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# REPRODUCTIVE EFFECTS OF HALOGENATED AROMATIC HYDROCARBONS ON NONHUMAN PRIMATES*

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During the past several years experiments have been conducted in this laboratory to determine the toxicologic effects in animals exposed to three halogenated hydrocarbons: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs). TCDD and PCBs are considered to be worldwide environmental contaminants. PBBs have caused major environmental problems in the state of Michigan. The health implications of these compounds are further complicated by their extremely slow biodegradation and their ability to accumulate in body tissues and remain there for an indefinite period.

As a result of their environmental and public health significance, a large amount of literature has been published on TCDD and PCB intoxication in humans and lower animals. There is also considerable research in progress to further clarify the toxicologic effects of the PBBs. However, there is a dearth of information on the effects of these halogenated hydrocarbons on reproduction in humans and closely related species, particularly when the exposure to these compounds is at low levels and occurs over an extended period.

The rhesus monkey is a suitable animal model with which to study the effects of exposure to halogenated hydrocarbons. Many of the toxicologic signs produced by these halogenated hydrocarbons are similar to those observed in humans. Thus, the data obtained from research conducted on the nonhuman primate are helpful in postulating the injurious effects that may arise in the human population following exposure to these compounds.

In the presently reported research, the three compounds were evaluated for their toxicologic effects on nonhuman primates. Attempts have been made to simulate the levels to which humans may be exposed. Data are presented which indicate that low-level, long-term exposure to these compounds gives rise to reproductive abnormalities in the adult primate as well as fetal and neonatal toxicity.

## POLYCHLORINATED BIPHENYLS

Adult female rhesus monkeys were fed a diet containing 2.5 and 5.0 ppm of a PCB mixture (Aroclor 1248). Within 4 months and after having consumed from 60 to 120 mg of the PCBs, their menstrual cycles were decidedly altered. Menses was 5 to 7 days

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longer in the experimental animals than in the controls. There was also an increase in menstrual bleeding. In addition, flattening and prolongation of the serum progesterone peak during the menstrual cycle was observed.

After being on the experimental diets for 7 months and having consumed from 105 to 210 mg of PCB,[2] attempts were made to breed the females to control males. All the animals fed 2.5 ppm PCB and the control animals conceived (TABLE 1). Six of the 8 animals on the 5.0 ppm diet conceived. Three of the 8 females on the 2.5 ppm diet and 4 of the 8 females on the 5.0 ppm diet experienced early abortions or resorptions. One of the females on the 5.0 ppm diet delivered a stillborn infant. Five females from the 2.5 ppm group and 1 female from the 5.0 ppm group were able to carry their infants to term.[2]

All of the infants born to the experimental animals were small (1 to 2 standard deviations from normal) but appeared normal in other respects. At birth there were detectable levels of PCB in the skin of these infants (1.0 to 4.8 μg/g tissue). In addition, analysis of the tissues of the stillborn infant of a female from the 5.0 ppm

TABLE 1

REPRODUCTIVE PERFORMANCE OF MONKEYS EXPOSED TO POLYCHLORINATED BIPHENYLS IN THEIR DIETS FOR 18 MONTHS

| | Breeding Performance | | | |
|---|---|---|---|---|
| | During PCB Exposure | | One Year After PCB Exposure | |
| | 2.5 ppm | 5.0 ppm | 2.5 ppm | 5.0 ppm |
| Total impregnated | 8/8 | 6/8 | 8/8 | 7/7 |
| Abortions/resorptions | 3/8 | 4/8 | 1/8 | 1/7 |
| Stillborn | 0/8 | 1/8 | 0/8 | 1/7 |
| Normal births | 5/8 | 1/8 | 7/8 | 5/7 |

group showed large amounts of PCBs in the adrenals (25 μg/g) and pancreas (64 μg/g).[4]

The surviving infants were permitted to remain with their mothers, and their sole source of nourishment was through nursing. During this period the infants showed a consistent weight gain although it was less than that of the controls (TABLE 2). Within 2 months the infants began to show signs of PCB intoxication that included the development of acne, swelling of the eyelids, loss of eyelashes and increased pigmentation of certain portions of the skin.[4]

