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Toxicological Consequences of Aroclor 1254 Ingestion by Female Rhesus (*Macaca mulatta*) Monkeys. Part 2. Reproduction and Infant Findings

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Abstract—A group of 80 menstruating rhesus (*Macaca mulatta*) monkeys were randomly allocated to four similar test rooms (20 monkeys/room) and then randomly allocated within each room to one of five dose groups (four females/dose group/room). Each day, the monkeys self-ingested capsules containing doses of 0, 5, 20, 40 or 80 μg Aroclor 1254/kg body weight. After 25 months of continuous dosing, approximately 90% of the treated females had attained a qualitative pharmacokinetic steady state with respect to the concentration of polychlorinated biphenyl (PCB) in their adipose tissue. Commencing on test month 37, each female was paired with an untreated male until either an impregnation occurred or the 29-month breeding phase of the study was completed. The females continued to receive their daily test dose during mating and gestation. To preclude an infant ingesting the mother's dosing capsule, dosing of the dam was discontinued when a nursing infant was approximately 7 wk old. Treatment was restarted when the infant was weaned at 22 wk of age. At parturition, and every 4 wk until weaning, milk and blood samples were obtained from the dam and a blood sample was obtained from the infant for PCB analysis. When the infant was 20 wk old, immunological testing was initiated and an adipose sample was obtained from the infant and dam for PCB analysis. Subsequently, further adipose and blood samples were obtained from the infant and blood specimens were obtained from the dam for PCB analysis. Concurrently, each infant was subjected to anthropometric measurements and detailed clinical examinations until it was approximately 122 wk old. At 122 wk some of the control and all of the treated infants were killed humanely and autopsied. A statistical analysis of the reproduction data provided evidence for a significant decreasing dose-related trend in conception rates and a significant increasing dose-related trend in foetal mortality. Several comparisons between impregnated and non-impregnated females did not implicate 'age' as a confounding factor regarding these results. The major findings with the infants involved some immunological test differences and mild clinical manifestations of PCB ingestion.

INTRODUCTION

Previously, we reported on the clinical health (Arnold *et al.*, 1993b), and analytical and clinical laboratory findings (Arnold *et al.*, 1993a; Bell *et al.*, 1994) associated with the daily ingestion of Aroclor 1254 by adult female rhesus (*Macaca mulatta*) monkeys during the 3-yr prebreeding phase of this study. The doses were comparable with exposure levels of specific human subpopulations (Dillion *et al.*, 1981; Kreiss, 1985; Lione, 1988). During this time, 90% of the treated monkeys had attained a satisfactory qualitative pharmacokinetic steady state regarding the concentration of PCB in their adipose tissue (K. Karpinski, R. Stapley and D. L. Arnold, unpublished data, 1985). This paper provides a summary of most of the adult and infant findings from the reproduction phase of the study. On completion of the breeding phase, the test females were again returned to the monitoring program previously described (Arnold *et al.*, 1993a). We also present limited results from the analysis of PCB levels in the milk samples and of the PCB levels in the blood samples of the dams and infants. However, a more extensive presentation and evaluation of these analytical data has been published elsewhere (Mes

Abbreviations: ANOVA = analysis of variance; Con A = concanavalin A; CPM = counts per minute; F = female; Ig = immunoglobulin; Ket-HCl = ketamine hydrochloride; M = male; MANOVA = multivariate analysis of variance; PCB = polychlorinated biphenyls; PHA-P = phytohaemagglutinin; PWM = pokeweed mitogen; SD = standard deviation; SRBC = sheep red blood cells; [*H*]TdR = [*methyl*-^{3}H]thymidine; WBC = white blood cells.

et al., 1994a and 1995), and the post-mortem analytical and pathological findings for all of the female monkeys and their infants will be reported in detail in future manuscripts.

MATERIALS AND METHODS

The composition, acclimatization, predosing evaluation and randomization of the 80 menstruating rhesus (*Macaca mulatta*) monkeys to the four test rooms and the randomization of the animals to the five dose groups were described previously (Arnold *et al.*, 1993b), as were the source and contaminant content of the Aroclor 1254 (Arnold *et al.*, 1990; Mes *et al.*, 1989). Briefly, daily doses of 0, 5, 20, 40 or 80 μg Aroclor 1254/kg body weight were provided in a gelatin capsule for self-ingestion by the female monkeys in dose groups A (Control), B, C, D and E, respectively. The monkeys were housed in individual stainless-steel cages in environmentally controlled rooms (temperature 22 ± 2°C; relative humidity 50 ± 10%; 12-hr light/dark cycle; 15 air changes/hr). The clinical, analytical and clinical laboratory monitoring undertaken before the start of the breeding, as well as an analysis of the data collected, have been previously described and discussed (Arnold *et al.*, 1993a,b; Bell *et al.*, 1994). Four females (one from group C, two from Group D and one from Group E) were killed humanely before the start of breeding for reasons that were not believed to be attributable to treatment; three of these monkeys had endometriosis and one had a coagulation factor deficiency. During the breeding phase of the study, two animals in Group 1, one in Group C and two in Group E were killed humanely. Two monkeys in Group E died unexpectedly despite having been extensively monitored throughout the study. The Group A and C monkeys, and one of the Group E monkeys that died unexpectedly, had endometriosis. The death of this Group E monkey was attributed to post-operative complications as a consequence of pneumonia and peritonitis. The second unexpected death was attributed to endocervical leiomyoma. The two Group E monkeys that were killed humanely were in poor health as a consequence of an apparent wasting syndrome which was attributed to PCB ingestion. Additional information concerning these monkeys will be contained in a future manuscript.

The average estimated age of the monkeys at study initiation was 11.5 ± 4.3 yr (mean $\pm$ SD). This estimate was derived using the following procedure. Three veterinarians from our facility were asked to estimate each monkey's age, primarily on the basis of dentition. These estimates were evaluated against the following information: nine monkeys were born in captivity, and 23 had data sheets that recorded when they were acquired as well as an age as estimated by the facility that received them as feral monkeys. Our estimates of the monkeys' ages were undertaken because age was used in some statistical analyses to examine its effect on the variable being studied. Since the age of 89% of the monkeys could not definitely be established, such results should be viewed with some caution. It is important to note, however, that given the effort and process used to obtain the age estimates, we have a high degree of confidence in them.

Previous studies had indicated that monkeys treated with PCBs had difficulty maintaining their pregnancy (Allen and Barsotti, 1976; Allen *et al.*, 1979 and 1980; Barsotti *et al.*, 1976; Barsotti and Van Miller, 1984). Consequently, a concerted effort was made to eliminate all unnecessary handling of the monkeys during this phase of the study. The primary monitoring of test monkeys undertaken during the breeding phase included: (1) general health—daily visual inspection; (2) feed and water consumption (daily); (3) determination of menstrual status by swabbing (daily); (4) body weight—the monkeys had been trained to enter a transfer cage for weighing and they were weighed once a week. None of these procedures required that the monkey be handled and they did not appear to be stressful to the monkeys. However, the occasional monkey had to be removed from her cage for health monitoring purposes. In addition, blood samples were obtained for pregnancy testing and once from each of the monkeys near the end of the breeding program for purposes of PCB analysis, and haematological and serum biochemistry evaluations (see Arnold *et al.*, 1993a).

Mating and breeding methodology

On the basis of the female's usual menstrual cycle length, and when the monkey last started to menstruate, the monkey would usually be scheduled for breeding 9–12 days after the start of menstruation. A random allocation of an untreated male from the available pool of 20 males would be made. Sperm evaluation (count, motility and morphology) was carried out on all males before the start of breeding. On the designated first day of mating, the female monkey was brought to the male's cage. If the monkeys appeared to be incompatible (i.e. they displayed 'signs' of hostility) before the female was introduced into the male's cage, or during the initial interval when the female was in the male's cage and their behaviour was continuously monitored, a second male was usually assigned. Whenever the male and female were 'compatible', it was assumed the mating session was 'successful' (i.e. copulation had occurred). The mating session lasted for approximately 2 hr for 5 consecutive days unless the monkeys were subsequently found to be incompatible, at which time the mating session was terminated.

To ascertain whether conception had occurred, at least three serum samples were obtained between 19 and 26 days after the first day of mating. The amount of chorionic gonadotropin in the serum was determined using the BIOCEPT-G (Wampole Laboratories, Canbury, NJ, USA) bovine corpus luteum

plasma membrane tube. All monkeys found to have a sufficient increase in gonadotropin levels were ...ned to be pregnant and were removed from the ...ding programme. One monkey with a dubious increase in gonadotropic levels was initially diagnosed as non-pregnant, but was subsequently found to be pregnant. Consequently, only one BIOCEPT-G result appeared to be a false negative.

Initially the mating scheme was designed to maintain a balance across dose groups (i.e. a male was not allowed to impregnate more than one female in any dose group and matings were scheduled to maintain an approximate synchronization across dose groups so that females within each dose group would have the same probability of being impregnated each month). In addition, concerns about possible dystocia precluded the mating of large males (>12 kg) with the small females (<5 kg). Approximately 1 yr into the 29-month breeding programme, some of these criteria were relaxed. For example, only the 10 proven sires were used for mating; a male would be allowed to impregnate two females per dose group; the mated pair were housed together continuously for 5 consecutive days, except for whatever time was required by the female to ingest her dose of PCB in her own cage, and the males were housed in the female's room (four males room) instead of the original 'male's' room.

