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Aroclor 1254: Reproduction Study with Rhesus Monkeys (Macaca mulatta)

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Abbreviated Title: Effects of PCBs on Primate Reproduction

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ABSTRACT

Aroclor 1254, a polychlorinated biphenyl mixture containing 54% chlorine, was fed to groups of male and female, adult rhesus monkeys (Macaca mulatta) at dosages of 0, 5, 25 and 100 µg/kg/day for 14 months. Animals were observed for another 7 months. After 6 months of compound administration, they were bred to untreated animals. Resulting infants were observed for 2 months. At various intervals, body weights and results of hematology, serum chemistry, urine and semen analyses and tissue PCB determinations were recorded. Gross and microscopic postmortem examinations were carried out on adults and infants. Some high- and a few mid-dose animals showed signs of intoxication after 9 to 15 months on test. These were mainly effects upon the skin and its adnexae. A few high-dose animals died or were euthanatized after 12 months. High-dose females lost weight and had a significant reduction in fertility. There were no significant differences in mean body weight of infants. Some high-dose animals had decreased red blood cell values, increased serum iron values and decreases in serum cholesterol. The incidence of histologic morphologic effects attributed to Aroclor 1254 dosing was dependent upon dose, age and sex, females being more sensitive. Among adults, the highest average incidence was in the high-dose group. No effects were seen in control or low-dose adults. Infants were more sensitive. Changes ranged from severe in the single offspring of high-dose females to a minimal morphologic effect in the mandible of one of 6 infants born to low-dose mothers. PCB levels in fat of adults were significantly increased after 1, 4, and 7 months for the 100, 25 and 5 µg/kg groups, respectively. From 9 months to the end of the study, no further increases in

the levels of PCB in fat were detected. PCB levels in livers of adults necropsied at the end of the study were found to be increased in proportion to the amounts of Aroclor 1254 which these animals had received.

INTRODUCTION

PCBs are a mixture of chlorinated homologues of biphenyl. Their dielectric properties made them useful in capacitors and transformers, and their thermal stability led them to be used as heat exchangers. The latter use led to an outbreak of poisoning in Japan in 1968 (Kuratsune, et al., 1972). This occurred when Kanechlor 400, a Japanese PCB with 48% chlorine, leaked into cooking oil made from rice hulls during a heat purification (deodorizing) step. The Japanese term for the illness which resulted from consumption of the contaminated rice oil is 'Yusho'.

The Food and Drug Administration (FDA, 1973, 1977) considered human experience during the Yusho incident in setting temporary tolerances for PCBs in foods for human consumption. Amounts "allowable [for] protracted ingestion" (FDA, 1973) were derived by the application of a safety factor to the amount of PCBs consumed and the duration of the Yusho incident. The calculated amounts were 1 µg/kg/day based on the lowest total dose producing an effect and 4 µg/kg/day from the average total dose producing an effect. The latter value also appears as 3 µg/kg/day (FDA, 1977). Subsequently, Allen (1975) and Barsotti, et al. (1976) reported that reproduction was markedly impaired in rhesus monkeys fed polychlorinated biphenyls (PCBs) at a level (5.0 ppm) permitted in some foods for human consumption, and even one-half this level (2.5 ppm). They did not observe a no-effect level. A resolution of the apparently contradictory conclusions drawn from data on humans and nonhuman primates was suggested by calculation of the dosages received by the nonhuman primates. The dosages used by Barsotti, et al. (1976) were estimated from mean body weight data and the amounts of Aroclor 1248 consumed by female monkeys. In that study, 5.0 ppm in the diet represented a dosage of about 185 µg/kg/dy. The 2.5 ppm group averaged one-half that amount, 98 µg/kg/day. Thus, it

appeared that when allowance was made for the different amounts of food eaten by these 2 species, the dosages received by the nonhuman primates were well in excess of those that FDA had calculated as "allowable [for] protracted ingestion" based on human experience. The present study was undertaken to determine the dosage of PCBs which affected reproduction in nonhuman primates.

PCBs are a mixture of chlorinated homologues of biphenyl. While the earlier study (Allen, 1975; Barsotti, et al., 1976) had used Aroclor 1248 which has an average chlorination level of 48%, a decision was made to conduct reproduction studies with Aroclor 1254, whose average chlorination level is 54%. The latter was selected because it had been produced in greater volume than Aroclor 1248 (Monsanto, 1980). The dosages selected for testing were multiples of those that FDA had concluded were "allowable [for] protracted ingestion" (FDA, 1973, 1977).

METHODS

Animals and Dosage

Adult rhesus monkeys (Macaca mulatta) were used in this study. Animals were selected on the basis of a successful reproductive history and were estimated to be at least 6 years of age. They had been in captivity for periods ranging from 2 to 9 years. Four groups, each containing 8 female and 4 male monkeys, received daily doses of 0 (control), 5 μg/kg (low-dose group), 25 μg/kg (medium-dose group) and 100 μg/kg (high-dose group) of Aroclor 1254. The test material was administered orally once daily in a corn oil base. Each animal was weighed every 2 weeks at approximately the same time of day to minimize the effects of feeding on body weight, and the dose of Aroclor 1254 was adjusted as necessary. Solutions of Aroclor 1254 were supplied monthly by Monsanto Company (MC). They contained test material dissolved in a corn oil base so that 0.5 ml of a supplied solution per kilogram of body weight provided the prescribed amount of Aroclor 1254. The corn oil solution was added to apple sauce and offered to the animals in a shallow bowl prior to the day's feeding. Control animals received 0.5 ml/kg of corn oil in apple sauce each day. Animals received the test material 7 days a week for 14 months. Surviving animals were held on a control diet for another 7 months before they were euthanatized. After a new supply of the corn oil solution was received, material remaining from the prior month was returned to MC for analysis. Samples of the commercial primate feed fed to the animals were obtained, frozen and sent to MC 3 times (prior to compound administration and once each during months 10 and 19 of the overall test period) for analysis of their PCB levels.

Aroclor 1254-treated animals were bred to untreated male or female rhesus monkeys from the same colony. These animals were comparable to the test animals, except that they were not fed Aroclor 1254. Untreated adults used for mating to animals fed Aroclor 1254 were not studied further since their sole

purpose was for impregnating test females or determining the fertility of test males.

