Litton BIONETICS

LBI PROJECT 20771

FINAL REPORT

EFFECTS OF PCB ON THE REPRODUCTIVE PERFORMANCE OF THE RHESUS MONKEY (*Macaca mulatta*)

APRIL 4, 1977 - DECEMBER 11, 1978

SUBMITTED TO

MONSANTO COMPANY
800 NORTH LINDBERGH BOULEVARD
ST. LOUIS, MISSOURI

SUBMITTED BY

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND

APRIL 30, 1981

Litton BIONETICS

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

I. OBJECTIVES

The primary objective of this program was to evaluate the effects of three dose levels (5, 25, and 100 µg/kg/day) of a polychlorinated biphenyl (PCB), Aroclor 1254, supplied by the Monsanto Company (MC) on the reproductive performance of the rhesus monkey (Macaca mulatta). The pathologic effects of these three dose levels were to be evaluated through gross and microscopic postmortem examinations. A further objective of the project was defined as monitoring hematology and clinical chemistry values during the experimental period as well as periodic examinations to survey organ systems which were known to be affected by PCB intoxication.

During the course of the study, further objectives were defined as follows:

- Evaluation of the pathologic effects of PCB's by the gross and microscopic postmortem evaluation of offspring of the females to which PCB's had been administered;
- Evaluation of the effects of PCB's on the hematological values of these offspring;
- Evaluation of the clinical effects of PCB's on these offspring.

II. SUMMARY

Thirty-two female and sixteen male adult rhesus monkeys were equally divided into four groups which received either a placebo, or 5, 25 or 100 µg/kg/day of Aroclor 1254 for 14 months. After 6 months of compound administration, the animals were bred to non-study counterparts of the opposite sex in order to determine the effects of the test compound on fertility. Infants of test females were allowed to nurse for two months and were then euthanatized if they were clinically normal or nursery reared if signs compatible with PCB toxicity were present. Body weights, serum chemistry, hematology, urine, and semen samples were obtained at various intervals for analysis. All adult test animals and infants of test females were subjected to gross and microscopic postmortem examination.

No effect upon fertility occurred among the adult males in any dose group. A significant effect upon fertility was observed in the females receiving 100 µg/kg/day of the test compound, but no effect upon fertility was observed in the lower dose groups. A depression in red blood cell values (PCV, RBC and Hgb) and platelets, probably the result of test compound effect on the bone marrow, occurred in the medium and high dose adult animals. A decrease in serum cholesterol values in high dose female animals and an increase in serum iron values in high dose males and females and in medium dose females was considered to be treatment related. Alterations in albumin, globulin, and A/G ratio were considered to have a causal relationship to test compound administration in adults of both sexes. A dose-dependent effect upon physical changes in the adults and infants was noted with the majority of all effects to be upon the skin and its adnexa. Body weight loss in the treated adult animals was considered to be dose dependent.

The incidence of histologic morphologic effects and mortality was dependent upon dose, sex, and age. Infants were more sensitive to the test compound than were the adults ranging from severe changes in the only offspring of high-dose level females to a minimal morphologic effect in the mandible of one of six infants born to low dose mothers (five live, one stillborn). No effects were seen in the control or low dose adults; however demonstrable morphologic effects were present at a higher average incidence in the high dose animals and among the females only in the medium dose group.

III. MATERIALS AND METHODS

A. Adults

1. Animals

Thirty-two female and 16 male, adult rhesus monkeys (Macaca mulatta) were selected from the Litton Bionetics, Inc., (LBI) commercial rhesus breeding colony for use in this study. The animals had been all wild-caught and had been at LBI for a period ranging from 2 to 9 years. At the beginning of the study, the animals ranged in weight from 4.4 to 13.4 kg (Females: 4.4 to 8.1 kg; Males: 5.7 to 13.4 kg). The animals were identified according to a unique numbering system by a tattoo on the skin of the thorax. All of the animals selected for this study had an established average or above-average reproductive history. Since the animals were wild-caught, no exact way of determining their age exists. Because of their reproductive history, all were probably at least 6 years of age.

2. Experimental Design

a. Introduction

The animals were divided into four groups, each consisting of eight females and four males: a control group receiving a placebo (Group I), a "low dose" group in which each animal received 5 µg/kg of test compound per day (Group II), a "medium dose" group in which each animal received 25 µg/kg of test compound per day (Group III), and a "high dose" group in which each animal received 100 µg/kg of test compound per day (Group IV). The animals were randomly divided into groups which were then adjusted so as to minimize differences between groups:

1. The criterion for males was body weight.

2. The primary criterion for females was the minimization of differences in the number of live births, abortions, and stillbirths, with minimization of body weight differences secondary.

Test animals were dosed for a period of 6 months before they were mated. Mating began according to the menstrual cycles of the females on or after October 1, 1977. This time period coincided with the expected period of highest conception for the LBI rhesus colony; that is, September through February. (Even though LBI has maintained colonies of rhesus monkeys for well over a decade in constant temperature rooms with 12-hour light cycles, and births occur during all months of the year, a definite breeding season continues to exist.) Mating was stopped as of May 31, 1978, after 8 months [Table 1, Appendix A, Protocol Change (PC) 20771-13]. (All protocol changes are presented in Appendix C.)

b. Fertility testing

1) Males

The reproductive function of the adult, male experimental animals was evaluated through breeding and semen analysis. The 16 males were to be mated to female non-test animals for a 4-month period which was estimated would involve 16 mating periods. The females used for these matings were from LBI breeding stock with average or above-average breeding records. The timed breeding technique utilized was that in which the female is placed in the cage of the male only during the 72 hours of the menstrual cycle when ovulation is expected to occur. The diagnosis of conception or lack of conception was made based on alterations in the menstrual cycle and a digital examination of the reproductive tract of the female. Based upon LBI colony records, each male would be expected to produce at least two pregnancies from 16 mating periods.

Examinations of semen obtained from electroejaculation of the test males were made to measure sperm concentration, total sperm, sperm motility, percent of abnormal cells, and live/dead ratios. [1] Baseline semen parameters from the experimental animals are available for comparison, but since a seasonal variation exists in parameters, comparison of test groups to the control group would provide more meaningful results. The semen of the experimental males was to be evaluated monthly throughout the experimental period. Analysis was done monthly from just before the beginning of the administration of test compound until April 1978 (14 months) when it was discontinued (PC 20771-11). A final semen analysis was performed in June 1978.

2) Females

The reproductive status of the adult female test animals was evaluated through breeding. Fifteen male rhesus monkeys not receiving test compound and from the LBI breeding colony were mated to the test females in the same manner as described above for the test males; that is, for 72-hour periods according to menstrual cycles. Breeding was to continue for a maximum of 12 months or until a conception was diagnosed by digital examination of the uterus and alterations in the menstrual cycle of the female. In light of the number of conceptions and the relative infertility normally occurring in the summer months, breeding was discontinued after May 31, 1978 (8 months) (PC 20771-13).

Although the result of each mating was recorded, for the purpose of statistical evaluation, each female was considered to have conceived or not conceived over the 8-month period of breeding regardless of the number of matings. If no affect upon fertility existed, each group of eight females would be expected to produce six live infants. The decision as to the disposition of the infants was made during the study (PC 20771-11 and PC 20771-13). Also, the decision to attempt collection of milk/colostrum from the mother as soon as practicable after the birth was made at the same time (PC 20771-11). These samples were frozen and sent to MC.

Any nonexperimental female impregnated by a test male was allowed to continue the pregnancy for its natural course. Body weight and measurements were taken from these infants at birth and are available in LBI records (PC 20771-11). Offspring of these pregnancies were not studied further as a part of this experimental design.

3. Test Compound Shipping Plan

In order to ensure the availability of adequate supplies of test compound which were received pre-mixed by MC, the following system was devised:

- Before the start of study: 2-month supply sent to LBI (control and three dose levels, each supplied in ½-gallon containers for each month).
- First month of study: Use supply for the first month (that is, half of original shipment); to receive supply for the third month by end of month.
- Second month of study: Use supply for second month (that is, second half of original shipment); to receive supply for fourth month by end of month.

In this manner LBI would always have a back-up supply 1 month in advance of actual use. Once each new supply had been received, the compound remaining from the prior month was to be returned to MC where it would be analyzed for content (see letter dated March 8, 1979, Appendix B).

Due to the nature of the test compound, there is no reason to believe that any instability existed in the test compound which would result in loss of strength under the conditions of this study.

