

An Update: A Forward-Thinking Approach to Tackling New Synthetic Opioid Nitazene Analogues by LC-QQQ-MS

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Disclosures

- I have no conflicts of interest to disclose.
- I am a scientist and employee of FRFF / CFSRE, a 501(c)(3) non-profit research and educational facility.
- This project was supported in part by:
 - National Institute of Justice, Office of Justice Programs, U.S. Department of Justice
 - Award Number 2020-DQ-BX-0007, “Real-Time Sample-Mining and Data-Mining Approaches for the Discovery of Novel Psychoactive Substances (NPS)”
 - The opinions, findings, conclusions and/or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect those of the Department of Justice

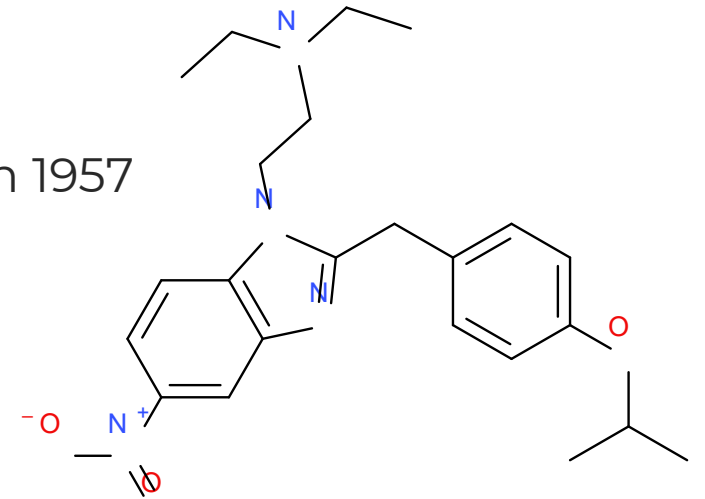


Recap of SOFT 2021



Background – Nitazene Analogues

- 2-Benzylbenzimidazole subclass of novel synthetic opioids (NSOs)
 - Structurally distinct from fentanyl analogues and other NSOs
 - Appeared on the illicit drug market in 2019
- Original patent in 1957
 - Nitazenes emerged that were not described in the original patent in 1957
 - *N*-pyrrolidino etonitazene
- Isotonitazene launched the rise of nitazenes
 - Potency 100-1000x that of morphine
- Adverse effects:
 - Respiratory depression
 - Combined use with benzodiazepines (**benzo-dope**) – increased CNS depressant effects



Isotonitazene

Potency of Nitazenes

N-Pyrrolidino Etonitazene
– potency comparable to
etonitazene



| | MOR-βarr2 | | |
|----------------------------|-----------------------|----------------------------------|----------------------------|
| | EC ₅₀ (nM) | E _{max} (% of fentanyl) | E _{max} (% of HM) |
| 1. isotonitazene | 1.63 (1.17–2.28) | 110 (105–115) | 179 (171–187) |
| 2. N-desethylisotonitazene | 0.614 (0.377–0.985) | 140 (131–149) | 229 (214–243) |
| 3. 4'-OH-nitazene | 176 (124–250) | 81.9 (76.4–87.5) | 133 (125–143) |
| 4. 5-aminoisotonitazene | 383 (263–554) | 115 (108–123) | 188 (176–201) |
| 5. metonitazene | 8.14 (5.12–12.8) | 113 (106–121) | 184 (172–197) |
| 6. etonitazene | 0.661 (0.338–1.26) | 134 (122–146) | 219 (199–238) |
| 7. N-desethyletonitazene | 1.81 (1.14–2.94) | 101 (94.7–107) | 164 (154–175) |
| 8. protonitazene | 3.95 (2.78–5.60) | 107 (102–111) | 174 (165–182) |
| 9. butonitazene | 36.2 (20.2–63.9) | 103 (92.8–113) | 167 (151–184) |
| 10. clonitazene | 140 (93.6–210) | 106 (98.0–114) | 173 (160–187) |
| 11. flunitazene | 377 (295–481) | 118 (113–124) | 192 (183–202) |
| 12. isotodesnitazene | 34.8 (22.1–54.4) | 94.9 (88.1–102) | 155 (144–166) |
| 13. metodesnitazene | 548 (365–811) | 91.2 (85.1–97.5) | 149 (139–159) |
| 14. etodesnitazene | 54.9 (36.1–82.0) | 96.8 (90.2–103) | 158 (147–169) |
| morphine | 338 (239–478) | 71.9 (68.3–75.4) | 117 (111–123) |
| fentanyl | 14.4 (11.5–18.0) | 100 (96.5–103) | 163 (157–169) |
| hydromorphone | 36.2 (27.9–47.0) | 61.3 (58.9–63.8) | 100 (95.9–104) |

Timeline



Schedules of Controlled Substances: Temporary Placement of Butonitazene, Etodesnitazene, Flunitazene, Metodesnitazene, Metonitazene, N-pyrrolidino etonitazene, and Protonitazene in Schedule I

