12/75 EPA Hearings in Chicago

PATHOBIOLOGICAL RESPONSES OF PRIMATES TO POLYCHLORINATED BIPHENYL EXPOSURE

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Abstract

Male and female rhesus monkeys received varying levels of polychlorinated biphenyls (PCB's) and were evaluated for toxic effects, reproductive dysfunctions, and metabolism of the compounds. Female rhesus monkeys exposed to dietary levels as low as 2.5 and 5.0 ppm of PCB (Aroclor 1248) developed facial acne, erythema, subcutaneous edema, conjunctivitis, and loss of eyelashes. Reproductive dysfunctions were manifested by irregular menstrual cycles, early abortions, and stillbirths. As a result of transplacental migration of the compounds, all infants born of PCB-exposed animals contained PCB's in their tissues at birth. The infants, which continued to be exposed to PCB's by ingestion of milk from their lactating mothers, developed skin lesions and 50 percent expired within 4 months.

Metabolic studies demonstrated 90 percent absorption of the PCB's from the gastrointestinal tract and distribution in organs of high lipid content. Hydroxylated metabolites were formed in the liver and excreted through the biliary and urinary routes. Lower chlorinated congeners were more rapidly metabolized and excreted, while concentrations of the highly chlorinated biphenyls persisted in the adipose tissue in excess of 2-1/2 years. The detection of the urinary metabolite trans-3,4-dihydro-3,4-dihydroxy-tetrachlorobiphenyl suggests that the mechanism of metabolism is through an arene oxide intermediate. In vivo and in vitro studies demonstrated binding of PCB's with macromolecules.

INTRODUCTION

Even though the polychlorinated biphenyls (PCB's) have been used extensively for various industrial purposes for the past 40 years, the health significance of human exposure to these compounds only recently has become of widespread concern. The scientific community was alerted to the potential environmental health problem by Jensen in 1966 (ref. 1) after PCB's were identified in tissue extracts of birds experiencing reproductive difficulties in Sweden. The magnitude of the problem was brought to the forefront by the "Yusho" incident when over 1,000 Japanese suffered prolonged ill effects from exposure to PCB-contaminated rice oil (ref. 2). Further concern about the potential danger of PCB's on human health followed disclosure of increasing levels of these compounds in various foods. The contamination has been attributed to incorporation of these compounds within the food chain or from packaging of food products in PCB-impregnated paper containers (ref. 3). Increasing levels of PCB's in human tissue samples attest to the magnitude of the human exposure (refs. 4,5).

Within this laboratory various animal models, including the nonhuman primate and rodent, have been evaluated following exposure to PCB's for the development of lesions which parallel those recorded in man. Emphasis has been placed on determining pathophysiological alterations that arise in animal models as a result of exposure to several levels of PCB's for variable periods of time. The absorption, tissue distribution, and rate of excretion of PCB's have been determined. The interaction of the PCB's or their metabolites with cellular macromolecules has also been a major area of investigation. The following report, which includes previously unpublished observations, presents a summary of the progress that has been made in this laboratory on the above mentioned areas of PCB research.

GENERAL EFFECTS OF PCB'S ON NONHUMAN PRIMATES

The investigation of toxicity produced by PCB's in various animal species demonstrated the limitations of rodents as animal models. Male Sprague-Dawley rats were able to survive for 1 year on diets containing 100 ppm PCB (Aroclor 1248, 1254, or 1262) without showing signs of illness (ref. 6). These observations substantiated those of Keplinger et al. (ref. 7). Increasing the PCB content of the rat diets to 1,000 ppm did not produce skin lesions; however, death occurred within 6 to 8 weeks due to widespread hepatic degeneration (ref. 8).

Male rhesus monkeys developed many of the signs

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experienced by humans that had been inadvertently exposed to PCB's. Monkeys fed diets containing 100 and 300 ppm PCB (Aroclor 1248) (table 1) developed facial edema, erythema, acne, and alopecia within 3 weeks. The lesions became progressively more severe with increased length of exposure (refs. 9,10). In addition, the animals developed anorexia, loss in weight, hypoproteinemia, hypolipidemia, and anemia. Within 3 months the majority of the monkeys had died or were moribund. Necropsies of these animals revealed decided mucosal gastric hyperplasia with penetration of the glandular epithelium into the underlying submucosa (ref. 10). Numerous ulcerations of the hyperplastic gastric mucosa were also present (ref. 11). In addition, there was a decided hypertrophy of the liver.

