ACUTE TOXICITY ASSESSMENT AND METABOLISM OF SYNTHETIC PYROVALERONE CATHINONES IN *DANIO RERIO* EMBRYO

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INTRODUCTION

Pyrovalerone cathinones are a large subgroup of designer drugs of the synthetic cathinones family, included in the new psychoactive substances (NPS) group. The most representative family member is 3,4-methylenedioxypyrovalerone (MDPV), which become the most frequently abused cathinone in Europe and the US. Due to the regulations imposed to MDPV, a new generation of pyrovalerone derivatives has been synthesized and commercialized, including α-PHP, α-PHPiP, 4-MePPP and TH-PVP. The use of zebrafish as an animal model for metabolism and toxicity studies has been proposed based on the compatibility of neurological pathways controlling behavior between fish and mammals. Fish embryos until the onset of independent feeding are considered unprotected life stages. In addition, zebrafish larvae at developmental stages as early as 72 h post fecundation (hpf) have already acquired mammalian-like metabolic detoxifying pathways. In summary, the main aim of this work was to determine the LC_{50} of the selected pyrovalerone cathinones in the zebrafish embryos and study their metabolism and toxicity using early zebrafish larvae.

EXPERIMENTAL

Acute toxicity test

Zebrafish eggs at developmental stage of 18 to 20 hpf

24-well plate with 1 embryo per well with 2 mL of cathinone solution

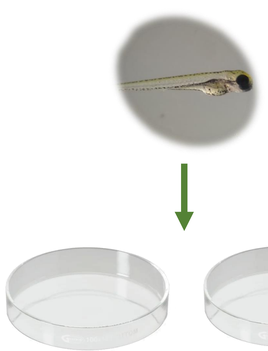
Kept in the benchtop incubator inside the acclimatized chamber

Embryos were observed each 24 h and dead embryos were noted. Selected endpoints were also observed

Data were processed using the Statistical Analysis System (SPSS 26.0)

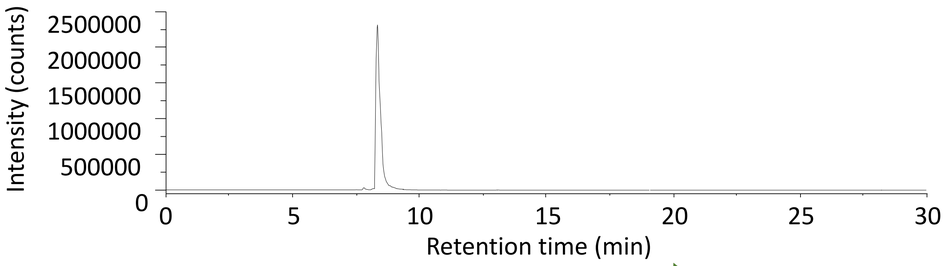
Metabolism study

Zebrafish larvae (72h old)



Larvae (n=10), were placed in two Petri dishes (n=5) and exposed to a sublethal concentration ($LC_{0.1}$-96h) of cathinone solution based on the previous acute toxicity test

