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The Center for Forensic Science Research & Education

NPS DISCOVERY

NPS Discovery – Evolution of an Open-Access Drug Early Warning System

Alex J Krotulski, PhD [1,*] & Barry K Logan, PhD, F-ABFT [1,2]

[1] *Center for Forensic Science Research and Education, Fredric Rieders Family Foundation, Willow Grove, PA,* [2] *NMS Labs, Horsham, PA*

The VIII International Conference on Novel Psychoactive Substances (NPS) – Wednesday November 17, 2021 (Virtual)

Disclosures & Funding

- 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.
- NPS Discovery is funded in part by the 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

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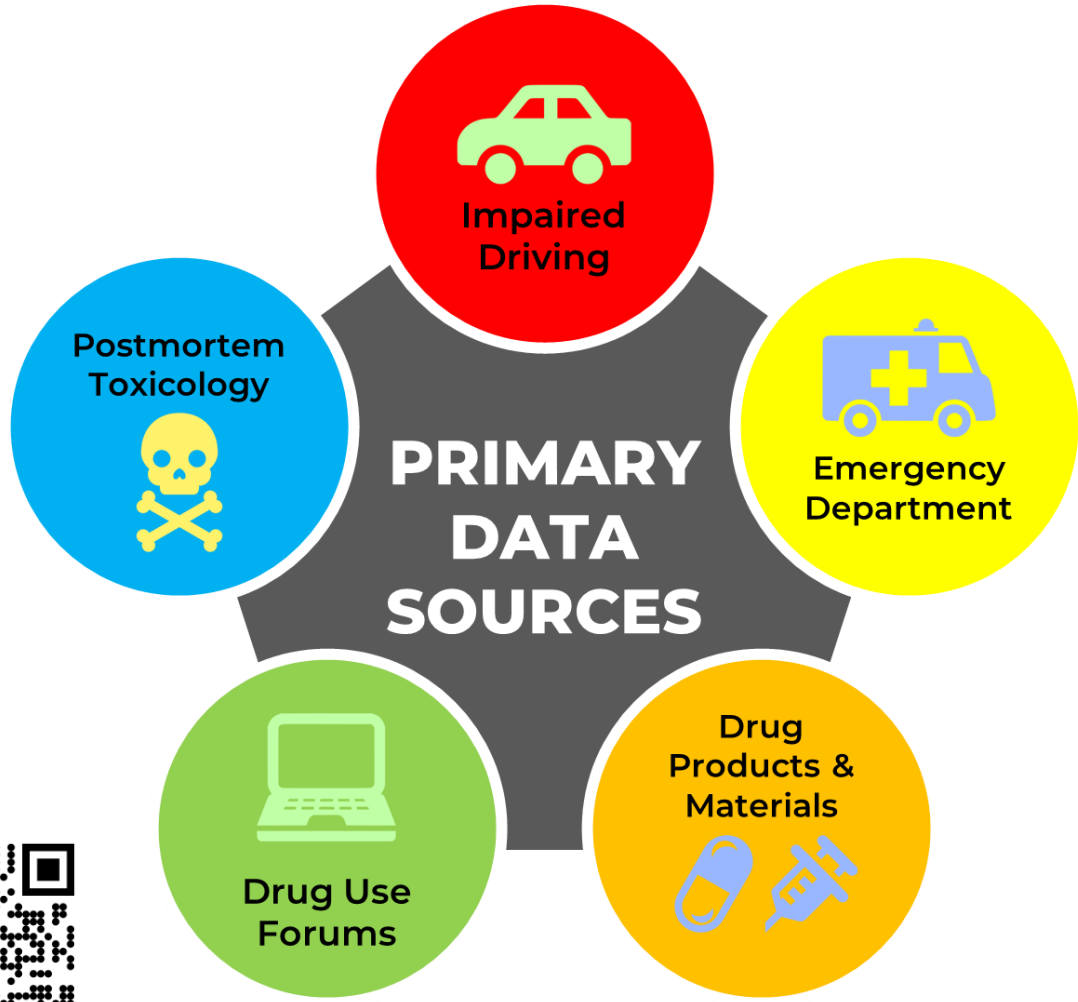
NIJ | National Institute of Justice
STRENGTHEN SCIENCE. ADVANCE JUSTICE.

What is NPS Discovery?