Breeding

Breeding began in October, a time that coincided with the expected period of highest conceptions for the colony, i.e., September through February. While births occur during all months of the year, a definite breeding cycle continues to exist even though colonies have been maintained for over a decade in constant temperature rooms with 12-hour light cycles. (Martin, in press). At that time, test animals had been dosed with Aroclor 1254 for 6 months. A timed breeding technique was used in which the female was placed in the cage of the male only during the 72 hours of the menstrual cycle when ovulation was expected to occur. Breeding was continued 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 summer months, breeding was discontinued after 8 months. 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. Semen from test males was obtained by electroejaculation to measure sperm concentration, total sperm, sperm motility, percent of abnormal cells, and live/dead ratios (Valerio, et al., 1970). These analyses were done monthly from just before administration of Aroclor 1254 until dosing was stopped (14 months). A final semen analysis was made 2 months later.

Hematology and Clinical Chemistry

A wide range of hematologic and clinical chemistry tests (Table 1) were conducted. These included common parameters used in toxicity studies and those reported to be affected by PCB exposure. They were performed on each animal before dosing with Aroclor 1254, at 2, 4, 5 and 6 months later, and bimonthly thereafter. After one year, the frequency for hematologic examinations was changed to monthly, and the following month clinical chemistry was similarly scheduled. During cytologic examinations for the first year, the occurrence of platelets in the field was subjectively noted and reported as "adequate" or otherwise. Subsequently, an actual count of platelets and reticulocytes was made and recorded. Serum vitamin A levels were determined just prior to necropsy.

Urinalysis

Urine was collected for urinalysis (Table 2) before compound administration and during the 3rd, 6th, 9th, 12th, 15th and 19th months. In each case, urine for urinary ketosteroid output was collected for a 24-hour period between days 22 and 26 of the menstrual cycle of the animal. Because pregnancy may affect the outcome of the 17-hydroxysteroid tests and because the pregnancy status of the female might not be known when the urine was obtained, results were not used if the animal was determined to have been pregnant at the time of urine collection. Pregnant animals were not tested for 17-hydroxysteroid until completion of the second menstrual cycle following birth.

Observations on Infants

Birth weight and somatic measurements were taken and recorded for all offspring born of both test males and females. Offspring of treated males were not studied further. Weighing and a complete blood cell count, including platelets and reticulocytes, were performed monthly on each infant born to a test female.

These infants were allowed to remain nursing on the mother. Infants not showing signs of Aroclor 1254 intoxication were euthanatized at 2 months of age. Those showing such signs were weaned from the mother and observed for reversal of those signs. Unless euthanatized earlier, all infants were killed at the end of the study (21st month) along with the adults.

Tissue Levels of Aroclor 1254

A biopsy of subcutaneous fat from the abdominal region of each adult was obtained before feeding of Aroclor 1254 began and at 2, 4, 5, 6, 8, 10, 12, 14, 15, 17, 19 and 21 months (necropsy). The last 4 samples were taken after dosing with Aroclor 1254 was stopped. When insufficient subcutaneous fat was available, a laparotomy was done to obtain omental fat. However, fat biopsies were not done on females of more than 100 days gestation, or on mothers rearing young during the first month of the infants' life to avoid interference with late gestational or neonatal periods. Due to insufficient amounts of subcutaneous fat for analysis, fat/skin biopsies of infants were taken at birth and at monthly intervals thereafter. These consisted of a segment of abdominal skin and the underlying fat. The decision of whether or not to take biopsy samples was made by the clinical veterinarian based upon the apparent health of the animal. Biopsy samples were individually marked in plastic bags which were then placed into larger bags by sex and treatment group, frozen, and sent in an insulated carton with dry ice to MC for analysis by the method of Kaley, et al. (1976). Some samples of colostrum and milk also were obtained for Aroclor 1254 analysis.

Necropsy and Microscopic Evaluation of Tissues

The 48 experimental adult animals were necropsied as they died or when eutha natized at the beginning of the 21st month of the study. Infants from test females were necropsied as they died or when euthanatized either at 2 months of age or the end of the study. Tissues (Table 3) from all adults and infants were

preserved in 10% buffered formalin solution (pH 7.0), dehydrated in ethanol, cleared in xylene and embedded in Paraplast®.[3] Bone specimens were subjected to prior decalcification in RDO decalcifying agent®.[4] Tissue blocks were routinely sectioned at a range of 5 to 6 microns in thickness except for brain which was sectioned at a range of 5 to 10 microns. 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.

Quality Assurance. This study was initiated before the effective date of FDA's Good Laboratory Practice Regulations for Nonclinical Laboratory Studies. However, it was audited by the Quality Assurance Unit of Litton Bionetics, Inc. in anticipation of and in conformity with such regulations as they became known. The Quality Assurance Unit stated that this study was performed according to acceptable laboratory practices. The discrepancies noted were minor in nature and would in no way invalidate the study.

RESULTS

Mortality

Table 6 shows the fate of all animals in this study. Causes unrelated to Aroclor 1254 exposure resulted in the deaths of 3 adult females. Acute gastric dilatation, a relatively common but poorly understood spontaneous affliction of macaques, was the cause of death of a low-dose female after 8 days on test and of a mid-dose female in the 17th month of the study. The low-dose female was replaced and is not discussed further. A control female died of hemorrhage and pulmonary aspiration following placental separation on the 94th day of pregnancy during the 13th month of the study.

Death or euthanasia in extremis occurred in 5 high-dose animals. A female died in the 10th month from a systemic Herpesvirus infection. In the 12th month a female with clinical signs of anemia was euthanatized and another died of intestinal bleeding. The 4th female, euthanatized in the 17th month, had severe biliary tract lesions. The single male was euthanatized in the 16th month because of extensive gangrenous stomatitis. The latter 4 deaths were probably related to Aroclor 1254 dosage.

Among the infants, a female born to a mid-dose mother died at 12 weeks of age of acute peritonitis. Another female, borne by a high-dose mother, died at 14 weeks of unknown causes.