Female monkeys fed 25 ppm PCB (Aroclor 1248) (table 1) in the diet developed facial lesions similar to those observed in the animals receiving higher levels of PCB's (ref. 12). After 2 months on the PCB diet it was necessary to discontinue the exposure due to the severity of intoxication. One of the six experimental animals died 4 months after the initial exposure to PCB's. Necropsy evaluation demonstrated severe gastric hyperplasia and ulceration. The surviving adult female monkeys continued to be devoid of eyelashes and displayed facial acneform lesions 2 years following exposure to the PCB's. Infants born to these females were small (350 vs. 450 g) and contained PCB's in their tissues at birth.

Female monkeys given 2.5 and 5.0 ppm PCB (Aroclor 1248) (table 2) in their diets developed facial edema, swollen eyelids, erythema, loss of hair, and acne within 2 months (ref. 13). By the fourth month, irregularities in the menstrual cycles and an increased level of urinary ketosteroids were recorded (ref. 14). Following 6 months of PCB exposure the female monkeys were bred to control males. Six of eight animals on the 5.0 ppm diet conceived (table 2). The remaining two were bred on five separate occasions without conceiving. Four of the six females experienced abortion early in gestation. Eight of eight of the 2.5 ppm PCB fed animals conceived; however, only five were able to carry their infants to term. As was the case with infants of animals given the higher levels of PCB's, all the infants were small and at birth their skin contained detectable levels of PCB's.

The infants were permitted to nurse their mothers for 4 months. Within 2 months focal areas of hyperpigmentation, swollen lips and eyelids, loss of eyelashes, and acneform lesions of the face developed. The skin of these infants showed a decided increase in the PCB level over this period. Within 4 months, 3 of the 6 infants died due to PCB intoxication. After weaning, the remaining three have shown improvement of the skin lesions during the 4-month period.

Four adult male rhesus monkeys were also exposed to a diet containing 5.0 ppm PCB's (Aroclor 1248) (table 1) for 17 months (average total intake of PCB's 460 mg). They began to develop a slight periorbital edema after 6 months of exposure; however, it was much less severe than in the female monkeys receiving a similar level of PCB. The morphological features and viability of the spermatozoa as well as the ability to fertilize control female rhesus monkeys was unaffected during the initial 12 months of PCB exposure. Subsequently one of the four males lost weight and developed alopecia, acne, periorbital edema and decreased libido. A testicular biopsy of this animal showed a decided hypoactivity of the seminiferous tubules. There was an absence of mature spermatozoa and a predominance of Sertoli cells of the tubules. The remaining three males have remained healthy and sexually active (ref. 15).

ABSORPTION, METABOLISM, TISSUE DEPOSITION, AND EXCRETION OF PCB's

Over 90 percent of a single oral dose (1.5 or 3.0 g per kg) of PCB's (Aroclor 1248) given to adult rhesus monkeys was absorbed from the gastrointestinal tract. Chromatographic analysis of the tissues 14 days after exposure revealed a predominance of higher chlorine isomers that had a predilection for the adipose tissue and organs containing a high fat content (ref. 16).

Rhesus monkeys fed 25 ppm PCB (Aroclor 1248) in the diet attained levels of 127 μg/g within the adipose tissue after 2 months. Eight months after discontinuation of exposure to PCB's the levels were 34 μg/g within the adipose tissue. After 33 months, the levels within the adipose tissue ranged from 3 to 14 μg/g; the residues contained greatly increased proportions of highly chlorinated congeners. Transplacental movement of the PCB's was demonstrated by the presence of PCB's in the infants born to exposed females. The tissues of an infant, born to a female 8 months following the discontinuation of PCB's in the diet, contained 25 μg/g of PCB in the fat and adrenal tissues at the time of birth. In an infant born to a female 29 months following the discontinuation of PCB's, the levels within the adipose tissue were 3.38 μg/g at 4 months of age.

