Toxicology, 6 (1976) 331—340
© Elsevier/North-Holland, Amsterdam — Printed in The Netherlands

THE EFFECTS OF TRANSPLACENTAL AND MAMMARY MOVEMENT OF PCBs ON INFANT RHESUS MONKEYS *

J. R. ALLEN and D. A. BARSOTTI

Department of Pathology, University of Wisconsin Medical School; and Regional Primate Research Center, University of Wisconsin, Madison, Wisconsin 53706 (U.S.A.)

(Received March 2nd, 1976)
(Revision received June 15th, 1976)
(Accepted June 20th, 1976)

SUMMARY

Sixteen adult female rhesus monkeys were fed diets containing 2.5 and 5.0 ppm PCB (Aroclor 1248) for approx. 1.5 years. 6 of the 8 animals on 5 ppm PCB and 8 of the 8 animals on 2.5 ppm PCB conceived when bred after 6 months' exposure to PCB. Only 1 live infant was born to the animals of the 5.0 ppm group and 5 infants to the animals in the 2.5 ppm PCB group. All of the infants had PCBs in their tissues at birth. Thereafter, there was a rapid increase in the PCB levels of the infants' tissues. This increase was attributed to consumption of PCB-containing milk from their mothers. Within 2 months following birth the infants had facial acne and edema, swelling of the eyelids, loss of facial hair including eyelashes, and hyperpigmentation of the skin. Three of the 6 infants had expired within 8 months following birth due to PCB intoxication. The three survivors were weaned and subsequently showed marked improvement in their physical state.

INTRODUCTION

In the last few years considerable interest has been shown regarding the possible effects of PCB exposure on the reproductive capability of various animal species. Women exposed to PCB in the "Yusho" incident had altered

* This investigation was supported in part by U.S. Public Health Service grants ES-00958, ES-00472, GM-00130 and RR-00167 from the National Institutes of Health, and the University of Wisconsin Sea Grant Program. A portion 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. Primate Center Publication No. 16—016.
Abbreviation: PCBs, polychlorobiphenyls.

NONS 207384

serum ketosteroid levels, dysmenorrhea and irregularities in their menstrual cycles [1]. Female rhesus monkeys have also shown irregularities in their menstrual cycles [2] and increased urinary ketosteroids following PCB exposure [3]. There are reports in mice [4] and rhesus monkeys [2] that suggest a decreased frequency of embryonic implantation in PCB-exposed females. Embryotoxic and teratogenic abnormalities have been observed in birds [5], rabbits and rats [6]. Following pregnancy there appears to be a ready transplacental movement of PCBs in humans [1], rhesus monkeys [7], bovine species [8], sea lions [9], mice [10] and rats (unpublished observations). Premature births resulting from PCB exposure occurred in sea lions [9]. Small hyperpigmented infants were born to humans and nonhuman primates exposed to PCBs during gestation [1,2]. Weak offspring have been recorded in mink [11] and birds [5] exposed to PCBs. The movement of PCBs from the body to the egg of avian species [12] and into the milk of lactating mammals [13] represents other possible sources of toxicity in the developing young. In this study it is shown that low level exposure to PCBs affects the general body health of adult female rhesus monkeys as well as intrauterine and neonatal viability of their offspring.

MATERIALS AND METHODS

16 adult female rhesus monkeys weighing approx. 6 kg and experiencing regular menstrual cycles were fed diets containing 2.5 and 5.0 ppm PCBs (Aroclor 1248; Monsanto Company, St. Lous, Missouri) for 6 months prior to breeding, throughout gestation, and for 3 months following delivery. Following 6 months exposure the females were bred to control males [2]. Abortions and stillbirths were evaluated grossly and microscopically. Levels of PCBs in the various infant tissues were determined by GLC analysis [2].

