

Embryos of the annual killifish *Nothobranchius furzeri* acutely exposed to 3,4-dichloroaniline show long-term elevation of embryonic malformations.

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INTRODUCTION: The 3,4-Dichloroaniline (3,4-DCA) is a contaminant from diuron degradation, an herbicide widely used in Brazil. In the aquatic environment, 3,4-DCA can be absorbed in sediments, posing a risk to non-target organisms. This is the case of annual fishes, endangered species with drought-resisting embryos that can survive in temporary ponds that dry out periodically. Due to its rapid growth, the annual fish *Nothobranchius furzeri* is emerging as a laboratory model, especially for chronic exposure and aging research. However, until this date, information regarding sublethal impacts of 3,4-DCA on *N. furzeri* embryonic development is lacking. **OBJECTIVE:** To evaluate the effects of 3,4-DCA on developmental morphological endpoints of *N. furzeri* during an exposure period in water and a post-exposure period in a dry substrate, mimicking their natural life-history. **MATERIALS AND METHODS:** The embryos were exposed to 1, 2, 4, and 8 mg/L of 3,4-DCA in a 24-well plate with complete medium renewal every 96h during 8 days, together with a control group. Following the exposure, embryos were transferred to a dry substrate for 14 days to complete their development. In both periods, images of the embryos (n=6) were taken in 3 and 7-day intervals for posterior analysis. **RESULTS AND CONCLUSION:** Our results showed that 3,4-DCA caused alterations in the normal development of embryos when compared to control animals ($p < 0.0001$). During the third and seventh exposure days, the percentage of malformed embryos increased from 8% (1 and 4mg/L) to >30% (1, 2, and 4mg/L). The malformations detected on the third day included early pigmentation of the body and eye, whereas on the seventh day, the malformations included abnormal eye development, low body pigmentation, pericardial edema, scoliosis, and abnormal pigmentation in the region of the lipid droplet and gut. Following the transition to the dry substrate, malformation percentage progressed to >60% (1, 2, and 4mg/L) on the third day and reached 100% (1, 4, and 8mg/L) on the seventh day. These results are the first to indicate the capacity of 3,4-DCA to cause long-term impacts on the embryonic development of *N. furzeri*, which may dramatically affect the life cycle of annual fishes.

KEYWORDS: sublethal effects; endangered species; pesticides.

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