4. Test Compound Administration

The test compound (Aroclor 1254) supplied by MC was administered orally once per day to each animal. To assure the proper quantity of test compound for its body weight, each animal was weighed every 2 weeks. Animals were weighed at approximately the same time each day to minimize the effects of feeding. The test compound was received from MC premeasured for each dose level in a corn oil base so that 0.5 ml of the material was the proper amount for each kilogram of body weight. MC performed all analyses on the test material both before and after use. The test compound was added to apple sauce and offered to the animals in a shallow bowl prior to the day's feeding. A palatability test was conducted in cull animals prior to the start of the test period. The control animals received 0.5 ml of corn oil/kg in apple sauce each day.

The original protocol was written so that treatment of the females that became pregnant was to be discontinued after parturition. In fact, the protocol was changed (PC 20771-13) so that test compound administration to all animals began April 4, 1977, and stopped on May 31, 1978, a total time period of 14 months.

5. Statistical Analysis

a. Student's t-test

The computation of the Student's t-test during the course of the study for the quarterly reports was made using a "degrees of freedom" number which was based upon the size of the test groups at the beginning of the study. No effort was made during the study by the LBI statistician to adjust the degrees of freedom for the reduction in group size that occurred as a result of deaths or other deleted determinations. As a result, the analyses of data using the Student's t-test, presented during the quarterly reports, were unduly conservative in the comparison of groups. By taking into account the animal deaths, the final report utilizes reduced degrees of freedom in some categories, and thus the level of significance may differ from the interim reports.

b. Effects of pregnancy

A potentially confounding variable occurred during the conduct of the study in that the majority of the female animals became pregnant during the study at various times. Since this variable could affect many of the measured physiological parameters as well as physical ones such as body weight, we identified hematology and biochemistry test results which were obtained during the first 3 months of pregnancy, the last 2 months of pregnancy, and the 2 months following pregnancy (when the body would still be adjusting to the changes of pregnancy). We then compared the values obtained during each of these months in the control animals against the values of nonpregnant control animals to see if, during the period of pregnancy or the 2 months following it, these values were significantly different from the nonpregnant controls. For body weight values, all periods of pregnancy were compared against nonpregnant animals. If the values of any period were statistically significantly different, a value judgment was made as to whether the difference was real or artifactual based upon the range of the values, how the value differed from the control value (above, below, within the range), and reported changes in that value in human pregnancy for which the only large body of comparable data exists. All values thought to represent real changes were deleted from all female animals for the affected time period of pregnancy prior to statistical analysis of the data. To effect consistency, females which experienced fetal wastage had their values analyzed as though the pregnancy had continued to term except for the body weight values, which were used only when the animal was actually pregnant.

6. Determination of PCB Levels

a. Tissue (fat biopsies)

A biopsy of subcutaneous fat from the ventral abdominal region was obtained before any test compound was fed. For this and all subsequent biopsy samples on adults, when insufficient fat was available in the subcutaneous location, a laparotomy was done and omental fat obtained. An attempt was made to obtain between 0.5 and 1.0 gm in all adult animals. When biopsies were made of the infants, they consisted of a segment of ventral abdominal skin and the underlying fat, since sufficiently large

samples of subcutaneous fat were not available. Fat biopsies of the adults were taken according to the schedule presented in Table 1, Appendix A; that is, prior to drug administration and during Months 2 (May 1977), 4 (July 1977), 5 (August 1977), 6 (September 1977), 8 (November 1977), 10 (January 1978), 12 (March 1978), 14 (May 1978), 15 (June 1978), 17 (August 1978), 19 (October 1978), and at necropsy.

Once the decision was made to allow the infants to nurse on the mothers, the fat biopsy schedule was amended (PC 20771-10) so that fat biopsies of the females were not performed on females of more than 100 days gestation or on mothers that were rearing their infants during the first month of the infant's life. This change was made to avoid interference with the late gestational and early postnatal periods.

Fat/skin biopsies of the infants were taken at birth, at 1 month, and at 2 months of age, as well as monthly thereafter if the infant was allowed to live. In all cases the determination of whether or not to take a fat or fat/skin biopsy was made by the clinical veterinarian based upon the apparent health of the animal.

The samples were placed into individually marked plastic bags, which were then placed into larger bags by sex and treatment groups. The samples were frozen and sent in an insulated carton containing dry ice to MC for analysis of PCB levels.

b. Diet

Feed samples were obtained prior to compound administration and at Months 10 and 19, frozen, and sent to MC (Section IV.D.).

7. Clinical Testing

Certain clinical tests were performed on each animal throughout the experimental period. These hematologic and clinical chemistry tests on the blood were scheduled to be performed prior to test compound administration, at 2, 4, 5, and 6 months, and bimonthly thereafter from the start of test compound administration. These tests included the following:

Red Blood Count	SGPT	Lactic Dehydrogenase
PCV	Total Protein	Total Iron
Hemoglobin	Albumin	Triglycerides
WBC and Differential	Calcium	Creatinine Phosphokinase
BUN	Phosphorus	Serum Potassium
Creatinine	A/G Ratio	Serum Sodium
Uric Acid	Globulin	Serum Chloride
Cholesterol	Total Bilirubin	Carbon Dioxide
SGOT	Alkaline Phosphatase	Glucose Fasting

During a routine cytologic examination at LBI, the technician subjectively notes the occurrence of platelets in the field, and this is reported as "adequate" or otherwise. Beginning April 1978 (PC 20771-7), an actual count of platelets and reticulocytes was made and recorded. The frequency of hematologic examinations was changed to monthly in the same protocol change. The frequency of the clinical chemistry testing was changed to monthly in May 1978 (PC 20771-13).

Urine was scheduled to be collected for urinalysis before compound administration was begun and during the 3d, 6th, 9th, 12th, 15th, and 18th months. This was extended to include Month 21 when the end of the study was finalized (PC 20771-13). To avoid conflict with the scheduled necropsy of the animals, the 18- and 21-month collections were cancelled and replaced with one during Month 19 (PC 20771-17). The following parameters were measured:

Total Volume	Urobilinogen, Random
Color	White Blood Cells
Appearance	Red Blood Cells
Specific Gravity	Epithelial Cells
pH	Amorphous Crystals
Protein	Triple Phosphate Crystals
Glucose	Calcium Oxalate Crystals
Ketone	Mucous Threads
Bilirubin	Casts
Blood	Bacteria
Bilirubin, qual.	Other
17-Ketosteroids	

In each case urine for the urinary ketosteroid output was collected for a 24-hour period between Day 22 and Day 26 of the menstrual cycle of the animal, with the first day counted as Day 1. The collection of the first urine sample was timed to occur during the month prior to the beginning of compound administration. In each succeeding month of collection, the urine was collected during the time frame mentioned above which first fell in the specified month; that is, the collection periods were as follows:

Month of Study	Time of Urine Collection
0	Prior to administration of compound.
3d	First appropriate time frame after 2 months of compound administration.
6th	First appropriate time frame after 5 months of compound administration.
9th	First appropriate time frame after 8 months of compound administration.
12th	First appropriate time frame after 11 months of compound administration.
15th	First appropriate time frame after 14 months of compound administration.
19th	First appropriate time frame after 18 months of compound administration.

Because pregnancy may affect the outcome of the 17-hydroxysteroid tests and because the pregnancy status of an animal might not be known when the urine was obtained, results were not used and the animal no longer tested for this parameter if it was determined to have been pregnant at the time of urine collection.

In order to account for the effects of pregnancy on the urinary 17-ketosteroid levels, these were not to be done until the second menstrual cycle following birth. If this second cycle fell within 60 days of the next regularly scheduled determination, testing was postponed until the scheduled month (PC 20771-14). A decision was made at the conclusion of the study to analyze serum for vitamin A levels just prior to necropsy (PC 20771-22).

8. Necropsy

The 48 experimental adult animals were necropsied either as they died or at the beginning of the twenty-first month of study (PC 20771-13). This was a modification of the original schedule, in which the males were to be necropsied immediately following the period of fertility testing and the female animals after parturition or after 12 unsuccessful matings.

Following is a list of tissues histologically evaluated on all 48 experimental animals.

