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Uncovering 4-Cl- α -PPP toxicity and mitigation strategies using *Caenorhabditis elegans* as a translational model

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Abstract

Background: Synthetic cathinones, commonly referred to as “bath salts”, are a class of New Psychoactive Substances designed to mimic the effects of traditional drugs of abuse. These compounds exert amphetamine-like effects by interacting with dopamine, serotonin, and noradrenaline transporters, leading to a range of adverse outcomes, including neurotoxicity [1, 2]. However, for several emerging synthetic cathinones, such as 4'-chloro-alpha-pyrrolidinopropiophenone (4-Cl- α -PPP), their toxicological profile remains poorly characterized, highlighting the need for further investigation. Additionally, exploring mitigation strategies to counteract their harmful effects may represent a promising approach. **Objective:** Using *C. elegans* as a discovery platform, this study aims to characterize the effects of 4-Cl- α -PPP on animal development, lifespan, reproductive behavior, and potential heritable toxicological signatures, as well as to identify novel strategies to mitigate its toxicity. **Methods:** Synchronized L1-stage animals of the DC19 [*bus-5(br19)*] strain (~200 per condition) were exposed in liquid medium to increasing concentrations of 4-Cl- α -PPP. After 72 h of incubation in M9 buffer supplemented with OP50 bacteria as a food source [3], survival was assessed by counting live and dead worms [2]. Further experiments were conducted to evaluate the protective effect of *N*-acetyl-cysteine (NAC; 1 mM) against 4-Cl- α -PPP-induced reductions in survival. **Results:** Exposure to increasing concentrations of 4-Cl- α -PPP resulted in a concentration-dependent decrease in animal survival. While no significant effects were observed at lower concentrations (≤ 0.05 mM), a marked reduction in survival was detected at 0.75 mM [survival percentage (mean \pm standard deviation): control (0 mM) = 98.47 ± 1.66 ; 0.75 mM = 18.78 ± 17.97 , $p < 0.01$], becoming more pronounced at higher concentrations (≥ 1.0 mM), with complete lethality observed at 2.5 mM [survival percentage (mean \pm standard deviation): 1.0 mM = 0.29 ± 0.87 , $p < 0.0001$; 2.5 mM = 0.00 ± 0.00 , $p < 0.0001$]. Notably, co-incubation with NAC (1 mM) attenuated the decrease in survival induced by 4-Cl- α -PPP. Additional studies addressing developmental, lifespan, reproductive, and heritable effects are currently ongoing. **Conclusions:** These findings show that 4-Cl- α -PPP causes a marked, concentration-dependent decrease in animal survival, partially prevented by NAC, indicating that oxidative stress plays a major role in 4-Cl- α -PPP toxicity.

Keywords: 4'-chloro-alpha-pyrrolidinopropiophenone; synthetic cathinones; *C. elegans*

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