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Reproductive Dysfunctions in Rhesus Monkeys Exposed to Low Levels of Polychlorinated Biphenyls (Aroclor 1248)

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Running Title: Effects of PCBs on Reproduction in Primates

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Exh. 4

Abstract-- Eighteen female and four male adult rhesus monkeys were fed the polychlorinated biphenyl (PCB) Aroclor 1248 at levels of either 2.5 or 5.0 ppm. These levels are equal to and one-half the concentration allowed in certain foods destined for human consumption. After feeding for 2 months, some of the females developed acne, alopecia, erythema and swelling of the eyelids. By six months all females exhibited these changes to some degree. Modifications in serum lipids developed gradually with a trend towards hypocholesteremia, hypolipidemia and decreased serum triglycerides. In addition there was a shift in the plasma albumin/globulin ratio and an increase in the SGPT concentrations. Analysis of subcutaneous fat showed an accumulation of the PCB isomers in the adipose tissue. The concentrations in the fat reached a plateau after which only slight variations were observed. Within 4 months, menstrual cycles were altered with menostaxis and menorrhagia occurring frequently and at times amenorrhea was apparent. The ability of the animals to maintain pregnancy was impaired as indicated by frequent resorptions and abortions. When infants were born they were small and transplacental movement of PCBs was evident in analysis of skin biopsies of neonates and autopsy tissue of a stillborn. Moreover, additional accumulation of PCBs occurred in infants after breast feeding. All of the males which were fed 5.0 ppm exhibited only slight periorbital edema and erythema after 14 months on the diet and were devoid of any alterations in their breeding capacities. The data presented indicate long term, low level exposure to PCBs in female nonhuman primates can affect many important biologic parameters.

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INTRODUCTION

The polychlorinated biphenyls (PCBs) are some of the more widespread and injurious environmental contaminants. These industrially produced compounds have been used extensively due to their heat resistance, dielectric properties and extreme stability. The properties which insure their usefulness to industry also make them very resistant to biodegradation. The main routes of environmental contamination by these compounds include open burning, vaporization, accidental spillage and waste disposal in sewage. In addition to acne, which is a rather constant complication in a majority of mammalian species exposed to PCBs, these compounds have caused reproductive failure in mink of the Great Lakes region (Ringer, Aulerich & Zabik, 1972; Aulerich, Ringer & Iwamoto, 1973), sea lions on the Pacific Coast (DeLong, Gilmartin & Simpson, 1973), birds in many areas of the world (Risebrough, Rieche, Peakall, Herman & Kirven, 1968) and man following the industrial contamination of rice oil in Japan (Kuratsune, Yoshimura, Matsuzaka & Yamaguchi, 1972). In the Japanese incident abortions, stillbirths and undersized infants were attributed to the transplacental movement of the PCBs. Subsequent research (Curley, Burse, Grim, Jennings & Linder, 1972; Allen, Carstens & Barsotti, 1974a)has shown that the PCBs do, in fact, pass across the placental barrier and are excreted in the milk of lactating mothers (Musial, Hutzinger, Zitko & Crocker, 1974; Miller & Fox, 1973).

Even though the utilization of the PCBs has been limited to closed systems, the level of contamination, particularly in bodies of water adjacent to densely populated areas, is not declining and in many instances appears to be increasing. Thus, the PCBs remain a major environmental con-

taminant of health significance. In the present report some of the possible ramifications of low level, long term exposure to PCBs are presented. Even at levels presently permitted in foods destined for human consumption the PCBs are capable of producing widespread harmful effects in female primates.

