following parameters is completely absent: skeletal findings, clinical observation data and data to document mating, dosing and environmental conditions. In the absence of in any raw data to support the conduct and findings of the study, it is recommended that this study be classified as Invalid.

Core Classification: Invalid

The submitted data, standing alone, is not adequate to support the validity of the reported study.

3. Teratology Study, Rabbits. Conducted by Ciba-Geigy, Basle, Switzerland Project No. 785565, report dated March 7, 1979 and submitted by Ciba-Geigy.

(Background Information:

This study was originally reviewed in the memo of April 22, 1982 by W. Woodrow of Toxicology Branch. At that time it was recommended that the study be classified as Supplementary Data on the basis of the following:

1) Inadequate study design i.e. lack of skeletal examination of the skull and lack of visceral examination of anything but the skull.

2) Inadequate data reporting i.e. only summary data were generally reported.

The memo of May 13, 1982 from L. Chitlik requested that a "complete and well-organized study report" be submitted by 7/1/82.

In response to that request, Ciba-Geigy has rewritten the Final Report to contain additional information concerning study design and findings for individual animals. The following is a review of the rewritten Final Report, submitted to the Agency on 7/29/82.)

Twenty Chinchilla rabbits per group were administered either 0, 5, 15 or 30 mg/kg of CGA-15324 technical (89.5%) in a 2% aqueous carboxymethylcellulose suspension on days 6 through 18 of gestation. Control rabbits received 4 ml of vehicle/kg of body weight, treated animals also received a total volume of 4 ml/kg. Weight gain and clinical observations were recorded daily. Food consumption was measured on days 6, 11, 15, 19, 24 and 28 of gestation. On day 28, dams were killed by cervical dislocation and fetuses removed by Caesarian section. The dams were necropsied, resorption sites counted and fetuses removed. All fetuses were externally examined and weighed. The following examination was then performed on each fetus:

1) "Assessment of the situs (content) of the body cavities (thorax, abdomen, pelvis) was carried out through careful autopsy. In the process, organs were removed one after another. Attention was paid not only to topography, but also to size, shape and macroscopic structure of the organs.

2) Heads were given initial inspections upon Caesarean delivery. Following separation from the trunks, they were skinned and reexamined, especially with regard to skull bones and sutures, particularly to assess the possible occurrence of "wide sutures" of the frontoparietal region of the skull.

Fetal heads then were fixed in a mixture of trichloroacetic acid (10 parts, 75 g/liter of distilled water) and formaldehyde 30% (5 parts) and preserved in 90% ethyl alcohol. The scanning of serial razor blade sections according to Wilson afforded the opportunity to identify abnormalities of all head structures, cephalic viscera as well as external structures.

3) Skeletal assessment of the fetal trunks (including limbs) following clearing in potassium hydroxide and staining with alizarine red S."

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Results:

No maternal mortality or toxicity was observed. Occasional adipsia, diarrhea and tarlike stools were noted in the control group but not in treated animals. Weight gain and food consumption were similar in all groups. A single fetus in each group exhibited one or more malformations. No abnormalities of the skull were noted in any fetus. The incidences of skeletal variations were similar in each group with unossification of phalanges II, digit 5 being the most common findings (observed in 43, 80, 56 and 49% of the 0, 5, 15 and 30 mg/kg groups, respectively). Visceral abnormalities consisted only of dystopia of the kidney (one fetus) and hypoplasia of the kidney (one fetus) found in the control group.