Since the daily dose of Aroclor 1254 ingested by each female was based on her body weight during the ...ding week, a procedure was devised so that ...ancy-induced weight gains would have a minimal effect on the quantity of PCB ingested. Once pregnancy had been confirmed with BIOCEPT-G, the body weights during the preceding 9 wk were averaged and the pregnant female's dose of PCB was based on her average '9-wk' body weight. A pregnant female continued to receive this dose until her infant was 7 wk old, at which time dosing was discontinued until the infant was weaned, to preclude the infant ingesting his her mother's dosing capsule. If the dam had a suspected resorption (i.e. a positive chorionic gonadotropin result, but no foetal tissue was ever observed), abortion, stillbirth or post partum infant death before the infant attained 7 wk of age, dosing was not stopped, but the dose was again based on the monkey's current body weight.

Each dam's feed allotment was approximately 180 g/day. Following the confirmation of pregnancy the feed allotment was increased to 220 g/day and was maintained at this level until a resorption, abortion, stillbirth, post partum infant death before weaning, or weaning occurred, at which time the feed allotment was decreased to approximately 180 g day.

On the day of parturition, all infants were X-rayed to ascertain osseus development. Also on the day of parturition, and at 4-wk intervals thereafter until weaning, the dam and her infant were separated for ...oximately 3 hr, during which time the dam and ...ifant's body weight were determined; the health of the dam and infant were evaluated by a detailed clinical examination (Arnold *et al.*, 1993b); blood samples from the dam and infant were obtained for haematological evaluation (Arnold *et al.*, 1993a) and PCB analysis (Mes *et al.*, 1983 and 1989); breast milk, manually expressed, was obtained for PCB analysis (Mes *et al.*, 1983 and 1989); tooth eruption was monitored and anthropometric measurements were taken. All specimens acquired for PCB analysis were obtained in the morning before the dam was given her daily dose of Aroclor 1254. When the infant was 16 wk of age, the first blood sample for serum biochemistry was taken. At 20 wk of age, immunological testing of the infant was started, and an adipose (nuchal fat pad) and skin sample (interscapular region) were obtained from the infant and dam for PCB analysis using a local anaesthetic. Zylocaine [2% lidocaine with epinephrine (ASTRA Pharmaceutical Inc., Westboro, MA, USA)].

At 22 wk of ...ge, the infant was weaned. For the subsequent 4–8 days, daily breast milk and blood samples were obtained from the dam for PCB analysis (Mes *et al.*, 1983 and 1989). On weaning, the dam's test dose was reinstated. The infant was weaned onto a diet of Certified Primate Chow (Ralston Purina, Richmond, IN, USA; Arnold *et al.*, 1993b for quality control procedures) and Municipality of Ottawa water with small amounts of fruit or vegetables being provided as treats (Arnold *et al.*, 1993b).

When the infant was 26 wk of age, and at 4-wk intervals thereafter until the infant was 78 wk of age, a blood sample for PCB analysis was obtained from its dam. In addition, blood samples for haematological evaluation (method of Arnold *et al.*, 1993a, b; Fernie *et al.*, 1994) were obtained from the dam when the infant was 46, 50 and 58 wk old. When the infant was 42 wk old, and every 4 wk thereafter until the infant was 86 wk old, as well as when the infant was 98, 110 and 122 wk old, blood samples for PCB analysis and haematological evaluation were obtained from the infant. The blood samples obtained from the infants for PCB analysis and haematological evaluation from 42 wk of age were collected after the administration of ketamine hydrochloride (Ket · HCl; 10 mg/kg body weight, im) which was used as a tranquillizer. The effect of Ket · HCl on haematological parameters in the control group has previously been reported (Fernie *et al.*, 1994). Usually, at the same time as the collection of the infant's blood samples, the following procedures were performed: an adipose sample from the nuchal fat pad and a sample of epithelium from the interscapular region were obtained for PCB analysis after administration of Zylocaine; a 24-hr faecal sample was collected for PCB analysis; tooth eruption was monitored; body weight and anthropometric measurements were taken and a detailed clinical examination was performed.

In addition, a blood sample for serum biochemistry evaluation (method of Arnold *et al.*, 1993a,b; Fernie

et al., 1994) was obtained when the infant was 44, 56 and 64 wk of age and at 4-wk intervals until the infant was 120 wk old.

At 122 wk of age most of the control infants and all of treated infants were killed humanely and autopsied.

Immunological testing of infants

The following four tests were carried out.

Antibodies to SRBCs. Infants were immunized with 1.0 ml 10% SRBCs (sheep red blood cells) iv at 20 wk of age and again at 60 wk of age. Sera collected at day 0 (pre-immunization) and at 3-wk intervals thereafter were divided into aliquots and stored at −80°C. Before use, the sera were heat-inactivated at 56°C for 30 min and IgM (immunoglobulin M) and IgG (immunoglobulin G) titres to SRBCs were determined as previously described (Tryphonas *et al.*, 1989).

Lymphocyte proliferation. The lymphocyte proliferation assay which measures [^{3}H]thymidine incorporation (Tryphonas *et al.*, 1991) was carried out when the infants were 20, 28 and 60 wk old. Briefly, Ficoll–Hypaque-isolated leucocytes were added to triplicate wells at a concentration of 1×10^5 cells/well. Mitogens were added to the appropriate wells at the following predetermined optimum concentrations: phytohaemagglutinin (PHA-P, DIFCO Laboratories, Detroit, MI, USA) at 5 μg/well; concanavalin A (Con A, DIFCO Laboratories) at 1 μg/well; pokeweed mitogen (PWM, GIBCO, Burlington, Ontario, Canada) at 1 μg/well. The plates were placed in a humidified incubator at 37°C and supplied with 5% CO_2 for 72 hr to optimize mitogen stimulation. Then 0.4 μCi [methyl-^{3}H]thymidine ([^{3}H]TdR) 6.7 Ci/mmol (NEN Research Products, Boston, MA, USA), was added and the cultures were incubated for an additional 18 hr at 37°C. Cells were harvested using the Titertek cell harvester system (Flow Laboratories, Mississauga, Ontario, Canada), transferred to vials containing 10.0 ml Econofluor (NEN Research Products), and the radioactivity was measured using a Beckman LS 3800 liquid scintillation system (Beckman Instruments, Palo Alto, CA, USA). The mean of quadruplicate cultures (counts per minute, cpm) was calculated and data were normalized by logarithmic transformation for statistical analysis.

Mixed lymphocyte culture assay (one-way). The method used was that described by Tryphonas *et al.* (1989). Briefly, responder cells [peripheral blood mononuclear leucocytes isolated by Ficoll–Hypaque gradient (Ficoll–Paque–Pharmacia AB, Montreal, Québec, Canada) centrifugation] at 0.5×10^6 cells/ml were incubated with inactivated stimulator cells (a pool of peripheral blood leucocytes collected from 12 monkeys and isolated using Ficoll–Hypaque gradient centrifugation) (5×10^6 cells/ml) in volumes of 0.1 ml responder and 0.1 ml stimulator cells in quadruplicate wells if a microplate. Controls included inactivated stimulator cells + medium, responder cells + medium, and stimulator cells + mitogen (PHA-P). Cultures were incubated at 37°C in 5% CO_2 for 96 hr, pulsed with 1 μCi of [^{3}H] TdR and incubated for a further 18 hr. Cultures were harvested, transferred to vials containing 10 ml Econofluor (NEN Research Products), and the radioactivity was measured using the Beckman LS 3800 liquid scintillation system. Net cpm was calculated as described previously (Tryphonas *et al.*, 1991). The monkeys were tested at 20, 28 and 60 wk of age.

Natural killer (NK) cell activity. As previously described, a 4-hr ^{51}Cr-release assay was used (Tryphonas *et al.*, 1989) when the infants were 20, 28 and 60 wk old. Briefly, effector (E) cells (peripheral blood leucocytes, isolated by Ficoll–Hypaque gradient centrifugation) were incubated with target (T) cells (^{51}C-labelled K562 tumour cells) at E:T ratios of 25:1, 50:1 and 75:1 in quadruplicate wells of a microplate. Following a 4-hr incubation, the cells were harvested and the radioactivity counted using a Gamma counter (Beckman Instruments, Fullerton, CA). The percentage cytotoxicity was calculated as follows:

$$\% \text{ cytotoxicity} = \frac{\text{mean cpm experimental} - \text{mean cpm spontaneous release}}{\text{mean cpm maximum release} - \text{mean cpm spontaneous release}} \times 100.$$

Statistical methodology

Adult females. The statistical methodologies used to compare conception rates, numbers of matings required for conception, gestation lengths and foetal mortality incidence rates are provided below. One factor that may have affected conception rates, foetal mortality or infant weights, besides the administered dose of Aroclor 1254, was the age of the female monkeys. Two-way analyses of variance (ANOVA) with the factors of dose and breeding outcome and with the factors of dose and gestation outcome were used to analyse estimated female ages to test for age differences across dose groups and between females who became pregnant and those that did not, and between those females who gave birth to live infants and those whose pregnancies resulted in dead foetuses/infants (i.e. suspected resorptions, abortions or stillbirths).