Brain, 3 sections
Pituitary (1)
Eyelids (2)
Lips (2)
Mandible (1)
Salivary glands (2) parotid with duct (1) and submaxillary (1)
Thyroid (2)
Lymph nodes (2), axillary (1) and mesenteric (1)
Heart (3)
Thymus (1), if present
Lungs (4) right and left of both apical and diaphragmatic
Tongue (1)
Esophagus
Stomach (1) **[add 1 section to include both pyloric and fundic regions]
Duodenum (1)
Liver (1)
Gallbladder (1)
Pancreas (1) with duct
Jejunum (1)
Ileum (1)
Colon (1)
Kidneys (2)
Adrenals (2)
Urinary bladder (1)
Ovaries (2)
Uterus (1)
Vagina (1)
Cervix (1)
Testes (2)

Epididymus (2)
Lesions (1)
*Bone marrow **[sterum (1) and femur (1)]
**Parathyroids, if present
*Spleen
**Extrahepatic bile duct
**Prostate
**Cecum
**Left nipple
**Foreskin
**Labium
*Skeletal muscle
*Sciatic nerve
**Distal tibial nerve
*Spinal cord, lumbar region
**Skin, left hip
**Skin, left thigh
**Fat biopsy to be sent to MC

Note: Those items not marked with an asterisk nor in brackets appeared on the original protocol.

*Additional tissues authorized per memorandum of June 21, 1978; Steve Johnson to David Martin, LBI. Sections of liver were to be stored for ultramicroscopic evaluation.

**Further additions made after final consultation in early December 1978 with Drs. P. Berteau and W. Ribelin, MC, just prior to necropsy of remaining adult animals. See PC-20771-22 to 24. Also at that same meeting the decision was made to obtain 30 cc of clotted blood from each adult and as much as possible from each infant just prior to euthanasia, from which determinations of vitamin A levels were to be made (PC 20771-22). A 5-gram sample of liver was also to be obtained at necropsy and frozen in a plastic bag for future use in determining vitamin A levels (PC 20771-22).

B. Infants

The question of the disposition of infants born to experimental females was to be made as the study progressed. On April 13, 1978, the following protocol was instituted by inter-office memorandum (see Appendix B):

1. A CBC with platelet and reticulocyte counts was performed on a monthly basis on each infant born to a study female.

2. Birth weights and somatic measurements of all offspring born of both study males and females were taken and recorded.

3. Offspring of study males were not to be retained as part of the study beyond No. 2 above.

4. Offspring of study females were to remain nursing on the mother. A skin/fat biopsy was to be taken of the infant if the health of the animal allowed it.

This was reiterated by a protocol change sheet (PC 20771-11).

A further modification followed (PC 20771-12) in which body weights and somatic measurements at 1 and 2 months of age were to be taken of offspring born to females assigned to the study. A physical examination of the infants at birth, 1 month, and 2 months of age was added, and signs of PCB toxicity in the infants were to be recorded (PC 20771-13). This same protocol change dictated that infants not showing PCB toxicity signs would be euthanatized at 2 months of age and those showing signs would be weaned from the mother and observed for the reversal of signs.* A further protocol change (PC 20771-15) indicated that infants showing signs would be given a physical examination at 2-week intervals with observations as necessary. This was to continue in those infants transferred to the Nursery at 2 months.

A clarification that all procedures (CBC with reticulocyte and platelet counts, body weight, somatic measurements, and fat [skin] biopsies) be done at monthly intervals in animals kept alive beyond 2 months was added (PC 20771-16).

The use of antibiotics in infants, with prior sponsor approval, was declared (PC 20771-18). Antibiotics had been in use when indicated prior to the assignment of Dr. Peter Berteau to the contract by MC, and, therefore, continuation of this policy was allowed.

Infants which died or were euthanatized prior to the revision of the necropsy protocol in December 1978 (PC 20771-24) were subjected to the same protocol as outlined for the adults in the original protocol (Section III.A.8.).

*Infants were to be given a physical examination 3 to 5 days prior to the scheduled euthanasia to determine if signs were present (memorandum of June 7, 1978).

IV. RESULTS

A. Adults

1. Fertility Testing

a. Males

Each male had been expected to produce at least two pregnancies from 16 mating periods if there was no effect of test compound upon fertility and the animals performed according to colony history. None of the males was able to be scheduled for the predicted 16 mating periods due to a lack of available females as a result of pregnancy. Extending the breeding period to May 31, 1978, to attempt to get more study females pregnant still did not allow 16 mating periods to occur for the study males. Nevertheless, the males exceeded the predicted conception rate, and each male in each group produced at least two pregnancies.

No statistically significant effect upon fertility was observed when any of the three groups receiving test compound was compared to the control group.

b. Females

If no effect upon fertility was seen, each group of eight females would have been expected to produce six live births. Two groups (the control and medium dose) met or exceeded that prediction with the production of six and seven live births each, respectively (Table 2, Appendix A). One additional female in the control group died during pregnancy after carrying an apparently normal fetus to 94 days of gestation. The low dose group produced five live births, one stillbirth, and one pregnancy which ended in fetal wastage. The high dose group produced one live birth and two pregnancies which ended in fetal wastage. The low dose female and one of the high dose females that experienced fetal wastage resumed menstrual cycles, which allowed further breeding but with no known pregnancy resulting. Three of the females in the high dose group died during the breeding phase of the program without being diagnosed as pregnant. Because pregnancy was diagnosed in this study by digital palpation of the uterus at approximately 28 gestational days (and later by alterations in the menstrual cycle), it is possible that some other pregnancies occurred which terminated prior to 28 days of gestation.

Statistical analysis of the data indicated that there was a significant difference in fertility between the control females and the high dose females only.

2. Test Compound Receipt

The test compound received, the dates of receipt, and the dates of return to MC are presented in Table 3, Appendix A.

As a result of late receipt of the shipment of test compound to be administered in July 1977, the animals received test compound meant for June 1977 in the first week of July until the July test compound arrived July 6, 1977 (Section IV.D.).

3. Test Compound Administration

The test compound was successfully administered in the apple sauce vehicle to the majority of animals. In the three animals in which refusal was a problem (569-H, control female; 432-J, low dose male; 427-J, high dose male), a crushed orange quarter, white bread, or a banana proved a successful substitute vehicle. The two male animals were given the test compound by stomach tube for 1 day early in the study.

4. Statistical Analysis

a. Mathematical vs. biological significance

Although the test group sizes were theoretically large enough to allow statistical analysis, several factors must be kept in mind.

(1) The group sizes were small, especially the groups of males which had only four animals at maximum.

(2) The death(s) of animal(s) in groups of this size could profoundly alter the statistical analysis of data, both by decreasing the group size and by removing the most seriously affected animals from future analyses.

Statistical analysis of the data showed levels of significance in numerous categories. Examination of these instances revealed that the deviations fell into one of four categories:

(1) A statistically significant change occurred, but the change was toward a more normal level than the control or baseline data.

(2) A statistically significant change occurred, but the test results were still within the range considered normal for that parameter.

(3) A statistically significant change occurred but was judged to be biologically inconsequential.

(4) A statistically significant change occurred but did not fall into any of the above categories.

Only those changes which fell into category 4 were considered further.

b. Analysis of variance

Although an analysis of variance was planned in order to determine changes within groups over time, this was not done due to the shifting of both control and test results which occurred, making such an analysis meaningless.

5. PCB Levels

Samples of subcutaneous fat and/or skin, placenta, milk, and liver (post-mortem), as well as diet, were collected and submitted to MC. Analysis was carried out by MC and will be presented as a separate report.

6. Body Weights

a. Significance of values during pregnancy

Body weight results were adjusted to account for pregnancy, but only weights actually obtained during pregnancy were checked for significance.

As expected, body weights of pregnant control animals when compared to nonpregnant controls were higher and these values were deleted from the analysis of body weight data.

b. Actual results

Analysis of body weight data showed a variably significant difference between the high dose females and the control females at numerous time periods. Because the study groups were chosen primarily based upon breeding history, the weights of the high dose females varied significantly at the 95% confidence level from the control females prior to drug administration. Visual analysis of the data and observation of individual animal weights, however, leaves little doubt that the loss of weight in the high dose females was truly significant.

