II. MATERIALS AND METHODS

A. Objective

The purpose of this study is to evaluate the effect of a chronic intake of 5, 25, and 100 µg/kg per day of a PCB mixture on the reproductive performance of the rhesus monkey (Macaca mulatta). These amounts are equivalent to 0.125, 0.62, and 2.5 ppm, respectively. During the total experimental period, various physiologic parameters will be monitored with periodic hematology and clinical chemistry evaluations and physical examinations. At the termination of the study, all animals will be euthanatized and examined for lesions. The basic experimental format is outlined in the Proposed Test Schedule in Appendix A (Proposal No. 1389 submitted February 13, 1976).

B. Experimental Design

Thirty-two female and 16 male animals will be selected from the Litton Bionetics, Inc., (LBI) commercial rhesus monkey breeding colony for use in this study. All of the animals selected for the study will have an established average or above-average reproductive history. Females with a history of recurrent stillbirths or abortions will not be selected. The selected animals will be divided into four groups, each group consisting of eight females and four males: a control group receiving a placebo, a group in which each animal receives 5 µg/kg of compound per day, a group in which each animal receives 25 µg/kg per day, and a group in which each animal receives 100 µg/kg per day. The females will be selected from a group of 60 available females and will be placed into the four test groups so as to minimize differences between groups in body weight and in the number of live births, abortions, and stillbirths. Females with abnormal cycle lengths will be excluded from the study.

C. Test Compound Administration

The test material (AROCLOR 1254) supplied by the Monsanto Company (MC) will be administered once per day to each animal. To assure that each animal receives the proper quantity of compound for its body weight, each animal will be weighed every two weeks. The animals will be weighed at the same time of day so as to minimize the effects of feeding. The test compound will be received from MC premeasured in an oil base so that 0.5 ml of the material will be the proper amount for each kilogram of body weight. This compound will be added to applesauce and offered to the animals in a shallow bowl. A palatability study will be conducted in cull animals prior to the start of the test.

Treatment of the females which become pregnant will be discontinued after parturition. With the concurrence of MC, treatment of those animals which do not become pregnant may be stopped at 12 months after the initiation of

treatment (April 1, 1978). These latter animals will be bred without compound administration for the next 6 months (April 1 to October 1, 1978). Because this latter time period is one of reduced natural fertility, MC may elect to continue breeding these animals beyond October 1, 1978, at additional cost.

D. Determination of PCB Tissue Levels

A biopsy of the hypodermal fat from the ventral abdominal region will be performed on each animal before any test compound is fed. This biopsy sample, as will all other biopsy samples taken for PCB analysis, will be sent to MC. The biopsy specimens which will weigh between 0.5 and 1.0 gm each will be placed into a properly marked plastic bag, and the bags will be placed into larger bags by sex and treatment group. The samples will be frozen and sent to MC in an insulated carton containing dry ice. Fat levels of PCB's in the rhesus monkey have been demonstrated to equilibrate with a daily dose of PCB's after approximately 6 months [2]. To determine when the monkeys in this study reach and maintain a constant level of PCB's in their adipose tissue and to minimize stress to the animals, biopsy samples will be taken before compound administration is begun and at 2, 4, 5, and 6 months and bimonthly thereafter from the start of compound administration.

E. Fertility Testing

Once the animals develop and maintain a constant concentration of PCB's in their adipose tissue, reproductive performance can be evaluated while the dosing schedule continues. An evaluation of the fertility of the rhesus monkey can be most accurately made during their breeding season, which extends from September through February. (Even though LBI has maintained colonies of rhesus monkeys for well over a decade in constant temperature rooms with 12-hour lighting cycles, a definite breeding season continues to exist.) We project that the assessment of reproductive function of the experimental animals can be made during the 1977-1978 breeding season with most or all births occurring within 18 months of study initiation.

The reproductive function of the male experimental animals will be evaluated through breeding studies. The 16 male animals will be mated for 16 mating periods over a 4-month period. The females used for these matings will be selected from the LBI breeding stock, all females chosen having an average or above-average breeding record. A 72-hour, natural breeding technique, successfully used at LBI for the past 13 years [22], will be employed for these matings. The conception or lack of conception which occurs from each mating will be diagnosed from alterations in the female's menstrual cycle and from a digital examination of the female's reproductive tract. The data generated can be statistically analyzed with a between- and within-group analysis of variance. In that LBI has records from the conceptions of over 6,000 pregnancies, comparison of the reproductive function

of each experimental group with the colony records will be possible. Additionally, the animals can be compared to peer animals during the same period of breeding by means of a student's "t" test. If no effect upon fertility occurs, we expect that each male will produce at least two pregnancies from 16 mating periods.

Semen quality is a good indicator of fertility. Alterations in spermatogenesis are reflected in sperm concentration, sperm motility, percent of abnormal cells, and live/dead ratios. These parameters will be evaluated during an examination of collected semen [27]. Baseline semen parameters for the experimental animals are available from their breeding records and can be compared to the experimental parameters. The semen of the experimental animals will be evaluated monthly beginning prior to the onset of drug administration and continuing throughout the experimental period.