- Program developed at the CFSRE
- Open-access national drug early warning system in United States
- Combines research & authentic cases
- Generate important data for the development of high impact reports
- Forensic toxicology, drug chemistry, clinical toxicology, and much more!
- **Website:** www.npsdiscovery.org



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Timeline of Our NPS Discovery Program

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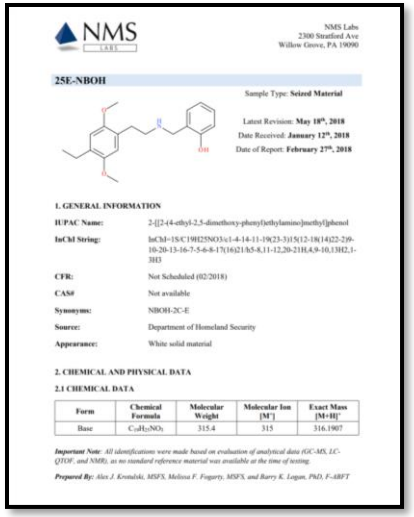

NMS LABS

NMS Labs
2300 Stratford Ave
Willow Grove, PA 19090

25E-NBOH

Sample Type: **Seized Material**

Latest Revision: **May 18th, 2018**
Date Received: **January 12th, 2018**
Date of Report: **February 27th, 2018**

1. GENERAL INFORMATION

IUPAC Name:	2-[[2-(4-ethyl-2,5-dimethoxy-phenyl)ethylamino]methyl]phenol
InChI String:	InChI=1S/C19H25NO3/c1-4-14-11-19(23-3)15(12-18(14)22-2)9-10-20-13-16-7-5-6-8-17(16)21/h5-8,11-12,20-21H,4,9-10,13H2,1-3H3
CFR:	Not Scheduled (02/2018)
CAS#	Not available
Synonyms:	NBOH-2C-E
Source:	Department of Homeland Security
Appearance:	White solid material

2. CHEMICAL AND PHYSICAL DATA

2.1 CHEMICAL DATA

Form	Chemical Formula	Molecular Weight	Molecular Ion [M+]	Exact Mass [M+H]+
Base	$C_{19}H_{25}NO_3$	315.4	315	316.1907

***Important Note**: All identifications were made based on evaluation of analytical data (GC-MS, LC-QTOF, and NMR), as no standard reference material was available at the time of testing.*

***Prepared By:** Alex J. Krotulski, MSFS, Melissa F. Fogarty, MSFS, and Barry K. Logan, PhD, F-ABFT*

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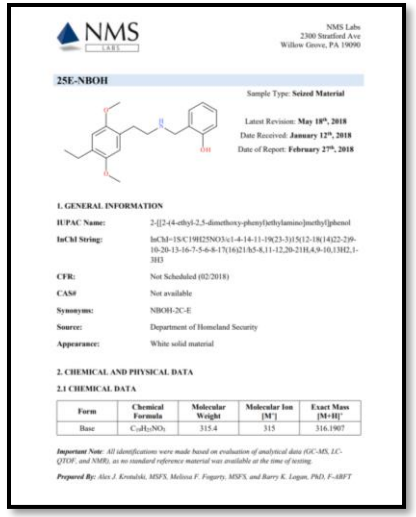
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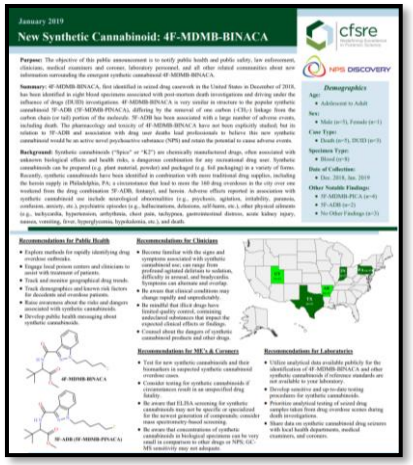
January 2019

New Synthetic Cannabinoid: 4F-MDMB-BINACA

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NPS DISCOVERY

Purpose: The objective of this public announcement is to notify public health and public safety, law enforcement, clinicians, medical examiners and coroner, laboratory personnel, and all other related communities about new information surrounding the emergent synthetic cannabinoid 4F-MDMB-BINACA.

Summary: 4F-MDMB-BINACA, first identified in seized drug casework in the United States in December of 2018, has been identified in eight blood specimens associated with post-mortem death investigations and driving under the influence of drugs (DUID) investigations. 4F-MDMB-BINACA is very similar in structure to the popular synthetic cannabinoid 5F-ADB (5F-MDMB-PINACA), differing by the removal of one carbon (-CH2-) linkage from the carbon chain (or tail) portion of the molecule. 5F-ADB has been associated with a large number of adverse events, including death. The pharmacology and toxicity of 4F-MDMB-BINACA have not been explicitly studied, but its relation to 5F-ADB and association with drug user deaths lead professionals to believe this new synthetic cannabinoid would be an active novel psychoactive substance (NPS) and retain the potential to cause adverse events.

Background: Synthetic cannabinoids ("Spice" or "K2") are chemically manufactured drugs, often associated with unknown biological effects and health risks, a dangerous combination for any recreational drug user. Synthetic cannabinoids can be prepared (e.g. plant material, powder) and packaged (e.g. foil packaging) in a variety of forms. Recently, synthetic cannabinoids have been identified in combination with more traditional drug supplies, including the heroin supply in Philadelphia, PA; a circumstance that lead to more the 160 drug overdoses in the city over one weekend from the drug combination 5F-ADB, fentanyl, and heroin. Adverse effects reported in association with synthetic cannabinoid use include neurological abnormalities (e.g., psychosis, agitation, irritability, paranoia, confusion, anxiety, etc.), psychiatric episodes (e.g., hallucinations, delusions, self-harm, etc.), other physical ailments (e.g., tachycardia, hypertension, arrhythmia, chest pain, tachypnea, gastrointestinal distress, acute kidney injury, nausea, vomiting, fever, hyperglycemia, hypokalemia, etc.), and death.

