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Reproductive Toxicology of 3,3',4,4' - Tetrachlorobiphenyl in Mice

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Environmental contamination by PCBs was first detected while screening environmental samples for DDT and related compounds (Jensen 1966). Since then, PCBs have been detected in almost every global environment including the Arctic and Antarctic. Accidental exposure of humans to PCBs has occurred in Japan (Higuchi 1976) and Taiwan (Chen *et al.* 1981). In both cases, the poisoning was a result of cooking rice in PCB-contaminated bran oil. It was believed that the symptoms derived from the poisoning were caused by contaminants of PCBs such as polychlorinated dibenzofurans (Masuda and Yoshimura 1984). Studies however, have since detected the presence of 3,3',4,4'-tetrachlorobiphenyl, an extremely toxic PCB congener, as a component of the PCB mixture (Kannan *et al.* 1987; Tanabe *et al.* 1987; Yoshimura and Yamamoto 1974).

The effects of PCBs on reproduction were first studied in the mink (Ringer *et al,* 1972). Since then the reproductive toxicity of many commercial and PCB congeners have been assessed. Ronnback (1991) found no observable effect of 1.5 mg/kg and 15.0 mg/kg TCB administered as a single dose to pregnant mice on day 13 of gestation. Lucier *et al.* (1978) reported that no observable effect appears in animals when the compound is administered at less than 16 mg/kg/day. Studies have evaluated the effects of TCB on reproduction, but none have studied its effects throughout the entire gestation period of mice. This study was undertaken to evaluate the effects of TCB on implantation, organogenesis, and embryogenesis in the developing mouse.

MATERIALS AND METHODS

Male DBA/2J (2-3 months old) and female C57BL/6J (7-9 weeks old) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) B6D2F1 hybrids were produced by mating DBA/2J males with C57BL/6J

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females at the Endocrine Research Center of Michigan State University. All animals were housed under a 12 hour light/dark photoperiod and maintained in an air conditioned room at 24±2°C. Feed (Mouse Chow@ #5015, Purina Mills, Inc.) and water were available *ad libitum* except where mentioned.

3,3',4,4'-tetrachlorobiphenyl, 99% pure by gas chromatography and flame ionization detector (GC/FID), was purchased in neat form (AccuStandard, Inc., New Haven, CT); lot numbers used were #70075, #10162, and #011893).

Each dose administered was based on the lowest lethal published dose (TD_{Lo}) of 7 mg/kg of body weight (b.w.) in mice. Four doses of 3,3',4,4'-tetrachlorobiphenyl in 0.1 ml sesame oil were used: control, low dose = 7 mg/kg, medium dose = 14 mg/kg, and high dose = 21 mg/kg. Each pregnant mouse received five consecutive daily oral gavages of either the control or treatment solutions beginning on day 1, 6, or 11 of pregnancy. Food was withheld for a period of 2-3 hours before each gavage.

On the day of arrival, female mice were grouped four to a box and allowed a week of stabalization. Males were housed individually on their day of arrival. Following this adaptation period female mice were put into a male's cage at a 1:1 or 2:1 ratio late in the afternoon. Females were observed for the presence of a vaginal plug the following morning. The presence of a vaginal plug denoted Day 1 of pregnancy. Mated females were caged together and randomly divided into groups on the first day of treatment. A minimum of 15 females were used per group, three replicate groups of 5 per treatment cage.

Dosed female C57BL/6J mice were weighed on Days 1, 8, and 15 of pregnancy and on Days 1, 8, 15 and 22 following parturition. The gestation length and litter size were recorded for each female. In addition, the total weight of the litter was recorded along with the number of males and females delivered. On Day 22 postpartum, each female was sacrificed by cervical dislocation. The liver and kidneys were examined for abnormalities, excised, and weights recorded. The spleen was also observed, excised, and its length measured and recorded. The liver and kidneys, one cut in a sagittal plane and the other in a frontal plane, were preserved in a 10% buffered formalin solution and catalogued for future studies.

On Day 1 of lactation, each pup was visually inspected for gross abnormalities and each litter was reduced to 6 pups, whenever possible on a 1:1 sex ratio. Pup weight and crown-rump length were recorded on

Days 1, 8, 15 and 22 of lactation. The day of lower incisor eruption, upper incisor eruption and eye opening was recorded. On Day 22, each litter was weaned, reduced to 2:2 and allowed to develop until Day 43. On Day 43, the pups were weighed and sacrificed. The ovaries and testes were excised, their weights recorded, preserved in Bouin's fixative solution, and catalogued for future studies.

Analysis of variance (ANOVA) for split-plot design was used to test for differences of treatment means with significance at the 5% level. Sex ratio data was analyzed by a binomial probability test.

RESULTS AND DISCUSSION

Data from replicate treatment sets was pooled for tests of statistical significance. A total of 300 female C57BL/6J mice were mated with a pool of fertile DBA/2J male mice. Of these, 180 were confirmed mated by the presence of a vaginal plug and were allocated randomly to one of the four groups.

The body weight of the pregnant dams was recorded on days 1, 8 and 15 of pregnancy and was recorded on days 1, 8, 15 and 22 postpartum

Table 1. Litter size ($\overline{X}$±SE) and total number of male and female mice delivered by C57BL/6J females exposed to TCB by gavage on days l-5, 6-10, or 11-15 of gestation.