Kept in the benchtop incubator inside the acclimatized chamber for 24 h



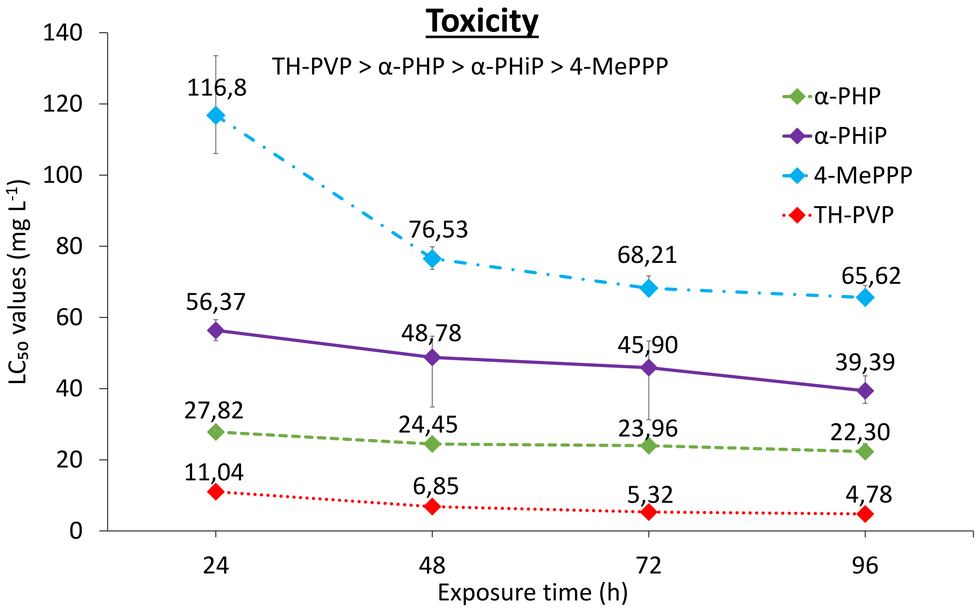
An aliquot of the water at the end of the experiment was filtered and transferred to an autosampler vial

Larvae were euthanized, extracted using 100 µL MeOH, shaken for 30 min and centrifuged for 2 min at 15000 rpm
The supernatants were transferred to autosampler vials

Metabolites were identified by LC-HRMS/MS

RESULTS AND DISCUSSION

Toxicity

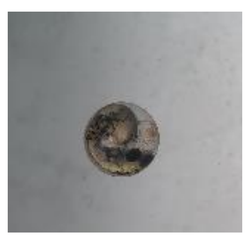


From the results obtained, it can be seen that the most toxic synthetic cathinone is TH-PVP and the less toxic is 4-MePPP. The toxicity of all the tested drugs increased over the experimental time, although 4-MePPP greatly increases its toxicity from 24 to 48 h. After 96 h exposure, TH-PVP induced the highest increase in toxicity compared to 24 h exposure.

