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Repeated in utero and lactational 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exposure affects male gonads in offspring, leading to sex ratio changes in F_2 progeny

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Abstract

The effects of in utero and lactational 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the reproductive system of male rat offspring (F_1) and the sex ratio of the subsequent generation (F_2) were examined. Female Holtzman rats were gavaged with an initial loading dose of 400 ng/kg TCDD prior to mating, followed by weekly maintenance doses of 80 ng/kg during mating, pregnancy, and the lactation period. Maternal exposure to TCDD had no significant effects on fetus/pup (F_1) mortality, litter size, or sex ratio on gestation day (GD) 20 or postnatal day (PND) 2. The TCDD concentration in maternal livers and adipose tissue on GD20 was 1.21 and 1.81 ng/kg, respectively, and decreased at weaning to 0.72 in the liver and 0.84 in the adipose tissue. In contrast, the TCDD concentration in pup livers was 1.32 ng/kg on PND2 and increased to 1.80 ng/kg at weaning. Ventral prostate weight of male offspring was significantly decreased by TCDD exposure on PND28 and 120 compared with that of controls. Weight of the testes, cauda epididymides, and seminal vesicle, and sperm number in the cauda epididymis were not changed by TCDD exposure at PND120. TCDD- or vehicle-exposed male offspring were mated with unexposed females. The sex ratio (percentage of male pups) of F_2 offspring was significantly reduced in the TCDD-exposed group compared with controls. These results suggest that in utero and lactational TCDD exposures affect the development of male gonads in offspring (F_1), leading to changes in the sex ratio of the subsequent generation (F_2).

Keywords: TCDD; Male reproductive system; Sex ratio

Introduction

High serum concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in parents from Seveso, Italy, were linked to their having more girls than boys after the parents were exposed to TCDD that was accidentally released into the environment in 1976 (Mocarelli et al., 1996). A more intensive analysis of the Seveso data indicated that the increased probability of female births is related to an increased TCDD concentration in the plasma of the fathers, and that fathers exposed at ages younger than 19 sired significantly more girls than boys (Mocarelli et al., 2000). Earlier studies in rats reported that the reproductive organs of male offspring are susceptible to a single exposure of TCDD on gestational day (GD) 15. Male offspring exhibited a decreased anogenital distance; reduced ventral prostate weight, daily sperm production, and cauda epididymal sperm numbers; decreased responsiveness of the adult prostate to androgenic stimulation; partially feminized and demasculinized sexual behavior; and feminized patterns of luteinizing hormone regulation (Gray et al., 1997; Mably et al., 1992a, 1992b, 1992c).

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The underlying mechanisms by which TCDD exerts such effects, however, are unclear. Further, adverse effects on the subsequent generation (F_2) following TCDD exposure in F_1 are also unclear.

The kinetics of TCDD vary among animal species during different stages of development. When rats are exposed daily to TCDD at a certain dose, equilibrium in plasma and various organs is reached after several weeks. Thus, the elimination half-life of TCDD in rats is calculated to be approximately 3 weeks. To keep the TCDD body burden as constant as possible, Faqi et al. (1998) gave rats an initial loading dose followed by weekly maintenance doses of TCDD. They reported that the number of sperm per cauda epididymis and daily sperm production was decreased by TCDD exposure at postnatal days (PND) 70 and 170. The effects of TCDD exposure on the subsequent generation (F_2), however, were not investigated.

In the present study, TCDD was administered with an initial loading dose of 400 ng/kg orally prior to mating, followed by weekly maintenance doses of 80 ng/kg during mating, pregnancy, and the lactation period, and the effect of TCDD exposure on the reproductive system in male offspring (F_1) and the sex ratio of the second generation (F_2) were evaluated.

Materials and methods

Materials. >2,3,7,8-Tetrachloro [U-^{14}C] dibenzo-*p*-dioxin ([^{14}C]-TCDD, specific activity; 47.7 mCi/mmol, purity ≥ 98%) was obtained form ChemSyn Laboratories (Lenexa, KS) and dissolved in corn oil. Solvable and Hionic-Flour liquid scintillation cocktails were obtained from Packard Instruments (Meriden, CT). Other reagents were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Animals and treatments. Holtzman rats were obtained from Harlan Sprague–Dawley Inc. (Indianapolis, IN) and maintained in the National Institute for Environmental Studies (NIES) facility. Animals were handled with humane care according to the Guidelines for the Care and Use of Laboratory Animals of the University of Shizuoka. Holtzman rats at 8 weeks of age were moved to the radioisotope center at the University of Shizuoka and maintained under controlled conditions with the temperature at 24 ± 1 °C, relative humidity at 45 ± 5%, and a 12-h lighting cycle. Animals were given distilled water and laboratory chow (CE-2, CLEA Japan, Inc., Tokyo, Japan) ad libitum. Rats (n = 12) at 10 weeks of age weighing approximately 250 g were given 400 ng/kg of [^{14}C]-TCDD orally by gavage, followed by weekly maintenance doses of 80 ng/kg of [^{14}C]-TCDD during mating, pregnancy, and lactational periods. Corn oil (2 ml/kg) was administered to control rats (n = 12). Two weeks after the start of exposure, females were mated with untreated males by a pair. The day the plug was positive was estimated to be GD0. Some dams (4 rats per each group) were sacrificed on GD20 to evaluate the in utero toxicity by TCDD and to measure TCDD concentration in dams. Remaining dams (8 rats per each group) delivered pups. Litter size, weight, and anogenital distance of F_1 pups were examined on PND2. Some offspring (F_1) were sacrificed for the measurement of TCDD concentration and remaining offspring (F_1) were weaned on PND28. Some male offspring (F_1) from TCDD- or vehicle-exposed dams were mated with untreated females on PND98. Litter size, sex ratio, weight, and anogenital distance of F_2 pups were examined on PND2. After mating, male offspring (F_1) and remaining unmated male offspring (F_1) were anesthetized with diethyl ether, and the testis, epididymis, seminal vesicle, and the ventral prostate were excised and weighed. The cauda epididymis was homogenized in 0.9% NaCl containing 0.05% Triton X-100, and the sperm number in homogenate was counted under a microscope using a counter chamber.

Measurement of TCDD concentration. To examine the accumulation of TCDD, dams on GD20 or at weaning and offspring (F_1) on PND2 and PND28 were anesthetized with diethyl ether. Blood was collected from the abdominal aorta. The liver and adipose tissue were excised and weighed. Some of the tissue (approximately 0.5 g) was incubated with Solvable (2 ml) at 60 °C overnight. Solubilized tissue was decolorized with hydrogen peroxide. Radioactivity was measured with a liquid scintillation counter following the addition of Hionic-Flour (10 ml).

Statistical analysis. Data from fetuses were collected as litter means and expressed as mean ± SEM for the number of dams. Data from pups or dams were collected individually and expressed as mean ± SD for the number of animals. Data were analyzed using Student's t test or the chi-square test (sex ratio), using StatView J5.0 (SAS Institute Inc., Cary, NC).

Results

Experimental parameters

Table 1 summarizes the effects of in utero TCDD exposure on litter size, sex ratio (percentage of male fetuses), and fetal weight on GD20. TCDD exposure had no significant effect on litter size, sex ratio, or fetal weight in either sex. All the fetuses in both groups survived. Table 2 summarizes the effects of TCDD exposure on PND2. Litter size and sex ratio on PND2 were unaltered by the exposure, whereas pup weight was significantly decreased by the exposure in both sexes. TCDD exposure did not influence anogenital distance.