Demographics

Age:
- Adolescent to Adult

Sex:
- Male (n=5), Female (n=1)

Case Type:
- Death (n=5), DUID (n=1)

Specimen Type:
- Blood (n=5)

Date of Collection:
- Oct. 2018, Jan. 2019

Other Notable Findings:
- 5F-MDMB-PICA (n=4)
- 5F-ADB (n=2)
- No Other Findings (n=2)

Recommendations for Public Health
- Explore methods for rapidly identifying drug overdose outbreaks.
- Engage local poison centers and clinicians to assist with treatment of patients.
- Track and monitor geographical drug trends.
- Track demographics and known risk factors for decedents and overdose patients.
- Raise awareness about the risks and dangers associated with synthetic cannabinoids.
- Develop public health messaging about synthetic cannabinoids.

Recommendations for Clinicians
- Become familiar with the signs and symptoms associated with synthetic cannabinoid use; can range from profound agitated delirium to sedation, difficulty in arousal, and bradycardia. Symptoms can alternate and overlap.
- Be aware that clinical conditions may change rapidly and unpredictably.
- Be mindful that illicit drugs have limited quality control, containing undeclared substances that expand the expected clinical effects or findings.
- Counsel about the dangers of synthetic cannabinoid products and other drugs.

Recommendations for MCs & Coroners
- Test for new synthetic cannabinoids and their biomarkers in suspected synthetic cannabinoid overdose cases.
- Consider testing for synthetic cannabinoids if circumstances result in an unspecified drug fatality.
- Be aware that ELISA screening for synthetic cannabinoids may not be specific or specialized for the newest generation of compounds; consider alternative testing methods.
- Be aware that concentrations of synthetic cannabinoids in biological specimens can be very small in comparison to other drugs or NPS; GC-MS sensitivity may not adequate.

Recommendations for Laboratories
- Utilize analytical data available publicly for the identification of 4F-MDMB-BINACA and other synthetic cannabinoids if reference standards are not available to your laboratory.
- Develop sensitive and up-to-date testing procedures for synthetic cannabinoids.
- Prioritize analytical testing of seized drug samples taken from drug overdose scenes during death investigations.
- Share data on synthetic cannabinoid drug seizures with local health departments, medical examiners, and coroners.

4F-MDMB-BINACA

5F-ADB (5F-MDMB-PINACA)

