CHLOROTHALONIL AS AN ENDOCRINE DISRUPTOR IN ZEBRAFISH: EFFECTS ON STEROID HORMONES THROUGH AN EX VIVO APPROACH

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INTRODUCTION: Chlorothalonil is the fourth most used active ingredient in Brazil, employed in agriculture to control foliar fungal diseases. However, its effects on reproduction remain poorly understood. Previous studies have demonstrated that chlorothalonil alters sperm quality and affects the steroidogenic pathway in fish. Although there is evidence suggesting its role as an endocrine disruptor, no study has directly examined its effect on the production of gonad steroid hormones. Ex vivo studies represent an important tool for analyzing mechanisms and tissue direct effects. **OBJECTIVE:** The aim of this study was to evaluate the direct effects of chlorothalonil on the ovaries and testes and its implications for steroid hormone levels. **MATERIALS AND METHODS:** Female and male zebrafish were weighed and dissected to obtain gonads. Gonadal explants were exposed to three concentrations of chlorothalonil (1, 10, and 100 µg/L) dissolved in DMSO and added to L-15 culture medium, along with a control group containing L-15 medium with DMSO only. The gonads were cultured by placing the pair of ovaries or the pair of testes in a well of 48-well and 96-well plates, with 600 µL (ovaries) and 200 µL (testes) of culture medium for 18 hours at 26°C. Subsequently, to evaluate the synthesis of sex steroids, the culture medium was collected, frozen at -80°C, and analyzed. Estradiol (E_2) and testosterone (T) levels were measured in ovaries, whereas 11-ketotestosterone (11-KT) and T were measured in testes. **RESULTS AND CONCLUSIONS:** The results show that in females, chlorothalonil increased E_2 concentration at 10µg/L compared to the control group. Regarding T, an increase was observed at the two highest concentrations tested (10 and 100 µg/L). On the other hand, for males, exposure to chlorothalonil did not alter the concentration of sexual hormones. In conclusion, chlorothalonil acts as an endocrine disruptor and exerts direct effects on the ovaries. However, in males, it is concluded that this contaminant had no direct effect on the testes, leading us to hypothesize that the previously observed impacts regarding sperm quality and gene expression appear to be a consequence of a generalized effect mediated by a broader systemic effect involving the hypothalamic-pituitary-gonadal axis.

KEYWORDS: Ecotoxicology; Reproduction; Pesticide; Hypothalamic-pituitary-gonad axis.