Female monkeys given 5.0 ppm PCB in their diets attained maximum levels of PCB's within their adipose tissue at 6 months (141 to 177 μg/g adipose tissue). However, it required approximately 14 months on the 2.5 ppm diet for the monkeys to reach similar maximum PCB levels in their adipose tissue (126 to 144 μg/g). Males which received 5.0 ppm PCB's attained levels

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Table 1. Experiments on exposure of primates to PCB's (Aroclor 1248*)

Level of PCB in diet (ppm)	No. of animals	Length of exposure (months)	Total PCB intake
300	6 males	3	3.6 to 5.4 g
100	6 males	2	0.8 to 1.0 g
25	6 females	2	250 to 400 mg
2.5	8 females	16-19	243 to 303 mg
5.0	8 females	16-19	460 to 614 mg
5.0	4 males	17	530 to 692 mg

*Monsanto Co., Inc., St. Louis, Missouri.

Table 2. 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

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ranging from 128 to 200 μg per g adipose tissue at 14 months. Infants born of mothers exposed to 2.5 and 5.0 ppm PCB's within the diet contained concentrations of PCB's ranging from 1.0 to 4.8 μg/g within the skin at birth. While nursing from mothers consuming PCB diets, the infants continued to accumulate the compound. At 3 months, the levels within the tissues ranged from 86 to 136 μg/g. The concentration of PCB's within the milk ranged from 0.15 to 0.40 μg/g. The tissues of the infants which died while nursing PCB-fed mothers contained high levels of PCB's within the thymus, ovaries, brain, kidneys, adrenal glands and pancreas (20-48 μg/g tissue). Lower levels were found in the liver, lymph nodes, and bone marrow (8-16 μg/g).

Monkeys and rats demonstrate species variation in the metabolic response to the PCB congener 2,5,2′,5′-tetrachlorobiphenyl (TCB). Over 66 percent of the single dose (500 mg/kg) administered to rats was recovered from the feces, and an additional 10 percent was present in the urine within the initial 72 hours (ref. 17). The material present in the body was concentrated within the adipose tissue. There was a transient high level of TCB within the blood at 24 hours. Other organs which contained significant quantities of TCB, however at lower concentrations, included the liver, skin, and muscle. The major urinary metabolite was identified as 3-OH-2,5,2′,5′-tetrachlorobiphenyl. Other monohydroxy TCB metabolites were present in minor quantities (ref. 17).

Over 90 percent of an oral dose (500 mg/kg) of ^{3}H TCB administered to infant rhesus monkeys was absorbed from the gastrointestinal tract and was highly concentrated in the skin, adrenal gland, liver, and adipose tissue. At 72 hours, less than 2 percent of the dose had been eliminated in the urine and 1 percent in the feces. Monohydroxy TCB, a major metabolite present in the rat urine, was a minor metabolite in the urine of the monkeys. The two major metabolites were dihydroxy-TCB and trans-3,4-dihydro-3,4-dihydroxy TCB (ref. 18). A second minor metabolite was hydroxy-3,4-dihydro-3,4-dihydroxy TCB.

Following the oral dose of ^{3}H-TCB (1 g/kg) to juvenile monkeys, a major percentage of the material was absorbed from the gastrointestinal tract and was highly concentrated in the adrenal, adipose tissue, and skin. Significant quantities were present within the liver, muscle, and uterus. After 4 weeks, 14.8 percent of the dose was secreted into the bile. Approximately 75 percent of the biliary material was reabsorbed by the gut and the nonabsorbed material was recovered from the feces during this period. Over 90 percent of the PCB's excreted in the bile was in the form of water soluble glucuronic acid conjugates. An additional 8 percent of the total dose was recovered from the urine.

