PCB Feeding Studies

AFFIDAVIT OF JAMES R. ALLEN, D.V.M., Ph.D.

James R. Allen, having been duly sworn, deposes and says:

My name is James R. Allen. I am a Professor in the Departments of Pathology and Food Microbiology and Toxicology at the University of Wisconsin-Madison and a Senior Scientist at the Wisconsin Regional Primate Research Center, Madison, Wisconsin.

I received my B.S. from the University of Tennessee in 1950 and an M.S. in Nutrition from the same institution in 1951. I received a D.V.M. degree from the University of Georgia in 1955 and the Ph.D. degree in Pathology from the University of Wisconsin-Madison in 1961. I have been on the faculty of the University of Wisconsin Department of Pathology since 1962 and became a full professor in 1969. I have been involved in research on environmental toxicants, namely the chlorinated hydrocarbons and pyrrolizidine alkaloids, since the late 1950's. I have published 44 papers on the toxicity of chlorinated hydrocarbons - polychlorinated biphenyls, polychlorinated terphenyls, and tetrachlorodibenzodioxins. A copy of my curriculum vitae, including a list of publications, is attached hereto as Exhibit A.

My staff and I at the University of Wisconsin-Madison first began to study PCBs in 1967. At that time we were concerned with the proliferation of a cell organelle called the endoplasmic reticulum in hepatic tissue (liver) because of its importance in generating enzymes (mixed-function oxidases) and its relation to the metabolism of various compounds. Such studies were important in establishing an understanding of how drugs and toxic agents interact in the body. As a result of these studies we found that PCBs produced a decided proliferation of the smooth endoplasmic reticulum and an increase in hepatic microsomal enzyme activity.

Shortly after commencing these studies we received reports of an outbreak in Japan of PCB intoxication that resulted from consumption of rice oil contaminated with PCBs. In this outbreak the Japanese consumed from 0.5 g to 5.0 g of the PCB mixture in their diet over approximately a six month period. As a result, the exposed persons developed, within a short period, nausea, lethargy, subcutaneous edema (fluid) of the face and pigmented skin pustules (chloracne). Infants born to mothers who ingested the PCB-contaminated rice oil were smaller than average, had discolored skin and an abnormal ocular discharge.

This outbreak caused us to expand our work to include studies on general body effects of PCBs in monkeys and rats. Initially we were concerned with establishing an animal model that would more closely simulate the lesions that occurred in the Japanese subjected to PCB intoxication. We determined that the nonhuman primate developed signs and lesions quite similar to those that occurred in man, including acne, loss of hair, loss of eyelashes, facial edema and puffy eyelids.

After these studies we embarked upon a series of experiments to establish the levels at which these compounds would be toxic to the nonhuman primate. In the initial study we fed doses of 300 ppm PCB on a continuous basis in the diet. Within a period of 3 to 4 weeks, these animals developed severe acne, swelling of the eyelids, swelling of the face and loss of hair. Additionally, there was a decided thickening of the gastric mucosa and the development of distinct ulcers in the stomach of these animals within 2 to 3 months (Allen and Norback, Science 179:498, 1973, attached to this testimony as Exhibit "2"). All of the animals died by the end of the third month.

The next study involved lowering the dosage in the diet of the nonhuman primate to a level of 100 ppm. In spite of the decreased PCB dosage, signs of PCB intoxication developed at the same rate as in the 300 ppm PCB study. The animals became obviously ill within a period of 3 weeks; death occurred within a period of 2-3 months.

The next phase of our continuing study involved feeding the nonhuman primates levels of PCBs at 25 ppm in the diet (Allen et al., Toxicol. Appl. Pharmacol. 30:440, 1974, attached to this testimony as Exhibit "3"). This level represents approximately 5 times that which is permitted in certain foods destined for human consumption. Within one month we began to observe signs of PCB intoxication and lesions. Primarily there was swelling of the eyelids, edema of the subcutaneous tissue of the face, loss of eyelashes and acne. After 2 months of exposure these animals became sufficiently ill that we deemed it necessary to remove them from the experimental diet. During this time these test animals had consumed approximately 250 to 400 mg of PCB. Two months after removal from the experimental diet, one of six animals died as a result of severe PCB intoxication. Here again, we observed the very distinct acne, edema and marked thickening and ulceration of the gastric mucosa characteristic of exposure to PCBs at much higher levels. We have permitted the remaining animals to survive for an additional 3 years. During this time 6 infants have been born, all of which weighed at least 150-200 g less than infants born to control animals. We have also determined that these infants have detectable levels of PCB in their tissues at birth, indicating a transplacental movement of PCBs from the mother through the placenta into the infant. Invariably these infants showed a decided increase in their PCB level following nursing undoubtedly as a result of the high levels of PCBs

in the mothers' milk (Barsotti. Marlar and Allen, Food Cosmet. Toxicol. 14:99, 1976, attached to this testimony as Exhibit "4").

The five surviving animals from this experiment have continued to manifest signs of PCB intoxication at least 3 years following the cessation of their two-month daily exposure to diets containing 25 ppm PCBs. Three years following exposure, the animals still do not have all their eyelashes, and continue to exhibit a considerable loss of hair and some reddening, particularly around the eyes. Fat biopsies of these animals continue to show detectable levels of PCB in their tissues. These data are consistent with the Japanese findings that humans exposed to PCBs continue to show signs of intoxication after four years (Kuratsune et al., Environ. Health Persp. 1:119, 1972, attached to this testimony as Exhibit "5").