7. Clinical Testing

a. Significance of values during pregnancy

Values obtained during pregnancy were analyzed according to the methods outlined in Section III.A.5.b. An explanation of those values considered to be related to pregnancy is presented in Appendix F.

b. Hematology

A depression in red cell values (PCV, RBC, and Hgb) which was statistically significant, was observed in many collection periods when the medium and high dose animals were compared to the control animals. Even though the significance is not present for all values and for all collection periods, one should note that the most profoundly affected animals died or were euthanatized, thus removing their results from all future analyses. Confounding the interpretation of this data is the fact that, due to the selection process, both the medium and high dose animals differed significantly from the control animals prior to test compound administration. Observation of individual animal data, however, shows that an effect on red cell values of certain high dose animals most certainly did occur. Platelet values which were only done by actual count for a portion of the study showed a significant change between high dose and control values in only a few time periods. Observation of the data, however, showed a profound depletion of platelets in certain high dose animals. Often these latter animals died, removing these values from future calcula-

tions. Based upon the postmortem findings, an effect of the test compound on the bone marrow seems to be the most likely mechanism of action.

c. Biochemistry

Occasional, and apparently random, statistically significant results were observed in many parameters in numerous time periods. Only the following were thought to be of biological significance.

1) Serum cholesterol

Serum cholesterol values were reduced significantly in the females of the high dose group during seven blood collection periods. Five of these time periods clustered around the time period just after compound administration stopped. Values were lowest in the time frame when the animals could be expected to be under the maximum influence of the compound. Although significantly lower levels were only observed in the females of the medium and low dose groups on two occasions each, visual observation of the data demonstrated a general lowering of values for both sexes following the end of compound administration and a slow return towards normal during the remainder of the study. This lowering of serum cholesterol values has been previously reported [3].

2) Serum iron

Serum iron levels were significantly higher for the high dose female animals during nine blood collection periods (seven for males) and for the medium dose females during seven blood collection periods (zero for males). This finding is consistent with alterations in hematology parameters.

3) Albumin, globulin, and A/G ratios

Alterations in these parameters were inconsistently present in all treatment groups, usually resulting in a slightly decreased A/G ratio. Although no pattern of change was apparent, the frequency with which significant change occurred was such that a causal relationship to compound administration seems likely.

d. Vitamin A

Analysis of vitamin A levels in the serum of animals just prior to necropsy showed a significant difference between the medium dose females and the control females. Because of the wide range of results in all dose levels of both sexes and because the high dose females are not significantly different, this difference is thought to be of no biological significance (Table 4, Appendix A).

e. Urinalysis

Urinalysis results are presented in Appendix D. No statistical analysis of these data has been done due to the nature of the data; however, examination of the data has revealed no dose-related trends.

8. Semen Evaluations

Semen evaluations are presented in Appendix E. No statistical analysis of these data has been done due to the nature of the values and the fact that no semen was obtained by electroejaculation from various animals in some time periods. Examination of the data has revealed no dose-related trends. Analysis of pregnancy data of females bred to these males substantiates the lack of effect since males of all dose groups impregnated females satisfactorily and with no significant difference between groups.

9. Physical Examinations--Adults

No toxic effects of PCB administration were observed until the fourth quarter of test compound administration when two of four males and three of five females in the high dose group were observed to have edema and wrinkling of the eyelids. One male was observed to have swelling of the lips as well.

Redness and/or edema and wrinkling (puffiness) of the eyelids continued and became more apparent in the fifth quarter when two of four males and four of five of the remaining females in the high dose group were observed to have this condition. Two males and one female were observed to have swelling of the lips during this quarter.

During the sixth quarter of the study, redness and/or edema and wrinkling of the eyelids continued in many high dose adult animals but became less severe in many animals and disappeared in the two males. All high dose animals and one medium dose female manifested swelling of the lips; however, this condition appeared to subside in severity in some animals during the quarter.

During the seventh quarter of study, the signs noted in the eyelids appeared to subside but did not disappear completely. All high dose animals and one medium dose female continued to manifest swelling of the lips, but the condition was reported as slight in many animals. One medium dose male was observed to have slightly swollen lips towards the end of the report period.

Alopecia is difficult to quantify and occurs occasionally in rhesus monkeys not receiving any treatment. Because daily changes are almost impossible to observe, the condition was noted only at the time of the monthly physical examination. Alopecia in varying degrees was observed more frequently and to a greater extent than previously in the medium and high dose animals during the fifth quarter.

During the sixth quarter, alopecia continued to be more severe in the medium and high dose animals of both sexes, but some animals appeared to experience a regrowth of hair.

Regrowth of hair continued during the seventh quarter among the medium and high dose animals which had been observed to have alopecia.

During the sixth quarter, two high dose male animals were observed to have a black coloration to the calculus present on their teeth. A high dose female was observed to have this same condition early in the seventh and final quarter of study..

Black coloration of the teeth continued in the seventh quarter in the two surviving animals which had manifested this condition. In the single male which survived with this condition, the coloration became somewhat browner than black by the end of the report period. Both of these animals were observed to bleed easily from the gums: the female while eating and the male near the lower incisors, when restrained for a physical examination. Frequent bleeding from the gums could account for the staining of the calculus of the teeth.

One high dose male experienced a dysenteric syndrome at the close of the sixth quarter of study from which Shigella was isolated. The animal was treated with supportive therapy in the form of fluids, but no antibiotics were used. The animal recovered early in the seventh quarter, and no relapse occurred.

Several medium and high dose group animals were observed to have an abnormal growth pattern of the fingernails and/or toenails. This had the appearance of an outward growth of the nail away from the nail bed with a seeming accumulation of debris under the nail.

Sparseness of the eyelashes of the high dose animals continued in the seventh quarter, but some regrowth was observed.

B. Infants

1. Analysis of Data

No statistical analysis has been performed on any values obtained from infants. Because the mothers became pregnant after receiving the test compound for various lengths of time and because the exposure of the offspring to PCB's, which only occurred via the mother during pregnancy or while nursing, was variable and unmeasurable, and because only one offspring was born to a high dose mother, statistical analysis of data would be meaningless.

2. PCB Tissue Levels

The analysis for PCB levels was carried out by MC and will be presented as a separate report.

3. Body Weights

Observation of infant body weights at birth and at 1 and 2 months of age showed them to be generally within acceptable limits (Table 5, Appendix A). The one high dose infant which was within an acceptable weight range at birth, however, failed to gain weight as expected during its 3-month life span. The one medium dose infant which survived, showing signs compatible with PCB toxicity, for 7 months gained weight beyond what was expected.

4. Clinical Testing

a. Hematology-cytology

Observation of the values of the formed elements of the blood are generally within normal limits for all dose groups for the periods for which samples are available. Blood was not taken when, in the opinion of the clinical veterinarian, it was potentially dangerous to the health of the infant. Infant values are presented in Table 6, Appendix A.

b. Vitamin A serum levels

Only three vitamin A serum level values are available (Table 7, Appendix A) coincident with the three infants alive at the time the samples were taken. These values have a range similar to those of the adults.

5. Physical Examinations

Births of offspring from study females began to occur in the fifth quarter of study with abnormal physical findings observed as follows: The offspring of the one high dose female (312-I) to give birth to a live infant (B-8542) was observed to have swelling of the eyelids and lips at birth and at 30 days of age during the fifth quarter of study.

During the sixth quarter, three infants (B-8546 from medium dose mother 757-I, B-8560 from medium dose mother 540-H, and B-8542 from high dose mother 312-I) were moved to the Nursery due to poor health (B-8546) and/or signs compatible with PCB toxicity (B-8560 and B-8542). All were moved after 2 months of age, which was a protocol discrepancy (Section IV.D.).

All three animals were born with or developed lesions while nursing on the mother compatible with PCB toxicity; that is, swollen lips and/or eyelids and/or scanty eyelashes. The lesions were more pronounced in the infant from the high dose mother (B-8542), which also failed to gain weight satisfactorily in the Nursery. The high dose animal and one of the medium dose animals (B-8546) died while in the Nursery, while the remaining infant (B-8560) manifested swollen lips and eyelids, scanty eyelashes, scaly skin, and a chronic respiratory wheezing. This latter infant remained alive and continued to demonstrate signs compatible with PCB toxicity until the end of the study. It had a weight gain which was greater than expected when compared to non-study animals. Some evidence of eyelash regrowth occurred toward the end of the study.

The animal continued to show the chronic respiratory wheezing until the end of the study. Although the deciduous teeth of this animal were observed to be brown, the new teeth which erupted in the seventh month of life were normal in color. A subjective observation was made that this animal became more excited when approached by personnel than similar, non-study animals.

One infant (B-8815 born to a low dose mother, 483-F) was moved to the Nursery early in the seventh quarter for health reasons.

All other infants were clinically normal.

