PROTOCOL FOR

A REPRODUCTION STUDY EMPLOYING

RATS AND RABBITS

#11,000

I. General

A reproduction study employing rats and rabbits will be initiated. Phase one, fertility and reproduction performance, will be conducted employing albino rats. Teratology, phase two, will employ two species, both the rat and the rabbit. Rats will again be the species used in phase three, perinatal and postnatal study. Two dosage levels which need not be the same for all three segments will be employed in each phase. In all phases, an untreated control group will be run concurrently. In addition, in the teratology segment employing rabbits, a fourth group, a positive control, will be used.

II. Procedure

A. Phase One - Fertility and Reproduction Performance

1. Experimental Animals

A total of 90 rats (30 male and 60 female animals) will be employed, subdivided into three groups of 10 males and 20 females each.

2. Diets and Feeding

All diets will be prepared in the central diet room of the

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laboratories. The diets for any given test group will be prepared by adding a calculated weight of test material to a pre-weighed portion of the stock diet*. Two dose levels will be employed. Control animals will receive the basic ration containing no added test material. All animals are to be fed ad libitum and checked for food requirements daily.

In this phase of the study, test males will be given the compound from 60 days prior to mating until breeding has been successfully accomplished Female animals will receive the test compound 14 days prior to mating and will continue on the test diet until the completion of this phase of the study.

3. Body Weights and Weight Gains

Initially, the body weight of each male rat in all groups will be determined and recorded. Thereafter, individual weighings will be made weekly until mating trials commence. Female animals will be weighed prior to the initial dosing. Individual weighings will then be conducted weekly until mating trials begin. While mating, females will be weighed periodically, not as an index of growth but rather to calculate dietary adjustments. All females will be weighed individually at weaning (21 days post-partum) and again one week post-weaning.

Total weight gains will be calculated on both male and female animals; males the gain during the 60-day feeding before mating trials, females the gain during the 14-day pre-mating feeding.

* Wayne Mouse Breeder Diet; Allied Mills, Chicago, Illinois.

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4. Mortality, Reactions and General Observations

Checks for mortality and abnormal behavioral reactions will be made daily throughout the investigation. In addition, the animals will be observed for fertility, length of gestation and lactation performance.

5. Mating Procedure

At 100 days of age, following 60 days of testing in male and 14 days of testing in female animals, all animals will commence mating trials. Males will be randomly placed with females from within the same group on a 1 to 2 basis. The exact day of mating will be determined by vaginal examination, followed by microscopic examination of a vaginal sample, noting the presence of spermatozoa and any abnormalities thereof. Any male failing to copulate during the first 10 days (two female estrus cycles) will be replaced by another male from within the same group. Each female will be given a maximum of three males. Records of fertility, mating ability, pregnancies and other reproductive parameters will be made.

6. Observations

Any apparent effect upon the breeding cycle (stimulation or suppression) will be noted throughout the breeding period. In addition, one-half of the female animals from each group will be sacrificed on Day 13 (14th day of pregnancy) and then subjected to gross examination. The ovaries, uterus and fetus of each animal will be examined with special attention being paid to corpora luteum, implantation sites,

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resorption, fetuses and any apparent abnormalities thereof. The remaining half of each group will be continued on treatment until the offspri have been delivered and weaned.

f. Progeny

a. Body Weight Data

Pups will be weighed at birth, 4 days post-partum and at weaning and the data recorded.

b. Mortality, Reactions and General Observations

All pups will be examined for physical abnormalities at birth and the number of viable and stillborn members of each litter recorded. Records of survival at periodic intervals during the weaning period will be maintained and a final examination of physical abnormalities will be made at the weaning of each litter.

c. Survival Data

Survival indices will be calculated for various points during the weaning period.

d. Pathologic Studies

Tissues will not normally be preserved or studied from either parent or offspring. However, if the results indicate such a need exists, histopathology will be done on stillborn or dying pups. In addition, 10 male and 10 female pups (chosen randomly) from the litters of each of the control and test groups of all studies will be held for the possible

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future examination of the following tissues and organs:

Brain, pituitary, thyroid, lung, heart, liver, spleen, kidney, adrenal, stomach, pancreas, small intestine, large intestine, urinary bladder, gonads, bone and bone marrow.

Note: If no unusual findings are noted, Phase One is complete. If abnormalities are observed, parent animals will be kept on the test compound and rebred, repeating the study without the interim sacrifice.

B. Phase Two - Teratology

1. Rats

a. Experimental Animals

A total of 60 female albino rats, impregnated at the supplier's laboratories, will be subdivided into three groups of 20 females each.

b. Diets and Feeding

All diets will be prepared in the fashion described in Phase One. In this phase, the compound will be fed to each pregnant female beginning on Day 6 and continuing through Day 15. (Day of breeding is considered Day 0.) During the remainder of the test, all females will receive the stock ration.

c. Mortality, Reactions and General Observations

Same as in Phase One.

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d. Observations

All females will be sacrificed on Day 19 and the pups will be delivered by Cesarian section. Both viable and stillborn pups

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will be counted, weighed and examined for gross abnormalities.

The ovaries and uterus of each parental female will be examined for corpora luteum, implantation sites, resorptions and other evidence of abnormal or normal reproduction. Following this, the surviving pups will be sacrificed and examined for visceral abnormalities. Two-thirds will be cleared and stained to evaluate skeletal development with the remaining third examined histologically using the method of Wilson.

2. Rabbits

a. Experimental Animals

Four groups of ten pregnant female New Zealand albino rabbits (between 12-15 females will be artificially inseminated to obtain the required 10 pregnant females per group) each will be utilized in the study. The four groups will correspond to the following:

Group I - Untreated Control

Group II - Treated Control (Thalidomide)

Group III - Low Dose.

Group IV - High Dose

b. Test Material Administration

The test and treated control material will be administered to the females via gelatin capsule on days 6 through 18 following conception. Prior to starting the teratology study, a group of female rabbits will be treated with test material at selected dose level for two weeks to establish tolerability as judged by weight gain.

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c. Observations

On the 29th or 30th day of pregnancy all pups will be delivered by C-section. The number, weight and viability will be recorded. The pups will be examined for gross abnormalities and placed in an incubator for observation of 24-hour survival.

Each parent female will be examined for corpora luteum, implantation sites, resorptions and any other evidence of normal or abnormal reproduction. The surviving pups will be sacrificed and examined for external and visceral abnormalities. All pups will be processed for microscopic evaluation of skeletal development.

C. Phase Three - Perinatal and Postnatal Study

1. Experimental Animals

Same as in Phase Two - Rats only.

2. Diets and Feeding

All diets will be prepared in the manner described in Phase One. The compound in this phase is administered to each pregnant female beginning on Day 15 and continuing through weaning.

3. Mortality, Reactions and General Observations

Same as in Phase One.

4. Observations

All females will be allowed to deliver and carry their litters through weaning. Records will be kept on progeny mortality, reactions, survival and body weights in the same manner as in Phase

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One. Some litters may be exchanged between control and high-dose animals to elucidate causes of poor survival, if such is observed.

III. Reports

Three copies of a complete, formal report will be submitted upon completion of the investigation.

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