

## Effects of *in ovo* exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the sexual development and sexual differentiation in chickens

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### Introduction

The female chicken is heterogametic sex while the male is homogametic. Sexual differentiation to female occurs as a result of aromatase expression in the left gonad at day 6.5 with the consequent production of estradiol from testosterone. Autosexing chicks, a cross between Rhode Island Red males and Barred Plymouth Rock females, indicate genetic sex by their specific color appearance at hatching. These features are useful for monitoring sex reversal. In this study, the effects of *in ovo* exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on sexual differentiation and gonadal aromatase activity were investigated.

### Methods

TCDD obtained from Cambridge Isotope Laboratories was dissolved in DMSO and diluted with propylene glycol (final DMSO concentration was 0.1%). *Experiment 1*: TCDD (2.5~25 ng/50  $\mu$ l/egg) were injected into the fertile eggs from Barred Plymouth Rock females crossed with Rhode Island Red males on day 0. The eggs were incubated at 37.6°C at a relative humidity of 53% in a SHYOWA FURANKI incubator (model AH3) while automatically turned once per hour. At hatching, phenotypic sex was determined by an experienced vent sexer, and genotype sex by the color of the head feathers, legs and mandible. Chicks were anesthetized with ether and blood was collected from the heart. Gonads were macroscopically examined and dissected under a microscope, then frozen with liquid N<sub>2</sub> and kept at -80°C until the assay for aromatase activity. *Experiment 2*: TCDD (5 ng/egg) or the vehicle was injected into the fertile eggs on day 0 and incubated as described above. Hatched chicks were raised in order to examine the size of comb and wattle, and masculine sexual behavior at mature. Further, the hatchability of the eggs fertilized by these TCDD-treated males, and sex ratio and gonadal aromatase activity in chicks of consequent generation were also examined.

### Results and Discussion

*Experiment 1*: The *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. The genetic sex ratio coincided with the phenotypic sex ratio in both control and TCDD treated groups. The gonadal aromatase activity did not change by TCDD exposure. These results suggest that *in ovo* TCDD exposure did not affect sexual differentiation in chicks at these concentrations. *Experiment 2*: *In ovo* TCDD exposure did not significantly change body weight at hatching in both males and females. In males, the comb and wattle tended to grow faster in TCDD-treated group than in control. The cocks in TCDD treated group crew and copulated much earlier than those in control. These results suggest that *in ovo* exposure to TCDD induced precocity in males. Sex ratio (male %) of chicks of consequent generation in TCDD treated group was greater than that those in control with a significant increase in the gonadal aromatase activity at hatching. These results suggest that *in ovo* exposure to TCDD at this concentration influenced sexual differentiation and the sexual development in the consequent generation.

In conclusion, *in ovo* exposure to TCDD affect not only the sexual development in chicks, but also sexual differentiation as well as sexual development in the consequent generation.