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Enantioseparation and ecotoxicological studies of a cathinone in *D. magna*

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Abstract

Background: The synthetic cathinone 3-chloromethcathinone (3-CMC) is among the most prevalent new psychoactive substances (NPS), accounting for 46% of NPS seized in Europe in 2023 [1]. Moreover, like most synthetic cathinones that are chiral, 3-CMC enantiomers may exhibit distinct toxicological effects [2,3]. Therefore, enantioselective studies are essential for the comprehensive characterization of their associated environmental risks. **Objective:** This study aimed to optimize a chromatographic method for the semi-preparative enantioresolution of 3-CMC and assess its ecotoxicity in *D. magna*. **Methods:** The enantioresolution of 3-CMC was performed by liquid chromatography (LC) coupled with a UV/Vis detector, using a semi-preparative CHIRALPAK® AD-H column. For the ecotoxicity assessment, neonates aged ≤ 24 h were exposed to 3-CMC at concentrations ranging from 3.13 to 50 mg L⁻¹ in a 48-h acute test, and to 260, 325, and 520 μ g L⁻¹ in a 9-day subchronic test. Various key endpoints were evaluated, including behavioural, morphophysiological, reproductive, and biochemical parameters. **Results:** The optimized method enabled the isolation of the enantiomers of 3-CMC with high purity (> 97.78%). Acute exposure assays to 3-CMC yielded an EC₅₀ (48 h) of 26.14 mg L⁻¹. The racemic mixture of 3-CMC induced significant alterations in behavioural and morphophysiological parameters, while no effects were observed on reproduction. Biochemical analyses revealed a significant increase in thiobarbituric acid reactive substances (TBARS) levels across all exposed groups. However, due to the low stereochemical stability of the isolated enantiomers, enantioselective toxicity assessment was not feasible. **Conclusions:** These findings demonstrate that 3-CMC can induce toxicity in aquatic organisms even under short-term exposure, emphasizing the need for routine monitoring of this contaminant in surface waters. Moreover, the racemization of the isolated 3-CMC enantiomers in the culture medium prevented the assessment of enantioselective toxicity, highlighting the importance of evaluating stereochemical stability to ensure accurate toxicity risk assessment.

Keywords: new psychoactive substances; 3-chloromethcathinone; ecotoxicity; enantioseparation

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