Conception rates were analysed using a life-table analysis (Thomas *et al.*, 1977) with adjustment for total number of matings and adjustment for number of matings with positive sires. A positive sire is defined as a male who impregnated at least one

Table 1. Reproductive data and incidence rates of stillbirths, abortions and suspected resorptions for female rhesus monkeys that ingested Aroclor 1254 for more than 3 yr before being mated with untreated rhesus males

Female dose groups*	No. impregnated (no. available)	No. of live infants	No. of post partum (infant) deaths	No. of abortions†	No. of suspected resorptions	No. of stillbirths‡	Incidence rate§
A	11 (16)	9	0	1	0	1	0.182
B	10 (16)	5	1‖	1	2	2	0.500
C	4 (15)	1	1*	3	0	0	0.750
D	6 (14)	4	1¶	1	1	0	0.333
E	5 (15)	1	1**	1	3	0	0.800
Total	36 (76)	16	4	7	6	3	0.444

*A = 0 μg Aroclor 1254/kg body weight/day; B = 5 μg; C = 20 μg; D = 40 μg, E = 80 μg.
†Abortion = gestation period less than 139 days (Hendrickx and Binkerd, 1980; Hendrickx and Henrickson, 1988).
‡Stillbirth = gestation period greater than 140 days (Hendrickx and Binkerd, 1980, Hendrickx and Henrickson, 1988).
§No. of abortions + no. suspected resorptions + no. stillbirths/no. impregnated.
‖Premature infant, born on day 133 of gestation; died 1 day post partum. Gestation length data for rhesus monkeys: range 135–171 days (Fujikura and Niemann, 1967), 144–197 days (Hendrickx and Kraemer, 1970), 158–173 days (Krohn, 1960); 145–185 (average 168.4) days for females, 139–194 (average 168.5) days for males (van Wagenen, 1972), 168 days (Jacobson and Windle, 1960); gestation length 164 ± 6 days (average ± SD) (Valerio and Dalgard, 1975); average gestation 168 ± 5 days (Conaway and Koford, 1965), 'full term' average gestation 168 ± 4 days (Kerr *et al.*, 1969a).
*Infant died 4 days post partum.
**Infant died 11 days post partum.

female during the breeding phase of the study. In each analysis, the number of matings for females who failed to conceive or were killed humanely or died before completion of the breeding phase were considered as censored observations.

For impregnated females, the total numbers of matings and the numbers of matings with positive sires were analysed during the Kruskal–Wallis test (Hollander and Wolfe, 1973, p. 115) and the Jonckheere–Terpstra test (Hollander and Wolfe, 1973, p. 120).

Gestation lengths for females giving birth to live infants were analysed using a one-way ANOVA. For each female, the length of gestation was calculated by assuming that the chorionic gonadotropin peak occurred 21 days after impregnation (Hodgen *et al.*, 1974 and 1975).

Foetal mortality incidence rates, defined here as the ratio of the number of stillbirths, abortions and suspected resorptions to the number impregnated were analysed for dose group homogeneity, dose-related trend and differences between treatment groups and control using Pearson's chi-squared statistic (Fleiss, 1981), the Cochran–Armitage test (Fleiss, 1981) and Fisher's exact test (Gart *et al.*, 1979), respectively.

Infants. Only the data for the infants born to females in dose groups A, B and D were statistically analysed since these infants were the only ones to survive more than 2 wk post partum.

Birth weights were analysed using a two-way ANOVA with the factors of dose and sex. The body weights measured between 4 and 122 wk of age were analysed by fitting orthogonal quadratic polynomials and using both ANOVA and multivariate analysis of variance (MANOVA) to analyse the estimated polynomial coefficients.

The 16 to 21 replicate anthropometric measurements were averaged at each measurement time for the purposes of the statistical analysis. The resulting data were analysed using principal components (Morrison, 1976, p. 266) and partitions of principal

Table 2. The number of mating sessions* for untreated male rhesus monkeys with females that had ingested Aroclor 1254 for more than 3 yr—each male produced at least one impregnation

Male identifier	No. of mating sessions (no. of different females) for female dose groups†: A	B	C	D	E	Total
750/850	3 (2)	10 (7)	15 (10)	9 (5)	13 (8)	50 (32)
626	7 (6)	17 (8)	12 (7)	7 (6)	8 (7)	51 (34)
927	8 (6)	15 (7)	8 (5)	7 (5)	18 (8)	56 (31)
754/852	1 (1)	3 (3)	4 (4)	4(4)	4 (4)	16 (16)
962§	5 (4)	7 (6)	3 (2)	1 (1)	5 (5)	21 (18)
961	10 (4)	18 (9)	14 (9)	3 (3)	27 (12)	72 (37)
74	7 (7)	10 (7)	17 (11)	25 (9)	10 (7)	69 (41)
965	6 (6)	7 (4)	17 (9)	21 (9)	15 (9)	66 (37)
956	4 (4)	12 (7)	19 (10)	15 (9)	16 (10)	66 (40)
55	4 (4)	13 (7)	16 (8)	14 (6)	17 (9)	64 (34)
70	7 (6)	6 (3)	8 (6)	8 (6)	7 (6)	36 (27)
Totals	62	118	133	114	140	567

*Matings started during study week 160, each session lasted approximately 5 days.
†A = 0 μg Aroclor 1254/kg body weight/day; B = 5 μg; C = 20 μg; D = 40 μg, E = 80 μg
‡Killed humanely during study wk 196 due to urinary tract problems.
§Removed from the breeding programme during study wk 216 due to aggressive behaviour.
¶Removed from the breeding programme during study wk 241 due to aggressive behaviour.

components. The principal components analysis provided a means of obtaining a few linear combinations of the anthropometric measurements that explain most of the variability between infants. The number of measurements considered in the analysis is thereby reduced.

Tooth eruption data were analysed using Wilcoxon's signed rank test (Hollander and Wolfe, 1973, p. 27) to test for left side versus right side differences in eruption times for each tooth type in each of the upper and lower jaws and the Jonckheere–Terpstra test to test for delayed eruption times due to PCB treatment.

A two-way repeated measures ANOVA with the factors of dose and immunization series was used to analyse the SRBC titres. A one-way ANOVA was used to analyse data from the lymphocyte proliferation and mixed lymphocyte culture assays. A two-way ANOVA with the factors of dose and sex was used to analyse the natural killer cell activity.

For the haematology parameters, the results for blood samples obtained after the administration of Ket · HCl were analysed separately, since it has been reported that Ket · HCl affects the level for some haematology parameters (Loomis *et al.*, 1980; Porter, 1982; Yoshida *et al.*, 1986). For the samples that were collected pre-weaning and the three post-weaning samples where no Ket · HCl was used, a two-way ANOVA with the factors of dose and sex were used to analyse the mean response levels for each parameter to test for differences across dose groups. For the post-weaning samples collected after the administration of Ket · HCl, an orthogonal quadratic polynomial was fitted to the response profile for each parameter, separately for each infant, and the estimated polynomial coefficients were analysed using both ANOVA and MANOVA with the factors of dose and sex.

Serum biochemistry parameters were analysed using ANOVA for the pre-weaning data, and using MANOVA to analyse the estimated polynomial coefficients from orthogonal polynomials fit to the remaining (post-weaning) data. Note that there was a problem here related to a change of equipment used to analyse the serum samples partway through the

Table 3. The number of matings sessions* for untreated male rhesus monkeys with females that had ingested Aroclor 1254 for more than 3 yr—nc impregnations resulted from these matings

Male identifier	No. of mating sessions† in female dose groups‡: A	B	C	D	E	Total
909	5	5	4	5	5	24
MC§	6	5	6	5	5	27
76-012	5	5	5	5	5	25
77-227	5	5	5	5	6	26
963	5	5	5	4	5	24
954	4	5	5	4	4	22
014	3	3	3	3	3	15
MI‖	3	4	3	4	3	17
770A58¶	5	5	4	4	5	23
Totals	41	42	40	39	41	203

*Mating started during study wk 160; each session lasted approximately 5 days.
†No. of mating sessions equals no. of different females: see Methods Section.
‡A = 0 μg Aroclor 1254/kg body weight day; B = 5 μg; C = 20 μg; D = 40 μg; E = 80 μg.
§Killed humanely during study wk 214 due to an acute health problem.
‖Killed humanely during study wk 208 due to poor health.
¶Killed humanely during study wk 214 due to dramatic behavioural changes.

Table 4. Concentration of PCB during the first 8 wk post partum (0–8), at weaning (wk 22) and post weaning (54–62 wk) in the blood of rhesus dams that received daily doses of 0 (A), 5 (B), 40 (D) or 80 (E) μg Aroclor 1254/kg body weight/day

Dam no.	PCB level, ppm (wet tissue wt) at wk*: 0–8†	22‡	54–62§	PCB level, ppm (lipids) at wk*: 0–8†	22‡	54–62§
FA 001	0.001	0.001	0.001	0.865	5.520	8.034
007	0.004	0.004	0.004	10.527	10.579	28.088
041	0.002	0.006	0.002	14.707	12.352	4.196
051	0.001	<0.001	<0.001	5.753	1.548	1.009
159	0.001	0.001	0.001	1.609	10.545	11.217
Geometric mean	0.002	0.002	0.001	4.156	6.519	6.397
FB 031	0.006	0.005	0.005	33.019	38.463	18.831
039	0.006	0.010	0.009	16.333	38.106	25.784
137	0.009	0.016	0.005	30.548	52.075	11.599
155	0.007	0.006	0.006	24.626	23.922	25.709
Geometric mean	0.007	0.008	0.006	25.238	36.759	19.507
FD 003	0.055	0.034	0.037	237.361	87.223	52.619
079	0.024	0.019	0.058	75.575	48.597	126.272
125	0.022	0.038	0.068	140.825	232.220	222.253
Geometric mean	0.030	0.029	0.053	136.193	99.475	113.876
FE 037‖	0.271			204.609		

*Weeks since parturition. Nursing dams did not receive a daily dose of Aroclor 1254 for parturition wk 8–22, inclusive.
†Geometric mean of three determinations (parturition, 4 and 8 wk post partum).
‡Geometric mean of four to eight daily determinations starting on the day of weaning.
§Geometric mean of 3 monthly determinations (wk 54, 58 and 62 post partum).
‖Infant died at 11 days of age.

study. Where exploratory analyses indicated significant machine differences, a two-way ANOVA with the factors of machine and dose was used.