Administration of the higher chlorine congener ^{3}H 2,4,5,2′,4′,5′-hexachlorobiphenyl (HCB) to rats or monkeys demonstrated low levels of exretion into the bile. An oral dose of HCB (1 g/kg) administered to rats resulted in excretion of 0.3-0.7 percent of the dose per day in the bile over a period of 14 days. The urine was free of detectable levels of radioactivity (ref. 19). At 14 days, 65 percent was recovered from the body tissues. The compound was highly concentrated within the adrenal glands, adipose tissue, and skin and in the female within the ovaries and uterus. Following the administration of a single dose of HCB (1 g/kg) to juvenile rhesus monkeys, less than 2 percent was excreted via the biliary-fecal route over a 3-week period. There was no detectable radioactivity within the urine. The organs with the highest concentration of the material included the adrenal, adipose tissue, and skin. Due to the large mass of muscular tissue, this was a major reservoir of the compound.

INTERACTION OF METABOLITES WITH CELLULAR MACROMOLECULES

Following the administration of ^{3}H 2,5,2′,5′-tetrachlorobiphenyl (TCB) to infant rhesus monkeys, interaction of TCB and macromolecules of cells and of serum was evaluated (ref. 20). Separation of the serum constituents by polyacrylamide gel electrophoresis demonstrated association of the TCB primarily with serum albumin. Over 90 percent of the radioactivity of liver homogenates eluted from a Sephadex G-25 column was in the protein and nucleic acid fractions. The majority of the macromolecular-associated HCB apparently was bound by hydrophobic association. Extraction of liver homogenates with hexane, precipitation of the hexane-extracted homogenate with TCA, and subsequent extraction of the TCA precipitate with methanol resulted in extraction of the majority of the radioactivity. In the extracted residue, 1.1 percent of the radioactivity remained which may represent covalently bonded material. Recent in vitro studies employing monkey microsomes incubated with an NADPH generating system demonstrated 20 percent of the metabolized ^{3}H-TCB was bound to microsomal protein and RNA in a nonextractable form. Binding of ^{3}H-TCB was prevented by heating the microsomes to 100°C prior to incubation (ref. 21).

DISCUSSION

These experiments employing nonhuman primates have demonstrated development of parallel signs and

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lesions of PCB intoxication in humans and rhesus monkeys exposed to similar levels over comparable periods of time. Acne, subcutaneous edema of the face, and edema of the eyelids were observed in man (ref. 22) and lower primates (ref. 15) exposed to PCB's. The facial signs of PCB exposure appear to be a sensitive indicator of PCB intoxication. The rhesus monkeys after exposure for 2 months to dietary levels of PCB's (2.5 and 5.0 ppm) developed facial alterations after a total consumption of 32-50 mg of PCB's. It is noteworthy that PCB levels of 5.0 ppm are presently permitted in foods destined for human consumption.

The most debilitating lesions in the monkeys were the severe hyperplasia and ulceration of the stomach. Whether similar changes occur in the stomach of man exposed to PCB's remains to be clarified. Nausea and anorexia described by the human subjects suggest potential gastric alterations. Liver hypertrophy, proliferation of the endoplasmic reticulum, and increased hepatic microsomal enzyme activities were observed in man (ref. 23) and in lower primates (ref. 11).

Menstrual irregularities, decreased libido, occurrence of stillborns, reduced birth weights, and transplacental movement of PCB's in humans and rhesus monkeys have been recorded (refs. 13,24). The reproductive failures of monkeys exposed to PCB's were due to inability to maintain a pregnant state. The majority of the animals did not experience appreciable difficulty in conception; however, a large percentage of the animals aborted during the first 45 days of pregnancy. These observations suggest an inability of implantation or inability to maintain the implanted embryo during the early stages of pregnancy.

Although the mechanism of reproductive dysfunction has not been clarified, there is some indication of hormonal modifications. Alterations in the urinary ketosteroids were reported in humans exposed to PCB's (ref. 22). Increased levels of urinary ketosteroids have been observed in the nonhuman primates that experienced reproductive failures (ref. 14). One mechanism of altered steroid metabolism may be secondary to the increase in the hepatic mixed function oxidases that are present in the hypertrophic livers of exposed animals. It has also been a consistent observation that the organs associated with steroid production, the adrenals and ovaries (particularly the corpora lutea), have contained relatively high concentrations of PCB's in exposed animals. Thus the compounds may possibly have a direct effect on these organs.