C. Intercurrent Deaths--Clinical Reports

1. Adults

a. Animal 296-I, female (low dose)

This animal was found dead on April 12, 1977. The death was caused by acute gastric dilatation which was not related to tha test compound administration. Because of the short time on study and the conclusiveness of the gross necropsy, a histopathological examination was not performed on this animal.

Acute gastric dilatation is a recognized clinical entity in many species of animals including the rhesus monkey. It occurs sporadically in many colonies, and although much studied, its etiology in the nonhuman primate remains obscure. An animal may recover if treated early in the course of the syndrome; however, the outcome depends on the quick relief of the gastric tympany and the ability to reverse the shock present when the condition is discovered.

Because the death occurred only 8 days after the start of the study, a substitute animal, 483-F, was assigned to the low dose group as a replacement. Death was probably not related to the test compound.

b. Animal 152-F, female (high dose)

The clinical changes that were noted in this animal during the study were moderately-elevated SGPT values in April through August 1977, alopecia of the chest and abdomen in June 1977, and generalized alopecia in January 1978. Slight reddening of both eyelids was observed in September 1977, and although this continued, it was thought to be within normal limits.

On the morning of January 22, 1978, this animal was found unable to stand in its cage. The left eye was swollen shut, and numerous petechiae were noted in both eyes. Epistaxis was present, mucous membranes were white, and the animal was markedly dehydrated.

It was treated with 500 ml of Ambex® intravenously, 100 mg of Solu-Cortef®, and 1 million units of potassium penicillin. The animal died at 11:15 a.m., January 22. Death was attributed to an indirect effect of the test compound.

c. Animal 486-H, female (high dose)

At the time of the regularly scheduled fat biopsy of this animal on March 7, 1978, it was noted to be extremely pale. Because the blood sample for hematology had been taken that morning, the results were available and indicated that the animal had a PCV of 14.5, a platelet count of 10,000, and a WBC of 1,400 with a differential count of 17 segmented neutrophils (seg), 82 lymphocytes (lym), and 1 monocyte (mon). The lymphocytes were noted by the reader as "very small."

A transfusion of whole blood and one of red blood cells was given to the animal. A hemogram taken the next morning indicated a PCV of 23.5 and a platelet count of 95,000. Over the next 2 weeks, the animal responded briefly to transfusions given on March 17 and 19, but its course was generally "downhill."

The fat biopsy incision did not heal properly, with bleeding noted on March 19 and dehiscence noted the following day; the eyelids appearing swollen on both of these days. Also on March 20 the animal was noted to have petechial hemorrhages on the ventrum and gums with a tendency toward bruising easily. Bruises were noted over the arms, legs, and face on March 21 and 22, as was weakness. On March 22 the animal appeared dehydrated and inactive, and on that day euthanasia was performed. The condition of the animal was attributed to the effect of the test compound.

Test Date	RBC ($mm^3 \times 10^6$)	S.R. (mm/hr)	Cell Vol(%)	Hgb (gm%)	Plate (mm^3)	WBC (mm^3)	Differential (%)			
							Ban	Seg	Lym	Mon
3/07/78	1.73	3.9	14.5	6.0	10,000	1,400	-	17	82	1
3/08/78	2.68	-	23.5	7.9	95,000	1,400	-	25	74	1
3/10/78	2.42	42.0	19.0	6.5	84,000	1,200	-	13	87	-
3/17/78	1.75	61.0	15.0	4.7	5,000	1,500	1	1	98	-
3/20/78	1.97	-	12.5	4.3	4,000	1,600	-	1	99	-
3/22/78*	0.73	>78.0	4.5	1.7	6,000	500	-	8	88	4

*Terminal sample.

d. Animal 87-F, female (control)

This animal was in apparent good health and 94 days pregnant. It was observed to have had profuse vomiting late on the afternoon of March 29, 1978, followed almost immediately by severe weakness and apparent anemia. Physical examination revealed a distended and very painful abdomen and pale gums and skin.

An exploratory laparotomy was performed that evening. The uterus was found to be very pale and turgid and much larger than expected for the number of gestation days. When opened, the placenta was found to have separated from the endometrium with a large amount of both clotted and unclotted blood between the placenta and myometrium. Some separation of each of the two placental discs was evident. The apparently normal fetus was dead at the time of surgery and was removed with the placenta. Uterine contraction was good.

Although the surgery was uneventful other than the findings noted, the animal did not respond to treatment and died on March 31. At necropsy, the findings were related to the postoperative condition of the uterus, severe cecitis and colitis, and aspiration.

Although relating the physical findings to their cause is speculative, we think the animal probably suffered an attack of acute gastric dilatation on the afternoon of March 29. This condition occurs with some frequency in singly caged, nonhuman primates, particularly macaques, and may end with relief by emesis or death from respiratory embarrassment

and/or endotoxic shock. Judging from the amount of vomited material, the distension must have been considerable and perhaps enough to cause placental separation by pressure. Another possibility is that the placental separation was the result of violent abdominal muscle contractions during emesis. Since this was a control animal, death was not related to the test compound.

e. Animal 770-I, female (high dose)

The results of the scheduled hematology testing on March 7, 1978, indicated that this animal had a low PCV (28%), slightly decreased number of platelets, and a low WBC (4,100) with an absolute decrease in segmented neutrophils.

After the scheduled fat biopsy that day, the animal was observed to be lethargic and dehydrated with some bleeding from the incision site. Transfusions were given on March 8 and 31; however, the animal did not respond and was found in a terminal state on April 2. Fluids were administered, but the animal died. The death of the animal was attributed to the effect of the test compound.

Test Date	RBC ($mm^3 \times 10^6$)	PCV (%)	Hgb (gm%)	Plate (mm^3)	WBC (mm^3)	Differential (%) Ban	Seg	Lym	Mon
3/07/78	3.81	28	9.8	SI Dec	4,100	-	11	87	2
3/08/78	3.03	21	7.7	26,000	3,700	1	52	47	-
3/09/78	3.62	28	9.1	91,000	4,000	4	36	60	-
3/10/78	3.73	26	8.5	37,000	3,400	-	36	63	1

f. Animal 706-E, male (high dose)

This animal experienced a gradual loss of condition and weight from March 1978. There was a weight loss of nearly 2 kg in this time with severe alopecia. Swelling of the eyelids and lips worsened, and the blood picture deteriorated with a reduction in PCV and platelets. There was non-healing of the biopsy site and the development of petechia. Numerous fingernails and toenails loosened and fell out and the animal sat hunched in its cage. Euthanasia was performed July 19, 1978. The condition of the animal was attributed to the effect of the test compound.

g. Animal 841-H, female (medium dose)

This animal was found dead from acute gastric dilatation at approximately 9:30 p.m. on August 30, 1978. Although it had been bled that morning, it had been fed by 9:30 a.m. and had appeared normal through the day. Death was probably not related to the test compound.

h. Animal 338-I, female (high dose)

This animal had been observed to have red and swollen eyelids from May 25, 1978. Dry, scaly skin had been observed from July 1978. A yellow discoloration of the skin and missing eyelashes were observed in August 1978. A hemogram collected on the day of death indicated bone marrow depression with a platelet count of 45,000 mm^3 and total WBC of

1,700 mm^3. It was inactive, dyspneic, severely depressed, and then comatose prior to euthanasia. The condition of the animal was attributed to the effect of the test compound.

2. Infants

a. Animal 8-8546, female, born of 757-I (medium dose)

This infant was observed to have signs compatible with PCB toxicity; that is, swollen eyelids and possibly swollen lips. It had some trouble coming out of anesthesia when a fat biopsy was taken at 2 months. Upon being brought to the Nursery on July 10, 1978, with a rough chest, it was put on 0.2 ml Erythrocin® tid and 0.2 ml Lixaminol® tid. On July 17 the eyes were reddened whereupon ophthalmic Chloromycetin® was administered tid; the condition improved. On July 19 Lixaminol® was discontinued.

In the evening of July 26 and continuing the next day, it was observed to be depressed and had a loose, green stool. Four ml of Donnagel® and 0.2 ml Biosol-M® were given by stomach tube, and an anal swab was taken in the evening of July 27. The animal was still very depressed and inactive and was given 5 ml lactated Ringer's solution sub-Q, 0.1 ml Lixaminol® per os, and 0.25 ml sodium bicarbonate IV. It was found dead at 7:30 a.m., July 28, 1978.

b. Animal 8-8542, female, born of 312-I (high dose)

Due to signs compatible with PCB toxicity, this animal was separated from its mother and brought to the Nursery on July 17, 1978. It was emaciated and had severely irritated eyes and a rough chest. Administration of 0.2 ml of Erythrocin® per os tid and ophthalmic Chloromycetin® tid was begun. The eyes improved, but the rough chest continued.

