Polychlorinated Biphenyl Toxicity in the Pregnant Cynomolgus Monkey: A Pilot Study

J. Truelove, D. Grant, J. Mes, H. Tryphonas, L. Tryphonas, and Z. Zawidzka

Health Protection Branch, Health and Welfare Canada, Ottawa, Canada, K1A 0L2

Abstract. Three pregnant cynomolgus monkeys (Macaca fascicularis) were dosed with 100 or 400 μg/kg/day of Aroclor 1254 from approximately 60 days of gestation. One additional pregnant monkey was given dose vehicle only. The two monkeys dosed with 100 μg/kg/day delivered stillborn infants and the 400 μg/kg/day dosed monkey delivered a term infant that had impaired immunologic function compared with the control infant, and died at 139 days post partum. The three dams also had impaired immunologic capacity assessed at approximately 50 days post partum (148 days treatment). With the exception of loss of fingernails in two monkeys, no overt clinical signs of toxicity were observed in the adults. Polychlorinated biphenyl (PCB) concentrations and peak ratios in breast milk and tissues are reported.

There is a potential threat of polychlorinated biphenyl (PCB) poisoning to man as a result of widespread environmental pollution (Wassermann et al. 1979). Since PCBs occurring in the environment are mixtures of many isomers, each one potentially having a different toxicity, and since they are often contaminated with chlorinated dibenzofurans, interpretation of toxicity data has been inconclusive; therefore, the significance of PCB contamination of the human food supply remains unclear. Several studies have demonstrated that because of their lipophilic nature, PCB residues are commonly found in human breast milk (Wickizer et al. 1981; Kodama and Ota 1980; Mes and Davies 1979; Currie et al. 1979; Masuda et al. 1978) and therefore may represent a most insidious hazard for nursing infants. However, data relating PCB concentration in maternal blood and breast milk with infant toxicity is lacking.

Using the rhesus monkey as a model, Barsotti et al. (1976) and Allen and Barsotti (1976) reported that infants exposed to PCBs in utero and postnatally from dams dosed with Aroclor 1248 (2.5 and 5 ppm in the diet) had low birth weights, developed facial acne and edema, alopecia, swelling of the eyelids, hyperpigmentation of the skin, and in some instances, died. While all infants had PCBs in their tissues at birth there was a rapid post partum increase in infant skin and subcutaneous tissue PCB concentration that was attributed to the consumption of breast milk. The clinical signs improved when the infants were weaned. PCB breast milk concentrations ranged from 0.154 to 0.397 ppm. In a later study the same females were rebred after consuming a control diet for one year. Infant tissue concentrations of PCB again increased postnatally and the infants developed hyperpigmentation about the hairline. Breast milk concentrations were 0.020 to 0.19 ppm (Allen et al. 1980). Bailey et al. (1980), also using rhesus monkeys, reported that only 1 out of 3 nursing mother-infant pairs developed signs of toxicity when dosed (by stomach tube) with 16 mg/kg/day of Clophen A-30 for 30 days beginning at 1 to 3 months post partum. However, after 30 days the PCB concentration in breast milk (approx. 11 ppm) was approximately 20 times that of the maternal serum, further demonstrating the tendency of PCB to become concentrated in monkey breast milk.

The pilot study described in this paper was the first step towards the initiation of a major PCB toxicity study designed to provide regulatory information concerning tolerable levels of PCB in human breast milk. In addition to testing some of the techniques required for the main study, this pilot was intended to provide clinical data concerning PCB...
follows: A stock PCB solution was prepared by dissolving an equal parts in disposable scintillation vials, capped and stored in the refrigerator. On dose days, each vial was warmed to room temperature, shaken vigorously, fitted with a rubber stopper and spigot and mounted on the door of each monkey's cage. When the dose was consumed, approximately 10 ml of gelatin/apple juice solution was sprayed into each vial through the spigot, shaken and reoffered to the test monkey. Prior to the initiation of the experiment, test monkeys were trained (by water deprivation when necessary) to consume the dose medium and in most cases drank it avidly (within 15-20 min). The control animal was dosed as above but without PCB.

**Table 1. Summary of pilot study design**

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Days treated prepartum</th>
<th>Total days dosed (µg/kg/day)</th>
<th>Aroclor® 1254</th>
<th>Approximate dietary equivalent (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>292</td>
<td>89</td>
<td>262</td>
<td>Control</td>
<td>--</td>
</tr>
<tr>
<td>218</td>
<td>108</td>
<td>238</td>
<td>100</td>
<td>2.5</td>
</tr>
<tr>
<td>836</td>
<td>105</td>
<td>238</td>
<td>100</td>
<td>2.5</td>
</tr>
<tr>
<td>285</td>
<td>95</td>
<td>267</td>
<td>400</td>
<td>10</td>
</tr>
</tbody>
</table>

* ^a Total 7 day dose was administered in 3 days (Mon., Wed., Fri.) (infants were left with their mothers and were not dosed)

* ^b Based on a 3 kg female cynomolgus monkey consuming 120 g of chow/day

Toxicity in cynomolgus monkeys and to describe the relationship between PCB dose and PCB concentration in breast milk and various tissues.