At birth the infants were weighed, evaluated grossly for abnormalities and skin biopsies were taken to determine PCB levels. Thereafter, the infants were examined daily and weighed weekly. On the third month after parturition the adult females were placed on control diets. The infants were permitted to remain with their mothers and nurse for a minimum of 4 months. One infant was allowed to remain with its mother for an additional 5 months. The remaining infants were placed on a milk supplement at 4 months of age.

Milk samples were obtained from four of the lactating females that were on the 2.5 ppm PCB diet on days 22, 118, 141 and 142 following delivery of their young and analyzed for PCB content by GLC analysis.

RESULTS

After 6 months of consuming a diet containing 2.5 or 5.0 ppm PCB, the monkeys were bred to control males. 8 of the 8 animals on the 2.5 ppm diet conceived; however, only 5 were able to carry their infants to term. 6 of 8 animals on the 5.0 ppm diet conceived. 4 of the 6 females experienced

MONS 207385

TABLE I

TISSUE LEVELS OF PCBs IN INFANT MONKEYS THAT DIED FOLLOWING IN UTERO AND TRANSMAMMARY EXPOSURE

Infant No.	1	4	7	Stillborn
Sex	Female	Male	Male	Male
Age (days)	239	44	112	0
Level of PCB fed to mother (ppm)	2.5	2.5	5.0	5.0
Organ	Tissue Level of PCB (μg/g)			
Adrenal	21.23	18.93	9.48	24.85
Bone marrow	16.40	50.79	157.00	not evaluated
Cerebrum	26.94	undetected	0.53	0.50
Kidney	26.70	17.07	14.98	3.00
Large intestine	6.11	9.43	3.02	1.35
Liver	6.12	0.62	12.17	2.47
Lung	1.67	49.48	6.03	99.36
Muscle	23.39	12.65	0.21	4.48
Pancreas	20.17	17.78	22.82	63.85
Spleen	14.54	14.00	2.03	12.52
Testes or ovary	48.80	0.51	0.82	not evaluated
Thymus	63.52	34.41	0.80	12.95

abortions early in gestation. There was one stillborn infant delivered by a female that had received 5.0 ppm PCB in her diet. In this case death was due to hypoxia that occurred as a result of a difficult delivery. Although the gross and microscopic appearance of the tissues of this infant were free of lesions attributable to PCBs, there were high levels of the more chlorinated isomers in its tissues as determined by gas chromatography. Organs containing considerable fat such as the pancreas and adrenals had 63.85 and 24.85 μg per g tissue, respectively (Table I). In addition, the lungs had 99.36 μg of PCB per g tissue.

TABLE II

BODY WEIGHTS OF INFANT MONKEYS BORN TO MOTHERS RECEIVING 2.5 OR 5.0 ppm AROCLOR 1248 [a] IN THEIR DIETS PRE- AND POSTPARTUM

Age (weeks)	Body weight (g)	
	Control	Experimental
0	507 ± 59	399 ± 22
2	601 ± 47	463 ± 40
4	711 ± 58	517 ± 12
5	763 ± 82	572 ± 39
6	796 ± 81	601 ± 38
12	1003 ± 71	765 ± 67

[a] Monsanto Company, St. Louis, Mo.

MONS 207386



Fig. 1. 4-month-old rhesus monkey showing signs of PCB intoxication. Note the absence of eyelashes, swelling of the eyelids and accumulation of secretions along the edge of upper eyelids and at the commissures. Numerous small acneform lesions are apparent on the temple and forehead. One larger lesions with ulceration is located above the right eye. The mother of this infant had received 2.5 ppm PCB prior to becoming pregnant, during gestation and for 3 months while nursing the depicted infant.

At birth the 6 infants were small (399 ± 22 vs. 507 ± 59 g) (Table II). Other than their small stature, low birth weights and focal areas of hyperpigmentation of the skin, their general appearance, hemograms and osseous development as evaluated radiographically were normal. Skin biopsies including the epidermis, dermis and the attached underlying subcutaneous tissue, which were taken from these infants soon after birth, revealed PCB levels of 1.0 to 4.8 μg per g tissue. By the third month the PCB levels in the skin had a range of 86.4 to 136.8 μg per g tissue.