A Proposed Rule by the Drug Enforcement Administration on 12/07/2021

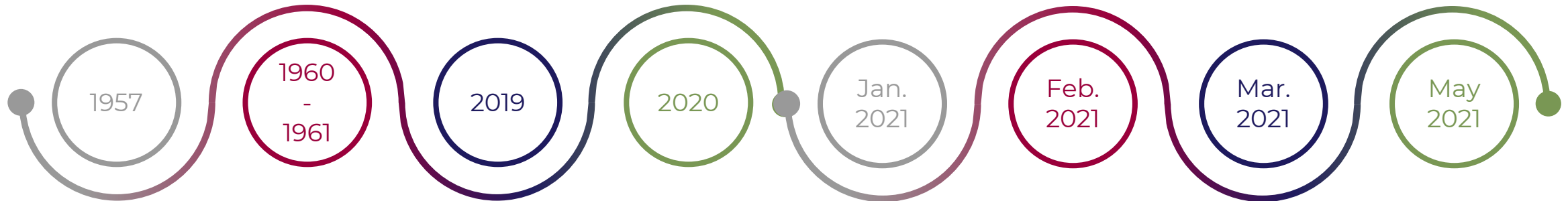


Synthesis of Benzimidazoles

Isotonitazene

Butonitazene

Flunitazene



Patent Issued
Etonitazene & Clonitazene Schedule I in US under CSA

Scheduling of Isotonitazene
Metonitazene

Etodesnitazene

N-Pyrrolidino Etonitazene & Protonitazene


Objective

Develop an innovative, sensitive assay to identify and quantitate the current and potential future nitazene analogues

- Method Development
- Method Validation
- 60-Day Stability Study
- Application to Authentic Samples