Following the development of PCB intoxication in the infants, milk samples were obtained from the females to determine PCB content. Levels in the whole milk ranged from 0.154 to 0.397 ppm PCB.[5]

The infant from the mother on the 5.0 ppm diet and two infants from mothers fed the 2.5 ppm diet died from PCB intoxication. 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 an absence of eyelashes. There were only rudimentary thymuses, and the spleen and lymph nodes were small. There was also a decided thickening of the gastric mucosa. Microscopically, thymocytes were sparse, the lymph nodules of the spleen were small with inapparent germinal centers, and there was a decided hypocellularity of the bone marrow. The Meibomian glands of the eyelids were hypertrophic, and the hair follicles of the eyelashes were keratinized. In addition, the stomach showed

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Performance

| One Year After PCB Exposure | |
|---|---|
| 2.5 ppm | 5.0 ppm |
| 8/8 | 7/7 |
| 1/8 | 1/7 |
| 0/8 | 1/7 |
| 7/8 | 5/7 |

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TABLE 2

BODY WEIGHTS OF INFANT MONKEYS BORN TO MOTHERS RECEIVING 2.5 OR 5.0 PPM PCB (AROCLOR 1248) IN THEIR DIETS PRE- AND POSTPARTUM

| Age (weeks) | Body Weight (grams)* Control | Experimental |
|---|---|---|
| 0 | 507 ± 59 | 399 ± 22 |
| 2 | 601 ± 47 | 463 ± 40 |
| 4 | 711 ± 58 | 517 ± 12 |
| 6 | 796 ± 81 | 601 ± 38 |
| 12 | 1003 ± 71 | 765 ± 57 |

*Mean ± one standard deviation

marked hyperplasia of the mucous-secreting cells of the gastric mucosa with glandular elements penetrating the underlying muscularis mucosa.[4]

The three surviving infants were weaned at 4 months of age and placed on a milk replacer. These infants showed improvement within 4 months after being removed from their mothers. Their eyelashes became more plentiful, the swelling of the eyelids disappeared, and the acne was less apparent. However, the hyperpigmentation particularly about the hair line of the face persisted.[4]

After being weaned the three infants were evaluated for their learning and behavioral development. It was found that these infants were hyperactive and did not learn reversal tasks as readily as control infants. These changes have persisted in the animals for over 2 years.[7]

After separation of the infants, all of the adult females were removed from the experimental diets. During the subsequent year there was marked improvement in their physical status. Their menstrual cycles became normal, and the level of serum estradiol and progesterone were similar to pretreatment levels. One year after their removal from the experimental diets the females were bred again to control males. All of the animals conceived (TABLE 1). Seven of the 8 animals from the 2.5 ppm group, and 5 of the 7 animals from the 5.0 ppm group gave birth to live infants. However, the infants were much smaller than the control infants. These infants have not grown as rapidly as the controls and have experienced a greater frequency of illnesses. Focal areas of hyperpigmentation have developed in these infants. Two infants from mothers in the 5.0 ppm group have died. The thymuses were small, and the lymph nodes and spleens were rudimentary in these animals.

In subsequent experiments female rhesus monkeys were fed diets containing 0.5 and 1.0 ppm of Aroclor 1248 three times per week. These animals showed no

TABLE 3

REPRODUCTIVE PERFORMANCE OF MONKEYS FED 0.5 AND 1.0 PPM POLYCHLORINATED BIPHENYLS (AROCLOR 1248) THREE TIMES PER WEEK DURING THE PRE- AND POSTPARTUM PERIODS

| No. Animals | Level in Diet (ppm) | No. Conceived | Live Births | Birth Weights | PCB Intake at Conception (mg) | PCB Intake After 4 Months Lactation (mg) |
|---|---|---|---|---|---|---|
| 8 | 0.5 | 8/8 | 6/8 | 463 | 12 | 16 |
| 8 | 1.0 | 8/8 | 7/8 | 466 | 18 | 46 |

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irregularities in their menstrual cycles or alterations in serum estradiol or progesterone. After being on the experimental diets for 7 months and having consumed 8 to 16 mg of PCBs, the females were bred to control males. All of the animals conceived (TABLE 3), and 6 of the 8 animals on the 0.5 ppm diet and 7 of the 8 animals on the 1.0 ppm diet gave birth to live infants. These infants were somewhat smaller than their controls and have gained weight less rapidly. During the nursing period they developed focal areas of skin hyperpigmentation. However, they were devoid of other signs of PCB exposure. The initial psychologic evaluations on these infants indicate behavioral patterns similar to infants exposed to higher levels of PCB.

