PROTOCOL FOR

TERATOGENIC STUDY IN

SWISS MICE

#1600

1. Experimental Animals

The animals employed will be Charles River Swiss Mice. All animals will be examined for general physical well-being and isolated two weeks prior to breeding. To insure the specified number of pregnancies, 300 females will be mated. Sixty of the females that have mated, as indicated by sperm-positive results of vaginal examinations, will be divided into four groups of twenty females each.

2. Organization of Groups

A structural outline of the experiment is given in Table I.

TABLE I

Group	Dose Level of Drug (mg/kg of body weight/day)	Number of Females/Group
C	None	20
T-I	X	20
T-II	Y	20
T-III	Z	20

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3. Dosages and Feeding

The test material will be administered orally by stomach tube. All test animals will be given the material daily from the sixth day of the gestation period through the fifteenth day inclusive (a total of ten doses). Control females will be treated in a similar manner with distilled water or other vehicle. All animals will be allowed food and water ad libitum.

4. Body Weight Data

Females are weighed daily during the dosing period and again at sacrifice. Data reported include the mean group body weight at Day 6 of gestation (initial dosing), Day 9, Day 12, Day 15 (final dosing) and sacrifice, Day 17.

5. Mortality and Reactions

Daily records of mortality and reactions will be maintained throughout the investigation.

6. Autopsy Examination

All females will be sacrificed by an overdose of ether on the seventeenth day of gestation. An incision will be made in the abdominal wall and the full extent of both uterine horns exposed immediately. Fetal swellings and implantation sites will be counted with special attention being paid to resorption sites or any other uterine abnormalities. The number of corpora lutea will be determined and recorded at this time.

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The number of fetuses present in the uterus and their viability will be determined (spontaneous movement and a more ruddy color distinguishing live from dead animals). Each fetus will be removed by placing a small hemostat on the umbilical cord, flush with the abdominal wall, and cutting the cord distal to the clamp. Blotting paper will be used to remove excess amniotic fluid and blood prior to the weighing of the animals.

An external examination of the fetuses will be conducted with special attention paid to detection of the following abnormalities: hydrocephaly, exencephaly, meningoencephalocele, simple meningocele, anophthalmia, microphthalmic, cleft lip, mandible or palate, oblique facial cleft, micrognathia, external ear abnormalities in size, shape or position, unusual size or position of the limbs, number and disposition of the digits, umbilical hernia, gastroschisis, myelomeningocele, spina bifida and scoliosis.

7. Skeletal Development

Two-thirds of the fetuses (from each group) will be used for evaluation of skeletal development. The method of alizarin staining of bone used will be that of Hurley*.

* Hurley, Lucille S., "Demonstration A-Alizarin Staining of Bone", (revised) Supplement To Teratology Workshop Manual, Berkeley, California, January 25-30, 1965, pp. 121-122.

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8. Internal Development

Internal development of the remaining third of the fetuses will be evaluated by comparing those from treated females to those fetuses from untreated females using the free-hand razor blade section technique of Wilson*.

* Wilson, James G., Warkany, Josef, Teratology Principles and Techniques, University of Chicago Press, Chicago, Illinois, 1965, pp. 271-277.

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