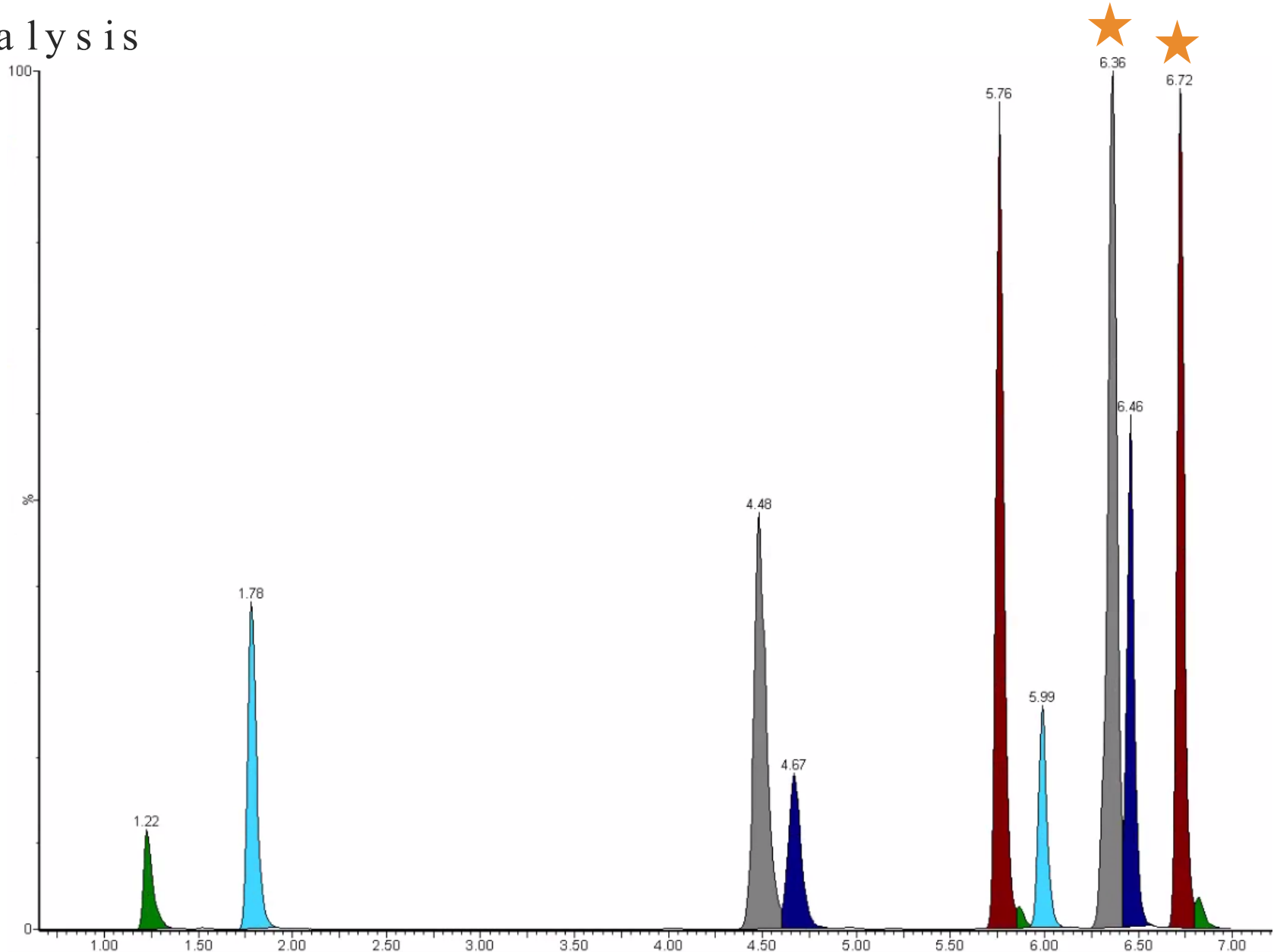
JOURNAL ARTICLE EDITOR'S CHOICE

A Forward-Thinking Approach to Addressing the New Synthetic Opioid 2-Benzylbenzimidazole Nitazene Analogs by Liquid Chromatography–Tandem Quadrupole Mass Spectrometry (LC–QQQ–MS) 

Sara E Walton, Alex J Krotulski , Barry K Logan

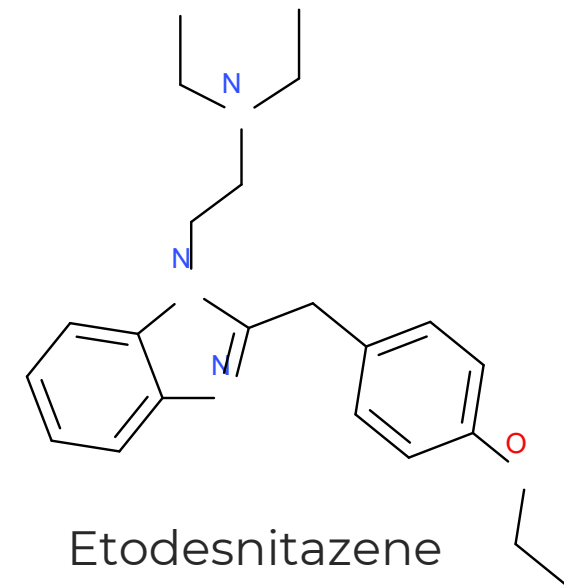
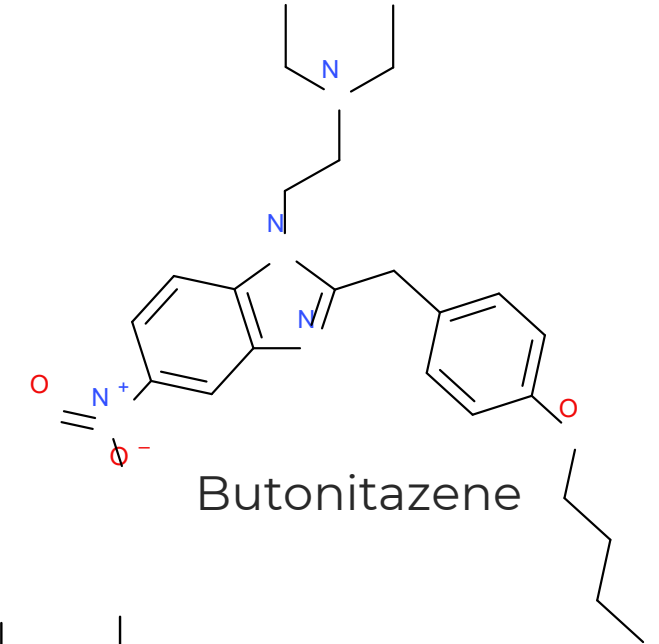
Original Scope of Analysis

- Order of elution
 - 5-Aminoisotonitazene
 - 4'-Hydroxy Nitazene
 - Metonitazene
 - Flunitazene
 - Etonitazene
 - Clonitazene
 - Isotonitazene*
 - N-Desethyl Isotonitazene
 - Protonitazene*



Validation & Application of Method

- Method validation performed and acceptable according to ASB standards
- 60-day stability study completed
 - All nitazenes stable in refrigerator for up to 2 months
 - Exception: 5-aminoisotonitazene
- Method applied to 171 authentic human samples
 - Postmortem: 47 cases
 - DUID: 67 cases
- Method adapted to include butonitazene and etodesnitazene



Previous Conclusions

- Isotonitazene (Oct. 2019 – Jan. 2020)
 - Mean & median concentrations low (high potency)
 - *N*-Desethyl isotonitazene – consistently higher concentrations in PM samples
 - Not found in combination with other nitazenes
- Metonitazene (Nov. 2020 – Feb. 2021)
 - Found with butonitazene, flunitazene, and protonitazene
- *N*-Pyrrolidino Etonitazene appears to be increasing in popularity (mid 2021)
- 4'-Hydroxy nitazene – universal metabolite
- Overall – nitazenes found with NPS benzodiazepines (e.g., flualprazolam), NPS opioids (other nitazenes), and fentanyl