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2020
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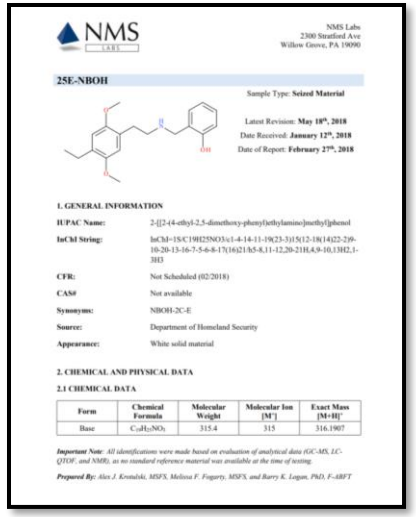
- Continued data collections through onset of COVID-19 pandemic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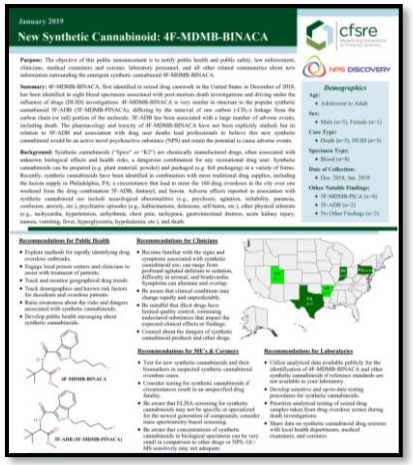
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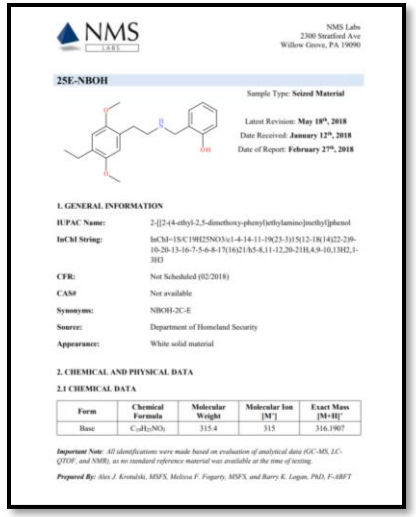
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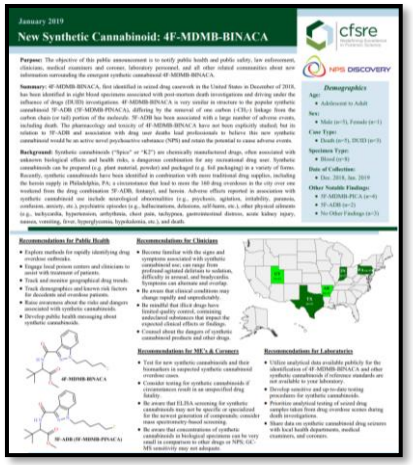
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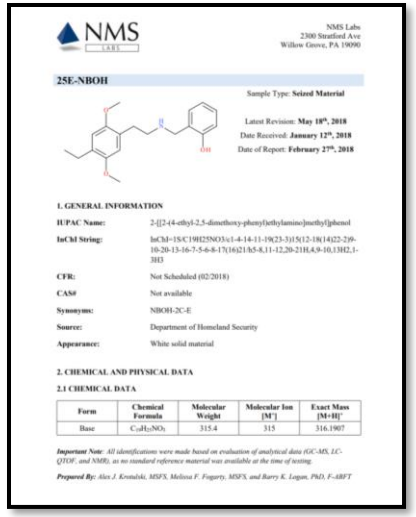
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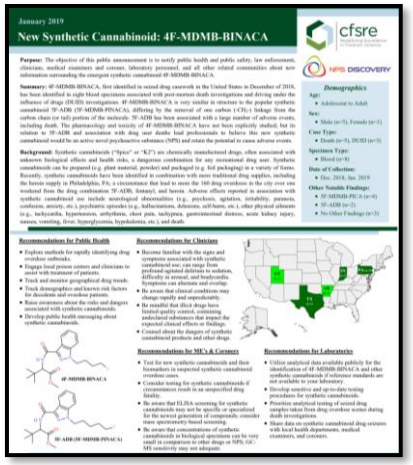
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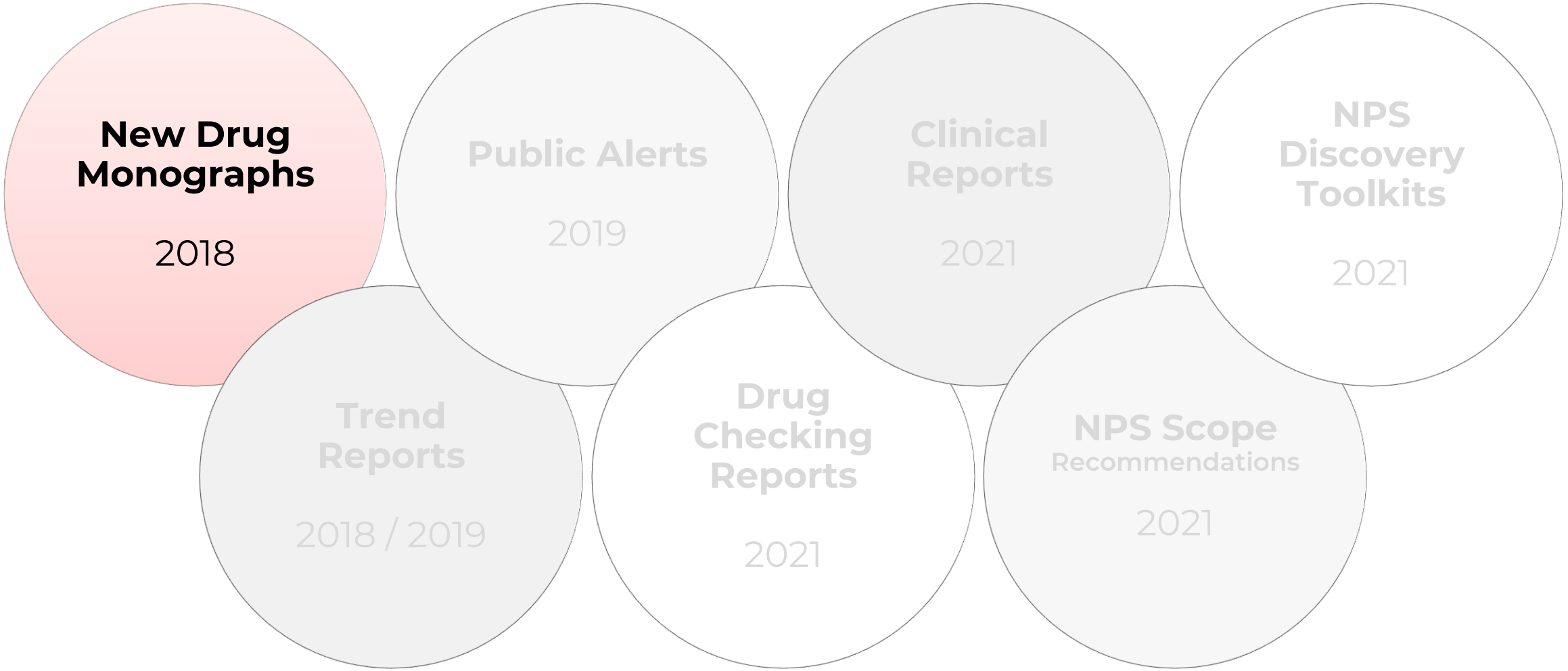
NPS DISCOVERY

