

(116)

**Determination of Threshold Toxicity of Chloride in Drinking Water Using
The Zebrafish as a Vertebrate Animal Model**

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Abstract

Chloride contamination in drinking water resulting from natural sources, industrial effluents, and disinfection processes, poses significant risks to aquatic life, particularly during early developmental stages making the evaluation of its ecological impact essential. Although chloride is essential in small amounts, elevated concentrations can adversely affect survival, growth, and physiological processes in aquatic organisms. This study aimed to determine the threshold toxicity of chloride in water and assess acute lethal effects on zebrafish (*Danio rerio*) embryos. Fertilized embryos at 2 hours post-fertilization (hpf) were exposed to five chloride concentrations (2,4,6,8 and, 10 mgL⁻¹), with tap water and distilled water serving as controls. Twenty embryos per each and duplicated. Toxicological endpoints including mortality, hatching rate, heart rate, and morphological abnormalities, were assessed until 96 hpf. The results demonstrate a clear dose-dependent increase in mortality, with the highest rate of 68.75±0.58% was observed at 10 mgL⁻¹ and the lowest rate of 25±0.58% was observed at 2 mgL⁻¹. The hatching rate declined gradually at higher concentrations, showing 50±0.00% at 2 mgL⁻¹, and further decreased to 12.5±1.2% at 10 mgL⁻¹, and heart rate was increased progressively, reaching 192±2 bpm at 10 mgL⁻¹ compared to 140±4 bpm in controls. Embryos in tap water exhibited 12.5±0.58 % mortality, 75±1.74% hatching, and a heart rate of 150±2 bpm at 96 hpf. Morphological abnormalities, including spinal curvature at 2 mgL⁻¹ and yolk sac oedema at 8 mgL⁻¹, were observed with an occurrence rate of 20% of the larvae. The calculated LC₅₀ value of chloride is 5.6 mgL⁻¹. These findings emphasize the need to reconsider water quality guidelines to protect vulnerable early developmental stages of aquatic life. Overall, this research demonstrates that even relatively low chloride concentrations can induce significant developmental and morphological effects in zebrafish embryos, supporting their application in environmental risk assessment and guiding safer drinking water management.

Keywords: *Chloride, Hatching rate, Morphological abnormalities, Mortality rate, Zebrafish*