New Information




Published Work

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Metonitazene in the United States—Forensic toxicology assessment of a potent new synthetic opioid using liquid chromatography mass spectrometry

Alex J. Krotulski , Donna M. Papsun, Sara E. Walton, Barry K. Logan


First published: 16 June 2021 | <https://doi.org/10.1002/dta.3115> | Citations: 6

Archives of Toxicology
<https://doi.org/10.1007/s00204-022-03276-4>

ORGAN TOXICITY AND MECHANISMS








Pharmacological evaluation and forensic case series of *N*-pyrrolidino etonitazene (etonitazepyne), a newly emerging 2-benzylbenzimidazole ‘nitazene’ synthetic opioid

Marthe M. Vandeputte¹ · Alex J. Krotulski² · Donna Walther³ · Grant C. Glatfelter³ · Donna Papsun⁴ · Sara E. Walton² · Barry K. Logan^{2,4} · Michael H. Baumann³ · Christophe P. Stove¹ 

Received: 7 February 2022 / Accepted: 14 March 2022
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Identification of a novel opioid, *N*-piperidinyl etonitazene (etonitazepipne), in patients with suspected opioid overdose

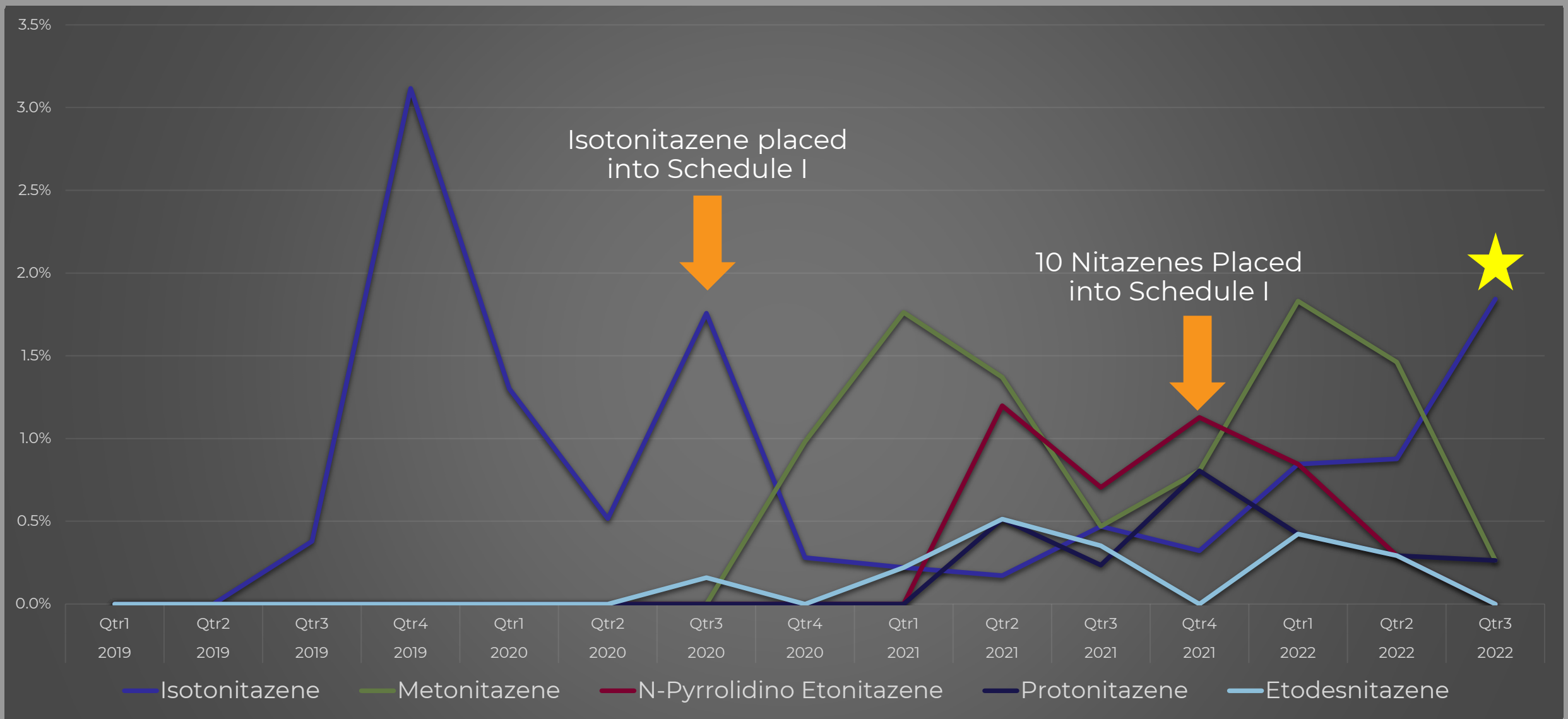
Diane P. Calello  , Kim Aldy, Mohamed Jefri, Tuyet-Anh T. Nguyen, Alex Krotulski, Barry Logan , Jeffrey Brent , Paul Wax, Sara Walton, Alex F. Manini  & The ToxIC Fentalog Study Group [... show less](#)