EXPERIMENTAL

Thirty female and ten male adult rhesus monkeys were housed in a controlled environment simulating the light, humidity and temperature conditions of the breeding season for these feral animals in India. During the six months prior to being placed on experimental diets, the menstrual cycles, sperm counts and morphological features of the spermatozoa were evaluated. These monkeys were subsequently placed in three groups: the control group consisted of 12 females and 6 males that consumed a commercial diet (Ralston-Purina Co., St. Louis, Mo.); the second group of 9 females received a similar diet to which 2.5 ppm of PCB (Aroclor 1248, Monsanto Co. Inc., St. Louis, Mo.) was added; and the third group of 9 females and 4 males received a similar diet containing 5.0 ppm PCB. The experimental diets were prepared by adding the appropriate levels of PCB to a finely powdered meal and subsequently pelleting the mixture into 1/2 x 1 1/4" cylindrical pellets. Periodically, samples were analyzed by GLC to affirm the PCB level. The females on the experimental and control diets received 200 g daily, while all males received 300 g daily of their respective diets. All diets were supplemented with fresh fruit and the animals received water ad libitum. The food left in the feeder was removed and weighed daily to calculate intake.

After 7 months on the experimental diets the animals were allowed to breed. Ovulation was predicted from observation of menses and previous menstrual cycle length (Valerio, 1969). In general, the experimental females were housed with control males for 5 days during the appropriate time of ovulation for breeding purposes, and the experimental males were housed with control females for a similar period. Pregnancy tests on

females were performed according to Wilson, Fradkin & Hardman (1972). The findings were confirmed by rectal palpation of the uterus at 40 days of gestation.

Throughout the experiment, animals were observed daily for any physical change. Monthly evaluation of the serum triglycerides, cholesterol, serum glutamic pyruvic transaminase (SGPT), albumin/globulin ratios, total lipid and hemograms were performed on all animals (Allen, Norback & Hsu, 1974b). In addition, every 3 months an inguinal subcutaneous fat biopsy was obtained from selected animals in each group (Allen et al., 1974a), extracted and evaluated for PCBs according to a modification of the procedure originally presented by Curley et al. (1972). For chromatographic analysis fat samples were homogenized in hexane (500 mg/20 ml) and subsequently evaporated under dry nitrogen in a 40°C waterbath. Cleanup of the extracted material prior to analysis was carried out in a disposable pipet microcolumn containing silica gel 60 (0.05-0.2 mm) by elution with 1:1 benzene-hexane mixture. Levels of PCBs were quantitated in a Hewlett-Packard 7620A chromatograph employing a ^{63}Ni electron capture detector. A pyrex column packed with Gaschrom Q (80-100 mesh) coated with 2% SE-30 was operated at 170°C with argon-methane (95:5%) as the carrier gas and at a flow rate of 40 ml per minute.

RESULTS

During the first 6 months that the females received PCBs in their diets, the intake was relatively constant with the animals ingesting an average of 180 and 364 mg of PCB on the 2.5 and 5.0 ppm diets, respectively. Despite normal food consumption, both experimental groups lost an average of 15.1% of their initial body weight (Table 1). Within 2 months after the start of the experiment and after having consumed 35 to 50 mg of PCB, individual animals began to show the typical signs of PCB intoxication, including loss of hair, acne of the face and neck, and erythema and swelling of the eyelids. At 6 months all females exhibited these signs to some degree. Skin biopsies revealed affected hair follicles with prominent keratinization. The hematologic changes developed more gradually. Hemograms remained relatively normal throughout the period. The concentrations of serum triglyceride, cholesterol and total lipids decreased progressively; however, only the decrease in total lipids was statistically significant. There was a shift in the albumin/globulin ratio and a progressive increase in the SGPT (Table 2).

The data obtained from the fat biopsies on the female animals (Table 3) showed an accumulation of PCB isomers in the adipose tissue. After a 6 month exposure period the PCB level in the adipose tissue of the animals receiving 5 ppm attained a plateau. These values varied little with continued consumption of the diet. A similar level was attained in 1 year by the 2.5 ppm PCB group.