Aroclor 1254 Dosing

Dosing solutions were prepared 13 times during the study. The initial analysis on one set of solutions was lost. During some intervals, all of the dosing solutions were used up, and no portions were available for analysis. Results of analyses of dosing solutions are shown in Table 4. The values confirm that the

solutions were properly prepared and that they were stable during the period of use.

Residues of PCBs in the 3 feed samples analyzed were below the limit of detection, i.e., below 0.05 μg/g.

Applesauce containing corn oil was refused only by 3 animals, a control female and one male each from the low- and high-dose groups. A crushed orange quarter, white bread or a banana were used as substitute carriers. Both males were given Aroclor 1254 by stomach tube once, early in the study. All other animals were dosed successfully using corn oil in applesauce.

Body Weight Changes

Body weight was a primary criterion in assigning males to the various groups. With females, it was a secondary factor. Primary emphasis was placed on past reproductive history to minimize differences in the number of live births, abortions and stillbirths. Consequently, there was a significant difference ($p<.05$) in mean body weight between high-dose and control females prior to dosing. Visual analysis of the data and observation of individual animal weights leads to a conclusion that high-dose females lost weight. Other adult groups showed about the same variability as the corresponding controls of that sex.

One-way analysis of variance was used to determine if mean body weights of infants were significantly different at birth and one and 2 months later. A significant difference in mean body weights was tested further by the Scheffe´ method for multiple comparisons to determine which mean body weights or combinations thereof were significantly different. The low-dose group gained weight more rapidly than the controls. At one and 2 months, there was a trend towards a difference between the mean weights of the control and low-dose groups

when compared to those of the mid- and high-dose groups. This trend was not statistically significant at any interval, but was almost significant ($.05<p<.10$) at 2 months (Table 5). The results were similar whether all body weights were included or whether 2 low-dose and one mid-dose weights were excluded, i.e., those of the stillborn infant (B8796), the 2-month weight of B8815, and B8860 on which only a 17-day weight was recorded. Mid-dose infant B8815 was moved to the nursery at 9 days of age because it had a low initial body weight and poor weight gain. Its weight gain in the nursery may not be representative of what it would have been had nursing on the mother continued.

The single high-dose infant (B8542) weighed 400 grams at 3 months, a few days before death. The medium-dose infant (B8560) showing signs compatible with those reported for polychlorinated biphenyl intoxication (see Signs of Intoxication) gained weight beyond what was expected and weighed 1267 grams at 7 months of age.

Effects on Fertility Testing

Each male had been expected to produce at least 2 pregnancies from the planned 16 mating periods if there were no effects of Aroclor 1254 upon fertility and if the animals performed according to colony history. Due to a lack of available females, it was not possible to schedule any test male for the planned 16 mating periods. Nevertheless, even with the reduced mating schedule, test males exceeded the anticipated conception rate. Each male in each group produced at least 2 pregnancies. There was no statistically significant effect on fertility of males between any test group and the controls.

No statistical analysis was done on sperm parameters due to the nature of the values and the fact that electroejaculation was not always effective in obtaining semen. Examination of the data revealed no dose-related trends. The lack of

an effect on sperm is substantiated by comparison to the pregnancy rates of untreated females bred to treated males.

Each group of 8 females would be expected to produce 6 live births if Aroclor 1254 had no effect on fertility. The control and medium-dose groups met or exceeded that level of performance with the production of 6 and 7 live births, respectively. An additional control female died during pregnancy after carrying an apparent normal fetus to 94 days of gestation. The low-dose group produced 5 live births, one stillbirth and one pregnancy which ended in fetal wastage. Three of the high-dose females died during the breeding phase without being diagnosed as pregnant. Among the other 5 high-dose group females, there was one live birth and 2 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. However, no known pregnancies resulted in those 2 animals. Since the earliest time that a pregnancy could be diagnosed by digital palpation of the uterus was at approximately 28 gestational days, it is possible that some other pregnancies could have occurred and been spontaneously terminated before that time. Statistical analysis of the data indicated a significant difference in fertility only between the control and high-dose females. Most of the births occurred within a 4-month span (Figure 1). Overall, there was no dose-related effect on the time of birth, even though some low-dose females delivered their young later than the other groups.

Signs of Intoxication In Adults

There were no signs of intoxication among adult animals referable to Aroclor 1254 dosing during the first 9 months of the study. After that time, about one-half of the high-dose group developed redness and/or edema and wrinkling (puffiness) of the eyelids. This condition continued and became more apparent as dosing with Aroclor 1254 continued. When dosing with Aroclor 1254 was

stopped after 14 months on test, the eyelid changes appeared to subside. They disappeared completely in 2 males and became less severe in many animals during the subsequent 7 months that the animals were observed. Swelling of the lips followed a similar pattern and was manifest in all high-dose animals at one time or another. This condition was reported as slight in many animals and also appeared to subside in some animals. After 15 months on test, 2 high-dose males and a high-dose female had a black coloration of the calculus on their teeth. The coloration became somewhat browner than black in one male who survived to the end of the test period. It was observed that 2 of these animals bled easily from their gums. Frequent bleeding from the gums could account for the staining of the calculi of the teeth. Several high-dose animals had an abnormal growth pattern of the fingernails and/or toenails. The nail appeared to grow outward away from the nail bed with an apparent accumulation of debris under the nail. Alopecia is difficult to quantify and occurs occasionally in rhesus monkeys not receiving any treatment. On the basis of observations made at the monthly physical examination, alopecia appeared to occur more frequently and to a greater extent in high-dose animals after a year on test. After Aroclor 1254 dosing was stopped, some regrowth of hair occurred, including eyelashes.

Among medium-dose adult animals, swelling of the lips occurred in one female and one male after 15 and 18 months on test, respectively. Animals of this group also had alopecia and the abnormal nail growth pattern described for high dose animals. The low-dose group adults did not show the preceding or any other signs attributable to Aroclor 1254 exposure.

