Fd Cosmet. Toxicol. Vol. 14, pp. 99–103. Pergamon Press 1976. Printed in Great Britain

REPRODUCTIVE DYSFUNCTION IN RHESUS MONKEYS EXPOSED TO LOW LEVELS OF POLYCHLORINATED BIPHENYLS (AROCLOR 1248)

D. A. Barsotti, R. J. Marlar and J. R. Allen

Department of Pathology, University of Wisconsin Medical School, Department of Food Microbiology and Toxicology, and Regional Primate Research Center, University of Wisconsin, Madison, Wisconsin 53706, USA

(*Received 5 September 1975*)

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 in the diet. These levels are equal to, and 50% of, the concentration allowed in certain foods destined for human consumption. After consuming these diets for 2 months, some of the females developed acne, alopecia, erythema and swelling of the eyelids, and by 6 months all females exhibited these changes to some degree. Modifications in serum lipids developed gradually, with a trend towards hypocholesterolaemia, hypolipidaemia and decreased serum triglycerides. In addition there was a shift in the plasma albumin/globulin ratio and an increase in serum glutamic-pyruvic transaminase activity. Analysis of subcutaneous fat showed an accumulation of the PCB isomers in the adipose tissue. The concentrations in this tissue reached a plateau, after which only slight variations were observed. Within 4 months, menstrual cycles were altered; menostaxis and menorrhagia occurred frequently and at times amenorrhoea 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 the transplacental movement of PCBs was evident from analyses of skin biopsies of neonates and of autopsy tissue from one stillborn. Moreover, additional accumulation of PCBs occurred in infants during breast feeding. All males fed 5·0 ppm PCB exhibited only slight periorbital oedema and erythema after 14 months on the diet and showed no alterations in their breeding capacities. The data presented indicate that long-term, low-level exposure of female non-human primates to PCBs can affect many important biological parameters.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are some of the more widespread and injurious environmental contaminants. These industrially produced compounds have been used extensively, because of their heat resistance, dielectric properties and extreme stability. The properties that ensure 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 fairly constant complication in most mammalian species exposed to PCBs, these compounds have caused reproductive failure in mink of the Great Lakes region (Aulerich, Ringer & Iwamoto, 1973; Ringer, Aulerich & Zabik, 1972), in sea lions on the Pacific Coast (DeLong, Gilmartin & Simpson, 1973), in birds in many areas of the world (Risebrough, Rieche, Peakall, Herman & Kirven, 1968) and in man following the industrial contamination of rice oil in Japan (Kuratsune, Yoshimura, Matsuzaka & Yamaguchi, 1972). In the Japanese incident, the occurrence of abortions, stillbirths and undersized infants was attributed to the transplacental movement of the PCBs. Subsequent research (Allen, Carstens & Barsotti, 1974a; Curley, Burse, Grim, Jennings & Linder, 1971) has shown that the PCBs do, in fact, pass across the placental barrier and are excreted in the milk of lactating mothers (Miller & Tox, 1973; Musial, Hutzinger, Zitko & Crocker, 1974).

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

EXPERIMENTAL

Animals and diets. 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 6 months prior to the administration of the experimental diets, the menstrual cycles, sperm counts and morphological features of the spermatozoa were evaluated in these animals. The monkeys were subsequently placed in three groups, a control group of 12 females and six males given a commercial diet (Ralston-Purina Co., St. Louis, Mo.), a second group of nine females receiving a similar diet to which 2·5 ppm PCB (Aroclor 1248, Monsanto Co. Inc., St. Louis, Mo.) was added, and a third group of nine females and four males receiving a similar diet containing 5·0 ppm PCB. The exper-

DSW 031481

STLCOPCB4015443

imental diets were prepared by adding the appropriate levels of PCB to a finely powdered meal and subsequently pelleting the mixture into 0·5 × 1·25 in. cylindrical pellets. Periodically, samples were analysed by gas–liquid chromatography to confirm the PCB level. The females on the experimental and control diets received 200 g daily, while all males received 300 g of their respective diets daily. All diets were supplemented with fresh fruit and the animals received water *ad lib.* The food left in the feeder was removed and weighed daily for calculation of intake.

Breeding study. After 7 months on the experimental diets, the eight surviving females from the 2.5-ppm group, eight females from the 5-ppm group and the 12 control females 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, 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.