Evolution of NPS Discovery Reports



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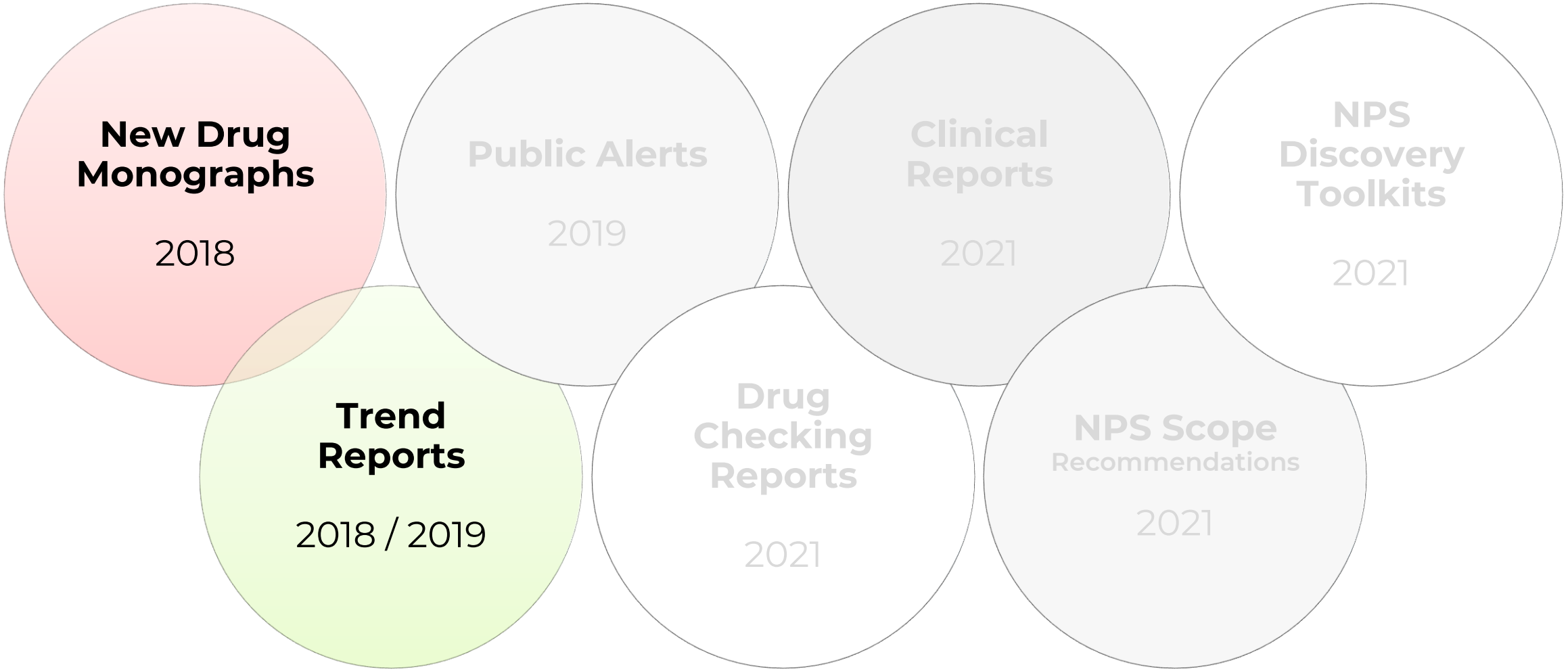

