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Using zebrafish to assess the impact of 3-CMC on embryonic and neural development

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Abstract

Background: Synthetic cathinones are a class of new psychoactive substances (NPS) increasingly detected in the environment due to their widespread human consumption. 3-Chloromethcathinone (3-CMC) is a halogenated and N-alkylated derivative of cathinone with a chiral centre [1][1]. It shares structural similarities with methcathinone and 4-chloromethcathinone (4-CMC, clephedrone). Like other cathinones, 3-CMC interacts with monoamine transporters, exerting psychostimulant effects by promoting the release of dopamine, norepinephrine, and serotonin [2][2]. The growing presence of NPS in wastewater and surface waters highlights the urgent need to investigate their potential toxic effects on aquatic organisms [3][3]. **Objective:** This study aimed to evaluate the effects of 3-CMC on embryonic development, neurotransmitter levels (dopamine, serotonin, and their metabolites) and apoptosis in zebrafish (*Danio rerio*). **Methods:** Embryos, approximately 3 h post-fertilisation, were exposed for 96 h to five concentrations of 3-CMC (0.02 to 200 µg/L) in triplicate. Mortality, spontaneous movements and heart rate were assessed during the exposure period. At 96 hpf, samples for apoptosis levels measurement were homogenised after exposure to acridine orange (10 µg/mL) for 15 min before measuring fluorescence (excitation/emission: 535/590 nm). Serotonin, 3,4-dihydroxyphenylacetic acid (DOPAC, a dopamine metabolite), and 5-hydroxyindolacetic acid (5-HIAA, a serotonin metabolite) were quantified by liquid chromatography coupled to a UV detector at 210 nm, while dopamine was assessed at 280 nm. **Results:** The results showed no significant effects on mortality, spontaneous movements or heart rate of zebrafish embryos. Likewise, no significant alterations were detected in neurotransmitter levels or apoptosis in exposed larvae compared to the control. **Conclusions:** These findings suggest that, under the tested concentrations, 3-CMC does not induce detectable developmental, neurochemical or apoptotic responses in zebrafish early life stages. Nevertheless, these results are preliminary. Given the increasing occurrence of NPS in aquatic environments, further research is needed to understand their impact (including 3-CMC) on other endpoints and the long-term effects of 3-CMC to improve environmental risk assessment and support the development of appropriate mitigation strategies.

Keywords: embryonic development; *Danio rerio*; synthetic cathinones

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