These infants were permitted to remain with their mothers, and their only source of nourishment was through nursing. During their period of nursing the animals showed a consistent weight gain, although it was below

MONS 207387

that of the controls (Table II). Within 2 months the infants began to show signs of PCB intoxication characterized by the development of acne, particularly of the face, swelling of the eyelids, loss of eyelashes and increased pigmentation of the skin (Fig. 1). There was also a decided difference in the hair color of the experimental and the control animals. In the control infants the hair that covered the posterior portion of the back and thighs had a distinctly yellow hue, whereas the hair of the experimental animals had a silvery appearance.

Following the clinical manifestations of PCB intoxication in the older infants, four 5 cc milk samples were obtained from the females to determine the PCB content. Three milk samples varied from 0.154 to 0.397 ppm PCB. The level of PCB in the milk fat was determined on the fourth sample and found to be 16.44 ppm.

3 of the 6 infants died from PCB intoxication. They included one male (No. 7) infant from the 5.0 ppm PCB group which expired on the 112th day and a male (No. 4) and a female (No. 1) from the 2.5 ppm group that expired on days 44 and 239 following birth. Infant No. 4 which died on the



Fig. 2. Thymus of an infant that died 44 days following birth due to PCB intoxication. Grossly there was only a remnant of the thymus. Microscopically, there was a loss of cortical thymocytes and a sparsity of medullary thymocytes. Note the abundance of epithelial cells in the medulla. The infant's mother had been fed 2.5 ppm PCB prior to and throughout gestation and while nursing this infant. Hematoxylin and eosin stain; x 30.

MONS 207388

44th day of life had the highest level of PCBs in the skin at birth. In addition, infant No. 1 survived for 239 days and experienced intermittent periods of vomiting during its terminal 3 months of life. Although the animals were showing clinical signs of PCB intoxication, their demise occurred suddenly.

At necropsy the animals were small for their age and exhibited focal hyperpigmented areas of the skin. The skin of the face was dry and crusty with isolated acneform lesions. The upper eyelids were swollen, and there was a sparsity of eyelashes. In addition, the conjunctivae were moderately congested. Internally there were only rudimentary thymuses, and the spleens were small. With the exception of the thickening of the gastric mucosa of the animal that experienced periods of vomiting, the other organs of the animals appeared normal grossly. Microscopically there was a near complete absence of thymocytes in the cortical and medullary portion of the thymus (Fig. 2). The lymph nodules of the spleen were extremely small with germinal centers being inapparent. A decided hypocellularity of the bone marrow was recorded in all of the infants (Fig. 3). There was hypertrophy of the Meibomian glands of the eyelids and keratinization of the hair follicles of the eyelashes. All of the livers showed a moderate fatty infiltration of the