Observations and analyses. Throughout the experiment, animals were observed daily for any physical change and were weighed monthly. Serum triglycerides, cholesterol, serum glutamic-pyruvic transaminase (SGPT), albumin/globulin ratios, total lipid and haemograms were determined monthly in all animals (Allen, Norback & Hsu, 1974b). In addition, every 3 months an inguinal subcutaneous fat biopsy taken from selected animals in each group (Allen *et al.* 1974a) was 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. Clean-up of the extracted material prior to analysis was carried out in a disposable pipette microcolumn containing silica gel 60 (0·05–0·2 mm) by elution with a benzene–hexane (1:1, v/v) mixture. Levels of PCBs were estimated 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/min.

Table 1. *Changes in the body weight of female monkeys receiving a diet containing 2·5 or 5·0 ppm Aroclor 1248*

Time on diet (months)	Mean body weight (kg)	
	Control group	Experimental group
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 are means ± standard deviation for groups of 12 control or 18 PCB-fed (2.5 or 5 ppm) animals.

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 90 and 180 mg 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 of the start of the experiment and when 30–60 mg PCB had been consumed, 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 haematological changes developed more gradually. Haemograms remained relatively normal throughout the period, but the concentrations of serum triglyceride, cholesterol and total lipids decreased progressively (Table 2), although only the decrease in total lipids was statistically significant. There was also a shift in the albumin/globulin ratio and a progressive increase in SGPT activity (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, subsequent values varying little with continued consumption of the diet. A similar level was attained at 1 yr by the group given 2·5 ppm PCB.

After 4 months on the experimental diets and consumption of a total of 60–120 mg PCB, menstrual

Table 2. *Modifications in the serum chemistry of female monkeys given a diet containing 2·5 or 5·0 ppm Aroclor 1248*

Time on diet (months)	Serum levels (mg/100 ml)			Albumin/ globulin ratio	SGPT activity (RFU)
	Triglycerides	Cholesterol	Total lipids		
0	75 ± 18	166 ± 28	582 ± 90	1·20 ± 0·19	14 ± 3
2	93 ± 48	152 ± 28	573 ± 98	1·23 ± 0·20	24 ± 7
3	75 ± 24	159 ± 24	581 ± 97	1·30 ± 0·21	21 ± 8
4	51 ± 20	156 ± 26	451 ± 74	1·23 ± 0·16	21 ± 9
8	61 ± 20	156 ± 22	470 ± 83†	1·08 ± 0·19*	31 ± 7**

SGPT = Serum glutamic-pyruvic transaminase RFU = Reitman-Frankel units

Values are means ± standard deviation for a group of 16 animals (eight given 2·5 ppm and eight 5·0 ppm PCB) and asterisks indicate those 8-month values that differed significantly from the value at month 0: *$P < 0·05$; **$P < 0·01$.

DSW 031482

STLCOPCB4015444

Table 3. *Total intake and adipose-tissue levels of PCBs in female monkeys consuming a diet containing 2·5 or 5·0 ppm Aroclor 1248*

Dietary level (ppm) of PCB	No. of animals	Values after administration of PCB diets* for 1 month: Total PCB intake (mg)	1 month: Tissue content of PCBs (μg/g)	6 months: Total PCB intake (mg)	6 months: Tissue content of PCBs (μg/g)
2·5	4†	15·63 ± 2·63	5·06 ± 3·50	85·29 ± 8·07	32·69 ± 15·97
5·0	3	30·95 ± 1·06	16·39 ± 4·49	175·36 ± 4·24	71·30 ± 31·56

*Data have not been included on the PCB levels of fat obtained from the control animals because in all instances the minor peaks recorded in the gas-chromatographic analysis could not be stated to be PCBs. Furthermore the amount they represented was infinitesimally small.

†One animal in this group showed lesions early in the experiment and gas-chromatography of its adispose tissue revealed that the PCB concentrations were closer to those of the animals fed 5·0 ppm despite the lower intake of PCB. This animal was not included in the calculations.