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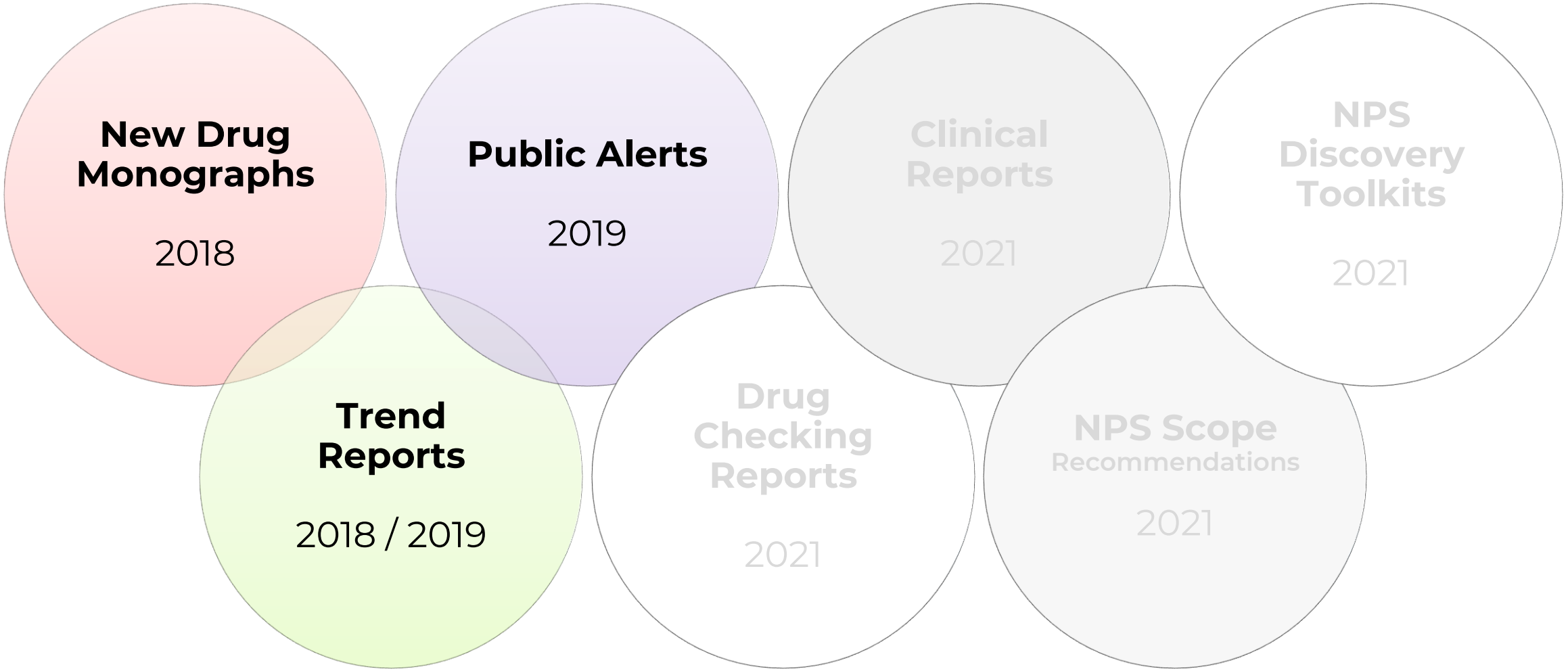

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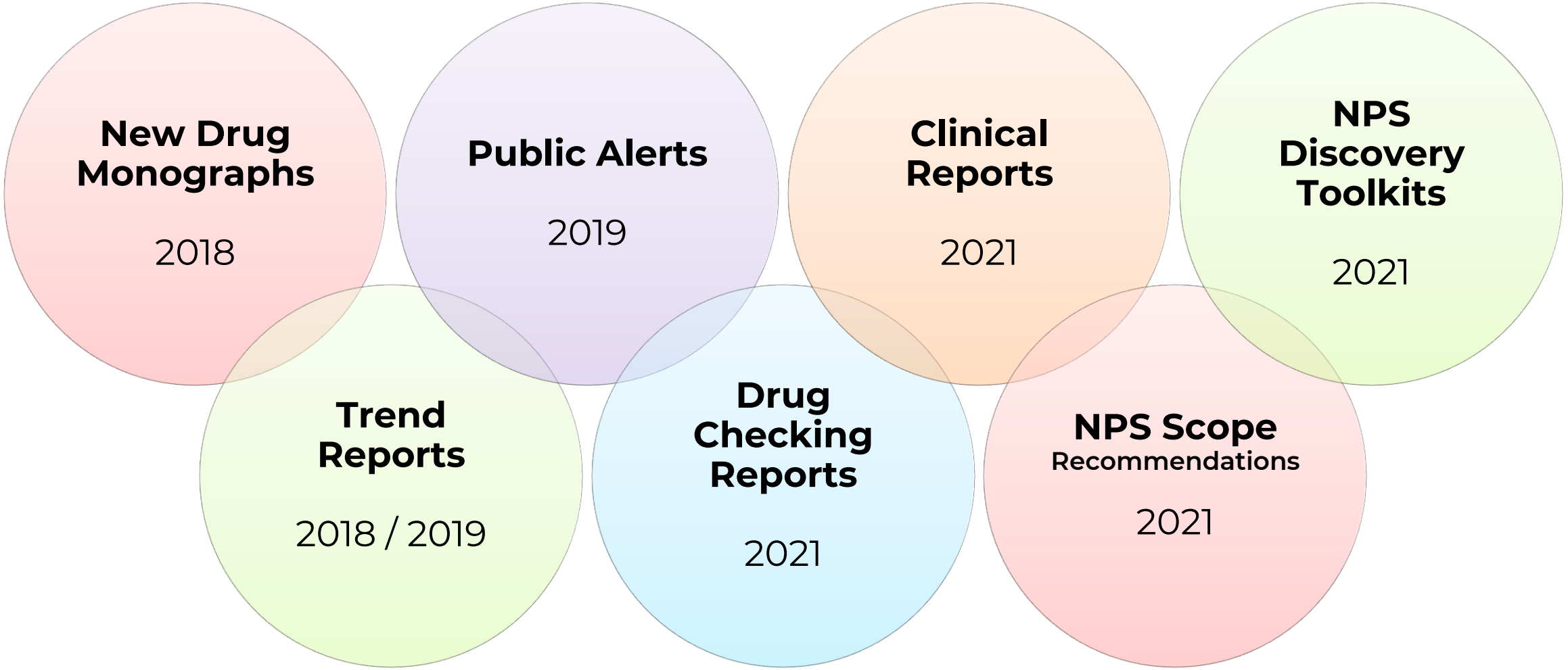

