# EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN EXPOSURE ON THE DEVELOPMENT IN CHIKENS

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## Introduction

The sex of the female chicken is the heterogametic (sex chromosome: ZW) while the male is homogametic (sex chromosome: ZZ). Chicken embryonic gonads are bipotential at an early stage. During development of the female, the left gonad differentiates into a single ovary / oviduct, and the right gonad regresses, developing a permanent female phenotype. This sexual differentiation occurs as a result of aromatase expression in the left gonad at day 6.5 and the production of estrogen from testosterone [1]. Autosexing chicks, a cross between Rhode Island Red males and Barred Plymouth Rock females, indicate genetic sex by their specific color appearance at hatching, and this feature is useful for monitoring sex reversal. To examine the embryonic toxicity of chemicals, *in ovo* exposure has several advantages: the concentration of chemicals can be easily controlled, the applied chemicals are not eliminated until hatching, and the embryonic toxicity can be assessed independently of maternal influence. We have reported that *in ovo* exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) had no effect on sexual differentiation in autosexing chickens [2]. We further found that *in ovo* exposure to TCDD less than 7.5 ng/egg on day 0 did not influence hatchability**,** whereas that of more than 10 ng TCDD/egg completely inhibited hatching [2].

TCDD has been shown to exert reproductive and teratogenic effects in several strains of mice, rats, and chickens [3]. Maternal exposure to TCDD in rats causes a variety of toxic responses such as a reduction in ventral prostate weight, in daily sperm production and in caudal epididymis sperm numbers, and partially feminized and demasculinized sexual behavior in male offspring.

In this study, 1) the effects of *in ovo* exposure to TCDD on the sexual development and the sexual behavior in male chicks, 2) the effects of *in ovo* exposure to TCDD on sexual differentiation and gonadal aromatase activity in chickens, 3) the effects of maternal exposure to TCDD on subsequent generation were examined.

## Materials and Methods

*Materials*

TCDD in nonane (10 % toluene) was obtained from Wellington Laboratories (Ontario, Canada). Solvent was removed by evaporation and TCDD was dissolved in DMSO. [1b-$^3$H]-androsto-4-ene-3, 17-dione ([$^3$H]-androstenedione) was obtained from NEN (Boston, MA).

*In ovo exposure*

TCDD in DMSO was diluted with propylene glycol (final DMSO concentration was 0.1 %). TCDD (5 ng/egg) or the vehicle was injected into fertile eggs on day 0, and incubated at 37.6 °C with a relative humidity of 53 % in a SHYOWA FURANKI incubator (model AH3)**.** The eggs were automatically turned once per hour. Hatched chicks were maintained to examine the size of chick comb and wattle,

and male sexual behavior (crow, mounting) beginning at 13 weeks of age. Further, TCDD-treated females were fertilized with the treated males, and vehicle-treated females with the treated males. The fertilized eggs were incubated as described above. At hatching, hatchability was examined, and phenotypic sex was determined by an experienced vent sexer. The chicks were anesthetized with ether, and blood was collected from the heart. Gonads were macroscopically examined; dissected under a microscope and frozen with liquid $N_2$. They were kept at -80 °C until the aromatase activity was assayed. A part of the liver was dissected and genomic DNA was prepared using DNeasy (QIAGEN). The genotype sex was determined by PCR through the detection of the female specific gene.

*Maternal exposure*

TCDD in DMSO was diluted with corn oil (final DMSO concentration was 0.1 %), and intramuscularly administered to Barred Plymouth Rock females at 50 ng/kg (50 ng group) or 200 ng/kg (200 ng group) once a week from 14 to 23 weeks of age. The vehicle was administered to control group. In females, egg production (%) was examined. Barred Plymouth Rock females at 26 weeks of age were crossed with Rhode Island Red males to obtain fertile eggs of autosexing chicks that indicate genetic sex by their specific color appearance at hatching. Some of the eggs were incubated as described above. Weight and shell thickness of the eggs laid at 31-34 weeks of age were measured.

At hatching, phenotypic sex was determined as described above, and genotype sex by the color of the head feathers, legs and mandible. The chicks were anesthetized with ether, and blood was collected from the heart. Gonads were macroscopically examined, and dissected gonads were treated as described as shown above until the aromatase activity was assayed.

*Assay for aromatase activity*

Gonads were homogenized in 10 mM potassium phosphate buffer (pH 7.4) containing 100 mM KCl, 1 mM EDTA, 10 mM dithiothreitol and a protease inhibitor cocktail by a glass homogenizer, and centrifuged for 10 min at 1800 g. Aromatase activity in the supernatant was assayed in terms of released $^3H_2O$ from $^3$H-androstenedione as described by Lephart and Simpson[4] and Roselli and Resko[5].

**Results and discussion**

*In ovo exposure*

In males, the comb and wattle tended to grow faster in the TCDD-treated group than in the control group. The cocks in the TCDD-treated group crew and copulated earlier than those in the control group. These results suggest that *in ovo* exposure to TCDD induced precocity in males. Sex ratio (% of males) of chicks hatched from the eggs of TCDD-treated females which were fertilized by the treated males, was greater than that from vehicle-treated chickens. Aromatase activity at hatching in the left ovary from TCDD-exposed chicks was significantly higher than that from control chicks. Two chicks of genetic females, which hatched from the treated eggs, were found to be phenotypically males. These results suggest that *in ovo* exposure to TCDD at this concentration influenced sexual differentiation, and the sexual development in the subsequent generation.

*Maternal exposure*

Egg laying started at 17 weeks of age. The 50 % egg production in 200 ng group was at 20 weeks of age, which was two weeks earlier than that of the control group (22 weeks of age). Egg laying in the 200 ng group, however, stopped between 23 and 24 weeks of age, and started again two weeks after TCDD administration was terminated at 23 weeks of age. In the 50 ng group, hens did not begin laying eggs even at 23 weeks of age, but they began laying two weeks after TCDD stopped at 23 weeks of age, suggesting that TCDD reversibly inhibits egg laying. The hatchability of the eggs from chicks in the

200 ng group was lower than that of the control group, which indicates that TCDD given maternally was transferred to eggs. The genetic sex completely coincided with the phenotypic sex in both control and TCDD treated groups. However, sex ratio (% of males) in the 200 ng group was slightly higher than that of the control group. Aromatase activity in the left ovary was not significantly different between the two groups. Eggshell thickness laid during 32~34 weeks of age in the 200 ng group was significantly thicker than that of the control group while egg weight was significantly lower than that of the control group. However, eggshell strength was not altered by TCDD exposure.

In conclusion, *in ovo* exposure to TCDD not only disturbs sexual development and sexual differentiation, but also sexual development in the subsequent generation. The maternal exposure to TCDD induced inhibition in laying eggs, and influenced the hatchability, eggshell thickness, and sexual ratio in the subsequent generation through transferring TCDD to the egg.

**References**

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