

## Chemical and *in vitro* pharmacological characterization of *N*-desethyl etonitazene in a powder sample handed in at a Swiss drug checking service

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Recently 2-benzylbenzimidazole “nitazene” opioids have emerged on the illicit drug market as new psychoactive substances (NPS). Nitazenes are considered a serious public health threat due to their generally high potency at the  $\mu$ -opioid receptor (MOR). Drug checking services are harm reduction instances where people who use drugs (PWUD) can have drugs from unregulated markets analyzed. The PWUD remain anonymous but are obliged to professional counseling. Such services are well established in Switzerland and are increasingly recognized to generate valuable insights into the unregulated drug market, such as early detection of novel compounds and trends.

Herein, we report the detection of *N*-desethyl etonitazene in a powder sample handed in at a Swiss drug checking service (Lucerne, Switzerland) in August 2023. The sample was bought by the drug checking visitor through a darknet vendor, where the sample was advertised as isotonitazene. *N*-Desethyl etonitazene, a nitazene analogue that had not been formally notified at that time (Q3 2023), was identified using nuclear magnetic resonance (NMR) spectroscopy and high-performance liquid chromatography coupled to high-resolution mass spectrometry (HPLC-HRMS). To gain further information on the MOR activation potential of *N*-desethyl etonitazene, the powder and a reference standard were investigated using two different *in vitro* bioassays (*i.e.* AequoScreen™ and  $\beta$ -arrestin 2 recruitment assays).

NMR and HPLC-HRMS confirmed the presence of *N*-desethyl etonitazene, which in the meanwhile had been notified by the EMCDDA in December 2023. *N*-Desethyl nitazenes have been detected before as metabolites of for instance isotonitazene. However, as first seen with *N*-desethyl isotonitazene, they are now also emerging as standalone drugs. *In vitro* activity profiling revealed a slightly increased efficacy and approximately 6 to 9-fold higher potency of *N*-desethyl etonitazene at MOR compared to fentanyl. Further, the powder sample was evaluated using the bioassays and showed good correlation to the reference standard. For forensic toxicological investigations, the emergence of other *N*-desethyl nitazenes must be considered to avoid misclassification of such analogues as metabolites. Finally, as demonstrated with the presented case, drug checking services are highly valuable for early warning purposes.