The presence of PCB's in the milk of other species has been previously reported (ref. 3). This avenue of infant exposure and the potential morbidity and mortality was vividly demonstrated in the infant monkeys who nursed from mothers exposed to 2.5 and 5.0 ppm in the maternal diets. The presence of relatively low levels of PCB's in the diets of lactating females represents a potential source of PCB intoxication to nursing infants.

Metabolic studies of the rhesus monkey demonstrate over 90 percent absorption of the PCB's from the gastrointestinal tract following oral administration. The material is concentrated in organs with high lipid content, including the adipose tissue, skin, adrenal, corpora lutea of the ovaries, and brain. Within the liver the greatest portion of the material is associated with the membranes of the endoplasmic reticulum.

Studies with the single congener TCB demonstrate the metabolism of the compound to hydroxylated forms which are conjugated with glucuronic acid and excreted into the bile. Enterohepatic circulation of the PCB's undoubtedly occurs as only 20 percent of the material secreted into the bile was recovered from the feces. The greatest portion of the metabolized TCB was excreted through the urinary system. The more highly chlorinated biphenyl HCB was more slowly eliminated from the body via the biliary-fecal route; HCB or metabolites were not detected within the urine. The facilitated metabolism and excretion of the lower chlorine congeners was also indicated by the relative decreased storage of these compounds, and conversely the accumulation of higher chlorinated congeners, within the adipose tissue.

Metabolic studies that have been conducted on nonhuman primates suggest mechanisms of interaction of PCB's with tissues and, more importantly, indicate potential mutagenic and carcinogenic effects of the PCB's. Metabolites have been isolated from rhesus monkeys exposed to the PCB congener 2,5,2′,5′-tetrachlorobiphenyl that are formed through an arene oxide intermediate (ref. 18). Similar metabolites of the PCB's and the potential for arene oxide formation has been demonstrated in rabbits (refs. 25,26). Arene oxides formed by the metabolism of other aromatic hydrocarbons have been shown to covalently bind with macromolecules and produce mutagenic and carcinogenic changes in mammalian cells (ref. 27). Dechlorination of the more highly chlorinated biphenyls, demonstrated by dechlorination of 2,4,5,2′,4′,5′-hexachlorobiphenyl (ref. 28), provides a mechanism through which metabolism of highly chlorinated biphenyls through an arene oxide intermediate would be facilitated.

Evidence demonstrating the association of PCB's with liver macromolecules supports the theoretical potential of the PCB's for covalent binding with cellular macromolecules. Thus, it appears that alkylation of macromolecules is one mechanism by which the PCB

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metabolites cause widespread injurious effects. Further credence for the ability of the compound to produce alterations in the macromolecules is presented in recent reports of hepatocellular tumors developing in rats and mice exposed to PCB's (refs. 29-31).

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DISCUSSION

VOICE: I'm from the Massachusetts Society. Have you examined the samples for minute contaminants?

DR. ALLEN: That is a good question. I presume your primary interests are in the dibenzofurans. The Monsanto Company has volunteered to analyze the Aroclor 1248 used in our experiments for the furans. In our discussion last week they were hopeful of having these data available for this conference. If Dr. Wright is in the audience perhaps he would give us a progress report on the subject. (No answer from the audience.) I can say that we have done some preliminary work in this area and have found undetectable levels of furans in the samples. However, more detailed studies to clarify this question are underway at the present time.

VOICE: How can you determine that the PCB's are covalently bound to macromolecules?

DR. ALLEN: Repeated extractions carefully monitored for radioactivity are the best methods of removing any absorbed material from the protein. Standard gel chromatography methods do not differentiate between adsorption and covalently bound materials. We hope to be able to generate enough PCB bound to macromolecules to permit the determination of the exact covalent nature of the bond following macromolecular digestion.

VOICE: Which macromolecule did you use?

DR. ALLEN: We were using protein and RNA from monkey microsomes. These microsomes were incubated with ^{3}H PCB in a NADPH generating system. The protein and RNA were isolated subsequently and their radioactivity determined.

VOICE: Could you tell us roughly how much PCB your animals consumed per kilogram of body weight?

DR. ALLEN: The female animals on the PCB experiments weighed between 6 and 7 kilograms. Table 1 gives the average total intake of PCB's by these animals during the various experiments.

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