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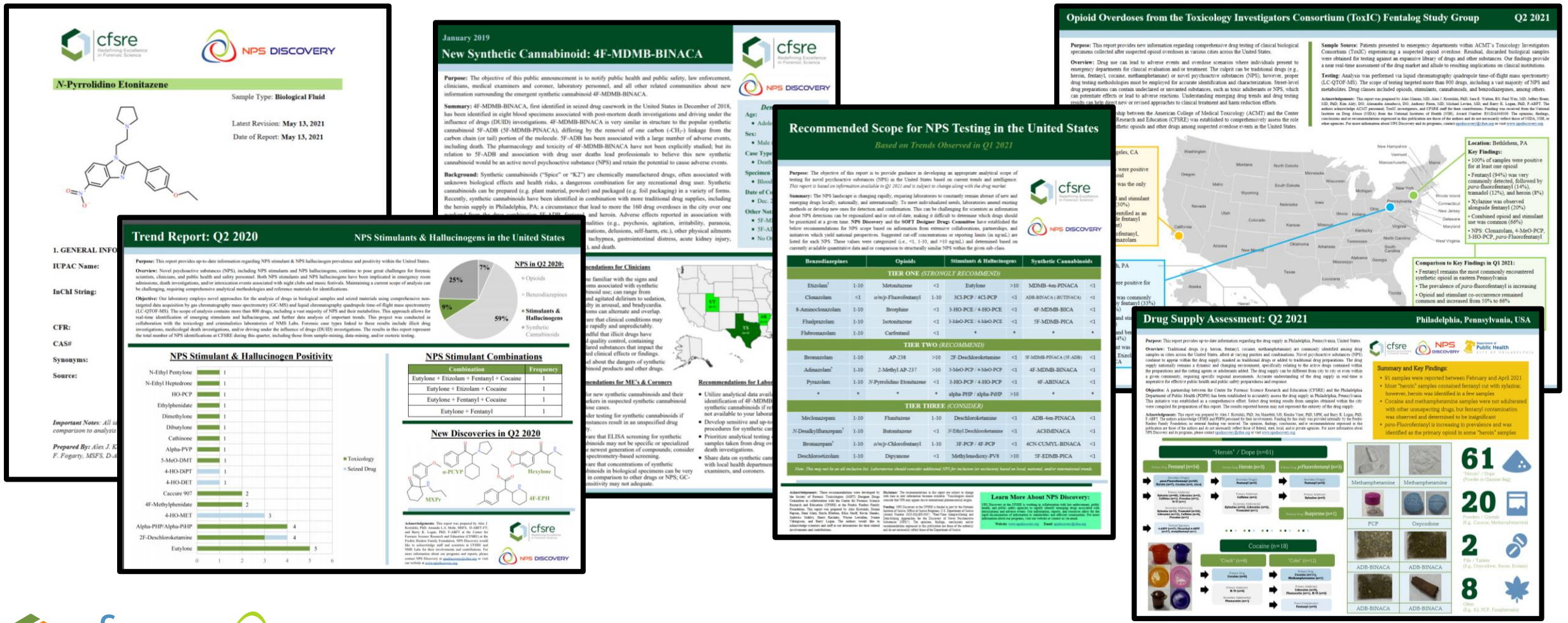
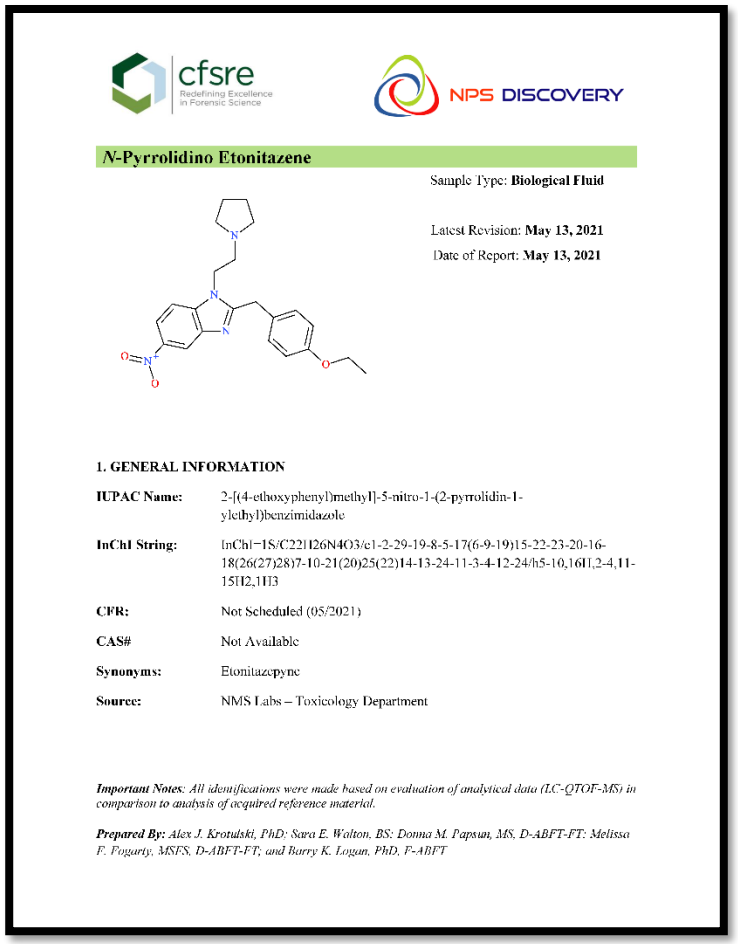
RESULTS & OUTCOMES