RESULTS

Adult females

The breeding phase lasted for 29 months. The breeding results and the outcome of all impregnations are shown in Table 1. As can be seen from these data, the combined incidence ratio of foetus and infant mortality in all of the treated groups (14 foetal deaths with 25 impregnations) was substantially higher than it was for the control group (two foetal deaths with 11 impregnations). If post partum infant deaths were added to the foetal deaths, the incidence ratio in the treated groups was higher yet (18/25 *v.* 2/11). Statistical analysis using Fisher's exact test showed a statistically significant greater incidence of foetal mortality in the treated groups than in the controls ($P = 0.039$); this difference became more significant if post partum infant deaths were included ($P = 0.004$). The results of the statistical analysis of these data were as follows. There was evidence of a significantly increasing dose-related trend in the foetal mortality incidence rates ($P = 0.040$). However, when only Groups B, C, D and E were compared there was no evidence of such a trend ($P = 0.220$), and this was due primarily to the smaller incidence rate for Group D in comparison with Groups B, C and E. The results from Fisher's exact test indicated a statistically significant increased incidence rate for Group E ($P = 0.036$) and a marginally non-significant increased rate for Group C ($P = 0.077$) relative to the control group.

Table 2 contains data pertaining to the number of mating sessions for untreated males with females that resulted in at least one impregnation by each male. Table 3 contains similar data for males that did not impregnate any of the females. Each non-impregnated female was mated an average of 13.6 times (range 4 to 23 times). (For more detailed information concerning the pairings, contact D. L. Arnold.)

For the 76 females included in the breeding phase of the study, the results from an ANOVA revealed no statistically significant differences ($P = 0.410$) in the average estimated ages across dose groups. Results from an ANOVA also revealed that there were no statistically significant differences ($P = 0.405$) between the average estimated ages of impregnated and non-impregnated females.

Results from an ANOVA of the estimated ages in each dose group for females who gave birth to live infants versus those with dead foetuses, revealed no statistically significant differences ($P = 0.259$) between these groups of females, nor were there any statistically significant differences ($P = 0.579$) in the average estimated ages across dose groups for the 36 impregnated females.

An analysis of conception rates, adjusting for either the total number of matings or for the number of matings with positive sires, was undertaken. In both situations there was a significant ($P = 0.017$) decrease in the rate of impregnation with increasing dose. However, when conception rates among the treated Groups (B, C, D and E) were compared, there was no evidence of a significant dose-related trend ($P = 0.143$ adjusting for total number of matings; $P = 0.155$ for matings with positive sires). Conception rates, adjusting for the total number of matings were marginally, but not significantly, lower for Group B ($P = 0.085$) and significantly lower for Groups C, D and E ($P = 0.009$, $P = 0.039$ and $P = 0.005$, respectively) relative to the control group. Similar results were noted after adjustment for the number of matings with positive sires.

For the 36 females that were impregnated, there was no evidence of an increasing dose-related trend in the number of matings ($P = 0.232$) or the number of matings with positive sires ($P = 0.215$) required for impregnation.

A review of the menses data files revealed that some females began menstruating during the mating session and that this menstruation was unexpected on

Table 5. Average concentration of PCB in breast milk samples from rhesus dams during the first 8 wk of lactation and at weaning*

Dam no.	PCB, ppm (wet tissue wt) at post partum wk: 0–8†	PCB, ppm (wet tissue wt) at post partum wk: 22‡	PCB, ppm (lipids) at post partum wk: 0–8†	PCB, ppm (lipids) at post partum wk: 22‡
FA 001	0.004		0.177	
007	0.031	0.028	0.660	0.443
041	0.005	0.025	0.061	0.360
051	0.089	0.014	3.372	0.362
077	0.004	0.075	0.100	0.696
081	0.059	0.135	0.925	1.577
133	0.026	0.017	0.593	0.466
159	0.016	0.097	0.849	1.011
Geometric mean	0.016	0.040	0.428	0.605
FB 031	0.236	0.275	11.046	6.424
039	0.294	0.233	7.816	5.928
137	0.406	1.233	9.952	15.582
155	0.744	0.169	8.404	5.593
Geometric mean	0.380	0.340	9.218	7.590
FC 121§	4.997		53.217	
FD 003	2.114	1.453	82.629	48.178
079	0.993	1.437	39.268	24.583
125	1.852	2.547	42.577	42.483
Geometric mean	1.573	1.745	51.695	36.918
FE 037‖	4.978		73.192	

*Data are for dams that received 0 (A), 5 (B) or 40 (D) μg of Aroclor 1254/kg of body weight/day and gave birth to infants that lived for at least 2 wk, and for dams that received 20 (C) or 80 (E) μg Aroclor 1254/kg of body weight/day and gave birth to infants that lived for less than 2 wk.

†Geometric mean of three monthly determinations (parturition, 4 and 8 wk post partum).

‡Geometric mean of 4–8 daily determinations starting on the day of weaning.

§Infant died at 4 days of age. Data represents average for breast milk collected at parturition and days 4–9 post partum, inclusive.

‖Infant died at 11 days of age. Data represents average for breast milk collected on post partum days 6, 11 and 12. No sample could be obtained on the day of parturition.

Table 6. A listing of all the live rhesus infants born to dams that received daily dosages of Aroclor 1254 and their untreated sires

Infant no.*	Gestation length† (days)	Sex	Birth weight (g)	Dam no.	Dam weight (kg)	Sire no.	Sire weight (kg)
FA002	169	M	457	FA001	5.64	75-162	12.30
FA008	162	F	341	FA007	6.40	626	16.36
FA020	170	M	508	FA019	7.58	956	12.82
FA042	174	M	465	FA041	5.28	74	13.37
FA052	174	M	454	FA051	6.50	965	12.84
FA078	169	F	458	FA077	6.80	961	9.07
FA082	164	M	454	FA081	6.40	750A50	11.83
FA134	167	F	420	FA133	9.15	70	11.19
FA160	160	F	421	FA159	6.90	55	10.15
Mean ± SD	167.7 ± 4.9		442.0 ± 45.8				
FB032	162	F	388	FB031	7.40	750A50	12.16
FB040	164	F	497	FB039	5.90	961	9.48
FB102‡	133	F	296	FB101	7.47	70	10.30
FB138	169	F	410	FB137	8.15	965	14.52
FB156	168	M	471	FB155	8.00	750A50	12.16
Mean ± SD	159.2 ± 14.9 (165.8 ± 3.34§)		412 ± 78.6 (441.5 ± 51.0§)				
FC122‖	169	F	435	FC121	6.98	74	13.37
FD004	166	F	326	FD003	6.10	961	8.66
FD080	170	F	515	FD079	7.82	927	7.50
FD100¶	173	M	461	FD099	9.61	70	11.19
FD126	164	F	493	FD125	5.10	961	9.43
Mean ± SD	168.3 ± 4.0.		448.8 ± 84.8				
FE038#	178	F	416	FE037	7.93	626	17.2

*A = 0 μg aroclor 1254/kg body weight/day; B = 5 μg; C = 20 μg; D = 40 μg; E = 80 μg.
†Day 21 of gestation was assumed to occur when the chorionic gonadotropin level peaked.
‡Premature infant, died 1 day post partum; therefore birth weight not included in group average (see Table 1).
§Does not include FB102.
‖Died 4 days post partum.
¶Died 11 days post partum.
#Died 11 days post partum.

the basis of their menstrual history. Valerio *et al.* (1969) has suggested that vaginal bleeding of 1 day's duration should be considered as true menstrual haemorrhage. However, menstrual changes/irregularities have been reported following PCB ingestion by rhesus monkeys (Allen *et al.*, 1979 and 1980; Arnold *et al.*, 1990; Barsotti *et al.*, 1976). Consequently, mating sessions where unexpected menstruations occurred were not included in the analysis of conception rates or in the analysis of the numbers of matings required for conception as impregnation was considered impossible in this circumstance. The review of the menses data files also revealed instances in which matings and conceptions

Table 7. Effect of Aroclor 1254 on Con A-stimulated lymphocyte proliferation ([^{3}H]thymidine incorporation) of peripheral blood leucocytes of rhesus infants whose dams had ingested Aroclor 1254 during gestation and the first 6 wk of lactation

Age of Infant (wk)*	Dose group† (no. of monkeys)	[^{3}H]Thymidine incorporation (cpm) Mean log scale	SEM	Geometric mean	Percentage change	*P* value
20	A (8)	10.33	0.59	30573		
	B (3)	9.62	1.71	15132	−50.5	0.62
	D (2)	8.64	2.00	5661	−81.5	0.28
	Test for linear trend§ ...					0.35
	Test for nonlinearity‖ ...					0.73
28	A (9)	10.62	0.31	40762		
	B (4)	10.83	0.96	50616	24.2	0.78
	D (2)	8.51	1.37	4948	−87.9	0.036
	Test for linear trend§ ...					0.062
	Test for nonlinearity‖ ...					0.55
60	A (9)	11.62	0.17	111688.9		
	B (4)	11.82	0.11	135538.8	21.4	0.50
	D (3)	10.38	0.92	32124.8	−71.2	0.053
	Test for linear trend§ ...					0.021
	Test for nonlinearity‖ ...					0.44

*The infants were weaned at 22 wk of age.
†A = 0 μg Aroclor 1254 kg body weight day; B = 5 μg; D = 40 μg.
‡Pairwise *t*-test with contol group (0 μg/kg body weight/day).
§Based on an ANOVA F-test for a linear dose-related trend in the mean response levels.
‖Based on an ANOVA F-test which provides an indication of how significantly the response curve deviated from linearity.

