Synthetic Psychoactive Cathinones (SPCs): Predicting Toxicity using *In Vitro* and *In Vivo* Models

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Background: In 2021 a staggering 33,000 American lives were lost to psychostimulant overdoses, accounting for over 30% of all drug overdoses that year. Synthetic psychoactive cathinones (SPCs) are novel psychoactive substances with effects like cocaine, methamphetamine, and methylenedioxymethamphetamine (MDMA). SPCs are of great concern because their abuse liability and potential for adverse effects, including lethal overdose, are largely unknown. Cell culture can help streamline toxicity assessment of new drugs of abuse.

Methods: For zebrafish studies, 5-day post fertilization (dpf) wildtype larval fish were exposed to various concentrations of SPCs to determine lethal dose 50 (LD50). For In vitro studies cell viability was assessed using 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT). HepG2 (hepatic), AC-16 (cardiac), and SH-SY5Y (neural) cells were exposed to each SPC to determine the half-maximal inhibitory concentration (IC50).

Results: The LC50 values in zebrafish exhibited a correlation with the IC50 values of both the HepG2 (R^2= 0.8653, F(1,6)= 38.53, p= 0.008) and SH-SY5Y (R^2= 0.5762, F(1,6)= 8.158, p= 0.0289) cells. However, there was no significant correlation between zebrafish lethality and AC-16 toxicity (R^2= 0.3182, F(1,6)= 2.801, p= 0.1452).

Conclusion: The toxicity evaluation of SPCs in HepG2 and SH-SH5Y cell lines predict lethal toxicity in zebrafish. The similarity in toxicity patterns between these cell lines and zebrafish strengthens the potential utility of predictive *in vitro* models of SPC-induced lethal toxicity. The correlation between the toxicity in both cell lines and zebrafish indicates that the adverse and lethal effects of SPCs involves cellular mechanisms rather than physiological factors. Therefore, cell culture can provide insights into the cause of *in vivo* lethality and reduce the number of vertebrate subjects necessary for the study of the toxicity of these drugs, in line with the 3R principle of animal research.