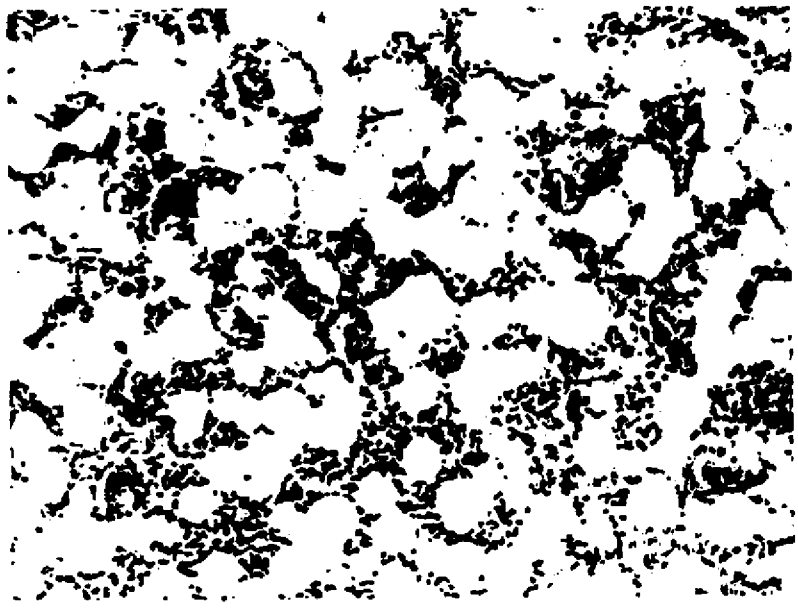

Fig. 3. Femoral bone marrow from an infant that died from PCB intoxication 112 days following birth. Note the hypocellularity in the marrow. The mother of this infant had been on 5.0 ppm PCB prior to breeding, throughout gestation and during the nursing period. Hematoxylin and eosin stain; × 30.

MONS 207389

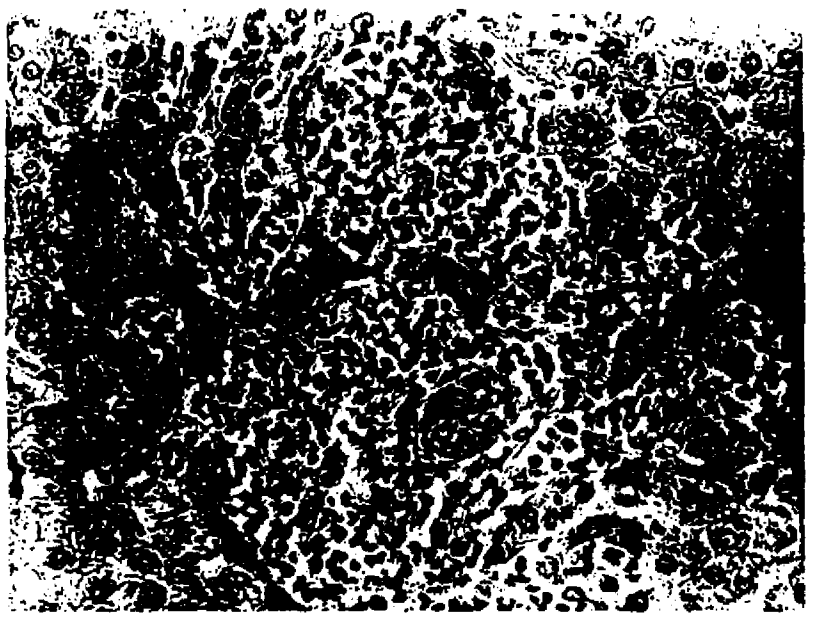

Fig. 4. Section of the liver from a PCB intoxicated infant monkey that died 239 days after birth. Dispersed throughout the liver were defined foci in which the hepatocytes had been replaced by large numbers of histiocytes. Acute inflammatory cells were randomly dispersed among the histiocytes as well as being the predominant cell in focal areas of necrosis adjacent to the more chronic area (-). This section was obtained from an infant whose mother had been fed 5 ppm PCB prior to breeding, throughout gestation and during its nursing. Hematoxylin and eosin; x 75.

hepatic parenchymal cells. The liver of infant No. 1 which survived for 239 days had focal well circumscribed areas of histiocytes and leukocytes that had replaced the hepatic cells (Fig. 4). In addition, there were widely dispersed areas where large sinusoids had developed following degeneration of hepatic cells. The older infant also had gastric mucosal hyperplasia with some glandular elements having penetrated the muscularis mucosa. Acute inflammatory cells were present in the edematous submucosa (Fig. 5).

In infant No. 1 that survived for 239 days, there were seven organs that had over 20 μg PCB per g tissue while in the two animals that survived for a shorter period, only 3 tissues in infant No. 4 and 2 in infant No. 7 exceeded this level (Table I). However, the latter animals had more tissues with PCB levels exceeding 10 μg PCB per g tissue. In addition, the older infant (No. 1) had much higher levels of PCB in the brain, thymus and lymph nodes. This infant also had a high concentration of PCB in her ovaries, while the PCB content of the testes of the two male infants was low.

