

PROTOCOL FOR
MONSANTO COMPANY
THREE-GENERATION REPRODUCTION STUDY
OF
AROCLOR
IN ALBINO RATS

I. General

A three-generation reproduction study will be conducted with Sprague-Dawley derived albino rats. A total of 96 weanlings (32 males and 64 females) will be equally subdivided into three test groups and one control group. The three test groups will correspond to the feeding of three graded dietary levels of the test material.

II. First Generation

A. Parental Animals

1. Diets and Feeding

All diets will be prepared in the central diet room of the laboratories. The basic ration with which all diets are prepared is a standard, pulverized rat stock diet*. The diets for any given test group will be prepared by adding a calculated weight of the test material to a pre-weighed portion of the stock diet.

The amount of food allotted per week to each rat is to be sufficient for ad libitum feeding. However, checks will be made daily to ensure that the food jars are not empty.

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* Purina Rat Chow, Ralston Purina Company, St. Louis, Missouri.

2. Body Weights and Weight Gains

The body weight of each rat in every group will be determined and recorded initially, and weekly thereafter until the animals are 100 days old.

3. Mortality, Reactions and General Observations

Mortality and abnormal behavioral reactions will be recorded daily. The animals also will be observed for fertility, length of gestation and lactation performance.

4. Mating Procedure

When the animals reach 100 days of age, each male will be randomly mated with two females from the same group. After successful copulation, determined by the presence of a copulation plug or blood in the vagina, the male will be removed from the females and returned to his original cage. Any male failing to copulate within two estrus cycles of the female will be replaced by another male of the same group. However, no more than two males will be used per female during a given breeding cycle. Records will be kept of the number of successful copulations observed, the number of estrus cycles required to obtain a mating, and the number of resulting pregnancies. These data will be utilized to calculate mating and fertility indices.

The Fl_a litters obtained will be weaned at 21 days post-partum. The females will then be given a 10-day rest period and again mated, the above procedure being repeated to obtain Fl_b weanlings.

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5. Pathologic Studies

After the second litter has been weaned (following approximately 33 weeks on test), 8 male and 8 female animals from each group will be sacrificed and gross pathologic observations will be made. Organ weights will be taken on the liver, kidneys, spleen, gonads, heart, brain and any organs which appear abnormal. A complete set of tissues will be removed and fixed in a ten per cent formalin solution.

In addition, microscopic examinations will be conducted upon five males and five females from both the control and the high test groups. If abnormalities are noted, the affected organs of the lower test group animals will also be examined.

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The following tissues and organs will be examined:

Tissues and Organs
Examined Grossly

Heart
Trachea
Lungs
Liver
Pancreas
Esophagus
Stomach
Intestinal tract
Spleen
Lymph nodes
Kidneys
Urinary bladder
Testes
Ovaries
Prostate
Seminal vesicles
Uterus
Pituitary gland
Adrenal glands
Salivary glands
Thyroid glands
Skeletal muscle
Bone
Peripheral nerve
Eyes
Brain
Thymus
Optic nerves
Aorta
Spinal cord

Tissues and Organs
Examined Microscopically

Heart (right and left ventricles)
Trachea
Lungs
Liver
Pancreas
Esophagus
Stomach (cardiac, fundic and
pyloric regions)
Small intestine (duodenum,
jejunum and ileum)
Caecum
Colon
Spleen
Lymph node (cervical and
mesenteric)
Kidney
Urinary bladder
Testis
Ovary
Prostate
Seminal vesicles
Uterus
Pituitary gland
Adrenal gland
Salivary gland (submaxillary)
Thyroid gland
Parathyroid gland
Bone marrow (sternum and femur)
Peripheral nerve (sciatic)
Brain (cerebrum, cerebellum
and pons)
Spinal cord (three levels)

Post-mortem animals will be examined in the same manner
but organ weights will not be recorded.

Throughout gross and microscopic examination, particular
attention will be paid to the reproductive organs.

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B. Progeny

1. Body Weight Data

Pups will be weighed at weaning.

2. 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 lactation period will be maintained and a final examination for physical abnormalities will be made at the weaning of each litter.

3. Survival Data

Survival indices will be calculated for various points during the lactation period. On the fifth day of lactation, litters of greater than 10 pups will be reduced to that number.

4. Pathologic Studies

A gross internal pathologic examination will be made upon any pup appearing abnormal. No such examinations will be made upon progeny which appear normal.

III. Second and Third Generations

The procedures followed for the second and third generations will be identical to those described for the first generation except that the parental animals for the F2 generation in each group will be selected from F1b weanlings of that group. Parents for the F3 generation will be selected from F2b litters.

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In addition, ten male and ten female pups from the F3b litters of each test group and the control group will be sacrificed at weaning (21 days post-partum) and subjected to complete gross and microscopic examination.

IV. Reports

A complete formal report will be submitted upon completion of each generation.

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