Values are means ± standard deviation for the numbers of animals shown.

cycles were decidedly altered, particularly in the group receiving the 5-ppm diet. There were increases in the duration of menses and in menstrual bleeding. Menses were 5 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 for those fed 2·5 ppm PCB were essentially the same, but, the conception rate in the animals receiving 5 ppm was decreased. As shown in Table 4, eight animals on the 2·5-ppm diet became pregnant, as determined by the level of chorionic gonadotropin in mouse bioassays, but shortly afterwards three of these animals resorbed their embryos. In these animals, implantation bleeding, which usually occurs 17 days after conception, was replaced by profuse uterine haemorrhage. In the 5·0-ppm group, six of the animals were impregnated. The remaining two 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, one resorption, one stillborn and one uncomplicated birth. Viable infants born to the experimental animals were small (1–2 standard deviations from the normal established by Kerr, Scheffler & Waisman, 1969), but were otherwise normal. These observations are in contrast to those in the control animals, all of which 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 died after 173 and 310 days of feeding, respectively. At autopsy, these animals showed the typical signs and tissue alterations of PCB intoxication. The major gross findings at autopsy included generalized alopecia, subcutaneous oedema 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 terminally a severe enteritis from which *Shigella flexneri* type IV was isolated. This organism is known to be a common pathogen of primates, but is generally sensitive to treatment. Even following treatment, the animals in this study experienced shigellosis on numerous occasions, a finding 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 died, as well as in 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 and a transplacental movement 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 mothers (275·0 ± 121·5 ng/g milk). The level of PCB in the skin obtained by biopsy on the day of birth from infants born to the mothers on the 2·5-ppm PCB diet

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

Reproduction parameter	No. recorded in groups fed diets containing PCB levels (ppm) of: 0	2·5	5·0
No. of animals/group...	12	8	8
Total impregnated	12	8	6
Resorptions or abortions	0	3	4
Stillborn	0	0	1
Normal births	12	5	1

DSW 031483

STLCOPCB4015445

Table 5. *PCB content of tissues obtained at autopsy from two adult female monkeys and a stillborn infant*

Tissue	PCB content (μg/g) in tissues from		
	Adults fed dietary levels (ppm) of		
	2·5*	5·0†	Stillborn infant‡
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 diet

was 2·8 ± 1·4 μg/g tissue. This value had increased to 111·6 ± 29·0 μg/g skin after breast-feeding had continued for 3 months.

One of the male animals given 5·0 ppm PCB exhibited a slight periorbital oedema and erythema of the eyes after 6 months (total PCB intake, 300 mg). Only the consumption of approximately 670 mg PCB over 14 months resulted in the development of slight to moderate periorbital oedema and congestion in all of the exposed males. Levels of PCB continued to increase in the adipose tissue of the males with time. In spite of these rises there was no alteration in their gross breeding capabilities (sperm count or breeding success), control females conceived at the same rate whether bred to experimental or control males.

DISCUSSION

The levels of PCB that were incorporated in the diets of experimental animals were selected to represent concentrations equal to or half as great as those permitted in certain foods destined for human consumption. Previous experiments reported from this laboratory have shown that PCB concentrations as low as 25 ppm in the diet of non-human 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 fed larger doses (Allen *et al.* 1974a; Allen & Norback, 1973). In fact, these skin changes were recorded in some animals after a total of only 30–50 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 month 8. 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.* (1974a) who recorded similar changes in the serum chemistry of monkeys fed 25 ppm PCB for 2 months.

Male monkeys fed a diet containing 5·0 ppm PCB 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 1 yr. One explanation for this difference in PCB response between 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 & 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 & Chura (1970) observed that heavier quail survived longer than lighter ones when treated with DDT.

One of the most serous results of PCB exposure in the adult female monkey was the effect on reproduction. Even with low dietary levels of PCBs, the menstrual cycles were irregular and there was excessive and prolonged menstrual bleeding. In addition, animals that conceived showed a higher incidence of resorption and early abortion even when compared with the 10% abortions and stillbirths recorded each year in the breeding colony. A likely explanation for these changes is related to an oestrogen-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 & Allen, 1975).

DSW 031484

STLCOPCB4015446

Another interesting observation was the reduced size of the infants born at term to PCB-fed monkeys. These findings are in agreement with 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 that in the control infants (unpublished observations). Histologically, there was also normal development of the tissues in 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 PCB exposure may have long-range effects 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 PCBs on a continuous basis in his diet. However, there may be instances when the intake of PCBs may exceed these concentrations for short periods or when exposure over a prolonged period may give rise to toxic concentrations in the tissues. Whether human exposure to the PCBs is sufficiently great to produce serous effects such as reproductive insufficiency 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.

