REVIEWER

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

~~SECTION II~~

FILE

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

PC 121301

SUBJECT: Protocol review for Larvadex in rabbits
EPA reg. No. 2F 2707/2H 5355
Caswell No. 167 B

TO: Adam Heyward, PM #17
Registration Division (TS-767C)

FROM: Quang Q. Bui, Ph.D. 7/9/85
Section V, Toxicology Branch
Hazard Evaluation Division (TS-769C)

THROUGH: Laurence D. Chitlik, D.A.B.T. for L.D.C. 7-9-85
Section Head
Toxicology Branch/HED (TS-769C) 7/12/85

and

Theodore M. Farber, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant

Ciba Geigy Corporation
Greensboro, N.C. 27419

Action Requested:

Review of a protocol to study the incidence of fetal malformations in the control population of BUK:(CRL)NZW fBr rabbits (WIL #82005) and a standard protocol for teratology study (segment II) in albino rabbits.

Recommendation

The two protocols submitted by the registrant are considered as adequate by the Agency. However, comments and suggestions are addressed for each protocol (see individual protocol review).

PROTOCOL REVIEW #1

entification:

"A study of the incidence of fetal malformations in the control population of BUK:(CRL)NZW fBr rabbits".

Testing Facility: WIL Research Lab., Inc.,
Ashand, Ohio 44805
Study Number: WIL-82005

Background Information

In a previous study with technical Larvadex in New Zealand rabbits (WIL #82001), several malformations were noted in the treated groups but not in the concurrent and historical control data (memo of 2/4/85). These malformations included, but were not restricted to, cyclopia and diaphragmatic hernia. The investigators stated that since most of the malformations found were from dams sired by the same buck (#2749), they may possibly be of genetic origin.

To justify and elucidate this possible genetically related effect, a proposal to investigate the spontaneous incidence of malformations in dams sired by that buck (#2749) was submitted by the registrant on June 4, 1985. A meeting between Ciba Geigy and the Agency representatives was held on June 11, 1985 to discuss the proposal (copy of the submitted proposal was not received by Toxicology Branch until June 12, 1985).

As per the meeting agreement, and with the consent of Registration Division (Tim Gardner, PM #17), several comments addressed in this memo were communicated previously to the registrant on June 12, 1985.

PROCEDURES

In general, the protocol submitted is not exceptionally different from a standard teratology study in rabbits relative to both maternal data (body weight, food consumption, necropsy) and fetal data collection (external, visceral, skeletal examination, fetal weight and length). The highlights of the protocol to be used are as follows:

1. Virgin New Zealand females (minimum 55) will be shipped from Hazleton-Dutchland (Pennsylvania).

2. Neither a test chemical nor vehicle will be used in this study.

3. All females will be artificially inseminated with semen collected from buck #2749 and will be divided randomly into three groups consisting of at least 18 females each.

a. Control
b. Control Sham 1 : gavaged with a 12-gauge cannula once daily
c. Control Sham 2 : gavaged with a 12-gauge cannula twice daily

. The ejaculate from buck #2749 will be evaluated for volume, motility, and concentration.

5. All animals will be sacrificed on day 29 of gestation. The reproductive ata at necropsy and fetal examination data (external, visceral, and skeletal) ill be recorded and reported according to routine teratology procedures.

COMMENTS AND DISCUSSION

This protocol was designed to study the possible spontaneous malformations that may be genetically related as well as to investigate any possible effects that may be exacerbated by additional maternal stress. The following comments are noted and should be considered in the study design:

1. In this proposal, rabbits from Hazleton-Dutchland will be used. However, New Zealand rabbits from Buckshire were used in the previous study (WIL #28001). Apparently, this is an oversight of the registrant in selecting the commercial supplier. It is this reviewer's opinion that to adequately investigate the genetic-related issue, animals from the same supplier (Buckshire) should be used.

2. The number of animals used per group (18) may not provide an adequate number of pregnant does and litters sufficient for statistical interpretation. Data from the previous study (WIL #28001) suggest that a high pregnancy rate would not be expected from animals inseminated with semen from buck #2749. The number of females per group should be increased to approximately 40 to assure 25 litters to improve statistical reliability of the data generated.

3. Gavage with a stainless cannula will be performed once daily (Control Sham I) or twice daily (Control Sham II). However, it is unclear as to whether this procedure will be conducted throughout the entire period of gestation or only during the period of major organogenesis. It is this reviewer's opinion that this gavage procedure should be performed as per the previous study, i.e. from days 7-20 of gestation. It should be emphasized that data generated from the Control Sham II group will not be useful for regulatory decision on the issue of genetically-related malformations.

4. Ejaculate from buck #2749 will be evaluated for volume, motility, and concentration. It is suggested that a microscopic examination of the semen must be performed with emphasis on sperm head morphology. Further, semen from untreated and proven fertile males should also be examined and reported for comparative purposes.