Pages 1067-1069 | Received 03 Feb 2022, Accepted 24 May 2022, Published online: 16 Jun 2022



NPSDISCOVERY.ORG

Nitazene Trends



CFSRE's Nitazene Scope

| | | | | | |
|---------------------------------|-----------------------------|-------------------------------|---------------------------------|-------------------------|--------------------------|
| Metodesnitazene | Etodesnitazene | Nitazene | Isotodesnitazene | 5-Methyl Etodesnitazene | Protodesnitazene |
| Menitazene | 4'-hydroxy nitazene | N-desethyl Etonitazene | Flunitazene | 5-Amino Isotonitazene | Metonitazene |
| Etonitazene | PropylNitazene | Nitazene Dihydrofuran Variant | N-Pyrrolidino Etonitazene | Clonitazene | N-Desethyl Isotonitazene |
| Methionitazene | N-Piperidiny Etonitazene | N-Pyrrolidino Isotonitazene | Pyrrolidino Variant Etonitazene | Isotonitazene | Protonitazene |
| Alpha-Methyl Etonitazene | Ethylene Etonitazene | N-Piperidiny Etonitazene | Butonitazene | Sec-Butonitazene | Isobutonitazene |
| 5-Trifluoromethyl Isotonitazene | N-Pyrrolidino Protonitazene | N-Pyrrolidino Metonitazene | Etoetonitazene | - | - |

CFSRE's Nitazene Methodology

- Fully validated nitazene analogue method
 - Isotonitazene, metonitazene, protonitazene, etonitazene, flunitazene, clonitazene, and metabolites
- Standard addition methods
 - Etodesnitazene
 - Butonitazene
 - N-Pyrrolidino Etonitazene
 - N-Piperidinyl Etonitazene

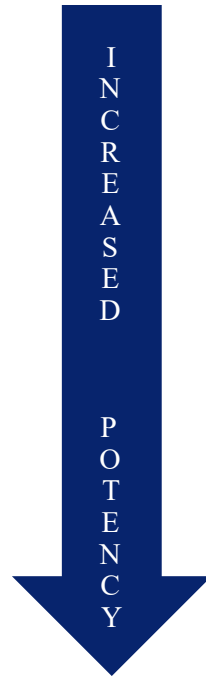


Waters Xevo TQ-S Micro LC-MS/MS

- Mobile phase compositions:
 - 0.1% Formic Acid in Water
 - 0.1% Formic Acid in Methanol
- Analytical Column
 - Agilent InfinityLab Poroshell 120 EC-C18
3.0 x 100mm, 2.7 μ m