Following 4 months on the experimental diets and after having consumed 60 to 120 mg of PCB, menstrual cycles were decidedly altered, particularly in the group receiving the 5 ppm diet. There were increases in the dura-

tion of menses and in menstrual bleeding. Menses was 5 to 7 days longer in the experimental group than in the controls. Several of the experimental animals exhibited occasional absence of menstrual bleeding.

Conception rates for the control animals and those fed 2.5 ppm PCB were essentially the same. However, the conception rate in the animals receiving 5 ppm was decreased. As shown in Table 4, 8 animals on the 2.5 ppm diet became pregnant as determined by the level of chorionic gonadotropin in mice bioassay tests. Shortly after this time 3 of the animals resorbed their embryos. In these animals implantation bleeding which usually occurs 17 days after conception was replaced by profuse uterine hemorrhage. In the 5.0 ppm group, 6 of the animals were impregnated. The remaining 2 animals were bred five times without success. Of the animals that conceived in this group, there were abortions at 46, 67 and 107 days of gestation, 1 resorption, 1 stillborn and 1 uncomplicated birth. Viable infants born to the experimental animals were small (1 to 2 standard deviations from the normal established by Kerr, Scheffler & Waisman [1969]), but were otherwise normal. These observations are in contrast to the control animals where all conceived and gave birth to normal infants (Table 4).

One adult female on the 2.5 ppm diet and one on the 5.0 ppm diet expired after 173 and 510 days of feeding, respectively. At necropsy, these animals exhibited typical signs and tissue alterations of PCB intoxication. The major gross findings at necropsy included generalized alopecia, subcutaneous edema and acne. Microscopically, in addition to hyperplasia of the follicular epithelium and inflammation of the surrounding tissue, hair follicles were filled with keratin. Throughout the liver there were focal areas of necrosis. Hepatocytes were enlarged and contained numerous small lipid droplets. Large lipid droplets prevailed in the cytoplasm of the Kupffer

cells. In addition, both animals developed a severe enteritis terminally from which Shigella flexneri type IV was isolated. This organism is known to be a common pathogen of primates, but in most instances is sensitive to treatment. Even following treatment these animals experienced Shigellosis on numerous occasions. This is consistent with previous observations that PCB-intoxicated animals appear to be more susceptible to this opportune pathogen.

The distribution of PCBs in the tissues of the two females that expired, as well as those of the dead infant born to a mother on the 5.0 ppm diet, is shown in Table 5. The affinity of PCB for lipid containing tissue is readily apparent. A transplacental accumulation of PCBs is indicated by the high concentration in the tissues of the stillborn infant. Furthermore the levels of PCBs in the other infants continued to increase after birth as a result of the high concentrations in the milk of the mother (267.0 ± 169.8 ng per g of milk). The level of PCB in the skin obtained by biopsies from infants born to the mothers on the 2.5 ppm PCB diet on the day of birth was 1.8 ± 1.4 µg per g of tissue. This value increased to 111.6 ± 29.0 µg per g of skin after breast feeding for 3 months.

One of the male animals that received 5.0 ppm PCB exhibited a slight periorbital edema and erythema of the eyes after 6 months (intake of 300 mg PCB). Only the consumption of approximately 670 mg of PCB over 14 months resulted in all of the exposed males developing slight to moderate periorbital edema and congestion. Levels of PCB continued to increase in the adipose tissue of the males with time. In spite of such elevations, there was no alteration in their gross breeding capabilities (sperm count or breeding success). Control females conceived at the same rate when bred to experimental males or control males.

DISCUSSION

The levels of PCB that were incorporated in the diets of experimental animals were selected to represent concentrations which are equal to or one-half of those permitted in certain foods that are destined for human consumption. Previous experiments reported from this laboratory have shown that concentrations as low as 25 ppm of PCB in the diet of nonhuman primates may produce morbidity and mortality after 2 months of exposure (Allen et al., 1974a).