On July 27 a clinical diagnosis of yeast infection was made, and 0.2 ml Mycostatin® tid per os was instituted. The 3-month biopsy was taken July 31 after which the animal had difficulty recovering from anesthesia. It was given 0.1 ml Dopram® sublingually, 0.1 ml Atropine® IM, and 0.1 ml Aminophyllin per os. Its condition improved on August 1.

On August 3 the infant was lethargic but demonstrated some periods of activity. The eye condition worsened, it had nasal discharge, and was dehydrated. Lactated Ringer's solution, 10 ml, was given tid sub-Q. Also on this day the infant stopped eating and had to be fed via stomach tube. The following day, August 4, it was holding fluids, and the Ringer's was discontinued, but stomach tubing continued throughout the day.

On the morning of August 5, the animal was found with a bloated abdomen, was inactive, and in shock. Ten ml of Lactated Ringer's was given IV as was a 5-ml soapy water enema and 0.2 ml of Solu-Cortef® IM. The stomach was aspirated, and due to shallow respirations, 0.1 ml Lixaminol® was given per os.

The animal was found dead at 12:30 p.m., August 5, 1978.

D. Protocol Discrepancies

The following deviations from protocol were known to have occurred during the course of the study:

- The project protocol called for the sending of a sample of primate diet to MC prior to compound administration, at 6 months, and at other time intervals as required by MC. The 6-month sample was inadvertently omitted and was obtained at 10 months (sent February 13, 1978). This was documented in a memorandum of January 24, 1978 (Appendix B). In addition, a sample was obtained at 19 months (sent March 7, 1979), as requested.

- PC 20771-13 (May 31, 1978) stated that infants demonstrating evidence of PCB toxicity would be weaned from the mother at 2 months of age. Three infants showing signs were inadvertently left nursing on the mother for periods longer than 2 months.

  These were as follows:

  B-8546 from Mother No. 757-I, medium dose, 7 days late.
  B-8560 from Mother No. 540-H, medium dose, 8 days late.
  B-8542 from Mother No. 312-I, high dose, 15 days late.

  This set of events was documented by a memorandum of July 18, 1978 (Appendix B).

- No CPK results were obtained in Month 19 (October 1978) of the study due to this test having been deleted from the SMAC 1000 series by the Bionetics Medical Laboratory without the knowledge of the study director. This change in the SMAC 1000 profile was instituted on September 5, 1978. A protocol change (PC 20771-21) was instituted to ensure that the CPK test was ordered as a separate test for the final two bleedings of the study.

- Final blood samples for hematology and biochemistry analysis were done in the last part of Month 20 (November 1978) in order to allow time to repeat any questionable tests. One female animal, No. 226-F (high dose), was easily stressed, and when obtaining the blood from her presented difficulties, a decision was made to wait and bleed her at the time of necropsy. This was not done due to a change in necropsy scheduling which allowed the animal to be killed while the person in charge of obtaining the blood was busy elsewhere. Serum obtained for a vitamin A level determination was used to obtain biochemistry values, but no hematology values were read. This was documented in a memorandum to the project file dated December 7, 1978.

- As a result of late receipt of the shipment of test compound to be administered in July 1977, the animals received test compound meant for June 1977 in the first week of July until the July test compound arrived July 6, 1977.

- The original protocol called for the collection of a 24-hour urine sample for urinary ketosteroid measurement to occur between Days 22 and 26 of the menstrual cycle of each female animal. For the first 9 months of the study (three collection periods), urine was collected between Days 20 and 26 of the menstrual cycle. This error was communicated to Dr. P. Wright of MC by telephone on January 23, 1978. Observation of data shows that the change in collection periods did not influence results.

E. Pathology

1. Necropsy Procedures

Live monkeys were euthanatized by intravenous injection with either Somlethol® (Med Tech Inc., Elwood, Kansas 66024) or Lethane® (Eli Lilly, Indianapolis, Indiana 46206), as indicated on the individual necropsy records. The euthanatizing agent was administered intravenously, 3 cc to each adult male, 2.5 cc to each adult female, and 0.5 cc to each infant. Gross anatomic abnormalities were recorded in descriptive or diagnostic terminology as determined by judgment of the pathologist in attendance.

Carcasses and live animals submitted for pathologic examination are listed and identified in text Table A.

Monkey 296-I, which was originally placed in Group II females, died of acute gastric dilatation on the eighth day of the experiment and was replaced by 483-F. Due to the timing and the fact that the cause of death (acute gastric dilatation) was unrelated to compound administration, tissues for microscopic examination were not collected. This animal is not included in the tables.

TABLE A
Animals Submitted for Pathologic Examination

Adults

Group I	Group II	Group III	Group IV
227-G-Male-T	119-H-Male-T	20-I-Male-T	425-J-Male-T
418-J-Male-T	428-J-Male-T	46-I-Male-T	427-J-Male-T
426-G-Male-T	432-J-Male-T	595-I-Male-T	600-I-Male-T
739-F-Male-T	516-G-Male-T	599-I-Male-T	706-E-Male-K
200-F-Female-T	284-I-Female-T	273-I-Female-T	226-F-Female-T
274-F-Female-T	309-I-Female-T	281-G-Female-T	269-I-Female-T
569-H-Female-T	483-F-Female-T	307-I-Female-T	287-I-Female-T
578-H-Female-T	605-F-Female-T	540-H-Female-T	312-I-Female-T
604-I-Female-T	624-I-Female-T	757-I-Female-T	152-F-Female-D
618-I-Female-T	646-E-Female-T	762-E-Female-T	338-I-Female-K
764-E-Female-T	701-E-Female-T	763-E-Female-T	486-H-Female-K
87-F-Female-D	839-H-Female-T	841-H-Female-D	770-I-Female-D

Infants

Group I	Group II	Group III	Group IV
B-8518-Male-T	B-8604-Male-T	B-8511-Male-T	B-8542-Female-D
B-8557-Male-T	B-8796-Male-S	B-8559-Male-T	
B-8574-Male-T	B-8527-Female-T	B-8603-Male-T	
B-8483-Female-FW	B-8562-Female-T	B-8860-Male-T	
B-8532-Female-T	B-8754-Female-T	B-8546-Female-D	
B-8533-Female-T	B-8815-Female-T	B-8560-Female-T	
B-8621-Female-T		B-8592-Female-T	

Key:

T = Terminal Euthanasia
K = Moribund Euthanasia
D = Found Dead
S = Stillborn
FW = Fetal Wastage

Tissues for microscopic examination were preserved (fixed) in 10% buffered formalin solution, pH 7.0, dehydrated in ethanol, cleared in xylene and embedded in Paraplast® brand of paraffin embedding medium (Lancer Division of Sherwood Medical, St. Louis, Missouri 63103).

Bone specimens were subjected to prior decalcification in RDO decalcifying agent® (Dupage Kinetic Laboratories, Inc., 25 W 550, North Aurora Road, Naperville, Illinois). Tissue blocks were routinely sectioned at a range of 5 to 6 microns in thickness with the exception of brain blocks, which were sectioned at a range of 5 to 10 microns in thickness. All tissue sections were stained routinely with hematoxylin and eosin. In several instances special stains were employed for histochemical demonstration of iron containing pigment (hemosiderin) and for mucin in mucin-secreting cells.

A complete listing of tissues for microscopic examination appears in Section III.A.8.

Stipulations made concurrent with additions appearing in PC 20771-22 were:

- Parotid gland and pancreas should be trimmed to include duct.
- The two lymph node specimens should be axillary and mesenteric lymph nodes.
- Two sections of stomach should be taken to include both pyloric and fundic regions.
- Lung specimens should include apical and diaphragmatic lobes of both lungs.

- After final discussion with MC representatives on selection of tissues for microscopic examination, it was decided to omit skeletal muscle and cecum from the list, but to harvest and preserve them in formalin should future reference be necessary.

Additions to the list of tissues to be examined microscopically that were made in accordance with approved modifications of protocol account for differences in the scope of microscopic examination in some monkeys that died or were euthanatized intercurrently and those that were euthanatized at termination of the study.