Table 8. Concentration of PCB in the blood of rhesus infants that were nursed for 22 wk by dams receiving daily doses of 0(A), 5(B) or 40(D) μg of Aroclor 1254/kg body weight/day

Infant no.	PCB, ppm (wet tissue wt) at post partum wk: 0*	21†	98–122‡	PCB, ppm (lipid) at post partum wk: 0*	21†	98–122*
FA 002	0.025	0.004	0.004	49.07	25.16	15.50
008	0.066	0.006	0.003	156.20	18.53	6.55
042	0.004	<0.001	0.001	13.58	0.27	4.14
052	0.006	0.001	0.001	14.22	8.78	4.35
160	0.002	0.006	0.003	5.03	61.50	13.00
Geometric mean	0.010	0.002	0.002	23.68	9.26	7.50
FB 032	0.008	0.031	0.006	59.54	132.94	12.21
040	0.018	0.045	0.003	5.11	70.50	9.04
138	0.098	0.157	0.003	199.72	441.36	7.11
156	0.027	0.023	0.003	37.55	79.17	8.15
Geometric mean	0.025	0.047	0.004	38.86	134.52	8.94
FD 004	0.049	0.698	0.010	10.95	1586.39	36.15
080	0.026	0.426	0.014	12.81	1005.66	35.84
126	0.023	0.222	0.005	56.94	1387.10	11.02
Geometric mean	0.031	0.404	0.009	19.99	1303.13	24.26

*One sample on the morning of parturition.
†One sample, 21 wk post partum, 1 wk before weaning.
‡Geometric mean of three determinations taken on wk 98, 110 and 122 post partum.

occurred during usually long or short menstrual cycles. These matings were not deleted from the analysis of conception rates or from the analysis of the number of matings required for conceptions. As there was no *a priori* knowledge of when the next menses would start following commencement of a mating session, deletion of these matings could have biased the results of the analysis as erratic behaviour of menstrual cycles for the dosed females could be indicative of a dose effect. (A detailed examination of the menstrual data will be the subject of a subsequent manuscript.)

The gestation lengths for females giving birth to live infants was examined for treatment effects. The results from this ANOVA revealed no statistically significant differences in the average gestation lengths across dose groups, with or without the premature infant in dose Group B (Table 1, footnote 5; Table 6, footnote 4) included in the analysis ($P = 0.276$ and $P = 0.240$, respectively).

The concentration of PCB, at three selected intervals, in the blood of several dams who gave birth to live infants and for which samples were available, is shown in Table 4. The concentration of PCB, at two selected intervals, in milk from several dams that gave birth to live infants is summarized in Table 5. Generally, PCB levels increased with dose.

Infants

Evaluation of the limited radiographic data did not reveal any dissimilarities between the offspring from control and treated dams. No statistically significant differences ($P > 0.500$) were found for the mean birth weights between control infants and those from treated dams. The growth curve for one infant in Group D (FD004) was an outlier ($P < 0.01$; Barnett and Lewis, 1978) with respect to that of all other infants. Since this infant was from the highest dose group for which data were available, this could be an effect of PCB or this may just be an aberrant infant. When the data for this infant were omitted from the analysis, the treatment effects were still non-significant.

Principal components were found for the following anthropometric measurements: neck to tail; shoulder to elbow; elbow to wrist; wrist to fingertip; hip to knee; knee to ankle; ankle to tip of toe; chest circumference; head, front to back; and head, side to side. The first two principal components found explained over 90% of the variation. Coefficients were calculated and used to compute the principal component scores, or linear combinations of the

Table 9. Concentration of PCB in the nuchal fat pad of infant rhesus monkeys that were nursed by dams receiving 0(A), 5(B) or 40(D) μg Aroclor 1254/kg body weight/day

Infant no.	PCB, ppm (wet tissue wt) at post partum wk: 20*	98–122†	PCB, ppm (lipid) at post partum wk: 20*	98–122†
FA 002	0.43	0.28	3.33	2.35
008	1.19	0.34	2.82	3.36
020	0.74	0.24	7.47	1.10
042	1.16	0.31	2.94	1.05
052	0.62	0.17	2.83	2.03
078	1.35	0.29	5.02	3.67
082	0.81	0.41	3.02	2.01
134	1.00	0.13	5.26	1.46
160	1.03	0.35	7.13	2.15
Geometric mean	0.88	0.27	4.11	1.96
FB 032		0.66		3.04
040	9.24	0.33	24.48	2.50
138	6.27	0.35	87.75	7.15
156	9.98	0.68	37.88	2.63
Geometric mean	8.33	0.47	43.17	3.46
FD 004	152.72	1.98	1266.26	15.09
080	67.78	5.40	485.03	24.16
126	30.54	0.93	382.29	4.73
Geometric mean	68.12	2.15	616.92	11.99

*One sample taken on wk 20 post partum; 2 wk before weaning.
†Geometric mean of three determinations taken on wk 98, 110 and 122 post partum. Infants were not dosed with PCB after weaning.

anthropometric measurements, and these were subjected to a statistical analysis. The first principal component was essentially a sum of the standardized anthropometric measurements and provided an overall measure of size. There was no significant treatment effect ($P > 0.050$) on the first principal component. The second principal component was made up of contrasts between the length from neck to the tail and the head measurements, and between the hand and foot sizes, and the forearm and leg length. The second principal component was partitioned into the contrast of the hand and foot measurements versus the forearm and leg measurements, and the contrast of the neck to tail measurements versus the two head measurements. These partitions were chosen as a reasonable split of the second principal component. This was somewhat subjective, but the partitions, like the second principal component, are approximately orthogonal to the first principal component and separate the limb measurements from the head and back measurements.

The second principal component was difficult to interpret since it was a combination of contrasts. For the first partition of the second principal component there was no evidence of a dose-related effect. But, for the intercept term for the second partition there was strong evidence of a treatment effect ($P = 0.0007$) and a linear dose related trend ($P = 0.0006$). When estimates of the coefficients for the intercept term for the second partition of the second principal component were plotted against dose, an increase in the coefficient estimates with an increase in dose was noted. This implies that the size of the head is smaller for a given back length with an increase in dose. A plot of the orthogonal intercepts for the side to side head measurement versus the orthogonal intercepts for the neck to tail measurement, revealed a generally smaller head size over the range of back lengths with increasing PCB dose.

Table 6 lists birth weights and parental weights for each live infant birth. Infant FB102 was a live birth—abortion (using the definition of Hendrickx and Henrickson, 1988) that lived for less than 1 day. Infants FC122, FD100 and FE038, which died on post partum days 4, 4 and 11, respectively, were neonatal deaths, using the definition of Hird *et al.* (1975). Infant FD004's growth rate, overall size and weight were significantly lower than the size and weight of the remaining infants. On the other hand, her birth weight, although the smallest, was not significantly lower ($P > 0.050$) than the weights for the other infants. FD004's parents were small but not the smallest; therefore, taking into account parental weights, FD004's weight was considered to be an outlier. Using dam weights and sire weights as covariates in the analysis of the birth weights and the orthogonal polynomial coefficients for the weights and principal components of the anthropometric measures did not affect the conclusions.

For the tooth eruption data, there was no evidence of a left versus right side bias in eruption times ($P > 0.400$). With the exception of a marginally non-significant ($P = 0.052$) delay in eruption due to PCB treatment for the second incisor in the upper jaw, there was no other evidence of an effect of PCB treatment on tooth eruption times.

For the immunological tests, there was a notable reduction in titres to SRBCs (IgM) at wk 22 ($P = 0.056$ and 0.023; Groups B and D, respectively), wk 23 ($P = 0.043$ and 0.029; Groups B and D, respectively), wk 61 ($P = 0.028$; Group B), wk 62 ($P = 0.043$; Group B), and wk 63 ($P = 0.056$; Group B). Although a statistically significant reduction in IgM levels was not found for Group D during wk 61, 62 or 63, it should be noted that the IgM levels were suppressed for the Group D infants,

Table 10. Clinical signs in infant monkeys that were nursed for 22 wk by dams that ingested 0–40 μg Aroclor 1254/kg body weight/day

Clinical signs	Dose of Aroclor 1254	No. of infants showing signs/ total no. in group	Onset of clinical signs (age in wk)
Inflammation and/or enlargement of tarsal glands	0	1/9	66
	5	4/4	42, 62, 74, 122
	40	3/3	Birth, 25, 28
Nail bed prominence*	0	0/9	—
	5	3/4	Birth, 4, 27
	40	3/3	8, 12, 23
Elevated nails*	0	0/9	—
	5	2/4	42, 86
	40	2/3	16, 42
Nails folding on themselves*	0	0/9	—
	5	1/4	42
	40	3/3	25, 42, 70
Gum recession	0	0/9	—
	5	1/4	27
	40	2/3	20, 28

*Arnold *et al.* (1993b) contains photographs illustrating this condition in the adult females in which the condition was more marked.

but the small number of infants in this group (n = 2) reduced the chance of finding a statistically significant result. Statistically significant reduced titres to SRBCs (IgG) were noted at wk 22 ($P = 0.0093$; Group D) only.