Effects on Offspring

The first viable offspring were born after test females had been receiving Aroclor 1254 for over a year. Except for the 4 infants discussed below, all were clinically normal. A high-dose (B8542) and 2 medium-dose (B8546 and B8560) level infants were born with or developed signs compatible with those reported for polychlorinated biphenyl intoxication while nursing on the mother, i.e., swollen lips and/or eyelids and/or scanty eyelashes. These signs were more severe in the infant from the high-dose mother. The high-dose and one of the medium-dose infants developed pulmonary signs and were moved to the nursery when 10-11 weeks of age. Both died about 3 weeks later. The other medium-dose infant (B8560) was taken to the nursery at 10 weeks of age and lived another 20 weeks until it was euthanatized at the end of the study. It had swollen lips and eyelids, scanty eyelashes, scaly skin and chronic wheezing. Some evidence of eyelash regrowth occurred toward the end of the study but the respiratory wheezing persisted. Although the deciduous teeth of this animal were brown, the new teeth that erupted in the 7th month of life were normal in color. A subjective observation was made that this animal became more excited than similar non-study animals when approached by personnel. This is the same animal that was mentioned earlier as having a greater than expected weight gain. A fourth infant (B8815) from a low-dose mother was moved to the nursery several days after birth because its initial body weight was low and it was not gaining weight. That animal was about 2½ months of age when killed at the end of the study. These 4 infants were the only ones which were kept on test longer than 2 months.

Effects on Hematology, Clinical Chemistry and Urinalysis

Evaluation of data

Students "t" test was used to evaluate numerical data. Differences which had

a 5%, or lower, probability of occurring by chance were considered significant. However, statistical significance was not the sole criterion for evaluating test results. While the initial size of the groups was theoretically large enough for statistical analysis, the actual number of animals in each was small, especially in the case of males. As the study progressed, the number of observations decreased because of death of some animals. In addition, the deletion of determinations for other reasons further decreased the number of observations on some parameters. A major cause of deleted determinations arose from the fact that females became pregnant at different times, and pregnancy affects physiological parameters as well as physical ones such as body weight. Therefore, changes in measured parameters deemed to have resulted from pregnancy alone were deleted when statistical analyses were performed. This determination was based on a comparison of hematology and biochemistry test results between pregnant and non-pregnant controls. Values obtained on control animals during the first 3 months of pregnancy, the last 2 months of pregnancy, and the 2 months following pregnancy (when the mother would still be adjusting to the changes of pregnancy) were identified. The values for each of those months were compared to corresponding measurements on control females. If the differences in the values for any period were statistically significant, a value judgement was made as to whether the difference was real or an artifact. The range of values for the measured parameter, its position relative to the control value (i.e., above, below, within the range) and changes for that value reported in human pregnancy (the only large body of comparable data) were considered in making such decisions. Data from females who experienced fetal wastage were treated as though the pregnancy had continued to term.

Analysis of the data showed numerous categories with significant differences. These statistically significant changes were grouped into 4 categories: changes toward a normal level even if they differed from the control group means;

changed values that were still within ranges considered normal; changes judged to be biologically inconsequential; and changes that did not fall into the preceding categories. Only the latter are discussed below.

Effects on hematology

Females were assigned to test groups on the basis of a successful reproductive history. Before dosing began, both high- and medium-dose adult female animals differed from the controls in red blood cell values (PCV, RBC and Hgb). Observation of individual animal data, however, showed that these values became depressed for some high-dose animals. Platelet values, which were only done by actual count for a portion of the study, also showed a profound depletion in some high-dose animals. The most severely affected animals died or were euthanatized, thus removing them from future calculations. Of the 5 animals from the high-dose group which died, 4 showed definite effects on blood parameters, particularly reduced platelet counts, and one had a frank pancytopenia. Three of these animals demonstrated prolonged bleeding from the sites of the last fat biopsies taken from them and the incisions did not heal properly. Based upon the postmortem findings, an effect of the test material on the bone marrow seems to have been a likely mechanism for the red blood cell changes.

Infant blood samples also were taken unless the clinical veterinarian judged such action to be potentially dangerous to the infant. Observation of the values for formed elements of the blood were generally within normal limits for all dose groups for the periods which were sampled.

Effects on clinical chemistry

Serum iron levels were consistent with the alterations in hematology parameters. Significantly elevated values were noted 10 times for high-dose females and 6 times each for high-dose males and medium-dose females. Serum cholesterol

levels also were more affected in females. Statistically significant decreases occurred 9 times in high-dose females and twice each in mid-and low-dose females. However, review of the serum cholesterol data revealed a general lowering of values for both sexes with a slow return towards normal after administration of the test material had ceased.

Except for a few sporadic values in males, serum albumin levels were not affected. Globulin levels were significantly increased among all male treated groups at several periods, particularly after administration of the test material had been stopped. Males also had decreased albumin/globulin ratios which roughly corresponded to the changes in serum globulin levels.

Serum vitamin A levels determined just prior to necropsy were quite variable at all dose levels. They were significantly decreased only for mid-dose females. No biological significance is attached to this difference. Since most infants had been euthanatized at 2 months of age, only 3 were alive when the study was terminated. Their serum vitamin A levels had a range similar to that of the adults.

Effects on urine parameters

No statistical analysis was done on these data. Examination of the various parameters did not reveal any dose-related trends.

Microscopic Changes in Tissues

Observations during microscopic examination of tissues were grouped into 3 categories: pathologic alterations incident to euthanasia and environment, e.g., terminal hemorrhages, minor mucosal lesions, etc.; spontaneous lesions of non-human primates; and pathologic alterations representing direct morphologic effects of the test material. Only the latter are discussed.

Classification of lesions as representing direct morphologic effects of the test material was based on the unique character of the lesions and their similarity to published observations on the effects of PCBs. These were alterations in Meibomian glands of non-human primates which have been designated "squamous metaplasia with conversion of the glands to a row of squamous cysts" (McNulty, 1977; McNulty and Griffin, 1976). Compound-related alteration in sebaceous glands of the skin has also been designated "squamous metaplasia" (McNulty, 1977).