In subsequent studies we have fed animals 2.5 and 5.0 ppm PCBs. These (3) levels are equal to (5.0) and one-half (2.5) those that are tolerated in many foods that man consumes (Barsotti, Marlar and Allen, Food Cosmet. Toxicol. 14:99, 1976, Exhibit "4"). In these studies we have found that within a period of less than two months, the test animals develop the classical signs of PCB intoxication, i.e. swelling of the eyelids, loss of eyelashes, edema of the face and the development of acne. After reaching a relatively steady state in the tissues at approximately 6 months on a diet containing 5.0 ppm PCBs, the test animals were allowed to breed with control male monkeys. We determined from this that the PCBs had a decided effect on the ability of these animals to maintain pregnancy. Of the 8 animals that had received the 5 ppm diet, only one infant was born, there were five abortions and 2 animals did not conceive even after repeated breedings.

Five infants have been born to the eight animals receiving the 2.5 ppm diet, and they, too, were extremely small. The remaining 4 animals aborted during early gestation. In addition to their difficulty in maintaining pregnancy, these animals exhibited irregular menstrual cycles as well as excessive menstrual bleeding.

Skin biopsies taken from the 6 infants that were born to the females that received 2.5 and 5.0 ppm PCB in their diet showed detectable levels of PCB. With time the levels of PCB in the infants' skin increased markedly due to the high levels in the milk obtained from their mothers. Levels in excess of 150 ng PCB per g milk were recovered from the milk that was being consumed by the infants. Within 2 months, the infants began showing signs of PCB intoxication that increased in severity with exposure time. They developed acne, loss of eyelashes and a decided swelling of the eyelids. Within 4 months following birth, 3 of the 6 infants had died as a result of PCB intoxication (Allen and Barsotti, Toxicology, in press; attached to this testimony as Exhibit "6"). Behavioral and learning experiments conducted on the 3 surviving infants for one year following weaning have revealed deficits in both areas (Bowman et al., personal communication).

The female rhesus monkeys that were given 2.5 and 5.0 ppm PCB in their diets for 16-18 months have been placed on normal diets for 1 year. They have subsequently been bred to control males. The ability of these animals to conceive and maintain pregnancy was markedly improved. However, at birth the infants were small and had PCBs in their tissues. After nursing for 4 to 6 weeks, the infants from the females that had received 5.0 ppm PCB were hyperpigmented. Analysis of the milk revealed detectable levels of PCB.

Experiments have shown that, in contrast to the nonhuman primate, the rat can tolerate doses of 100 ppm in the diet for 52 weeks without showing any obvious toxic effects (Allen et al., Arch. Environ. Contam. Toxicol. 4:in press, 1976; attached to this testimony as Exhibit "7"). In recent studies it has been shown that the rat is able to hydroxylate (metabolize) the lower chlorine isomers of PCB (2,5,2',5'-tetrachlorobiphenyl) rather readily and excrete them from the body (Van Miller et al. Proc. Soc. Exp. Biol. Med. 148:682, 1975; attached to this testimony as Exhibit "8"). However, our studies demonstrate that in the nonhuman primate there is a different metabolic pathway that predominates for metabolism of this PCB isomer. In the nonhuman primate at least a portion of the PCB goes through an intermediate metabolic pathway, i.e., the formation of very strong electrophilic agents known as arene oxides (Hsu et al., Bull. Environ. Contam. Toxicol. 14:233, 1975; attached to this testimony as Exhibit "9"). These oxides are capable of combining nucleic acids (DNA and RNA) and cell proteins. The altered cell components have been repeatedly shown to be linked with necrogenic, mutagenic and carcinogenic effects. Since the isomer 2,5,2',5'-tetrachlorobiphenyl is present in many of the commercially available PCBs containing tetrachlorobiphenyl, e.g. Aroclor 1016, 1242 and 1248, they would be metabolized in a similar manner by the nonhuman primate. Whether other PCB isomers have a similar metabolic pathway has not yet been determined. However, research in this area is currently in progress.

Our studies have demonstrated a striking similarity between the signs and lesions produced by PCBs in man and nonhuman primates. As is the case with man, we have found that the PCBs are extremely toxic to nonhuman primates over a wide range of doses. Even at levels accepted in certain foods destined for human consumption, PCBs are capable of producing obvious skin changes within two months, and reproductive abnormalities within four to six months. In addition, it has been shown that PCBs transfer through the placenta and are deposited in the fetus. Infants born to exposed mothers are smaller than average and show increasing tissue levels of PCBs following birth due to the consumption of mothers' milk that contains relatively high levels of the compounds. Sufficient PCBs were consumed via their mothers' milk to cause morbidity and mortality in infants as well as produce alterations in their learning and behavioral development. In addition, toxic metabolites produced from the PCBs are capable of combining with tissue macromolecules such as protein and nucleic acids creating the potential for necrogenic, mutagenic and carcinogenic effects. This PCB metabolite interaction with cell components is also likely responsible for many of the lesions produced by the PCBs in mammalian tissues.

James R. Allen

JAMES R. ALLEN

Subscribed and sworn to before me
this 7th day of September, 1976.

Carol J. Sommerman

Notary Public