Results of the lymphocyte proliferation experiments were as follows: for the Con A mitogen, a decreased response by the treated infants compared with the controls was noted at 28 and 60 wk ($P = 0.036$; $P = 0.053$; Group D; Table 7). The test for linear trend was significant ($P = 0.021$) only at test wk 60. There were no statistically significant differences between the treated and control for the PHA-P or PWM stimulation (data not shown). Similarly there were no statistically significant differences between the control and treated groups for the mixed lymphocyte culture or the natural killer cell assays (data not shown).

For the haematology data, the results from the ANOVA of the mean levels of each parameter for the pre-weaning and the three post-weaning samples revealed a significant treatment effect for reticulocytes ($P < 0.020$, pre-weaning) and no significant treatment effects ($P > 0.050$) for the post-weaning data. Dunnett's test revealed that Group B infants had significantly higher reticulocyte levels than control infants ($P < 0.050$), whereas the average level for Group D infants was slightly lower than controls.

For the post-weaning samples collected after the administration of Ket·HCl, results of the MANOVA on the estimated orthogonal polynomial coefficients revealed no significant treatment effects ($P > 0.050$ based on Wilks's Λ). The ANOVA of the estimated orthogonal polynomial coefficients indicated that for the linear coefficient, which is a measure of the general trend across time, treatment differences were significant for white blood cells (WBC) ($P < 0.010$) and basophils ($P < 0.050$). For WBC, there was evidence of a significantly larger positive trend ($P < 0.050$) for infants in Group D in comparison with the controls. For basophils, there was a slight negative trend over time for Group B infants and a slight positive trend for Group D infants, although neither trend was significantly different ($P > 0.050$) from the controls.

Table 8 contains data concerning the concentration of PCB in the blood of infants at the time of parturition, 1 week before weaning, and just before the infant's autopsy. Table 9 contains data pertaining to the concentration of PCB in several infants' nuchal fat pad just before weaning and just before autopsy. The data clearly demonstrate that the concentration of PCB in the infants blood and nuchal fat pad decreased following weaning, at which time their PCB exposure was only from the feed (i.e. < 0.05 ppm PCB).

For the serum biochemistry data, a single data point (wk 16) was analysed before weaning. In general, there was no evidence of statistically significant dose group differences and the few differences that were found were not consistent with a dose-related trend. Albumin was an exception, in that the average level for Group D infants was significantly higher ($P < 0.050$) than the average levels of the control or Group B infants.

For the analysis of post-weaning data, there was little evidence of any effects related to treatment. Exploratory analyses into the effects of the equipment change indicated that there were some differences due to equipment for the following measurements: albumin, albumin/total protein-albumin, amylase, cholesterol, indirect bilirubin, total protein and alanine aminotransferase. There was no evidence of a dose-related effect for any of these measures. For the other measures, where polynomial models were fit to data across time, there was no evidence of dose-related effects.

A summary of the clinical manifestations attributable to the PCB ingested by the suckling infants during their first 22 wk before weaning are shown in Table 10. Inflammation and/or enlargement of the tarsal (Meibomian) gland, nail lesions and gum recession were the major findings. In addition to the tarsal gland changes, infant FD004 had developed inflammation of both the upper and lower eyelids by 25 wk of age. At 27 wk of age, white pinpoint pustules were evident on both the upper and lower eyelids. There was a dramatic improvement in the eyelids by wk 34 (12 wk after weaning); however, the tarsal glands remained slightly inflamed for the majority of the time up to 86 wk of age. All other treated infants had only mild inflammation or enlargement of the tarsal glands on a short-term or intermittent basis.

Nail lesions consisted of the nail bed becoming 'fleshy' or prominent followed by some nails becoming elevated or folding on themselves [see Arnold *et al.* (1993b) for photographic illustrations]. There appeared to be an earlier onset of nail elevation or folding in Group D infants. All nail lesions were mild in the treated infants but the nails were noticeably different from those of the controls. The observed gum recession in three of the treated infants was not severe, but was consistently observed after onset.

DISCUSSION

The results of the current study clearly demonstrate that Aroclor 1254 ingestion by female rhesus monkeys adversely affected their impregnation by the untreated males. Statistical analysis of the conception rates revealed that the conception rates were significantly lowered for those females ingesting 20, 40 or 80 μg Aroclor 1254/kg body weight/day, and approached significance for those females ingesting 5 μg Aroclor 1254/kg body weight/day [P-values 0.007, 0.032, 0.003 and 0.059, respectively, using a one-tailed test (based on Peto's log rank statistic) for a decreased rate of conception over the control]. Previously, Allen's group (Allen and Barsotti, 1976; Barsotti and Allen, 1975; Barsotti *et al.*, 1976) have

reported a similar, but less dramatic effect, on the impregnation of rhesus females fed Aroclor 1248 at doses of 2.5 or 5.0 ppm. (To facilitate comparison of the dose levels, a dose of 80 μg/kg body weight was roughly equivalent to a dietary concentration of 2.0 ppm.) Barsotti and Van Miller (1984) also reported that in a study in which rhesus monkeys were fed Aroclor 1016 at doses of 0.25 or 1.0 ppm, a greater number of matings were required before the treated females were impregnated.

Contrary to our findings that Aroclor 1254 had no effect on the body weight of the females during the 3-yr prebreeding phase (Arnold *et al.*, 1993b), Allen's group reported that their treated females lost an average of 15.1% of their initial body weight during the 7-month prebreeding phase of their study in which Aroclor 1248 was fed at levels of 2.5 or 5.0 ppm (Barsotti and Allen, 1975; Barsotti *et al.*, 1976). Such a loss in body weight suggests that the doses of Aroclor 1248 chosen by Allen's group were, using the definition of Khera (1984 and 1985), maternotoxic.

One could speculate that the failure of the untreated males to impregnate the treated females was associated with maternal toxicity. While we are unaware of any direct information pertaining to such a relationship regarding PCBs, Khera (1984 and 1985) did undertake a review of the non-primate literature to evaluate whether a possible relationship between maternal toxicity and foetal malformations existed. His criteria for maternal toxicity included the occurrence of one of the following events: significant reduction in maternal body weight, clinical signs of toxicity, pharmacological activity, or death. Recently, Peterson *et al.* (1993), in a review of the tetrachlorodibenzodioxin (TCDD) toxicology literature, defined overt maternal toxicity as being a decrease in maternal weight gain and/or a marked subcutaneous oedema of the dam. [It is worth noting that several lines of evidence confirm that a common receptor-mediated mechanism exists for the TCDDs and PCBs (Safe, 1990 and 1994).] Using these definitions, the doses of 2.5 and 5.0 ppm Aroclor 1248 used in the diets by Allen's group (Barsotti and Allen, 1975; Barsotti *et al.*, 1976), which resulted in a 15% reduction in body weight as well as subcutaneous oedema, were overtly toxic. The toxicity observed in our monkeys before the breeding phase of the study included finger-nail changes, inflammation and/or prominence of Meibomian glands, eye exudate and several haematological and clinical chemistry changes (Arnold *et al.*, 1993a, b; Bell *et al.*, 1994). In short, the signs of maternal toxicity observed with our females was markedly less severe than those reported by Barsotti and Allen (1975) and Barsotti *et al.* (1976).

A statistical analysis of the effect of Aroclor 1254 on conception did not appear to be confounded by the age of the female monkeys in our study, although the ages of all the test monkeys could not be rigorously established (data not presented). Due to circumstances which precluded our ability to rigorously establish the age of each test monkey, we attempted to assess the reproductive performance of our monkeys in relation to information in the literature. Valerio (1969) described the reproductive performance of the rhesus monkeys in their breeding laboratory over a 3-yr period, during which the percentage of female conceptions increased from 73.5% in the first year to 92.7% in the third year. Previously, Eckstein and Kelly (1966) reported that the annual conception rate in the eight rhesus colonies they surveyed ranged from 36% to 94.5%; with an overall average of 60–70%. However, three of the eight colonies were 'breeding' colonies. The percentage of control females conceiving in our study was 69%. While the latter figure is based on the 29-month breeding phase period of our study, in comparison with the 12-month data period used by the other authors, our monkeys were not bred as rigorously as they would have been in a breeding colony due to the constraints of trying to maintain approximate synchronization of matings across dose groups. In view of these differences, a more appropriate basis for comparison may be the number of matings required to attain pregnancy. Valerio (1969) reported that 69% of their impregnated monkeys required five matings or less. Similarly, Martin (1984) reported that the mean number of matings necessary for pregnancy to occur in his rhesus colony was 4.2 for wild-caught and 4.4 for house-born monkeys. These mean numbers translated into 78% of the wild-caught and 74% of the house-born monkeys becoming pregnant within five mating periods. For the 11 control monkeys that were impregnated in our study, an average of slightly less than five matings per monkey were required for impregnation. Consequently, these data indicate that the reproductive performance of our control monkeys was within the limits expected for rhesus monkeys that had not been bred for at least 4 yr.