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 (Bloom and Fawcett, 1968). There is disagreement concerning replacement of cells that disintegrate into oily secretion. It has been stated that the regenerative activity occurs (McNulty, 1977) in the basal layer of epithelial cells lining the secretory alveoli and (McNulty and Griffin, 1976) in "cells close to the walls of the ducts, where the new cells move into the secretory regions" (Bloom and Fawcett, 1968; Ham, 1974).

In this report, terminology was selected to indicate the morphologic appearance of compound-related alterations without regard to the 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'. Two instances with less than total atrophy of the sebaceous elements were designated 'subtotal atrophy-sebaceous elements'. An appearance of squamous metaplasia also was recognized and so designated. Round and elongated structures with thick epithelial walls and large central

areas packed with epithelial squames were generally present. The round structures had the appearance of squamous cysts, but a majority were elongated and could be identified as Meibomian gland ducts impacted with squamous debris. These were designated 'squamous impaction-ducts'.

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'.

There were characteristic changes associated with loss of secretory epithelium of the Meibomian glands in the eyelids and the sebaceous glands in the skin. These changes occurred in 11 adults, (8 females and 2 males from the high-dose group and the female in the mid-dose group which died) and 5 infants (B8542 from a high-dose and B8546, B8559, B8560 and B8603 from mid-dose mothers).

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 effects 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'.

Subtotal or total atrophy of the sebaceous glands of the skin were noted in 3 male and 8 female adults from the high-dose, in one male and 2 female adults

from the mid-dose group and in an infant (B8542), born to a high-dose mother and another (B8546) from a mid-dose mother. Most of these animals also had atrophy of the sebaceous glands of the lips. Animals with follicular keratosis and squamous cysts are included in the totals with sebaceous gland atrophy.

Among adult animals, squamous cysts of the skin were observed in a mid-dose female (labium) and in a male and 2 females from the high dose. One of the latter and a second high-dose female also had squames in the deep mammary duct.

Follicular keratosis was observed in various sections of skin taken from 2 adult males and one adult female in the high dose group and the infant (B8542) born to a high-dose mother.

The system of nomenclature used in publications on the effects of polychlorinated biphenyls in nonhuman primates was adopted for compound-related lesions observed in the biliary tract, liver, gastric mucosa, and mandible (Allen, 1975; Allen and Barsotti, 1976; Allen, et al., 1974; Barsotti, et al., 1976; Becker, et al., 1979; McNulty, 1977; McNulty and Griffin, 1976).

Unique, apparently compound-related lesions were seen in the pancreas and kidney of infants. 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. It occurred in a mid-dose (B8603) and the high-dose (B8542) infant. The latter also had kidney lesions with the microscopic appearance of areas in the cortex where there was retarded cortical maturation.

A high-dose adult female had epithelial hyperplasia of the intrahepatic bile duct and of the main duct of the pancreas. She also showed mucosal hyperplasia in the extrahepatic bile duct and the gall bladder. The infant (B8542) born to a high-dose mother also had epithelial hyperplasia of the intrahepatic bile ducts. That infant and B8603, a male from the mid-dose group, also had ductal hyperplasia and acinar atrophy of the pancreas.

B8542, the infant born to a high-dose female, had cystic mucinous metaplasia of the mucosal glands and submucosal glandular cysts in the stomach, crypt metaplasia of Brunner's glands in the small intestine, and 'toxic' involution and cystic Hassall's corpuscles in the thymus. The designation 'toxic involution' was used for compound-related atrophy of the thymus in infants to differentiate it from the physiologic involution that was invariably present in adults to a greater or lesser degree. An infant from the mid-dose group (B8546) had similar alterations in the thymus only and another infant from that group (B8603) had cystic Hassall's corpuscles.

Subgingival epithelial cysts or microcysts were seen in the mandibles of 3 adults, a high-dose male and female and a mid-dose male. Three infant females, B8542, B8560 and B8815 from the high, medium and low groups, respectively, had subgingival squamous cysts of the mandible and the low-dose infant also had an intraosseous squamous cyst of the mandible.

There were single occurrences of squamous metaplasia among adults: in a glandular duct of the tongue of 2 high-dose females, in a glandular duct of the lip of a high-dose female, and in the thyroid of a high-dose male. A high-dose male had squamous metaplasia in the sebaceous glands of the lip and of the epicardium.

Non-specific bone marrow alterations were observed, in particular, decreased cellularity and/or decreased granulocyte maturation. In adults, these changes occurred in 5 females and one male from the high-dose group. They were also seen in infants B8542 and B8546 borne by high- and mid-dose mothers. The bone marrow alterations may have been compound related because they correlated with hematologic changes observed in the animals during the study.

Table 6 identifies each animal in relation to the test and tabulates the number of pathologic alterations observed for each animal. This table illustrates the fact that the incidence of direct morphologic effects of the test material was dose-related. This incidence was highest in the high-dose group and substantially lower in the mid-dose group. There was a minor morphologic effect, a minimal alteration in the mandible, in a single infant born to a low-dose mother. Insofar as comparisons can be made, since there was only one infant in the high-dose group, the incidence of direct morphologic effects of Aroclor 1254 was greater in infants than in adults.

Since animals with morphologic changes attributed to PCBs may show lesions in several tissues, Table 7 was prepared to summarize the incidence of morphologic effects attributed to the test material. Overall, there were dose-related and, to a lesser degree, sex-related and age-related morphologic effects of the material in high- and medium-dose animals.

Tissue Residues of PCBs

An existing procedure for detecting PCBs in animal lipids (Kaley, et al., 1976) provided the basic methodology for PCB tissue analyses in this study. However, the following facts should be considered in reviewing and interpreting the reported values: the necessity to modify extraction procedures for the variety of tissues involved, and the small sample sizes, precluded statistical evaluation of

the accuracy (recovery) and precision of the PCB determinations. Thus, it is extremely difficult to determine whether variations in PCB tissue concentrations for an individual animal, or between different animals, are significant differences or the result of analytical variability. The limited size of the samples introduces its own difficulties. On a relative basis, it tends to increase analytical variability, particularly as the concentration of the material sought decreases. Small sample sizes also increase the probability of nonhomogeneity, which contributes to greater variability.