2. Microscopic Examination

Compound (polychlorinated biphenyls)-related alteration in Meibomian glands of nonhuman primates has been designated "squamous metaplasia with conversion of the glands to a row of squamous cysts...."[4, 5] Compound-related alteration in sebaceous glands of the skin has also been designated "squamous metaplasia."[4]

The microscopic character of sebaceous gland alteration observed in the present study posed questions of interpretation and nomenclature. The long ducts of the Meibomian glands and the short ducts of the sebaceous glands in the skin are lined by stratified squamous epithelium. The secretory alveoli are rounded sacs consisting of epithelial cells undergoing breakdown to fatty detritus which constitutes the holocrine-type oily secretion.[6] There is disagreement concerning replacement of cells that disintegrate into oily secretion. It has been stated that the regenerative activity occurs [4] in the basal layer of epithelial cells lining the secretory alveoli and [5] in "cells close to the walls of the ducts, where the new cells move into the secretory regions."[6,7]

In this report terminology was selected to indicate the morphologic appearances of compound-related alterations without regard to kinetics of their development. In a majority of animals with Meibomian gland alterations, there was total absence of recognizable secretory alveoli. This was designated atrophy-sebaceous elements. In two instances with less than total atrophy of the sebaceous elements (designated subtotal atrophy-sebaceous elements), an appearance of squamous metaplasia also was recognized, and the designation squamous metaplasia was used. Round and elongated structures with thick epithelial walls and large central areas packed with epithelial squames were generally present. The round structures had the appearence of squamous cysts, but a majority were elongated and could be identified as Meibomian gland ducts impacted with squamous debris. The designation squamous impaction-ducts was used.

Another Meibomian gland alteration, not reported in literature that was reviewed, was the presence of large mucinous vacuoles in cells lining some of the ducts. The mucinous character of the vacuoles was confirmed by staining with Alcian blue and Periodic acid-Schiff techniques. Ducts so affected contained a mixture of squamous and mucinous materials. This alteration was designated mucinous metaplasia-ducts.

Changes in sebaceous glands of the skin were designated according to microscopic appearance as either atrophy-sebaceous glands or subtotal atrophy-sebaceous glands. Since only small areas of skin were routinely examined, a conservative interpretation of the microscopic appearance was made to avoid overstatement of the affects of the compound. Distention of hair follicles with keratinized material was designated follicular keratosis. Squamous cysts in the dermis (without demonstrable connection with overlying epidermis and without definite indication of origin) were designated squamous cysts.

Compound-related atrophy of the thymus in infants was designated "accidental involution" to differentiate it from the physiologic involution that was invariably present in adults to a greater or lesser degree.

The system of nomenclature used in publications on the effects of polychlorinated biphenyls in nonhuman primates was used for compound-related lesions observed in the biliary tract, liver, gastric mucosa, and mandible. [3,4,5,8,9,10,11]

Descriptive designations were given to unique, apparently compound-related lesions seen in the pancreas of two infants and kidneys of one infant. The pancreatic lesion was characterized by deficiency or diminution of acinar epithelial cells and increase of centroacinar cells and small ducts. It was designated ductal hyperplasia-acinar atrophy since both the ductal and acinar elements were involved. The lesions in the kidneys had the microscopic appearance of areas in the cortex where there was retarded cortical maturation and were so designated.

3. Results

A list of all pathologic alterations recognized on microscopic examination is given in the tables presented in Appendix G and identified as follows:

Table 3A: Summary of Histologic Findings - Adult Males - Terminal Kill.

Table 3B: Summary of Histologic Findings - Adult Females - Terminal Kill.

Table 3C: Summary of Histologic Findings - Adults - Males and Females - Intercurrent Death.

Table 3D: Summary of Histologic Findings - Infants - Males and Females.

The pathologic alterations can be grouped into the following categories:

- Pathologic alterations incident to euthanasia and environment; for example, terminal hemorrhages and minor mucosal lesions, etc.
- Spontaneous lesions of nonhuman primates.
- Pathologic alterations representing direct morphologic effects of the test compound.

In this study the pathologic alterations representing direct morphologic effects of the test compound could be identified because of their unique character and their similarity to lesions described in literature on the effects of polychlorinated biphenyls.

These alterations include the following:

- Characteristic changes associated with loss of secretory epithelium of the Meibomian glands in the eyelids and the sebaceous glands in the skin. There also were instances of mucinous metaplasia in the Meibomian gland ducts (previously unreported).
- Follicular keratosis--skin.
- Squamous cysts--skin.
- Subgingival squamous cysts (microcysts) and intraosseous squamous cysts in mandible.
- Squamous metaplasia in ducts of oral mucosal glands and in such unusual places as thyroid, epicardium, and mammary tissue.
- Hyperplastic epithelial changes involving the biliary duct system and the main pancreatic duct.
- Nonsquamous metaplastic changes in gastric glands and Brunner's glands.
- Non-specific bone marrow alterations that were compound related on the basis of experimental conditions.
- Previously unreported lesions of infants.
  - (a) Areas of retarded cortical maturation--kidneys.
  - (b) Acinar atrophy and ductal hyperplasia (increase of centroacinar cells)--pancreas.

Accordingly, the set of tables presented in Appendix G was prepared listing only the pathologic alterations representing direct morphologic effects of the compound:

Table 2A: Summary of Direct Morphologic Effects of Compound - Adult Males - Terminal Kill.

Table 2B: Summary of Direct Morphologic Effects of Compound - Adult Females - Terminal Kill.

Table 2C: Summary of Direct Morphologic Effects of Compound - Adults - Males and Females - Intercurrent Deaths.

Table 2D: Summary of Direct Morphologic Effects of Compound - Infants - Males and Females.

At the end of each table, a numerical score of the number of pathologic alterations listed for each animal was entered. Each pathologic alteration was given a value of 1 regardless of whether or not the item was recorded as present (x) or was recorded by a numerical grading symbol (1 through 5).

A final table was prepared (Table I) entitled "Survey of Direct Morphologic Effects of Compound." This table identifies each animal in relation to the test and furnishes individual and average group numerical scores of pathologic alterations for use in final interpretation of the pathologic examination. Incidence of direct morphologic effects of the compound was dose related, being highest in Group IV animals and substantially lower in Group III animals. There was a trace morphologic effect in one Group II infant and absence of effects in Group I animals (controls). Only 1 of the 18 Group II animals showed direct morphologic effect of the compound; it was an infant with minimal pathologic alteration in the mandible. In Groups IV and III, where comparisons could be made, the incidence of direct morphologic effects of the compound was greatest in the infants and least in the adult males.

Average scores and incidences of direct morphologic effects of the compound are summarized in text Tables B and C, respectively.

TABLE B

Average Score of Pathologic Alterations
Representing Direct Morphologic Effects of the Compound

	Group IV	Group III	Group II	Group I
Adult males	7.25	0.75	0	0
Adult females	9.13	0.88	0	0
Infants*	20.00	3.14	0.33	0

*This score has apparent validity because the single infant on which it is based represents the only conceptus in Group IV adult females that developed to infancy.

TABLE C

Numbers of Animals with Direct Morphologic Effects of the Compound / Numbers Examined

	Group IV		Group III		Group II		Group I	
	M	F	M	F	M	F	M	F
Adult<	3/4	8/8	1/4	3/8	0/4	0/8	0/4	0/8
Infants	-	1/1	2/4	2/3	0/2	1/4	0/3	0/4

Intercurrent deaths, including animals killed in extremis, in adults occurred in a single Group I female, a single Group III female, a single Group IV male, and four Group IV females. The Group I female died of hemorrhage and the effects of surgical treatment following placental separation. The Group III female died of acute gastric dilatation, a relatively common but poorly understood spontaneous affliction of macaques. There was no apparent connection between the cause of death and the test compound in either of these two cases.

Two of the Group IV animals, a male and a female, developed isolated cases of severe infectious disease. The male had extensive gangrenous stomatitis and was euthanatized when moribund; the female died of systemic herpesvirus infection. Under the prevailing status of spontaneous diseases in the primate colony, one might speculate that the unusual severity of infection in these two animals is a function of compound effect on the immunologic competence of the animals.

A second high dose female died of intestinal bleeding and a third was euthanatized when moribund with clinical signs of anemia. In both of these cases, hematologic effects of the compound were apparently responsible. The fourth high dose adult female that died had severe biliary tract lesions related to the compound with secondary hepatic necrosis, abscess formation, and periportal inflammation.