Acknowledgements This investigation (Primate Center Publication no. 15-017) was supported in part by US Public Health Service grants ES-00472, ES-00958 and RR-00167 and Pathology Graduate Training Grant GM-00130 from the National Institutes of Health, and by the University of Wisconsin Sea Grant Program. Part of this research was conducted in the University of Wisconsin-Madison Biotron, a controlled environmental research facility supported by the National Science Foundation and the University of Wisconsin. The authors thank Ms. J. Scheffler and Ms. L. Carstens for their technical assistance.

REFERENCES

Allen, J. R., Carstens, L. A. & Barsotti, D. A. (1974a). Residual effects of short-term, low-level exposure of nonhuman primates to polychlorinated biphenyls. *Toxic. appl. Pharmac.* **30**, 440.

Allen, J. R. & Norback D. H. (1973). Polychlorinated biphenyl- and triphenyl-induced gastric mucosal hyperplasia in primates. *Science, N.Y.* **179**, 498.

Allen, J. R., Norback, D. H. & Hsu, I. C. (1974b). Tissue modifications in monkeys as related to absorption, distribution and excretion of polychlorinated biphenyls. *Arch. Environ. Contam. & Toxicol.* **2**, 86.

Aulerich, R. J., Ringer, R. K. & Iwamoto, S. (1973). Reproductive failure and mortality in mink fed on Great Lakes fish. *J. Reprod. Fert. Suppl.* **19**, p. 365.

Barsotti, D. A. & Allen, J. R. (1975). Effects of polychlorinated biphenyls on reproduction in the primate. *Fedn Proc. Fedn Am. Socs exp. Biol.* **34**, 338.

Curley, A., Burse, V. W., Grim, M. E., Jennings, R. W. & Linder, R. E. (1971). Polychlorinated biphenyls: Distribution and storage in body fluids and tissues of Sherman rats. *Envir. Res.* **4**, 481.

DeLong, R. L., Gilmartin, W. G. & Simpson, J. G. (1973). Premature births in California sea lions: Association with high organochlorine pollutant residue levels. *Science, N.Y.* **181**, 1168.

Gish, C. D. & Chura, N. J. (1970). Toxicity of DDT to Japanese quail as influenced by body weight, breeding condition, and sex. *Toxic. appl. Pharmac.* **17**, 740.

Hsu, I. C., Van Miller, J. P. & Allen, J. R. (1975). Metabolic fate of ^{3}H 2,5,2′,5′-tetrachlorobiphenyl in infant nonhuman primates. *Bull. env. contam. & Toxicol. (U.S.)* **14**, 233.

Kerr, G. R., Scheffler, G. & Waisman, H. A. (1969). Growth and development of infant *Macaca mulatta* fed standardized diet. *Growth* **33**, 185.

Kuratsune, M., Yoshimura, T., Matsuzaka, J. & Yamaguchi, A. (1972). Epidemiologic study on Yusho, a poisoning caused by ingestion of rice oil contaminated with a commercial brand of polychlorinated biphenyls. *Environ. Health Perspect.* **1**, 119.

Miller, G. J. & Fox, J. A. (1973). Chlorinated hydrocarbon pesticide residues in Queensland human milks. *Med. J. Aust.* **2**, 261.

Musial, C. J., Hutzinger, O., Zitko, V. & Crocker, J. (1974). Presence of PCB, DDE and DDT in human milk in th provinces of New Brunswick and Nova Scotia, Canada. *Bull. env. contam. & Toxicol. (U.S.)* **12**, 258.

Platonow, N. S. & Karstad, L. H. (1973). Dietary effects of polychlorinated biphenyl on mink. *Can. J. comp. Med.* **37**, 391.

Ringer, R. K., Aulerich, R. J. & Zabik, M. (1972). Effect of dietary polychlorinated biphenyls on growth and reproduction of mink. *Proc. Am. chem. Soc.* **12**, 149.

Risebrough, R. W., Rieche, P., Peakall, D. B., Herman, S. G. & Kirven, M. N. (1968). Polychlorinated biphenyls in the global ecosystem. *Nature, Lond.* **220**, 1098.

Valerio, D. A. (1969). Breeding *Macaca mulatta* in a laboratory environment. *Lab. Anim. Handb.* **4**, 223.

Wilson, J. G., Fradkin, R. & Hardman, A. (1972). Breeding and pregnancy in rhesus monkeys used for teratological testing. *Teratology* **3**, 59.

Yoshimura, H. & Oshima, M. (1971). Studies on the tissue distribution and elimination of several components of KC-400 (chlorobiphenyls) in mice. *Fukuoka Acta med.* **62**, 5.

DSW 031485

STLCOPCB4015447