**Materials and Methods**

**Test Animals and Dosing:** Four pregnant cynomolgus monkeys (*Macaca fascicularis*) who had previously exhibited normal menstrual cycles and had delivered normal infants were dosed with Aroclor® 1254 (Lot 634, Monsanto Corp., USA) or dose vehicle beginning at approximately day 60 of gestation. Two monkeys (#218 and #836) were given 100 µg/kg/day, one monkey (#285) received 400 µg/kg/day and one monkey (#292) served as a control (Table 1). The test monkeys were housed in individual stainless steel cages in a breeding colony and were fed a commercial primate diet (High Protein Monkey Chow,Ralston Purina, USA) and water *ad libitum*, except on dosing days when no water was allowed from approximately 8 a.m. to 1 p.m. The monkeys received half an orange twice per week and 0.6 ml of a vitamin supplement (ABDEC Drops, Parke-Davis Ltd., Canada) in their water bottle once per week. The monkeys were weighed weekly and observed daily for signs of toxicity. No blood or tissue samples were collected and the monkeys were handled as little as possible prior to parturition in order to reduce the possibility of abortions caused by the stress of capture. Following parturition, infants were left with their mothers. When the infants began to move actively around the cage at approximately three months of age, it became obvious that the infants could and would consume the mothers PCB dose. In order to prevent this, the infants were fitted with fine chain collars and during dosing periods, a leash made of fine chain and elastic cord was attached to the collar with a snap fastener and the other end fastened to a bar on the cage. The length of the leash was adjusted so that the infant was prevented from reaching the mothers dosing vial.

The PCB dose was administered three times per week (Mon., Wed., Fri.) at approximately 1 p.m. The dose was prepared as follows: A stock PCB solution was prepared by dissolving an aliquot of PCB in corn oil so that the weekly dose for each animal was contained in approximately one ml of corn oil stock solution. Doses were prepared once per week by adding the appropriate volume (based on a weekly body weight measurement) of stock corn oil solution to approximately 45 ml of a solution of 0.25% gelatin (B.P., The British Drug Houses, Canada) in apple juice (Sun Pac Foods Ltd., Canada). The resulting mixture was emulsified with an homogenizer (Silverson Machines Ltd., England) for approximately 30 sec. The emulsion was divided into three equal parts in disposable scintillation vials, capped and stored in the refrigerator. On dose days, each vial was warmed to room temperature, shaken vigorously, fitted with a rubber stopper and spigot and mounted on the door of each monkey's cage. When the dose was consumed, approximately 10 ml of gelatin/apple juice solution was sprayed into each vial through the spigot, shaken and reoffered to the test monkey. Prior to the initiation of the experiment, test monkeys were trained (by water deprivation when necessary) to consume the dose medium and in most cases drank it avidly (within 15-20 min). The control animal was dosed as above but without PCB.

**PCB Analysis:** Methodology for PCB analysis in small tissue samples (blood, adipose tissue, liver, and kidney) and milk has been described previously (Mes et al. 1980). Fat and skin biopsies for PCB analysis were collected as follows: The test monkey was anesthetized with an intramuscular injection (7 to 12 mg/kg) of ketamine hydrochloride (Ketaset, BTI Products Inc., Canada) and an incision approximately two cm in length was made in the skin between the scapulae; the fat sample (up to 100 mg) was excised from the underlying fat pad and the skin sample (up to 100 mg) was excised from the edge of the incision. The incision was closed with a silk suture. In order to collect milk samples, nursing infants were separated from their mothers and placed in polycarbonate bins (Willes et al. 1977) for 4 to 5 hr before milk sample collection. Milk samples were obtained by manually expressing milk from the breast into hexane washed glass scintillation vials while the mother was restrained. About two ml could normally be collected in this fashion. Blood samples were collected by femoral venipuncture into glass hexane washed syringes using ethylenediaminetetraacetic acid as an anticoagulant.

**Hematology:** Hematologic parameters measured were red blood cell count, hematocrit, hemoglobin, mean corpuscular volume, platelet count, reticulocyte count, white blood cell count and evaluation of cell morphology. Blood samples were collected every two weeks following parturition for hematologic analysis.

**Immunology:** To test for immunocompetence, adult monkeys were immunized with two T-lymphocyte dependent antigens: sheep erythrocytes (SRBC) administered intravenously at a concentration of 10%, 0.5 ml/kg body weight, and tetanus toxoid (TT) 1.0 ml (5 LF units), given intramuscularly. The immunization and serum collection schedule is shown in Table 2. Day 1 of the immunization schedule corresponded to treatment day 148 (post partum days 59, 40, 43 and 53 for monkeys 292, 218, 836 and 285, respectively) for the adult monkeys and post partum days 75 and 69 for infants OM292 and OF285, respectively. During the immunization period, adults 292 and 285 were nursing infants whereas 218 and 836 were not. Infant monkeys received a single dose of 1 ml (10%) SRBC administered intravenously. Sera collected at the designated times were assayed for specific antibodies to SRBC using the direct tube agglutination method (Kabat and Mayez 1948). Hemagglutination titers were expressed as the reciprocal of the highest dilution of serum giving visible agglutination. Antibody titers to TT were determined by the single radial immunodiffusion test (Mancini et al. 1965). Antibody titers to TT were expressed in LF units/ml as compared to a standard antiserum.

**Results**

**Clinical:** The two 100 µg/kg/day dosed monkeys delivered dead male infants after 105 and 108 days