	Time of treatment		
	Day l-5	Day 6-10	Day 11-15
Control	8.1±0.36	8.00±0.17	8.0±0.32
Males	65	65	60
Females	56	57	52
7 mg/kg		8.3±0.21	8.0±0.22
Males	61	63	67
Females	55	61	54
14 mg/kg	7.40±0.40	8.1±0.24	8.3±0.25
Males	56	60	60
Females	55	61	64
21 mg/kg	7.9±0.27	7.5±0.55	8.0±0.26
Males	60	51	58
Females	56	61	62

for each treatment per period. No effect of dose over treatment period for each treatment was indicated by analysis of variance for split-plot design. In addition, there was not an interactive effect between the time period of dose administration and the dose administered. There were no significant maternal weight differences between the three periods of TCB administration.

No dose, time, or dose/time interaction effect was found for maternal liver or kidney weights.

No significant difference was seen in litter size or sex ratio of litters (Table 1).

No dose, time period, or dose/time period interaction effect was noted for pup weight and crown rump length. (Table 2) No significant difference was noted for day of eye opening or lower and upper incisor eruption.

Table 2. Crown-rump length (X±SE) of B6D2F1 mice at 1 to 22 days of age after dams were exposed to TCB by oral gavage on days 1-5, 6-10, or 11-15 of gestation.

		Dam treated Days l-5		
Postpartum	Day 1	Day 8	Day 15	Day 22
Control	28.6±0.2	38.0±0.8	48.6±0.5	54.8±0.3
7 mg/kg	27.6±0.1	38.8±0.2	48.1±0.3	54.0±0.3
14 mg/kg	27.5±0.l	37.3±0.3	48.9±0.3	54.8±0.5
21 mg/kg	28.8±0.8	37.0±0.3	48.4±0.5	54.9±0.6
		Dam treated Days 6-10		
Postpartum	Day 1	Day 8	Day 15	Day 22
Control	27.1±0.3	39.0±0.4	48.5±0.3	56.9±0.6
7 mg/kg	27.2±0.3	38.6±0.2	48.4±0.3	56.2±0.1
14 mg/kg	27.6±0.3	38.8±0.1	48.0±0.3	56.5±0.4
21 mg/kg	27.7±0.3	38.9±0.5	48.6±0.9	56.9±0.3
		Dam treated Days 11-15		
Postpartum	Day 1	Day 8	Day 15	Day 22
Control	28.4±0.4	39.3±0.6	44.2±0.9	56.3±0.4
7 mg/kg	28.3±0.4	39.3±0.5	44.4±0.6	56.3±0.6
14 mg/kg	27.4±0.2	40.3±0.5	45.7±0.7	56.4±0.8
21 mg/kg	27.4±0.2	40.2±0.4	45.1±0.3	57.2±0.8

The results of this study demonstrate that female C57BL/6J mice administered a single dose of TCB for five consecutive days at 7, 14, and 21 mg/kg of body weight by gavage will not experience a significantly different gain in body weight during gestation. The results of this study did not demonstrate a dose, period, or dose/time interaction effect.

Marks *et al.* (1989) reported that CD-1 mice exposed to TCB by gavage at doses of 16, 32, and 64 mg/kg of body weight experienced decreased weight gain between days 6 to 10 of gestation, but that mice exposed to doses below 16 mg/kg between days 6 to 17 of gestation did not experience a significantly different pattern of weight gain. The data of

the present study, are consistent (Marks *et al.* 1989), not having been significantly lower than the control at 7 and 14 mg/kg, however, the present study did not demonstrate a significantly difference in weight gain at the 21 mg/kg dose level. A similar dose dependent pattern of weight gain was reported by d'Argy *et al.* (1987) who found, on average, C57BL/6 mice administered a single intraperitoneal dose of 6 mg/kg or 16 mg/kg of TCB on day 12 were found to weigh less on day 18 at the higher dose.

In rat studies, Chen *et al.,* (1992) found no difference in weight gain when they were exposed to a single intraperitoneal injection of 150 µmol/kg of body weight of TCB. Previously however, Leece *et al.* (1985) had reported an ED_{50} of 3.3 µmol/kg and an ED_{25} of 2.0 µmol/kg body weight to induce weight loss when exposed to TCB by a single intraperitoneal injection.

No significant difference in liver or kidney weight was found between the control and treatment groups in our study. Buchmann *et a/.* (1991) found a significant difference in mean liver weight of TCB-treated rats compared to controls at dose levels of 150 µmol/kg and 15 µmol/kg of body weight after a nine week period of treatment.

Earlier results from our laboratory (Kholkute *et al.* 1994) have demonstrated that TCB adversely affects in vitro fertilization in the mouse. Treatment reduced the IVF rate significantly at the 0.1, 1.0 and 10.0 µg/ml dose levels. Furthermore it increased the number of degenerative ova and increased the number of abnormal 2-cell embryos.

No other information on the in vitro fertilization toxicity of TCB is available. Many environmental toxins have been detected in human follicular fluid and because of TCBs lipophilic nature, it could have serious effects on the fertilization ability of the oocytes (Trapp *et al.* 1984).

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