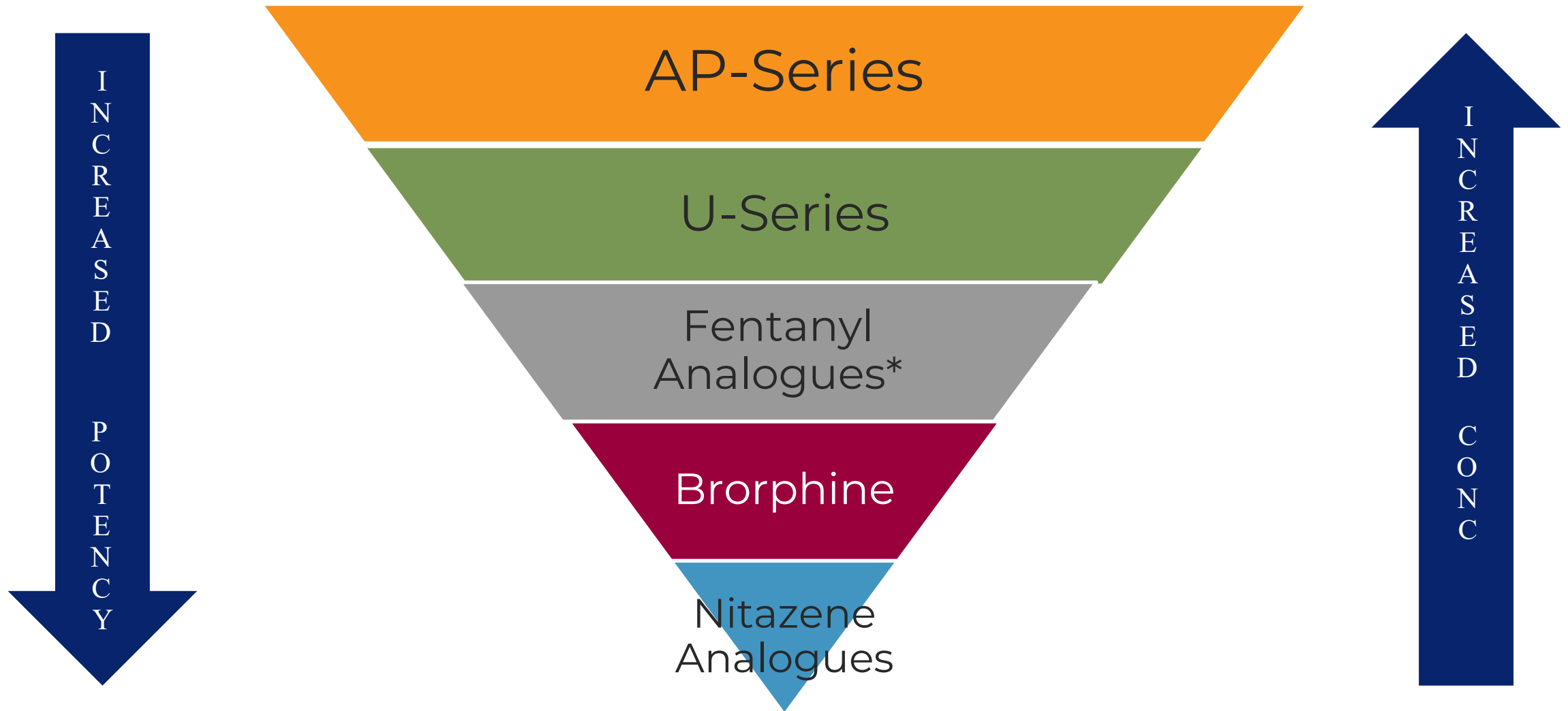
Comparing Concentrations in Death Cases

**Order similar to reported in vitro potency*



| Drug | N | Mean (\pm SD) (ng/mL) | Median (ng/mL) | Range (ng/mL) |
|----------------------------|----|-----------------------------|-------------------|------------------|
| Etodesnitazene | 15 | 40 \pm 61 | 5.2 | 0.53 - 230 |
| Protonitazene* | 3 | 11 \pm 9.9 | 5 | 3.1 - 25 |
| Metonitazene | 18 | 6.3 \pm 7.5 | 3.8 | 0.5 - 33 |
| Butonitazene | 1 | 3.2 | N/A | N/A |
| N-Pyrrolidino Etonitazene* | 15 | 3.9 \pm 5.9 | 2.4 | 0.3 - 25 |
| Isotonitazene* | 69 | 1.59 \pm 1.81 | 1.0 | 0.5 - 9 |

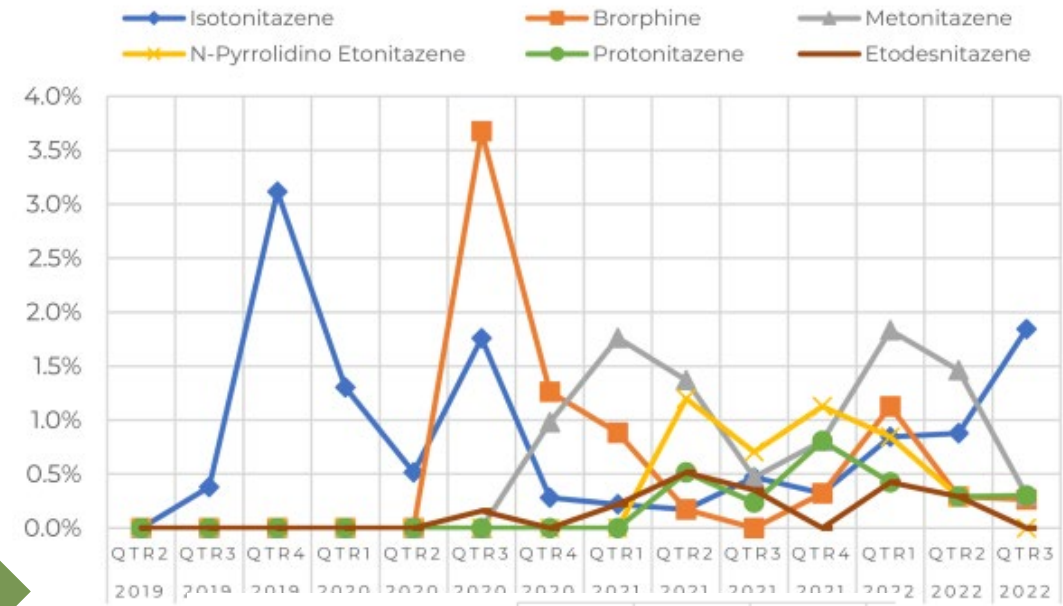
Comparative Concentrations to Other NPS Opioids



Nitazene Popularity Today

- Etodesnitazene
- **Isotonitazene**
- Metonitazene
- N-Pyrrolidino etonitazene
 - Extremely potent

