

*****Do not exceed 600 words including tables and charts.*****

TITLE: *Emergence of the Novel Opioid N-Desethyl Isotonitazene in the Recreational Drug Supply*

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ABSTRACT:

Introduction: The dynamic conditions surrounding novel psychoactive substances (NPS) continue to challenge the analytical and interpretive efforts of forensic toxicologists. Since the first identifications of NPS decades ago, the landscape of new drugs in the United States has continued to change rapidly, often initiated by national or international drug scheduling. These control actions often result in simple to complex chemical modifications spawning new drugs. The same is true for the recent evolution of the novel synthetic opioid subclass of nitazene analogues from the emergence of isotonitazene in early 2019 to the most recent emergence of *N*-desethyl isotonitazene in late 2022. While a metabolite of isotonitazene, *N*-desethyl isotonitazene itself has recently been encountered as a parent drug. *In vitro* pharmacological data show that *N*-desethyl isotonitazene is approximately 20 times more potent than fentanyl. The continued proliferation of high potency opioids has become of significant public health and safety concern, requiring maintained surveillance and response efforts to combat their impacts.

Objective: This presentation will focus on known information about *N*-desethyl isotonitazene, including cases where the drug was found in drug materials from market surveillance, urine samples from drug treatment programs, postmortem blood and urine samples from medicolegal death investigations, and other circumstances.

Methods: Initial forensic analysis was performed via liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) using a SCIEX TripleTOF 5600+ and a SCIEX X500R. Biological samples were prepared using a basic [pH] liquid-liquid extraction. The resulting datafiles were compared against an in-house library database of more than 1,000 analytes. Comprehensive drug screening resulted in qualitative identification for *N*-desethyl isotonitazene and characterization of other drugs in the samples. Secondary analysis was performed via liquid chromatography tandem quadrupole mass spectrometry (LC-QQQ-MS) using a Waters TQ-S Micro. Quantitative confirmation was completed using a previously validated method for ten nitazene analogues. Drug materials were initially prepared by solvent dilution and analyzed qualitatively using LC-QTOF-MS, followed by basic extraction and quantitative analysis by gas chromatography mass spectrometry (GC-MS). Corresponding case histories were compiled when available.

Results: *N*-Desethyl isotonitazene was first reported on its own in December 2022 after analysis of a counterfeit tablet from Florida; however, retrospective analyses determined that the first identification was observed as early as September 2022 in a urine sample collected from an individual in Philadelphia. Ten drug material samples collected in Philadelphia from late 2022 through early 2023 tested positive for *N*-desethyl isotonitazene (estimated average purity: <1%) alongside fentanyl (average purity: 3.3%), xylazine (average purity: 59%), bromazolam, and other drugs. To date, four forensic toxicology cases

have been quantitatively confirmed to contain *N*-desethyl isotonitazene at <0.2, 0.82, 5.0 and 5.1 ng/mL. Interestingly, some of the toxicology casework had common combinations of NPS benzodiazepines and opioids that were also observed in the tested drug materials. In a case from New Jersey, an individual with a history of drug use and prior drug related incidences was found unresponsive in bed. Investigators found a mirror with white residue near the decedent. Following autopsy and toxicology testing, the manner of death was ruled accident and the cause of death was attributed to multiple drug toxicity including *N*-desethyl isotonitazene (0.82 ng/mL), bromazolam, cocaine, methamphetamine, and ethanol.

Discussion: The novel synthetic opioid market continues to transform and evolve, as predicted based on prior experience with NPS. *N*-Desethyl isotonitazene is a highly potent opioid and one of the newest nitazene analogues to emerge in fatal drug overdoses. Data from drug materials and forensic toxicology casework show that this drug is impacting public health and that testing is necessary in overdoses containing or suspected to contain this new drug.