Values for tissue levels of PCBs were analyzed using a computer program which converts the data to logarithms and calculates the mean and standard deviation of PCBs for each level at each sampling time. It then calculates 95% confidence limits of the means of the logarithms and converts these back to ppm. The test for significance was based on the degree to which the 95% confidence limits of the means overlapped. The least significant difference (95% level) between the means is approximately 2.828 standard deviations. When there is no overlap of the 95% confidence limits, the means are approximately 4 standard deviations apart.

Over 500 individual assays were performed on fat from adults. Within each group, at all time intervals, there was considerable variation in the levels of PCB detected. Generally, the 95% confidence limits for the mean ranged from 50% to 200% of the mean. Nevertheless, some generalizations are possible.

The high-dose group had significantly elevated PCB levels from one month through the end of the study, averaging 28.2 ppm. In the medium-dose group, significant elevations were present from 4 months to the end of the study. Mean PCB levels were 13.2 ppm. The low-dose group averaged 2.8 ppm from 7 months to the end of the study, the interval during which there were significant

elevations. Fat samples from controls averaged 0.3 ppm. All values are expressed on a wet weight basis. From 9 months to the end of the study, there were no further increases in the levels of PCB detected.

PCB levels in the livers of 41 adults necropsied at the end of the study increased in proportion to the amounts of Aroclor 1254 which they had received. On a wet weight basis, the mean levels were 0.076, 0.42, 0.81 and 2.43 ppm for the control, low-, mid-, and high-dose groups, respectively.

Analytical results from infant skin and fat and mother's milk were insufficient for statistical analysis. Review of the small number of samples taken at different times showed a tendency for PCB levels to increase with increasing exposure of the mother.

DISCUSSION

As indicated in the INTRODUCTION, rhesus monkeys fed 2.5 ppm and 5.0 ppm of Aroclor 1248 by Barsotti, et al. (1976) received dosages of approximately 93 µg/kg/day and 185 µg/kg/day, respectively. Female rhesus monkeys have been given 0.5 ppm or 1.0 ppm of Aroclor 1248 in the diet 3 times per week (Allen, et al., 1979). Assuming similarity to their earlier study, since body weights were not given, those levels would give dosages of approximatley 7.9 µg/kg/day and 16 µg/kg/day. Thus, there is sufficient overlap between those dosages and the ones used in the present study to permit comparisons.

Among the similarities was the absence of effects on reproduction of male rhesus monkeys at any dosage of Aroclor 1248 or Aroclor 1254. In adult females, both Aroclor products at dosages of about 100 µg/kg/day produced some deaths, body weight loss, and lowered cholesterol values. At that dosage, both studies reported that viable offspring were smaller in size, and their number was reduced. Several similar morphologic effects also were observed in tissues taken from adults and infants. At lower dosages, 25 µg/kg/day and below, neither PCB produced menstrual cycle abnormalities, there were no alterations in serum estrogen levels, and no adverse effects on reproduction were observed. At these dosages, infants borne by mothers exposed to Aroclor 1248 were reported to be somewhat smaller and to gain weight less rapidly than their controls. Infants from mothers receiving 25 µg/kg/day of Aroclor 1254 presented a similar picture, but those from the 5 µg/kg/day group gained weight more rapidly than the controls. However, the numerical values for mean initial body weights and mean weight gains for Aroclor 1254 infants did not differ significantly from those of their controls.

Dosages around and above 100 µg/kg/day of Aroclor 1248 were reported to produce decreased A/G ratios in females. With Aroclor 1254, a decrease in this ratio was observed only in males. Changes in SGPT activity, urinary 17-ketosteroid excretion, and vitamin A levels reported for Aroclor 1248 were not seen after exposure to Aroclor 1254.

Another difference was that the rapid onset (1-2 months) of signs of intoxication reported for Aroclor 1248 was not seen with Aroclor 1254. The delayed onset and less severe signs of intoxication observed in the present study are consistent with limited data from other reports of nonhuman primates fed PCBs. Truelove, et al. (1982) dosed 3 cynomolgus monkeys with Aroclor 1248 for about 100 days; 2 at 100 µg/kg/day and one at 400 µg/kg/day. They commented that "It is particularly noteworthy that no overt signs of toxicity were observed in the adult animals with the exception of fingernail loss, observed in 2 out of 3 of the treated monkeys. This is in contrast to the work of Allen and Barsotti (1976)." Among the possible factors that could account for the lack of clinical toxicity observed in their experiment, Truelove, et al. (1982) mentioned differences in response or sensitivity of the 2 species of macaque to PCB insult.

Kataoka, et al. (1980) dosed 2 female African green monkeys (Cercopithecus aethops) with 500 µg of Kanechlor 400 per kg of body weight 3 times per week for 21 weeks. They reported that body weights did not change notably. While one monkey died at the end of 18 weeks with signs of facial acne, hair loss, and diarrhea, the authors did not consider PCB to be directly related to the death. The other animal was unaffected. Hori, et al. (1981) administered Kanechlor 400, from which polychlorinated dibenzofurans (PCDFs) had been removed, to 2 female cynomolgus monkeys (M. fascicularis). They gave 5 mg doses to each monkey, 6 times/week, for 20 weeks. Since initial body weights were about 2.5

kg, the administered dosage was about 2000 μg/kg. The only adverse sign reported was a body weight reduction.

Some of the different findings reported in nonhuman primates treated with PCBs appear to be related to severity of exposure and to sex and age of test subjects. Others may reflect variations in susceptibility between various genera of primates. Thus, a review of the results of the present study leads to a conclusion that a no-effect level in adult rhesus monkeys has been demonstrated at 5 μg/kg/day. The minimal morphologic effect in the mandible of one of 6 infants born to low-dose females treated with Aroclor 1254 is considered to be a reflection of the greater sensitivity of infant rhesus monkeys to PCBs. It may also be a reflection of the apparently greater sensitivity to PCBs of rhesus monkeys, as compared to other primates. Nevertheless, data from nonhuman primates are consistent with human experience in the Yusho incident, even though results with rhesus monkeys suggest that the safety factor may be somewhat less than FDA had originally estimated.

Studies with Aroclor 1248 and Aroclor 1254 revealed an apparent plateau of PCB levels in fat with time. However, we believe that no meaningful comparisons of tissue levels can be made because of uncertainties regarding the comparability of the analytical procedures used.