The average estimated age of our monkeys on commencement of the study's breeding phase was 14.1 ± 3.9 yr (mean $\pm$ SD). Dyke *et al.* (1986) reported that the fertility rate in their rhesus colony peaked between 10 and 12 yr of age, and declined gradually thereafter, but they concluded that peak fertility persisted until about age 20. Both van Wagenen (1972) and Hodgen *et al.* (1977) reported that the incidence of vaginal bleeding in rhesus monkeys declined progressively before ceasing in the monkey's third decade of life. Van Wagenen (1970) suggested that rhesus females may cycle regularly until they reach an age of 22–25 yr, followed soon after by menopause. Subsequently, Lapin *et al.* (1979) reported that the number of pregnancies in their rhesus monkeys declined once they reached an age of 13–17 yr, that menstrual irregularities or menopause occurred when their monkeys were in their early twenties, and that pregnancies after age 18 were often complicated or ended in abortion. Another factor that may have affected the reproduc-

tive performance of monkeys was what Pickering (1968) called a 'fatiguing of the (female) reproductive apparatus (which) becomes apparent at about the sixth pregnancy' in his rhesus colony. However, Martin (1984) reported that neither pregnancy nor number of matings necessary for conception was affected by increasing parity or prior occurrence of foetal wastage or hysterotomy. A few of our monkeys were known to have had five previous pregnancies, although the reproductive history for most of our monkeys was unknown. Additionally, most of our monkeys were not born in captivity, and Bernstein and Gordon (1977) have reported that females born and reared in colonies had a significantly higher reproductive efficiency.

Once our monkeys were impregnated, there was an increasing dose-related trend in foetal mortality. However, only the female monkeys receiving 80 μg Aroclor 1254 were significantly different from the controls in this regard ($P = 0.036$) while the difference for the females given 20 μg was approaching statistical significance ($P = 0.077$). Factors that may lead to foetal mortality are not well understood. However, Allen *et al.* (1980), Allen and Barsotti (1976) and Barsotti *et al.* (1976), have previously reported that Aroclor 1248 increased foetal mortality. Hendrickx and Nelson (1971) have suggested that the apparent time of ovulation may be the most important factor leading to defective ova and early mortality. Hertig (1967) had previously shown that there was a seven-fold difference in favour of women's ova developing normally if they had ovulated on or before day 14 of the menstrual cycle, in comparison with ovulation after day 14. We determined that 20 of our study monkeys were impregnated on day 14 or before and 16 were impregnated after day 14. Of the 20 monkeys that were impregnated before day 15 of the menstrual cycle, two were from the control group and 18 from the treated groups. Both of the control animals subsequently had live births. Only nine of the 18 treated monkeys gave birth to live infants. There were nine impregnations in the control group after day 14 of the menstrual cycle, and there were seven live births (one delivered by caesarean section). There were seven impregnations in the treated groups after day 14, but only two live births. One of these infants died at 4 days of age, and the second infant, on the basis of the gestational age definition of Hendrickx and Henrickson (1988), was a 'live' abortion that died within 24 hr.

Another factor that has been associated with foetal mortality in women is endometriosis (Damewood, 1989; Metzger *et al.*, 1986). A detailed examination of any possible relationship between the ingestion of Aroclor 1254 and the incidence and severity of endometriosis in our monkeys will be the subject of a forthcoming manuscript. However, the incidence of endometriosis in our study monkeys was similar to that reported by Rier *et al.* (1993) and the severity of the endometrial lesions was similar in all test groups. Additionally, there did not appear to be any relationship between the incidence of endometriosis or adenomyosis and foetal mortality in our test monkeys. This observation is in agreement with that of Schenken *et al.* (1984) who reported that 'based on our limited observation, the presence of endometriosis in [Cynomolgos (*Macaca fascicularis*)] monkeys did not appear to increase the incidence of spontaneous abortion'. Previously, Pickering (1968) had reported that nine out of 11 rhesus monkeys found to be infertile had developed severe endometriosis; however, the author did not comment about any association between endometriosis and foetal mortality.

In an attempt to quantify foetal mortality in relative terms, Barsotti *et al.* (1976) reported that their breeding colony had a 10% incidence rate for abortions and stillbirths. Hendrickx and Nelson (1971) reported on 612 rhesus births from their colony, of which the incidence rate for abortion was 17.2% while the incidence for stillbirths was 5.9%. In a summary of 15 studies, which do not include the two preceding findings, Small (1982) reported on more than 5800 rhesus monkey pregnancies, and found that the average abortion rate was 16.3%, while the average stillbirth rate was 9.9%. Binkerd *et al.* (1988) reported a 14.4% incidence of abortion and a 6.5% incidence of stillbirth with 262 rhesus births. Korte *et al.* (1988) reported an average rate of 11.0% for abortions/stillbirths over a 3-yr reporting period in which the number of live births per year ranged from 145 to 183. During a 15-yr period at the Yerkes Regional Primate Research Center, Tigges *et al.* (1988) reported that stillbirths and spontaneous abortions accounted for 5–8% of outcomes for their 627 rhesus pregnancies. The terms abortion and stillbirth are somewhat meaningless without a definition, but unfortunately, none of the preceding citations defined these terms. The definition we chose, as previously indicated, was the one used by van Wagenen (1972), Hendrickx and Binkerd (1980) and Hendrickx and Henrickson (1988): an abortion consists of a gestation period of less than 139 days while a stillbirth or live birth is a gestation period of more than 140 days. Using this definition for our control monkeys, one out of 11 births was a stillbirth (9%) and one out of 11 births was an abortion (9%). In the treated groups, two out of 25 births were stillbirths (8%) and six out of 25 births were abortions (24%). While the value of comparison of our data with those of other studies is limited because of the lack of standardized terminology, the data from our control monkeys appear not to be dissimilar from the cited studies. However, our findings with the treated monkeys suggest that foetal mortality may be dramatically increased as a result of Aroclor 1254 ingestion, and this effect was enhanced when impregnation occurs more than 14 days after menstruation. It should also be noted that Hendrickx and Binkerd's (1980) review of the literature found that the incidence of abortions and stillbirths was highest

with primigravid monkeys, but decreased after the first pregnancy and increased in later pregnancies. The stillbirth rate, however, was generally higher for winter births than for summer births and the rate was lower for monkeys housed in large pens than for those in individual cages.

The gestation lengths for all of our test groups (Table 6) were compared with literature values for the rhesus monkey, since female capacitor workers with a history of exposure to PCBs were found to have a shorter gestation period (Taylor *et al.*, 1984 and 1989). Krohn (1960) reported that the length of gestation for rhesus monkeys ranged from 158 to 173 days; Fleischman (1963) reported 136 to 190 days with an average of 164.5 days; Fujikura and Niemann (1967) and Hendrickx and Kraemer (1970) reported 135–171 and 144–197 days, respectively. Van Wagenen (1972) reported the length of gestation to be 145–185 (average 168.4) days for female offspring and 139–194 (average 168.5) days for male offspring. The average length of gestation has been reported to be 164 ± 6 days (Valerio and Dalgard, 1975), 168.5 days (van Wagenen, 1972), 168 ± 4 days (Kerr *et al.*, 1969a) and 168 ± 5 days (Conaway and Koford, 1965). These gestation lengths are similar to those in our monkeys (Table 6). Martin (1984) reported that gestation length for the rhesus monkey increased with parity only for wild-caught females and only when the data for both sexes of the offspring were combined.

Contrary to the findings of Allen and Barsotti (1976) and Barsotti *et al.* (1976), who fed Aroclor 1248 at levels of 2.5 or 5.0 ppm, and those of Barsotti and Van Miller (1984), who fed Aroclor 1016 at levels of 0.25 or 1.0 ppm, we did not find any differences in the average birth weights of those infants that survived for at least 2 wk post partum that could be attributed to the dams' ingestion of Aroclor 1254 (Table 6). However, for some unexplainable reason, the birth weights of our infant monkeys, which averaged slightly over 440 g, were generally less than those reported by other researchers. Schultz (1933) reported the average birth weight for rhesus males to be 438 g, and the average birth weight for the females to be 432 g (12 males and 12 females), while Jacobson and Windle (1960) reported the average birth weight of rhesus monkeys born vaginally at 150 days of gestation or more to be 430.8 ± 48.9 g (25 infants, sex unspecified). Kerr *et al.* (1969b) reported an average birth weight of 472 ± 56 g (nine females and 18 males). In a study of the foetal growth rate of rhesus monkeys, Kerr *et al.* (1969a) reported an average body weight of 544.4 ± 101.6 g at 175 days of gestation. The sample size consisted of three female and five male infants: five of these monkeys had been born by spontaneous vaginal delivery, and the other three (sex unspecified) were delivered by caesarean section. The markedly heavier birth weight of the latter monkeys was not surprising, since Hartman (1932) had previously reported that for the rhesus monkey, a 6% longer time *in utero* was associated with a 26.5% greater birth weight, and Schultz (1933) as well as Valerio *et al.* (1968 and 1969) have reported a partial dependency and/or a correlation between birth weight and gestational length. Hendrickx and Kraemer (1970) reported that the birth weight of rhesus monkeys ranged from 500 to 700 g. Price *et al.* (1972) calculated a mean birth weight of 470 g (SD, 80 g) for 94 births, while van Wagenen (1972) reported the birth weight range of 161 infant female rhesus monkeys to be 260–670 g (av. 457.7 g) and the birth weight range of 150 infant males to be 300–770 g (av. 479.1 g). Previously, Valerio *et al.* (1970), and more recently, Valerio and Dalgard (1975), reported that the average birth weight of their males was 500 ± 75 g (average ± SD) and the average female birth weight was 477 ± 77 g, while the colony average for 967 rhesus monkeys was 488 ± 76 g. One aspect of reproduction which is known to affect birth weight, but cannot be evaluated for our monkeys because of the lack of historical data, is the finding of Broadhurst and Jinks (1965) and Martin (1984), that the birth weight of rhesus monkeys increases with parity. However, it should be noted that one of the most prominent effects when humans inadvertently ingested rice oil contaminated with PCBs and other polychlorinated compounds, was a reduction in birth weight as well as small-for-date (length of gestation) babies (Funatsu *et al.*, 1972; Popp *et al.*, 1993; Yamashita and Hayashi, 1985).