4. Summary

Compound-related morphologic alterations were identified in adult males, adult females, and in infants of either sex that were born to the adult females. The effects of the compound were greatest in Group IV animals and were slightly more evident in adult females than adult males. They were most evident in the single animal representing the only conceptus in Group IV adult females that developed to infancy. Four intercurrent deaths in Group IV adult females (50%) and one in Group IV males (25%) could be attributed to lesions that were either direct or indirect effects of the compound.

The method for scoring lesions which was used indicated a considerably lesser amount of morphologic effect of the compound in Group III animals as compared with Group IV animals. Here again, the compound-related morphologic effects were slightly more evident in adult females than adult males and most evident in the infants. An adult female (Group III) that died intercurrently had acute gastric dilatation, apparently unrelated to compound administration. The Group III infant that died intercurrently had bacterial meningitis and septicemia. In this animal the score of pathologic alterations representing direct morphologic effects of the compound was approximately four times greater than the average score of Group III infants, indicating a probable relationship between death and administration of the compound.

Group II animals (adults and infants) were entirely free from morphologic effects of the compound, except one infant which had a single intraosseous squamous cyst in the mandible and several minute subgingival squamous cysts.

5. Conclusion

Pathologic examination revealed dose-related and, to a lesser degree, sex-related and age-related morphologic effects of the compound in high and medium dose animals.

Section III.E., Pathology, Submitted by:

Herman R. Selbold, V.M.D. Date
Veterinary Pathologist

Reviewed by:

Richard H. Cardy, D.V.M. 3/10/81 Date
Director
Department of Pathology

V. CONCLUSIONS

A. Adults

1. Fertility

No effect upon fertility occurred among the males in any dose group.

A significant effect upon fertility was observed in the females receiving 100 µg/kg of the test compound, but no effect upon fertility was observed in the lower dose groups.

2. Pathologic Effects

Pathologic effects were determined by identification and tabulation of the direct morphologic effects of the compound. The incidence of these morphologic effects and mortality was dependent on dose and sex. Group IV monkeys had a higher average score of morphologic effects than Group III monkeys. No effects were seen in Group II and Group I monkeys. In both groups that had demonstrable morphologic effects of the compound, the incidence of these effects was higher in females than males. Four intercurrent deaths in Group IV adult females (50%) and one in Group IV males (25%) could be attributed to lesions that were either direct or indirect effects of the compound.

3. Hematology-Cytology

A statistically significant depression in red cell values (PCV, RBC, and Hgb) and platelets, was observed in variable numbers of collection periods when the medium and high dose animals were compared to the control animals. Although all values were not depressed significantly for all collection periods, and the interpretation of these observations is confounded by the fact that the medium and high dose animals varied significantly from the controls in the pre-test period one should note that the most profoundly affected animals died or were euthanatized, thus removing their results from all future analyses. The postmortem findings suggest that this was a compound-related effect on the bone marrow.

4. Biochemistry

Statistical changes thought to be of biological significance were observed in the following three parameters:

- A decrease in cholesterol values appeared to be dose related and sex related with the females significantly different from controls more often than the males. A slow return towards normal values was observed after the cessation of compound administration.
- An increase in serum iron values appeared to be dose related and sex related, with the female animals significantly different from controls more often than the male animals.

- Alterations in albumin, globulin, and A/G ratio, usually resulting in a slightly decreased A/G ratio, were present in all treatment groups. Although the changes were not consistent and there was no apparent pattern of change, the frequency with which significant changes occurred was such that a causel relationship to compound administration seems likely.

5. Physical Effects

Physical changes attributable to test compound administretion were observed in all animals receiving the high dose of the tast compound. Changes of a lass severe nature were observed in some medium_dose animals. The majority of effects observed upon physical examination were attributed to the effect of the tast compound upon the skin and its adnexa. Significant weight loss was reported aven in the faca of a good appetite.

B. Infants

1. Pathologic Effects

Pathologic effects were determined by identification and tabulation of direct morphologic effects of the compound. The single high dose infant had the highest score of direct morphologic effects. This score also was higher than the average score of medium dose infants. This comparison appears to be valid because the single high dose infant that was examined represents the only conceptus of the high dosa adult females that developed to infancy. Minimal morphologic effect was seen in the mandible of one low dose infant. Thus a dose-dependent effect on infants was apparent. The higher score of direct morphologic effects among infants, as compared with adult females and adult males in the corresponding groups, indicates that the infants were more sensitive to the compound than were the adults.

2. Hematology

No statistical evaluation of the values of the infants was attempted due to the variable and unknown exposure of the infants to PCB's, which occurred only via the mother during pregnancy or while nursing. The birth of only one animal in the high dosa group also made statistical comparisons meaningless.

3. Physicel Effects

Physical effects observed in the one infant born to a high dose mother and in severel of the infants born to medium dosa mothers were dose dependent. These effects were similar in nature to those in the adult test animals but were more profound.

VI. KEY PERSONNEL

Key personnel assigned to this program were David P. Martin, V.M.D., Study Director; John L. Cicmanec, D.V.M., Study Veterinarian; and Herman R. Seibold, V.M.D., Pathologist.

VII. QUALITY ASSURANCE

Q.A. Inspection Statement

PROJECT 20771 LBI COMPOUND NO. Aroclor 1254

TYPE OF STUDY PCB EFFECTS ON RHESUS REPRODUCTION

This study was inspected by the LBI Quality Assurance Unit and findings were reported to the study director and management.

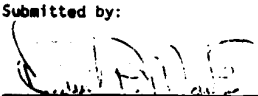
In-process audits were performed on two occasions - 9/21/78 and 11/14/78. The final pathology report was audited in detail on 5/11-5/15, 1979 and the entire final report was audited on 5/16/-5/22, 1979 and a follow-up audit was done on 8/7/80. Study records were inspected on 9/12-9/29, 1978, 10/20/78, 11/14/78 and 5/16/79. The protocol was reviewed early in the study and all protocol changes were reviewed as they were implemented. This study was performed according to acceptable laboratory practices. Those discrepancies noted were minor in nature and would in no way invalidate the study.

Robert Cypher
QA Manager

VIII. RECORDS RETENTION

All original data, including this report, will be stored at the LBI Archives at 1330 Piccard Drive, Rockville, Maryland.

Submitted by:



David P. Martin, V.M.D., Study Director — Date
Director
Laboratory Animal Medicine and Science

IX. REFERENCES

[1] Valerio, D.A., Leverage, W.E., and Munster, J.H.: Semen evaluation in macaques. Lab. Ani. Care, 20:734-740, 1970.

[2] Switzer, J.W., Valerio, D.A., Martin, D.P., Valerio, M.G., and Rininger, B.: Hematologic changes associated with pregnancy and parturition in Macaca mulatta. Lab. Ani. Care, 20:930, 1970.

[3] Barsotti, D.A., Marlar, R.J., and Allen, J.R.: Reproductive dysfunction in rhesus monkeys exposed to low levels of polychlorinated biphenyls (Aroclor 1248). Food Cosmet. Toxicol., 14:99-103, 1976.

[4] McNulty, W.P.: PCB poisoning in rhesus monkeys. Primate News. Publication of Oregon Regional Primate Center, 15(1):2-7, 1977.

[5] McNulty, W.P. and Griffin, D.A.: Possible polychlorinated biphenyl poisoning in rhesus monkeys. J. Med. Primatol., 5:237-246, 1976.

[6] Bloom, W. and Fawcett, D.W.: A Textbook of Histology, W.B. Saunders Company, (Ninth Ed.), p. 497, 1968.

[7] Ham, A.W.: Histology, J.B. Lippincott Company, (Seventh Ed.), p. 609, 1974.

[8] Allen, J.R.: Response of the nonhuman primate to polychlorinated biphenyl exposure. Research Activities at Regional Primate Centers. Fed. Proc., 34(8), 1975.

[9] Allen, J.R., Carstens, L.A., and Barsotti, D.A.: Residual effects of short-term, low-level exposure of nonhuman primates to polychlorinated biphenyls. Toxicol. Appl. Pharmacol., 30:440-451, 1974.

[10] Allen, J.R. and Barsotti, D.A.: The effects of transplacental and mammary movement of PCBs on infant rhesus monkeys. Toxicology, 6:331-340, 1976.

[11] Becker, G.M., McNulty, W.P., and Bell, M.: Polychlorinated biphenyl-induced morphologic changes in the gastric mucosa of the rhesus monkey. Lab. Invest., 40:373-383, 1979.