Skin changes appeared in animals receiving 2.5 or 5.0 ppm PCB as rapidly as in the animals which were fed larger doses (Allen et al., 1974a; Allen & Norback, 1973). In fact, these skin changes were recorded in some animals after only a total of 30 to 50 mg PCB had been ingested. These data suggest that only short exposure to PCBs is required to stimulate follicular epithelial hyperplasia, blockage of the sebaceous ducts and keratinization of the hair follicles. The early heavy localization of PCB in the skin of affected animals (Yoshimura & Oshima, 1971) is undoubtedly responsible for such tissue changes.

The values for SGPT, albumin/globulin ratios and total lipids were significantly different from the controls at 8 months. Although there were indications much earlier, it required 12 months on the experimental diets before changes in the serum triglyceride and cholesterol concentrations were statistically significant. These observations substantiate the findings of Allen et al. (1974) where similar changes were recorded in the serum chemistry of monkeys fed 25 ppm PCB for 2 months.

Male monkeys fed a diet containing 5.0 ppm invariably ate more food and attained higher levels of PCB in the adipose tissue than their female counterparts. However, the female monkeys developed signs of PCB intoxication within 2 months whereas only minor skin alterations developed in males after one year. One explanation for this difference in PCB response of the two sexes may be related to the total reservoir of fat tissue. Since the males were much larger than the females they had a greater quantity of adipose tissue in which the ingested PCB could be sequestered. Platonow and Karstad (1973) described similar differences in the toxic response of mink to PCB, noting that male mink at all treatment levels were less severely affected, survived longer and produced abundant, viable spermatozoa throughout the experiment. Gish and Chura (1970) observed that heavier quail survived longer than lighter ones when treated with DDT.

One of the most serious effects of PCB exposure in the adult female monkey was on reproduction. Even at low dietary levels of PCBs, the menstrual cycles were irregular and there was excessive and prolonged menstrual bleeding. In addition when the animals conceived they showed a higher incidence of resorption and early abortion even when compared to the 10% abortions and stillbirths that are recorded per year in the breeding colony. A likely explanation for these changes is related to an estrogen-progesterone imbalance in these animals, these hormones being responsible for menstrual flow and the maintenance of pregnancy. Preliminary observations from this laboratory suggest an endocrine disturbance resulting from PCB intoxication in females. When females are fed PCB in their diets they invariably have much higher levels of urinary ketosteroids than control monkeys (Barsotti and Allen, 1975).

Another interesting observation was the reduced size of the term infants born to PCB fed monkeys. These findings are in agreement with the reports from Japan where the children born to exposed mothers were reduced in size (Kuratsune et al., 1972). Even though the infant monkeys had shorter long bones, smaller head circumference and reduced crown to rump lengths, the osseus development as viewed radiographically was similar to the control infants (unpublished observations). Histologically, there was also normal development of the tissues of day old infants born to PCB fed mothers (Allen et al., 1974a).

Recently it has been shown that at least a portion of the PCBs are metabolized through an arene oxide intermediate (Hsu, Van Miller & Allen, 1975). There is a distinct possibility that many of the effects that have been recorded in this experiment may be related to an interaction of these epoxides with cellular macromolecules. These findings also suggest that long range effects of PCB exposure may occur in primate species since epoxides are known to be capable of producing necrogenic, carcinogenic and mutagenic changes in mammalian tissues.

It is unlikely that man will be exposed to levels of 2.5 or 5.0 ppm on a continuous basis in his diet. However, there may be instances when the intake of PCBs may exceed the above concentrations for short periods or exposure over a lengthy period may give rise to toxic tissue concentrations. Whether human exposure to the PCBs is sufficiently great to produce serious effects such as reproductive insufficiencies or modifications in the functional capacities of certain cellular macromolecules has not been determined. However, the data presented in this report suggest that present levels of PCB contamination may be sufficient to cause such effects.