Neonatal monkey deaths, as defined by Hird *et al.* (1975), were those deaths that occurred before the infant was 31 days of age. A post-neonatal death occurs when an infant attains 31 to 183 days of age. Hird *et al.* (1975) reported that their rhesus neonatal mortality rate was 8.0% (49 deaths/609 births) for monkeys housed indoors. Tigges *et al.* (1988) reported an incidence of 37 deaths with 618 (5.9%) live rhesus monkey births. There were no neonatal deaths in our control group but there were three neonatal deaths (3/9 = 33%) in the treated groups. Although the latter was a small sample size, it does suggest that Aroclor 1254 may adversely affect post-natal survivability.

The lack of complete clinical and experimental histories for our monkeys raises the possibility that some unknown factor(s) may have affected their reproductive performance, even though precautions were taken to minimize such potential happenings by randomly distributing the monkeys to cage locations and the test treatments were likewise randomly distributed to cage locations. In addition, previous experimental testing may not only have affected reproduction *per se* but may have contributed to the incidence of endometriosis observed at the time of autopsy. (Autopsy findings will be the subject of a future manuscript.) The latter is of particular concern in view of the findings of Rier *et al.* (1993) that there was a correlation between previous exposure to dioxins and the incidence and severity of endometriosis in

rhesus monkeys; since, as has been mentioned previously, dioxins and PCBs have a common receptor-mediated mechanism (Safe, 1990). Unfortunately, the cause or causes and extent of occurrence of endometriosis in non-human primates—and in humans—is largely unknown (Olive and Schwartz, 1993). The Oregon Regional Primate Research Center has found varying degrees of endometriosis at an incidence rate of between 10 and 20% following appropriate examination of the lower abdominal viscera. The incidence increases with age and multiple surgeries involving the endometrium (D. L. Hess, personal communication, 1993). The incidence of endometriosis observed in our study was quite variable among test groups, with the highest incidence being observed in the control group. The prevalence of endometriosis in our monkeys was very similar to the prevalence of 30% reported by Rier *et al.* (1993) for their colony (51 monkeys with endometriosis *v.* 169 monkeys examined).

Regarding the reproductive performance of our untreated male monkeys, only 11 of the 20 (58%) untreated males assigned to the study impregnated at least one female. While the reproductive history of our males was largely unknown, Eckstein and Kelly (1966) reported that 56.5% of their males were responsible for a conception after two matings with fertile females. Consequently, the reproductive performance of our males, especially regarding the control group, could be considered to be within the limits expected for rhesus monkeys in a non-breeding colony.

Barsotti *et al.* (1976) reported that the infants from dams receiving diets with 2.5 or 5.0 ppm Aroclor 1248 had shorter long bones, smaller head circumference and reduced crown-to-rump lengths, even though the osseus development, as viewed radiographically, was similar to that of the control infants. These findings were similar to ours. However, Funatsu *et al.* (1972) and Yamashita and Hayashi (1985) have both reported abnormal calcification of the skulls of babies born to patients who had ingested rice oil contaminated with PCBs and other polychlorinated compounds.

Barsotti *et al.* (1976) reported that the concentration of PCBs in the breast milk of dams that were given diets containing 2.5 or 5.0 ppm Aroclor 1248 was 275.0 ± 121.5 ng/g milk, but gave no further details. In another study, where the rhesus dams received 2.5 or 5.0 ppm Aroclor 1248 in their diet, Allen and Barsotti (1976) reported that for the four oldest infants (group unspecified), three milk samples contained 0.154–0.397 ppm PCB and the milk fat for the fourth sample contained 16.44 ppm PCB. The geometric mean, on a lipid basis, of the concentration of PCB in the breast milk of our dams whose infants survived for at least 2 wk ranged from 5.6 to 15.6 ppm for the dams given 5 μg/kg and from 24.6 to 82.6 ppm for the dams given 40 μg/kg during lactation (Table 5).

In addition to the depressed birth weight as a result of feeding Aroclor 1248 to rhesus monkeys, Allen's group also reported other clinical manifestations of toxicity. For example, 2 months after birth, infants from dams fed diets containing 2.5 or 5.0 ppm Aroclor 1248 had acne, particularly of the face, swelling of the eyelids, loss of eyelashes, and hyperpigmentation of the skin (Allen and Barsotti, 1976). Even after the dams had their Aroclor-containing diets replaced with control feed for approximately 1 yr before rebreeding, the infants from the formerly treated dams had a lower birth weight and still developed hyperpigmentation (Allen *et al.*, 1980). Before the infants died they usually became anorexic, developed swollen eyelids, had a thinning of eyelashes, scaly skin, acne and alopecia. Decreasing the dietary Aroclor 1248 to 0.5 and 1.0 ppm, the infants still grew at a slower rate and they had focal areas of hyperpigmentation (Allen *et al.*, 1979). While the infants in our study displayed clinical signs of PCB intoxication (Table 10) similar to those previously reported for their dams (Arnold *et al.*, 1993a,b), the infants' clinical signs were less severe than those of their dams and generally appeared after weaning. In addition, neither acne nor hyperpigmentation were ever observed in any of our test monkeys (Arnold *et al.*, 1993a, b; also see Arnold *et al.*, 1990; Truelove *et al.*, 1982; Tryphonas *et al.*, 1986a,b).

Published data regarding the effects of PCBs on the immune system of infant monkeys were scanty. Abrahamson and Allen (1973) histopathologically examined the tissues from infant monkeys exposed to Aroclor 1248 for 30 days at a dose of 35 mg/kg body weight/day and reported a regression of the thymic cortical areas and hypoplastic bone marrow in comparison with control monkeys. Barsotti *et al.* (1976) reported hypocellularity of the cortical and medullary zones of the thymus, hypocellular lymph nodes devoid of germinal centres, reduced spleen size with only a few lymph nodules, and hypoactive bone marrow in the offspring of dams that ingested diets containing 5.0 ppm Aroclor 1248.

In a study conducted by Truelove *et al.* (1982), one infant Cynomolgus monkey which was exposed *in utero* while its dam received 400 μg Aroclor 1254/kg body weight/day starting at 60 days of gestation, died at 139 days of age. Before its death, and following immunization with SRBC, a reduced antibody level (IgM) to this antigen was observed compared with the levels in a control monkey. Results of the present study indicate a possible immunosuppressive effect of Aroclor 1254 in the infant monkeys. Generally, the significant treatment-related suppression in the antibody levels to SRBC and the reduced mitogen-induced lymphocyte transformation ([^{3}H]thymidine incorporation) paralleled previously reported results for the dams of these infants (Tryphonas *et al.*, 1991). However, the number of surviving infants in the treated groups was small in comparison to that for the control group.

The marked effect that Aroclor 1254 had on the reproductive processes of our female rhesus monkeys at doses below those reported in previous non-human primate studies for PCBs with a similar degree of chlorination (see reviews by Golub *et al.*, 1991; Lione, 1988; Tilson *et al.*, 1990), was toxicologically significant. The doses used in our study were similar to those that Lione (1988) calculated had been ingested by Yusho patients, and which produced overt abnormalities in their offspring. However, the Aroclor 1254 used in our study did not contain the quantity of toxic contaminants found in the rice oil ingested by Yusho patients (Masuda and Yoshimura, 1984). Some have suggested that the contaminants in the rice oil ingested by the Yusho patients was of greater toxicological significance than the PCB *per se* (Bandiera *et al.*, 1984; Kashimoto *et al.*, 1981; Kunita *et al.*, 1984; Masuda and Yoshimura, 1984; Safe, 1984). Tilson *et al.* (1990) reviewed the data on the effects of PCBs on non-human primates and the results of human epidemiological studies and concluded that the rhesus monkeys are more sensitive than humans to PCBs. If the toxicological effects in the Yusho patients are primarily attributable to the contaminants in the ingested rice oil, and the toxicological effects observed in our female monkeys following the ingestion of Aroclor 1254 are analogous to those observed in the Yusho patients at a similar level of PCB ingestion, then our findings appear to support the conclusion of Tilson *et al.* (1990).

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