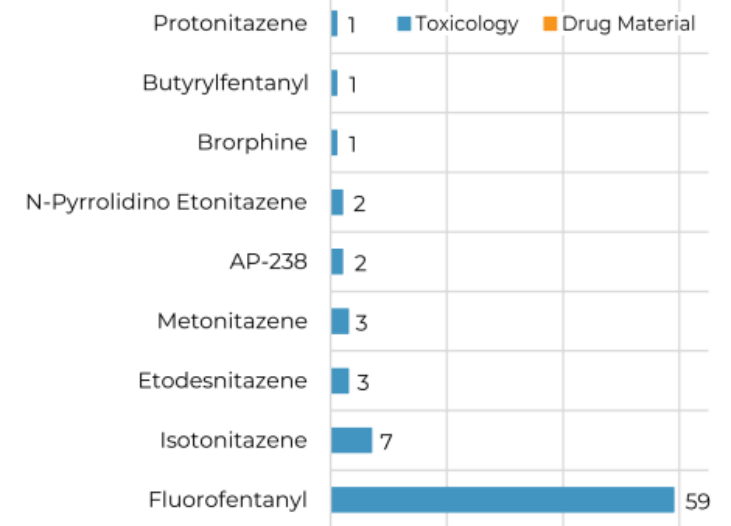
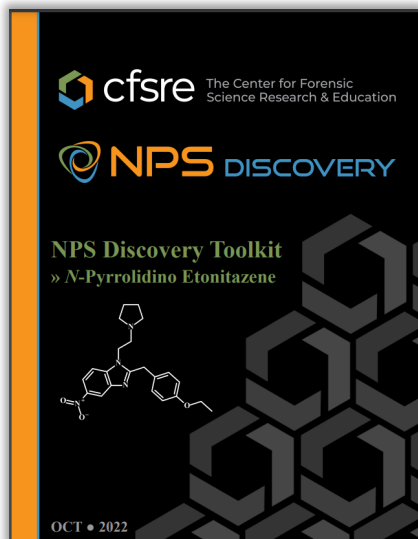
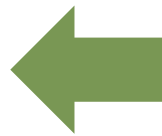
SELECT POSITIVITY: Q2 2019 to Q3 2022