### 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD)

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) has been fed to adult female rhesus monkeys at a level of 500 parts per trillion (ppt).[8] After 7 months of exposure, the length of the menstrual cycle as well as the intensity and duration of menstruation were not altered appreciably. However, decreases in serum estradiol and progesterone were observed in 5 of the animals.[9] The females were bred to control males starting in the seventh month of treatment. The two animals with normal serum estradiol and progesterone at the time of breeding conceived. However, only one of these animals was able to carry her infant to term. A third animal conceived and aborted early in gestation. The remaining two animals failed to conceive (TABLE 4).[10] All of the controls conceived and gave birth to normal infants.

In a second experiment 8 female rhesus monkeys were fed diets containing 50 ppt TCDD. During the initial 6 months of the experiment the animals experienced normal menstrual cycles, and the levels of serum estradiol and progesterone were unaltered. Following 7 months of TCDD exposure when the animals had consumed approximately 1.8 μg of TCDD, attempts were made to breed the animals. Four of the animals conceived but aborted their fetuses (TABLE 3). Two of the animals have been bred on repeated occasions and have not conceived. Two of the 8 animals conceived and were able to carry their infants to term. All of the 8 control animals conceived and gave birth to normal infants.

### POLYBROMINATED BIPHENYLS

Seven rhesus monkeys were fed a diet containing 0.3 ppm of a mixture of polybrominated biphenyls (Firemaster® FF-1). After being on the experimental diet for 6

TABLE 4

REPRODUCTIVE PERFORMANCE OF RHESUS MONKEYS FED TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) AND POLYBROMINATED BIPHENYLS (PBBs) IN THE DIET FOR SEVEN MONTHS PRIOR TO CONCEPTION AND DURING PREGNANCY

| | TCDD | | PBB |
|---|---|---|---|
| | 50 ppt* | 500 ppt | 0.3 ppm |
| Total impregnated | 6/8 | 1/8 | 7/7 |
| Absorptions/resorptions | 4/8 | 2/8 | 1/7 |
| Stillborn | 0/8 | 0/8 | 1/7 |
| Normal births | 2/8 | 1/8 | 5/7 |

*ppt = parts per trillion

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TABLE 3

GROWTH RATE OF INFANT MONKEYS BORN TO FEMALE MONKEYS THAT CONSUMED 0.3 ppm POLYBROMINATED BIPHENYL (PBB) FOR SEVEN MONTHS PRIOR TO CONCEPTION AND DURING PREGNANCY

| Age | Control (grams) | PBB (grams) |
|---|---|---|
| Birth | 391 ± 52 (5)* | 455 ± 34 (5) |
| 4 weeks | 666 ± 85 (5) | 518 ± 67 (5) |
| 8 weeks | 750 ± 65 (5) | 605 ± 78 (5) |
| 12 weeks | 901 ± 45 (5) | 706 ± 106 (5) |
| 16 weeks | 987 ± 67 (5) | 807 ± 116 (5) |

*Mean ± one standard deviation
†Number in parentheses = number of infants

months. Five of the animals showed a flattening of the serum progesterone peak and a lengthening of the menstrual cycle.[9] After 7 months on the diet (total consumption per animal, 1.7 mg) the animals were bred to control males. All of the experimental animals conceived after 1 to 4 breedings (TABLE 4). Two of the animals showed a prolonged implantation bleeding. One animal aborted a mummified fetus at 146 days gestation and a second animal had a stillborn infant on the 154th day. The remaining 5 animals delivered small, normal appearing infants. These infants did not gain weight as rapidly as the control infants (TABLE 3).

