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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460
April 5, 1985

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMO

SUBJECT: Review of Rabbit Teratology Study on Larvadex

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The following points were noted in the review of this study:

METHODS

1. Animals were quarantined for an adequate period of at least 30 days and evaluated by a veterinarian before being assigned to dose groups. No information is provided concerning the frequency of disease in animals from this source.

2. Diet, water, lighting cycle, air exchange, and temperature were all appropriate for the animals. Humidity was maintained at 40% or above, but an upper limit was not indicated.

3. Animals were assigned to treatment groups using a stratified weight design. However, the weight range was extremely wide (3 to 5 kg.). There were four treatment groups, a vehicle control and an untreated control.

4. Insemination was done using males from the same supplier. All males were equally represented in each treatment group except that male #2745 was used to inseminate one less female (6 instead of 7) in the 30 mg/kg/day dose group and male #2749 was used to inseminate one additional female (8 instead of 7) in the same dose group.

5. On page 8 (F.1.) the statement is made that "the test article was adjusted for purity..." How was this done?

6. Dosing suspensions were prepared daily during treatment. From the report on analytical chemistry in Appendix B, all samples were homogeneous within the appropriate dosing concentrations, and all but one of the dosing suspensions was within the acceptable concentration range. On the last day of dosing, the analysis of the sample for the

10 mg/ml suspension was 136-141% of the claimed concentration. Since dosing suspensions were only analyzed once each week, errors in the concentrations could have occurred on other days without detection.

7. Animals were weighed on days 0, 7, 10, 14, 20, 24 and 29. Dosing was done on days 7-19 based on the most recent body weight; therefore, on days 7-9 the dose was based on the day 7 weight, on days 10-13 it was based on the day 10 weight and on days 14-19 it was based on the day 14 weight.

8. Clinical observations were made daily on days 0-29 and females which aborted or died were necropsied that day. Fetuses from dead or aborted dams were examined and saved. Food consumption was recorded daily throughout gestation.

9. Fetal evaluations included weights, external examination, internal sexing, visceral examinations and skeletal examinations on every fetus. The examination of the head by a mid-coronal slice only is less than desireable since other parts of the brain, the eyes and the internal nares are not examined. Also, it is possible to miss cleft palates only by opening the mouth.

10. Statistical analyses were performed on the number of litters or number of fetuses, resorptions, etc. Were the mean number of fetuses, resorptions, etc., based on the mean number per litter? Were the percents of fetuses malformed (Table 9) based on the incidence per litter? It is unclear from the report as to how this calculation was done.

RESULTS

1. Three animals died, 1 control due to an open wound, 1 at 30 mg/kg and 1 at 60 mg/kg due to intubation errors.

2. Four animals aborted, 1 untreated control on day 27, 1 at 30 mg/kg on day 25, and 2 at 60 mg/kg on days 21 and 27. The report attributes the abortion in the 60 mg/kg group on day 21 to treatment, but not the other abortions. It is unclear how the abortions in both the 30 mg/kg and 60 mg/kg groups can be said not to be due to treatment, even though one abortion occurred in the untreated group.

3. The incidence of pregnancy in the 10 mg/kg group (38.9%) is extremely low; in fact, it is lower than that for any historical control group. However, it is probably not attributable to treatment since there was no dose-related effect.

4. The clinical signs data are not remarkable for any dose group.

5. The maternal weight data indicate significant reductions in maternal weight gain in the 60 mg/kg group. The change in the mean for net body weight change (minus gravid uterine weight) should not be attributed to treatment because of the extemely large standard deviations. If one looks at the standard error of the mean (SD - -/n-1) for these groups, there is still an overlap among all the groups. For example the vehicle control mean and SEM would be 139 + 96, and the mean (including #2629) for the 30 mg/kg group (the group with the lowest mean) would

be -74 + 164. Thus, it is difficult to justify attributing any of the differences between means to an effect of treatment because of the tremendous variability among animals within each group.

6. Food consumption was decreased in the 30 and 60 mg/kg groups, but not in the 5 and 10 mg/kg groups.

7. The significant differences in sex ratios are not considered to be biologically meaningful, because with only 4 to 6 fetuses per litter in 7-13 litters, the sex ratio could easily be affected by other than an equal number of fetuses in each litter.

8. The mean number of early resorptions and incidence of postimplantation loss do appear to be increased in the 30 and 60 mg/kg groups. However, the mean number of implantation sites was also somewhat higher in the 10, 30 and 60 mg/kg groups. As a result, the mean number of viable fetuses in the 30 and 60 mg/kg groups was about the same as for controls, while the mean number for the 10 mg/kg group was somewhat higher. Although all groups except the 10 mg/kg group had a smaller number of viable fetuses/litter than historical controls, none of these differences should be attributed to treatment.

9. There appears to be a significantly increased overall incidence of malformations in the 60 mg/kg dose group, and the incidence at 30 mg/kg also appears to be increased over vehicle controls. Although the overall incidence of malformations at 10 mg/kg is not increased, the fact that several of the same types of major defects (e.g., cyclopia, diaphragmatic hernia and hydrocephaly) occurred at this dose level as well as at the higher doses makes the 10 mg/kg dose suspect. Since the 10 mg/kg dose group had only about half the number of litters as in the vehicle control group, it may be possible that with an equal number of litters, the incidence of malformations would have been higher. The report makes a case for having almost as many fetuses to examine in the 10 mg/kg group as in controls, even though the number of litters was reduced. However, the litter should be considered the unit of measure, and in this case, the number of litters at 10 mg/kg is probably too small to determine accurately whether or not there was a treatment effect. Furthermore, there were two major defects that occurred at 5 mg/kg which did not occur in either control group (i.e., absent tail and coarctation of the left carotid). However, since neither of these occurred in any higher dose group, it would be difficult to attribute them to treatment.

10. The incidence of variations was not remarkably increased with dose except for 13th rudimentary ribs. Since the number of 13th ribs varies considerably in rabbits (as indicated in the historical control data), this effect is probably not related to treatment.

CONCLUSIONS

The data from this study clearly indicate a NOEL for maternal effects at 10 mg/kg/day. The data on fetal effects are not as clear because of the small number of litters at the 10 mg/kg dose level. I think that both the 30 and 60 mg/kg dose groups show an increased incidence of malformations. Since there are several malformations at 10 mg/kg which also occur at higher doses, this

dose is somewhat suspect. Therefore, under these circumstances it would seem appropriate to set 5 mg/kg as the fetal NOEL.

COMMENT

In general, the number of pregnant animals used in this study was small. Although the guidelines call for a minimum of 12 pregnant animals per dose group, only the vehicle control, untreated control and the 5 mg/kg dose groups achieved the minimum number of pregnant animals at scheduled sacrifice. I think that the minimum number of 12 pregnant animals per dose group should be considered the absolute minimum and that an agent which gives equivocal results should be tested using a larger number of animals where the power of the study would be greater, thereby providing more confidence in establishing the NOEL. For rodent studies (which tend to have lower background variability than rabbit studies) where 20 pregnant animals are used per dose group, an increase of 5-12 times in the overall malformation rate is required to detect a statistically significant increase. Thus, the power of the rabbit study reviewed here is probably very low.

I have not reviewed any other developmental data on Larvadex. Therefor my comments apply only to this study.