The three surviving infants were weaned at 4 months of age and placed

MONS 207890



Fig. 5. Gastric mucosal hyperplasia with penetration of the muscularis mucosa by glandular elements can be observed in a section from a PCB intoxicated infant that died 239 days after birth. Note the acute inflammatory cells (•) and epithelium in the submucosa. Hematoxylin and eosin; x 30.

on a synthetic milk replacer (Similac, Ross Laboratories, Columbus, Ohio). These infants showed a decided improvement within 4 months after being removed from their mothers. Their eyelashes became more plentiful, the swelling of the eyelids less obvious, and the acneform lesions of the face disappeared. The characteristic pattern of pigmentation about the hair line of the face that was present in all 6 of the infants remains discernible in the surviving young. Skin and subcutaneous fat biopsies of these animals at 8 months of age showed 19.71 ± 5.10 μg in the fat and 4.19 ± 1.43 μg of PCB per g of tissue in the skin.

DISCUSSION

The need for additional information on the potential injurious effects of PCB exposure in the human population has been emphasized in a recent report which states that approx. 5% of a randomly selected population in the United States had levels of PCB in excess of 2 ppm and an additional 30% had levels of PCB that ranged from 1 to 2 ppm in their adipose tissue [14]. Since data are available which indicate transplacental movement of the PCBs as well as the presence of high levels in the milk fat of lactating

MONS - 207391

mammals there is a distinct likelihood that a large percentage of the infant population has been exposed to a considerable amount of the PCBs during critical developmental stages. In order to clarify questions regarding the potential danger that the fetus or neonatal infant may experience from PCB exposure female rhesus monkeys were exposed to low levels of PCBs prior to conception, during gestation and following delivery.

As a result of the PCB exposure the female rhesus monkeys had difficulty in maintaining pregnancy. Preliminary data from this laboratory indicate that excessive hydroxylation of steroid hormones may be in part responsible for the early abortions. Once the monkey fetuses have progressed beyond the second month of gestation the likelihood of abortion seems to be much less. However, the inhibiting effects that PCBs have on fetal growth are apparent at birth indicating a persistence of fetal toxicity. The reduced size of the infants cannot be attributed to a decrease in food consumption by the mothers in that the intake was the same for the control and experimental animals. Small infants were also recorded in the "Yusho" outbreak where exposure to PCBs occurred during gestation [1].

Hyperpigmentation of the skin was also a common feature of infants born to PCB-exposed mothers. 9 of 13 infants born to 13 women in the "Yusho" outbreak had dark brown skin [1]. In nonhuman primates the areas of the skin normally having more pigment were much darker and assumed a pattern common to all 6 of the PCB-exposed infants. The relationship of PCB exposure to excessive melanin production remains to be clarified. However, the skin and subcutaneous tissue are major sites of PCB localization, thus placing a high concentration of these compounds in close proximity to the melanocytes.

These is considerable transplacental movement of PCBs in exposed mammals. A stillborn infant born following the "Yusho" outbreak had detectable levels of PCB in its tissue [15]. Similar observations have also been recorded in infant nonhuman primates whose mothers were exposed to PCB prior to and during gestation [7]. The fetal tissues of mice [8] and rats (unpublished observations) also accumulate PCBs via transplacental movement.

Compounding the direct exposure of the fetus to PCBs through placental movement is that the amniotic fluid appears to be a source of additional exposure for the fetus. This is suggested by the high levels of PCB in the lungs and gastric contents of stillborn infants (99.36 and 18.96 μg/g, respectively). However, soon after birth when the fluid has disappeared from the alveoli of the lungs and the meconium has been eliminated, the PCB levels of these tissues decreased markedly [7]. In the rat the gastric and intestinal contents and unexpanded lungs of the fetus were 10 to 20 times greater in PCB content than the amniotic fluid, thus suggesting a PCB concentrating ability of these tissue in the fetus (unpublished observations).