Subsequently, it has been reported that Yusho oil was highly contaminated with other chlorine-containing materials, i.e., PCDFs and polychlorinated quarterphenyls (PCQs), and that PCDFs and PCQs have been detected in tissues of Yusho patients (Kuratsune, 1980). PCDFs are more toxic than PCBs to rats (Oishi, et al., 1978). Little is known about the toxicity of PCQs. Reports of these impurities raise questions as to the actual causative agent(s) of Yusho disease. In a recent article commenting on the 1968 Yusho episode and a very

similar outbreak in Taiwan in 1979, Rogan (1982) remarked that "measurable PCBs may be only a surrogate for what is actually the toxic agent." If this proves to be correct, FDA may have overestimated the toxicity of PCBs to humans in setting temporary tolerances for them in foods.

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Table 1

Aroclor 1254: Reproduction Study With Rhesus Monkeys

Hematological and Clinical Parameters

RBC	Triglycerides	Serum potassium
PCV	Bilirubin, total	Serum sodium
Hemoglobin	BUN	Serum chloride
Platelets	Creatinine	Carbon dioxide
WBC & differential	Uric acid	SGOT
Total protein	Glucose, fasting	SGPT
Albumin	Calcium	Alkaline phosphatase
Globulin	Phosphorus	Creatinine phosphokinase
A/G ratio	Total iron	Lactic dehydrogenase
Cholesterol	Serum Vitamin A	

Table 2

Aroclor 1254: Reproduction Study With Rhesus Monkeys

Urinalysis Parameters

Total volume	Ketone	Calcium oxalate crystals
Color	Bilirubin	Triple phosphate crystals
Appearance	Blood	Amorphous crystals
Specific gravity	RBC	Mucous threads
pH	WBC	Casts
Glucose	Epithelial cells	Bacteria
Protein	Urobilinogen	Other
	17-Ketosteroids	

Table 3

Aroclor 1254: Reproduction Study With Rhesus Monkeys

Tissues Examined Histologically

Brain (3 sections)	Esophagus	Cervix
Pituitary	Stomach,	**Labium
Eyelids	**pylorus	Testes
Lips	**fundus	Epididymus
Mandible	Duodenum	**Prostate
Salivary glands,	Jejunum	**Foreskin
parotid with duct	Ileum	*Skeletal muscle
submaxillary	**Cecum	*Spinal cord, lumbar
Thyroid	Colon	Nerve,
**Parathyroid (if present)	Liver	* sciatic
Lymph nodes,	**Extrahepatic bile duct	**distal tibial
axillary	Gall bladder	*Bone marrow,
mesenteric	*Spleen	**femur
Heart	Pancreas with duct	**sternum
Thymus (if present)	Kidneys	**Skin,
Lungs,	Adrenals	hip
apical	Urinary bladder	thigh
diaphragmatic	Ovaries	**Nipple, left
Tongue	Uterus	Lesions
	Vagina	

* Added after 14 months on test after tissues taken on control and 3 high dose females.

** Added after 20 months on test after tissues taken on additional mid dose female, high dose male and female and 2 infants which died.

Table 4

Aroclor 1254: Reproduction Study with Rhesus Monkeys

Analysis of Corn Oil Dosing Solutions

	As Prepared		After Use	
Solution[a]	No.	µg/ml[b]	No.	µg/ml
Control	9	0.42 ± 0.34	8	0.31 ± 0.29
Low dosage (5 µg/kg)	12	11.9 ± 2.3	10	11.8 ± 2.3
Medium dosage (25 µg/kg)	12	53.5 ± 7.3	11	51.2 ± 7.2
High dosage (100 µg/kg)	12	199.5 ± 21.2	12	201.8 ± 25.9

[a] Administered at 0.5 ml/kg

[b] Mean value ± S.D.

Table 5

Aroclor 1254: Reproduction Study with Rhesus Monkeys

Body Weights of Infants

Parental Aroclor 1254 µg/kg/day		Infant No.	Sex	Body wt. (g) Birth	1 Month	2 Months*
0		B8518	M	482.5	* *	656.0
		B8532	F	440.7	* *	675.0
		B8533	F	514.0	* *	669.0
		B8557	M	473.0	623.0	756.0
		B8574	M	500.0	506.0	573.0
		B8621	F	561.0	697.0	918.0
	Mean			495.2	608.7	707.8
5		B8527	F	470.0	* *	748.0
		B8562	F	600.0	680.0	863.0
		B8604	M	471.0	613.0	671.0
		B8754	F	593.0	753.0	858.0
		B8796	M	460.0[a]	-	-
		B8815	F	349.0	438.0	592.0[b]
	Mean			490.5	621.0	746.4
25		B8511	M	328.0	326.0	397.7
		B8546	F	326.0	406.0	473.0[c]
		B8559	M	603.5	639.0	789.0
		B8560	F	394.0	426.0	452.0[d]
		B8592	F	442.0	513.0	690.0
		B8603	M	396.0	420.0	485.0
		B8860	M	467.0	(493.0)[e]	-
	Mean			422.4	460.4	547.8
100		B8542	F	365.0	391.0	425.0[f]

Table 5

(continued)

* Infants left to nurse on mother and euthanatized at 2 months unless otherwise noted.

** 1-Month body weight measurement added to protocol after first 4 infants had passed that age.

[a] Stillbirth

[b] Taken to nursery at 9 days of age. Euthanatized at 91 at end of study.

[c] Taken to nursery at 68 days of age. Died at 86.

[d] Taken to nursery at 69 days of age. Euthanatized at 211 at end of study.

[e] 17 days of age. Euthanatized at 31 at end of study.

[f] Taken to nursery at 78 days of age. Died at 97.