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Table 1. Changes in body weight of female monkeys receiving a diet containing 2.5 and 5.0 ppm Aroclor 1248*

Time on Diet (months)	Control Weight (kgs) (12 animals)	Experimental Weight (kgs) (18 animals)
0	4.50 ± 0.68	5.57 ± 0.55
1	4.89 ± 0.60	5.47 ± 0.58
2	4.86 ± 0.41	5.28 ± 0.55
3	5.00 ± 0.55	5.38 ± 0.60
4	5.00 ± 0.51	5.38 ± 0.60
5	5.01 ± 0.59	4.84 ± 0.48

* Values represent mean ± standard deviation.

Table 2. Modifications in the serum chemistry of female monkeys receiving a diet containing 2.5 and 5.0 ppm Aroclor 1248*

	Time on diet (months)				
	0	2	3	4	8
Triglycerides (mg/100 ml)	75 ± 18	93 ± 48	75 ± 24	51 ± 20	61 ± 20
Cholesterol (mg/100 ml)	166 ± 28	152 ± 28	159 ± 24	156 ± 26	156 ± 22
Total lipids (mg/100 ml)	582 ± 90	573 ± 98	581 ± 97	451 ± 74	470 ± 83
A/G ratio**	1.20 ± 0.19	1.23 ± 0.20	1.30 ± 0.21	1.23 ± 0.16	1.08 ± 0.19
SGPT (R-F units)†	14 ± 3	24 ± 7	21 ± 8	21 ± 9	31 ± 7

* Values represent mean ± standard deviation; sixteen animals- 8-2.5 ppm, 8-5.0 ppm PCB.

† Difference between month 0 and month 8 significant, $p < .001$.

**Difference between month 0 and month 8 significant, $p < .05$.

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Table 3. Total intake and adipose tissue levels of polychlorinated biphenyls in female monkeys consuming diets containing 2.5 and 5.0 ppm Aroclor 1248*

	One month on diet		Six months on diet	
	PCB intake (mgs)	Tissue Content (µg/g)	PCB intake (mgs)	Tissue Content (µg/g)
2.5 ppm group (4)†	15.63±2.63	5.06±3.50	85.29±8.07	32.69±15.97
5.0 ppm group (3)	30.95±1.06	16.39±4.49	175.36±4.24	71.30±31.56

* Values represent mean ± standard deviation.

† One animal in this group exhibited lesions early after commencement of experiment and GC analysis of adipose tissue revealed that the concentrations were closer to those of the 5.0 ppm animals despite the lower intake of PCB. This animal is not included in the calculations.

Table 4. Modification in reproduction in primates that were exposed to Aroclor 1248 in the diet

	Control	2.5 ppm	5.0 ppm
Total impregnated (no./no. animals)	12/12	8/8	6/8
Resorptions or abortions (no./no. animals)	0/12	3/8	4/8
Stillborn (no./no. animals)	0/12	0/8	1/8
Normal births (no./no. animals)	12/12	5/8	1/8

Table 5. Polychlorinated biphenyl content of tissues obtained at necropsy from two adult female monkeys and a stillborn infant

Tissue	2.5 ppm* (µg/gm)	5.0 ppm† (µg/gm)	Infant** (µg/gm)
Pancreas	0.16	53.89	63.85
Liver	5.61	24.38	2.47
Adrenal	3.09	40.18	24.85
Kidney	0.60	52.77	2.47
Spleen	1.55	30.15	12.52
Small intestine	0.77	3.22	15.00
Stomach	2.68	47.45	18.96
Brain	1.58	21.41	0.34
Lung	3.20	8.69	99.36
Muscle	0.19	17.66	4.48
Skin	0.98	10.03	0.001

* Aroclor 1248 intake-75.3 mg over 173 days.

† Aroclor 1248 intake-284.56 mg over 310 days.

** Aroclor 1248 intake by mother 394.3 mg over 401 days on 5.0 ppm Aroclor 1248 diet.