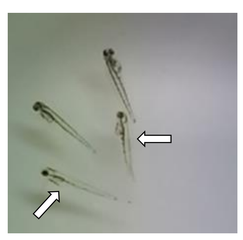
Observed symptomatology

Pericardial edema

Curved spinal cord

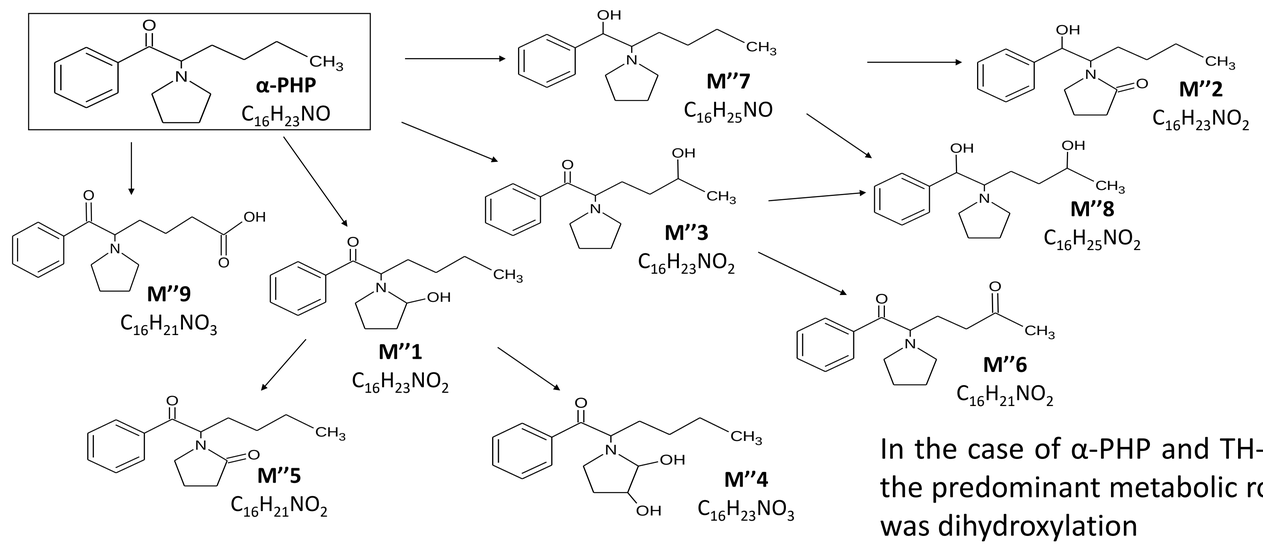


Non-hatched embryos



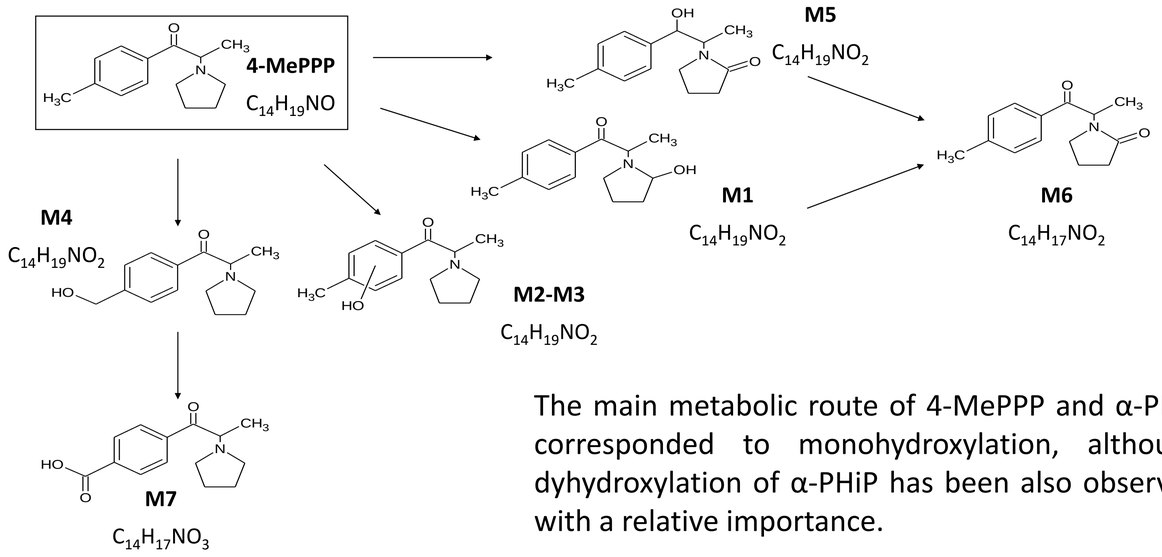
Lateral resting with neither movement nor escape response

α-PHP metabolism



In the case of α-PHP and TH-PVP the predominant metabolic route was dihydroxylation

4-MePPP metabolism



The main metabolic route of 4-MePPP and α-PHiP corresponded to monohydroxylation, although dyhydroxylation of α-PHiP has been also observed with a relative importance.

CONCLUSIONS

LC_{50} results from the four selected pyrovalerone derivative cathinones has been obtained in zebrafish embryos to compare its acute toxicities. As it is derived from the degree of slope of these curves, we can conclude that TH-PVP could be a dangerous substance, due to the narrow range between concentrations and toxic effect, while 4-MePPP showed the lowest observed toxicity. On the other hand, the metabolism of these second generation of pyrovalerone cathinones has been studied using early zebrafish larvae as in vivo model. It was observed that the higher the aliphatic side chain, the higher the concentration ratio of dihydroxylated metabolite to the parent drug, being a predominant metabolism route for TH-PVP and α-PHP. In the case of 4-MePPP and α-PHiP, the major metabolic route corresponded to monohydroxylation, although for α-PHiP dihydroxylation has been also observed with a relative importance. Moreover, a low incidence of phase II transformations in the elimination of pyrovalerone derivative cathinones in zebrafish embryos was observed.

The obtained results have demonstrated the utility of early zebrafish larvae as in vivo model to early predict the metabolism of abuse substances in order to select the analytical target to evaluate human abuse.

Acknowledgements

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