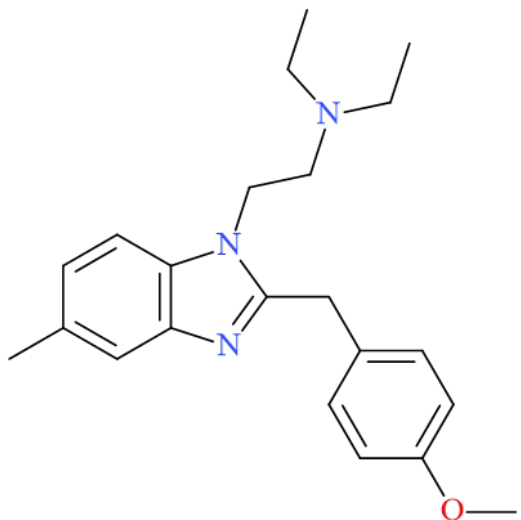
NEW
Q3 2022
Trend Report



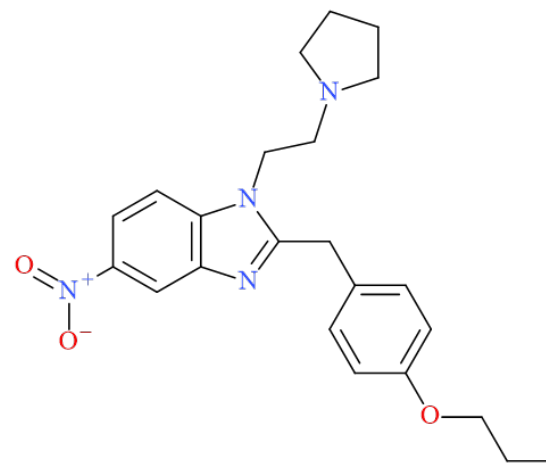
NEW
N-Pyrrolidino
Etonitazene Toolkit



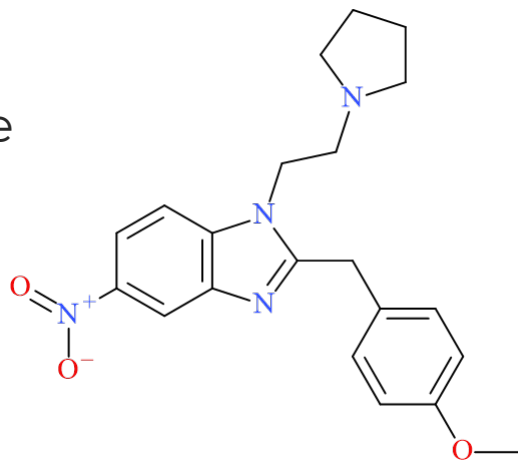
Nitazenes (Possibly) on the Horizon



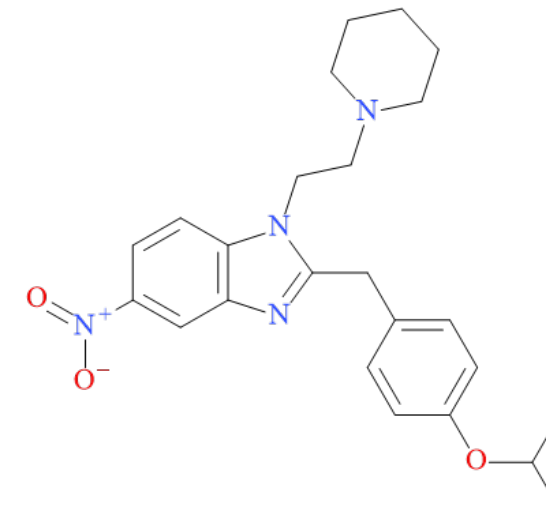
5-Methyl Etodesnitazene



N-Pyrrolidino Protonitazene



N-Pyrrolidino Metonitazene



N-Piperidinyl Isotonitazene



Conclusion



Conclusion

- Nitazenes continue to proliferate in the United States
 - Most popular: etodesnitazene, metonitazene, and *N*-pyrrolidino etonitazene
 - **Isotonitazene** appears to be making a comeback
- Quantitation of nitazenes indicates low ng/mL concentrations
 - Exception: “desnitazenes”
 - Sensitive methodology necessary
 - High potency opioids
 - *N*-Pyrrolidino etonitazene potency comparable to etonitazene
 - Found alongside novel benzodiazepines (**benzo-dope**)
 - Also discovered with stimulants, opioids, and many other NPS

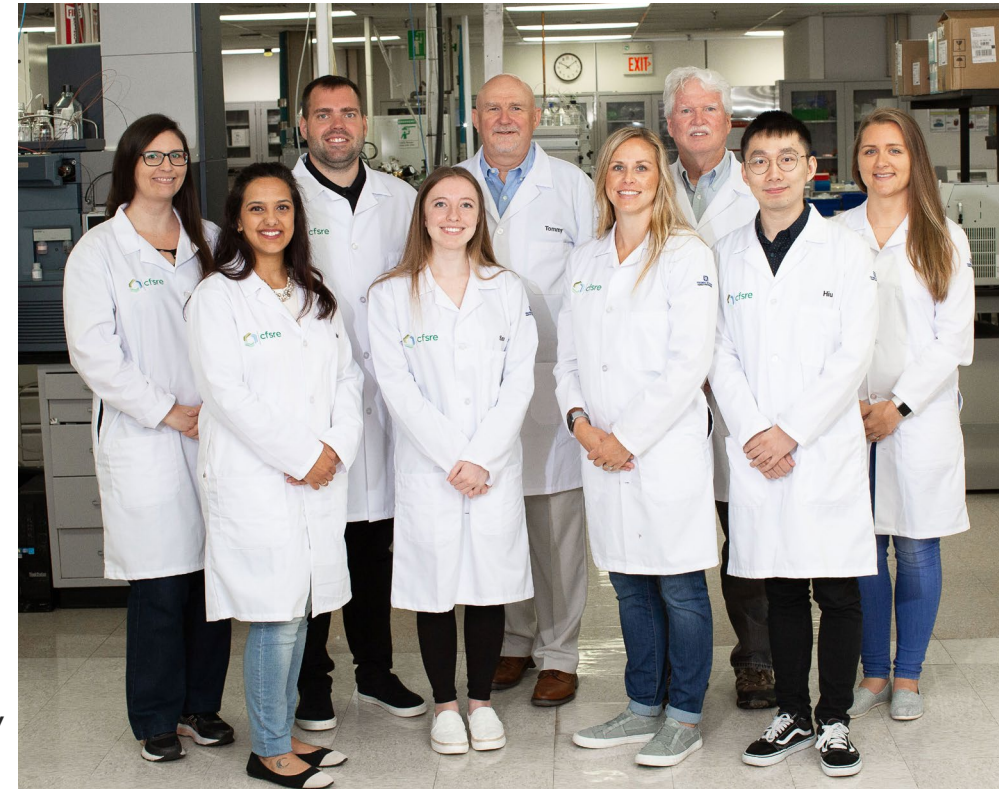
Learn More about *N*-Pyrrolidino Etonitazene

- Thursday November 3rd
 - Platform presentation **S36 – 2:15 - 2:30**

Pharmacology and Toxicology of *N*-Pyrrolidino Etonitazene – a New Nitazene Synthetic Opioid Increasingly Observed in Forensic Cases

Acknowledgements

- **Leo dal Cortivo Family**
- Our MANY Collaborators
 - Amy Miles & Aaron Zane from WSLH
 - Eric Lavins & Szabi Sofalvi from CCME
 - Donna Papsun & Kyle Miller from NMS Labs
 - Mike Baumann & team from DDRU at NIDA
 - Christophe Stove & Marthe Vandeputte at Ghent University
 - Toxic Fentanyl Study Group from ACMT
- CFSRE Staff
- NMS Labs
- Collaborations with medical examiner and coroner offices





Thank you!

Questions?

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