EFFECTS OF THE THREE HALOGENATED HYDROCARBONS ON MALE RHESUS MONKEYS

*Polychlorinated Biphenyls*

Four adult male rhesus monkeys were given a diet containing 5.0 ppm PCB (Aroclor 1248) for 18 months. During the time the animals consumed from 530 to 692 mg of PCB. During the initial year the animals showed only minor gross signs of PCB exposure. The sperm counts and breeding performance of these four males were similar to those of the control males. However, between the 12th and 18th month one of the four animals developed clinical signs of PCB intoxication. During this period there was a marked decrease in the sperm counts and he failed to impregnate females after repeated breedings. A testicular biopsy revealed a hypocellularity of the seminiferous tubules and absence of spermatozoa. A second biopsy taken one year after the animals were removed from the experimental diet showed a complete recovery of spermatogenesis.

*TCDD*

Male rhesus monkeys fed diets containing a mixture of dioxins including TCDD for periods in excess of 1 year experienced a decrease in spermatogenesis. Tissue samples of the testes showed an absence of gametocytes or mature spermatozoa.[12]

*Polybrominated Biphenyls*

There are no data available on the effects of the PBBs on the reproductive capability of the male nonhuman primate.

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## Discussion

All three of the halogenated hydrocarbons when administered at levels ranging as low as parts per billion in the case of the PBBs and PCBs and parts per trillion of TCDD in the diet are capable of causing reproductive abnormalities. The menstrual cycles become irregular and in most instances prolonged. There is prolonged and excessive hemorrhage during menstruation. These changes are associated with a reduced conception rate as well as a high incidence of early abortions usually following extremely heavy postconceptional bleeding.

All of the above alterations are thought to be related to the changes in the sex hormones. The serum progesterone peak is flattened and lengthened in most cases. Those animals showing no alterations in serum estradiol and progesterone experienced none of the reproductive problems. However, in the animals with the most dramatic changes in sex hormones the reproductive alterations were most severe and in some instances progressed to an anovulatory state. The specific reasons for the modifications in the sex hormones are not presently known. Alterations in levels of enzymes involved in the breakdown of these hormones may, at least in part, be involved. Such enzyme alterations are characteristic in chlorinated hydrocarbon toxicity.

Female humans exposed to PCB-contaminated rice oil also showed symptoms suggesting abnormal ovarian function.[13,15] Over 60% of the patients showed abnormalities in the menstrual cycle, with the interval being prolonged, shortened or irregular. In approximately 55% of the patients, abnormal durations of menstrual flow were observed. In addition, the quantity of menstrual flow was either increased or decreased. The basal body temperature showed an abnormal pattern in 85% of the patients as characterized by a poor high phase and a prolonged low phase, suggesting the existence of corpus luteum insufficiency, retarded follicular maturation, or no ovular cycle. There are also preliminary reports that suggest menstrual irregularities in PBB-exposed persons.[16]

Reduced birth weight of infant nonhuman primates born to mothers treated with halogenated hydrocarbons is a consistent observation. In addition, toxic effects typical of these compounds have been observed in neonates. This latter finding is probably due to ingestion of milk containing the compounds since the infants recover rapidly after weaning. Similar findings have been reported in human infants born to mothers exposed to PCBs through consumption of contaminated rice oil.[17] It is of interest that fetotoxicity is observed in PCB-treated monkeys even when the mothers are removed from the experimental diets and all overt signs of reproductive dysfunction have disappeared.

Further complications of exposure to PCBs *in utero* and from mothers' milk have been suggested in the studies on nonhuman primates. In addition to the development of acne, swollen eyelids, loss of eyelashes, and reduced growth rate, these infants have inadequately developed lymphoid tissues which seemingly were responsible for their increased susceptibility to infection. More subtle effects of PCB exposure on the fetus and neonate occurred in the central nervous system. There are data which indicate that permanent learning and behavioral deficits are complications on PCB exposure during fetal and neonatal development in nonhuman primates.

There is only a limited amount of information on the effects of the halogenated hydrocarbons on reproductive capabilities of the male primate. In nonhuman primate studies one of four males that consumed approximately 600 mg of PCBs over 18 months became sexually inactive and aspermatic. The limited amount of data available on dioxin toxicity in nonhuman primates suggest that acute as well as chronic exposure results in a marked reduction in spermatogenesis. The Japanese have reported decreased libido in human males that were exposed to the PCB-contaminated

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rice oil.[15] In addition, there are reports of decreased libido in some Michigan farmers that were exposed to the PBBs.

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