5. To clearly demonstrate that the malformations, if any, may be genetically related, a "study-control" group apparently is also needed. Females from this group will be inseminated with semen collected from fertile males (other than buck #2749), and housed under the same conditions as the other groups. Data from this "study-control" group will serve as baseline data. The number of females in this group should be equivalent to the other groups.

6. The protocol indicates that the brain will be examined by a mid-coronal slice. This procedure is inadequate. It would be better to examine the intracranial tructures in serial coronal planes. A single coronal section would not permit he visualization of a number of malformations and variations.

7. Method for fetal euthanasia was not described.

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8. The summary of mean fetal weight and length at time of laparotomy must be reported separately for each sex as well as combined.

9. Statistical methods to be used were not presented.

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PROTOCOL REVIEW #2

Identification:

"Ciba Geigy Corporation standard protocol for Teratology study (segment II) in albino rabbits".

Testing Facility: Undecided
Project No.: To be assigned

PROCEDURES

Cyromazine (unknown purity) will be dissolved in 0.5% carboxymethyl cellulose and given, by gavage, to groups of artificially inseminated New Zealand rabbits (source to be determined) at 0, 5, 10, and 30 mg/kg of body weight from days 7-19 of gestation. Control animals will receive the vehicle only by the same route of administration (gavage) and will be housed under the same experimental conditions as the treated animals.

All animals will be observed twice daily and the body weight changes will be recorded on gestational day 0, 7, 10, 14, 20, 24, and 29. Individual food consumption will be measured weekly.

On day 29 of gestation, at least 25 pregnant rabbits per group will be sacrificed. The uteri will be removed and all reproductive parameters will be recorded. Fetal observations will be performed according to routine teratological procedures. The authors state that cephalic visceral examination will be conducted after the technique of Staples. All lesions will be described and photographed.

A post-natal phase will also be conducted with at least 10 dams per dosage level. These animals will be allowed to deliver their offspring. Pups will be evaluated 30 days after parturition for body weight and malformations.

COMMENTS:

1. Number of animals

The investigators must assure that at least twenty litters will be collected for each dosage level and control group at C-section to permit meaningful statistical analysis of the data.

The protocol indicates that 35 and 15 inseminated females per dosage level will be assigned respectively to the teratogenic phase and post-natal phase. The authors should understand that by pre-assigning the dams to each respective phase, the number of litters in the teratology phase may become inadequate for statistical evaluation in the presence of low fertility index. Therefore, it is suggested that a total of 60 inseminated animals should be assigned to each group. On day 29 of gestation, a randomly selected number of pregnant females (minimum 20) will be sacrificed for teratogenic evaluation. Once these 20 pregnant animals (minimum) are obtained, surviving rabbits from the selected population will be allowed to deliver. This approach will ensure enough animals for meaningful analysis of the data, at least for the teratology phase.

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2. Dosage levels

In terms of the dosage levels selected (0, 5, 10, and 30 mg/kg), the Agency does not comment on their adequacy other than relative comments already disseminated in the 1982 FIFRA Guidelines. However, the methods of calculating the doses, whether based on the technical material or corrected to 100% active compound, should be stated in the final report.

3. Necropsy data

The reproductive status of all dams which abort or die during the entire investigation should be recorded and reported. Further, these dams should be examined thoroughly for any pathological and/or morphological changes which may affect pregnancy.

4. Artificial insemination

The artificial insemination procedures should contain information relative to the buck number, semen collection and characteristics, volume of semen used, volume and dose of the HCG solution, as well as any other relevant data.

5. Teratology fetal data

In addition to the routine fetal data collected, it is suggested that all findings, including skeletal variations, should also be tabulated on a per litter basis. Individual fetal variation data should be submitted with the final report.

6. Post-natal phase

Information relative to the post-natal phase is scarce in this submitted protocol. The authors indicate that pup weight will be recorded only on day 30 post-partum and all litters will be randomly culled to six (3 males and 3 females whenever possible) on post-natal day 4. Apparently, all pups will be examined for visceral and skeletal malformations and variations on post-natal day 30.

It is suggested that:

a. Maternal body weight should be recorded at several intervals during the post-natal phase.

b. All dams which do not deliver by post-natal day 30 should be subjected to a gross necropsy and the uteri removed and examined for possible signs of implantation.

c. The length of gestation for each group should be reported.

d. Since cyclopia and diaphragmatic hernia were previously observed in rabbits treated with Cyromazine, parameters to evaluate these developmental signs and careful evaluation of the diaphragm and liver should be considered.

7. Historical control data

Historical control data within 2 years of the study from the same rabbit strain obtained from the same testing facility should be submitted. The historical control data should include findings on a per litter basis as well as individual fetal incidence. Untreated and vehicle treated control data should be reported separately.