Table 6

Aroclor 1254: Reproduction Study With Rhesus Monkeys

Tabulation of Direct Morphologic Effects

Aroclor 1254 (µg/kg/day)																			
0					5					25					100				
		Anim. No.,	Pathologic Alterations				Anim. No.,	Pathologic Alterations				Anim. No.,	Pathologic Alterations				Anim. No.,	Pathologic Alterations	
Sex	Age	Fate	No.	Mean	Sex	Age	Fate	No.	Mean	Sex	Age	Fate	No.	Mean	Sex	Age	Fate	No.	Mean
M	A	227G T	0		M	A	119H T	0		M	A	20I T	0		M	A	425J T	4	
M	A	418J T	0		M	A	428J T	0		M	A	46I T	0		M	A	427J T	0	
M	A	426G T	0		M	A	432J T	0		M	A	595I T	3		M	A	600I T	9	
M	A	739F T	0		M	A	516G T	0		M	A	599I T	0		M	A	706E K	16	
				0					0					0.75					7.25
F	A	200F T	0		F	A	241I T	0		F	A	273I T	0		F	A	226F T	10	
F	A	274F T	0		F	A	309I T	0		F	A	281G T	0		F	A	269I T	5	
F	A	569H T	0		F	A	483F T	0		F	A	307I T	2		F	A	287I T	7	
F	A	578H T	0		F	A	605F T	0		F	A	540H T	0		F	A	312I T	6	
F	A	604I T	0		F	A	624I T	0		F	A	757I T	0		F	A	152F D	8	
F	A	618I T	0		F	A	646E T	0		F	A	762E T	2		F	A	338I K	21	
F	A	764E T	0		F	A	701E T	0		F	A	763E T	0		F	A	486H K	10	
F	A	87F D	0		F	A	839H T	0		F	A	841H D	3		F	A	770I D	6	
				0					0					0.88					9.13

Table 6

(continued)

F	I	B8443	FW	0	F	I	B8527	T	0	M	I	B8511	T	0	F	I	B8542	D	20
M	I	B8518	T	0	F	I	B8562	T	0	F	I	B8546	D	11					
F	I	B8532	T	0	M	I	B8604	T	0	M	I	B8559	T	2					
F	I	B8533	T	0	F	I	B8754	T	0	F	I	B8560	T	4					
M	I	B8557	T	0	M	I	B8796	S	0	F	I	B8592	T	0					
M	I	B8574	T	0	F	I	B8815	T	2	M	I	B8603	T	5					
F	I	B8621	T	0						M	I	B8860	T	0					
				0					0.33					3.14					20

A = Adult

D = Found dead

FW = Fetal wastage (abortion before 151 days gestation)

I = Infant

K = Killed in moribund state

S = Stillborn

T = Terminal kill

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Table 7

Aroclor 1254: Reproduction Study With Rhesus Monkeys

Incidence of Animals with Morphologic Effects

Attributed to the Test Material*

Aroclor 1254 (µg/kg/day)**	0		5		25		100	
Sex	M	F	M	F	M	F	M	F
Adults	0/4	0/8	0/4	0/8	1/4	3/8	3/4	8/8
Infants	0/3	0/4	0/2	1/4	2/4	2/3	-	1/1

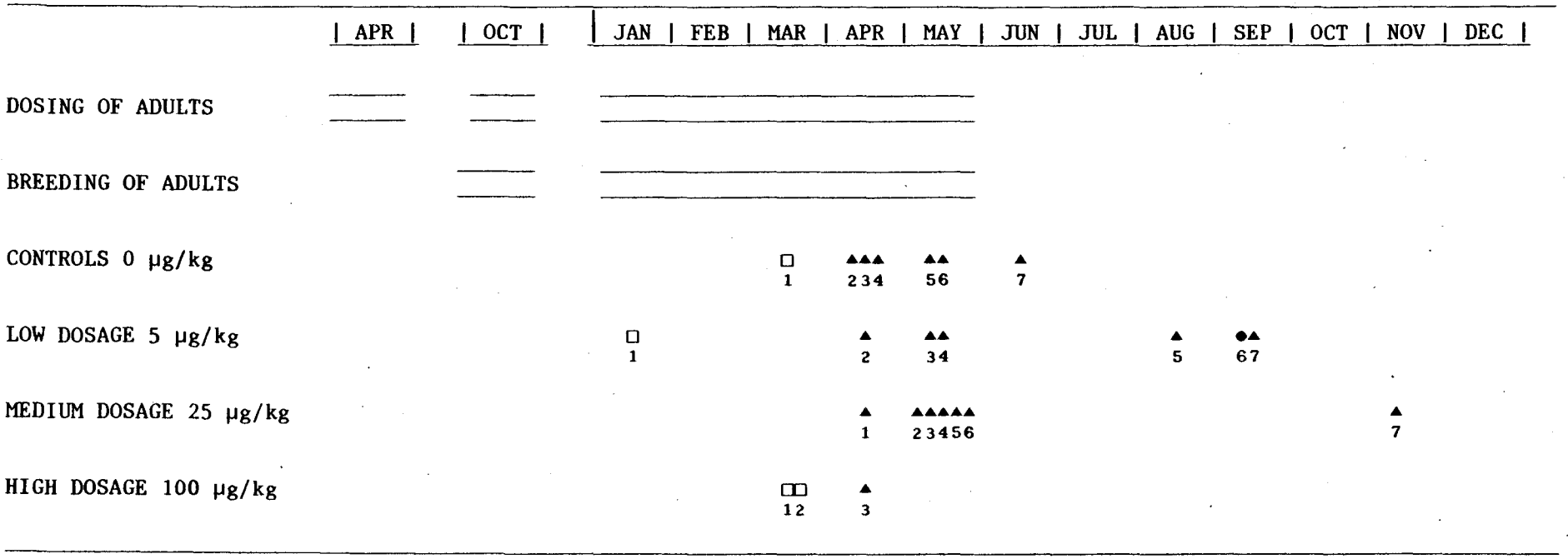
* No. affected/No. examined

** Only parental animals dosed

FIGURE 1

AROCLOR 1254: REPRODUCTION STUDY WITH RHESUS MONKEYS

TIME OF BIRTH OF INFANTS



▲ Live Birth

● Still Birth

□ Fetal Wastage

Symbols show time, relative to start of dosing and breeding of adults, at which result of pregnancy was determined.

Numbers beneath symbols represent cumulative known pregnancies of each group.

Footnotes

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