

Suspect and non-target screening by high resolution mass spectrometry for the detection of new psychoactive substances in wastewater and pooled urine

Alexander L.N. van Nuijs¹, Juliet Kinyua¹, Noelia Negreira¹, Lies Gremeaux², Peter Blanckaert², Adrian Covaci¹

¹ Toxicological Centre, University of Antwerp, Antwerp, Belgium. Adrian.Covaci@uantwerpen.be

² Scientific Institute of Public Health, Brussels, Belgium

New Psychoactive Substances (NPS) are compounds that mimic effects of illicit drugs, such as cocaine, cannabis and amphetamine-like stimulants and are synthesized to evade law enforcement by introducing slight modifications to chemical structures of controlled substances. Currently, there are more than 400 NPS detected worldwide. Their use is sporadic depending on popularity amongst users or on specific settings (e.g., festivals, nightlife), but can cause serious negative health effects due to unknown side-effects and chance of overdosing. The detection of NPS is a challenge due to their quick transience on the drug scene, creating a continually moving analytical target. Our recent efforts have focused on the development of innovative strategies based on advanced mass spectrometric techniques that can deliver important information on the actual use of NPS through collecting wastewater and pooled urine samples and screening them for NPS biomarkers (parent compounds and/or metabolites). Seven wastewater and seven pooled urine samples were collected during a three-day festival in Belgium. The main objectives of the study were: (a) the identification of NPS (parent compounds or metabolites) by applying suspect and non-target screening strategies based on liquid chromatography quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS), comparison with a home-made database containing approximately 1000 NPS and their metabolites, and an in-house designed data analysis workflow scheme and (b) the structure elucidation of candidate structures, based on accurate mass and isotopic pattern measurements by QTOF-MS and interpretation of MS/MS spectra, using *in silico* fragmentation tools and the literature.

Overall, from the analysis, 50 and 28 compounds of interest were detected in the wastewater and pooled urine samples, respectively. The results indicated that NPS were used by festival attendees with the phenylethylamines and synthetic cathinones as the most popular classes. 4-fluoro-amphetamine (4-FA) and α -pyrrolidinopentiophenone (α -PVP) were detected in the majority of the samples suggesting a substantial use of these substances. Furthermore, several metabolites from synthetic cathinones (α -PVP, MDPV, mephedrone) were detected for the first time in wastewater and pooled urine. The presented work demonstrates that advanced mass spectrometric techniques can be used to detect the actual use of NPS in populations through the analysis of pooled urine and wastewater.