The reproductive function of the female experimental animals will be evaluated through breeding studies and alterations in menstrual history. As is done with all females in the LBI colony to gain this information, the experimental female animals will be submitted to a daily vaginal swab, thereby making any alteration from the normal cycle readily apparent. Information regarding the menstrual cycles prior to this toxicology study will be available from the individual animal's record.

The fertility of the 32 experimental female animals will be evaluated through a breeding study. Five male rhesus monkeys of known fertility from the LBI breeding colony will be used for this purpose. The experimental females will be placed with one of the five males during the appropriate 72 hours of its menstrual cycle. This process will be repeated as the cycle of the animal warrants for 12 months or until a conception has been diagnosed. The result of each mating will be recorded, but for the purpose of statistical evaluation, the animal will be considered to have conceived or not conceived over the 12-month period regardless of the number of matings. These data can be analyzed with a chi square test for random distribution to determine if a relationship exists between the dose of the received compound and fertility. The conception rate of each group can be compared to the conception rate of the colony using an appropriate statistical model. If no effect upon fertility is observed, each group of eight females should produce six live infants during the study. The decision as to the disposition of the offspring will be made as the study progresses. If requested by MC, LBI will attempt to obtain a maternal milk sample after parturition.

Although the Request for a Proposal asks only that male fertility be evaluated, any females impregnated by experimental males would be allowed to continue the pregnancy in line with the goals of LBI. Data resulting from these pregnancies, along with the weights and measurements taken from neonates born to females bred to the experimental males, will be available to MC. If so desired, these neonates may be purchased.

Add for histology
* if present in place of section we examine
Parathyroid

F. Clinical Testing

Certain clinical tests will be performed on each animal throughout the experimental period. The hematologic and clinical chemistry tests on the blood will be performed at the time of the tissue biopsies (that is, before compound administration is begun) and at 2, 4, 5, and 6 months and bimonthly thereafter from the start of compound administration. These tests will include RBC, PCV, hemoglobin, WBC and differential, total protein, SGOT, SGPT, BUN, serum bilirubin, and serum cholesterol. Urine will be collected for urinalysis, urobilinogen, urinary bilirubin, and 24-hour urinary ketosteroid output before compound administration is begun and at 3, 5, 8, 11, and 14 months. Feed samples will be obtained prior to compound administration at 6 months and can be obtained at time intervals beyond that time if required by MC. Because pregnancy may affect the outcome of the 17-hydroxysteroid tests and because the pregnancy status of an animal may not be known when the urine is obtained, results will not be used and the animal no longer tested for this parameter if it is determined to have been pregnant at the time of urine collection.

G. Necropsy

The 48 experimental animals will be necropsied at some time following the period of fertility testing. The male animals will be necropsied immediately following the period of fertility testing and the female animals after parturition or after 12 unsuccessful matings. This schedule may be modified during the course of the study as results become available.

A necropsy which will include histopathologic evaluation of listed tissue will be performed on all 48 experimental animals. The necropsy protocol and associated costs for the neonates will be discussed at a later time.

Sections of the liver will be stored for ultramicroscopic evaluation. Tissues to be saved for histopathology are as follows:

- Brain (3 sections)
- Pituitary (1)
- Eyelids (2)
- Lips (2)
- Mandible (1)
- Salivary glands (2) parotid submandibular
- Thyroid (2) *
- Lymph nodes (2) axillary mesenteric
- Heart (3)
- Thymus (1) if present (or [illegible] in state [illegible] insulation)
- Lungs (4) over
- Tongue (1)
- Esophagus (1)
- Stomach (1) (2) over
- Duodenum (1)
- Liver (1)
- Skin left hip thigh
- Gall bladder (1) separate unless impossible
- Pancreas (1) [through the duct]
- Jejunum (1)
- Ilium (1)
- Colon (1) cecum (save in formalin) no mine
- Kidneys (2)
- Adrenals (2)
- Urinary bladder (1)
- Ovaries (2)
- Uterus (1)
- Vagina (1)
- Cervix (1)
- Testes (2)
- Epididymis (2) section
- Bone marrow - sternum (1)
- Bone marrow - femur (1)
- Lesions (1) section

MONS 014814

BIONETICS Litton

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Bone marrow: smear femur.

lungs

right and left apical

" " diformatic

4,000 or less

stomach

{ fundus and pelvic cord

extra-hepatic bile duct – add

ADD

Nipples (1) female
male } left part of mammary [illegible]

prepuce (foreskin) – male

vulva (labium) – female

sciatic nerve (proximal) – longitudinal
Tibial nerve (distal) } die back

spleen (1)

skeletal muscle [illegible] – no mice

spinal cord (1) – lumbar region

prostate (1)

liver – vitamin A $10.00
(freezer)

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