The rates of implantation, resorption and live fetuses per litter were as follows:

	0 mg/kg	5 mg/kg	15 mg/kg	30 mg/kg	Historical
Mean Implants/Litter ± SD	10.6±1.8	9.1±1.6	8.7±2.5	8.8±2.1	8.3±2.4
Mean Live Fetuses/Litter	9.7	8.4	7.6	7.8	7.5*
Mean Resorptions/Litter	.79	.64	1.07	1.00	.50*

*Estimate

The mean live fetal weights were significantly increased ($p < .01$) in the high dose group and were elevated in a dose related manner for other groups:

Mean Live Fetal Weight SD (g)

	0 mg/kg	5 mg/kg	15 mg/kg	30 mg/kg
Combined	33.1±6.2	34.0±5.6	35.7±6.8	37.7±5.7
Males	33.9±5.0	33.8±5.9	35.8±6.9	37.0±6.3
Females	32.9±6.0	34.2±5.2	35.6±6.8	38.1±5.3

The increased fetal weights of the high dose animals are also clearly greater than the reported historical control value (34.3 ± 5.9 grams for combined male/female). Although the registrant has noted that differences in fetal body weight are not significant when corrected for litter size using Analysis of Convariance, the examination of historical control data confounds this attempt to explain away the increased fetal body weights. The number of live fetuses/litter for the cumulative control group is similar to that of the mid and high dose groups (7.5, 7.6, 7.8 for the historical control, 15 mg/kg and 30 mg/kg groups, respectively). However, mean live fetal weights are clearly greater (34.3, 35.7 and 37.7 grams for the historical, 15 mg/kg and 30 mg/kg groups, respectively). Thus, litter size alone is insufficient to account for the trend toward higher fetal weights in treated animals.

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Although the rate of implantation appears to decrease with dose level, the historical control value is similar to that of the mid and high dose groups (8.7, 8.8 and 8.3 mean implants per litter for the 15 mg/kg, 30 mg/kg and historical control groups, respectively). Furthermore, implantation rate is not normally expected to be a function of treatment as dosing occurs after implantation is expected to take place in the rabbit. This suggests that a variable other than test compound may have influenced the rate of implantation. On October 20, 1982, this reviewer telephoned Dr. Ada Kung of Ciba-Geigy and inquired as to whether the groups on test were truly concurrent. According to Dr. Kung, day one of pregnancy occurred during the following time intervals:

Control	December 14, 1978 - January 15, 1979
5 mg/kg	January 4, 1979 - January 11, 1979
15 mg/kg	December 19, 1978 - January 2, 1979
30 mg/kg	December 11, 1978 - December 18, 1978

No explanation was offered for the significantly differing starting dates for each group.

Core Classification: Supplementary Data.

Unresolved issues remain regarding study execution and findings. These questions can be summarized as follows:

1) Why were test groups started on test at different times? Because the dates of mating and gestation varied between groups, it is likely that test conditions may have also varied. What assurance does the Agency have that test conditions were comparable for all dose groups and for all dams?

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2) Why are fetal weights significantly greater in high dose animals in comparison to (reportedly) concurrent or historical controls? Although edema was not reported among fetuses examined in the high dose group, it cannot be ruled out as a cause of the increased fetal weights. As Grabowski* has noted, "edemic changes in mammalian embryos have been well documented in relation to a variety of causes...".

3) Maternal toxicity at the high dose level was not demonstrated, indicating that dose levels were inadequate for the intent of the study. What justification exists for the selection of dose levels, aside from an acute oral study in the rabbit?

4) What was the justification for sacrifice of dams on day 28 of gestation rather than day 29 or 30?

5) What assurance does the Agency have that the "careful autopsy" of pups was conducted in a manner which would detect internal as well external organ abnormalities? The referenced W.H.O. protocol is very general and only requires that fetuses in each test group be examined "for external and internal malformations, including skeletal defects." From the description of the autopsy and the reported findings, it appears that the visceral examination was limited to the external appearance of the organ. This would not be considered by this reviewer to constitute a thorough visceral examination.

Resolution of all the above issues is necessary before this study can be accepted by the Agency. Furthermore, given the issues noted above (especially with regard to noncurrency of test groups), submission of supporting raw data for the study must also be a requirement prior to Agency reconsideration of the study.

*C.T. Grabowski, in "Handbook of Teratology, Vol. II" pp. 160-162, J.G. Wilson and F.C. Fraser, editors, Plenum Press, New York, 1977.

Gary J Burin 10/26/82

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