In addition to the transplacental movement of PCB, nursing of PCB-exposed females serves as a means of increasing the level of PCB in the infants. PCBs have been detected in human milk samples from many areas

MONS 207392

of the world, ranging from 0.018 to 0.103 ppm in whole milk and 1.86 to 3.50 in milk fat [16]. A milk sample from a Yusho patient contained 0.06 ppm PCB [13]. Nonhuman primates exposed to 2.5 ppm PCB in the diet for over one year had levels ranging from 0.154 to 0.397 ppm (16.44 ppm in milk fat) in their milk. Cows exposed to 200 mg per day for 60 days had levels of approx. 3 ppm (66.7 ± 84 ppm in milk fat) in whole milk [17]. The milk of rats fed a diet containing 100 ppm PCB (Aroclor 1248) for the 3 weeks of gestation had 80 ppm PCB (unpublished observations).

Although there is little doubt that transmammary movement of PCB is a major source of exposure for infant mammels, it is only in the nonhuman primate that infant deaths have been attributed to ingestion of PCB-containing milk. If it is assumed that the monkeys of the presently reported experiment consumed 100 ml of milk per day with a 3.5% fat level per day before death the infants would have ingested approx. 2.5, 6.0 and 14.0 mg PCB via the milk. This level, in addition to the body load already accumulated in utero, was sufficiently great to cause morbidity and mortality. The importance of exposure of infants to PCBs through nursing is further emphasized in the 3 surviving infant monkeys. Once removed from their mothers and placed on a synthetic milk replacer, they showed a rapid improvement with the signs of PCB exposure becoming less apparent. A retarded growth rate has also persisted in the infants born to the female monkeys exposed to 2.5 ppm PCBs prior to and throughout gestation.

REFERENCES

1 M. Kuratsune, T. Yoshimura, J. Matsuzaka and A. Yamaguchi, Environ. Health Persp., 1 (1972) 119.
2 D.A. Barsotti, R.J. Marlar and J.R. Allen, Food Cosmet. Toxicol., 14 (1976) 99.
3 D.A. Barsotti and J.R. Allen, Fed. Proc., 34 (1975) 338.
4 J. Orberg and J.E. Kihlstrom, Environ. Res., 6 (1973) 176.
5 H.C. Cecil, J. Bitman, R.J. Lillie, G.F. Fries and J. Verrett, Bull. Environ. Contam. Toxicol., 11 (1974) 489.
6 D.C. Villenauve, D.L. Grant, K. Khera, D.J. Clegg, H. Baer and W.E.J. Phillips, Exp. Physiol., 1 (1971) 67.
7 J.R. Allen, L.A. Carstens and D.A. Barsotti, Toxicol. Appl. Pharmacol., 30 (1974) 440.
8 N. Platonow and N.Y. Chen, Vet. Rec., 92 (1973) 69.
9 R.L. DeLong, W.G. Gilmartin and J.G. Simpson, Science, 181 (1973) 1168.
10 P. Torok and H. Kriegel, Arch. Toxicol., 33 (1975) 199.
11 R.K. Ringer, R.J. Aulerich and M. Zabik, Proc. Amer. Chem. Soc., 12 (1972) 149.
12 N.S. Platonow and B.S. Reinhart, Canad. J. Comp. Med., 37 (1973) 341.
13 Y. Masuda and R. Kagawa, Bull. Environ. Contam. Toxicol., 11 (1974) 213.
14 F.W. Kutz and S.C. Strassman, National Conference on Polychlorinated Biphenyls (Nov. 19–21, 1975, Chicago, Illinois) Proceedings (1976) 139.
15 H. Yoshimura, Fukuoka Acta Med., 62 (1971) 109.
16 C.J. Musial, O. Hutzinger, V. Zitko and J. Crocker, Bull. Environ. Contam. Toxicol., 12 (1974) 258.
17 G.F. Fries, Environ. Health Persp., 1 (1972) 55.

MONS 207393