Reports Available Now

www.npsdiscovery.org



New Drug Monographs



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NPS DISCOVERY

***N*-Pyrrolidino Etonitazene**

Sample Type: **Biological Fluid**

Latest Revision: **May 13, 2021**

Date of Report: **May 13, 2021**

1. GENERAL INFORMATION

IUPAC Name: 2-[(4-ethoxyphenyl)methyl]-5-nitro-1-(2-pyrrolidin-1-ylethyl)benzimidazole

InChI String: InChI=1S/C22H26N4O3/c1-2-29-19-8-5-17(6-9-19)15-22-23-20-16-18(26(27)28)7-10-21(20)25(22)14-13-24-11-3-4-12-24/h5-10,16H,2-4,11-15H2,1H3

CFR: Not Scheduled (05/2021)

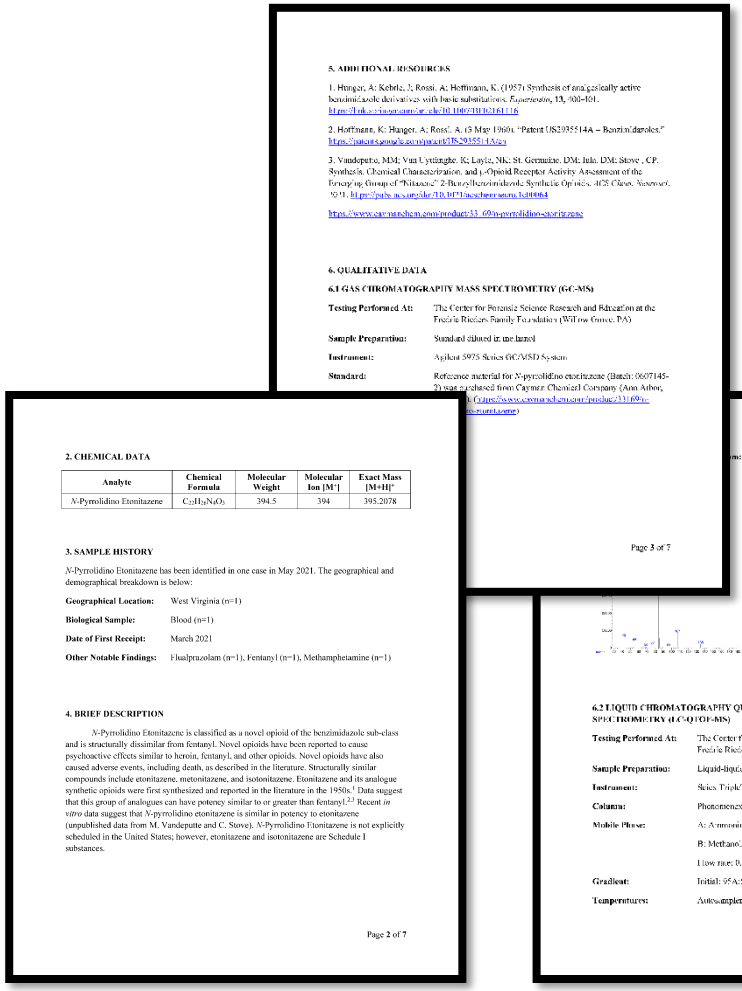
CAS# Not Available

Synonyms: Etonitazepyne

Source: NMS Labs – Toxicology Department

***Important Notes**: All identifications were made based on evaluation of analytical data (LC-QTOF-MS) in comparison to analysis of acquired reference material.*

***Prepared By:** Alex J. Krotulski, PhD; Sara E. Walton, BS; Donna M. Papsun, MS, D-ABFT-FT; Melissa F. Fogarty, MSFS, D-ABFT-FT; and Barry K. Logan, PhD, F-ABFT*



5. ADDITIONAL RESOURCES

1. Hunger, A; Kebrle, J.; Rossi, A; Hoffmann, K. (1957) Synthesis of analgesically active benzimidazole derivatives with basic substituents. *Experientia*, 13, 400–401. https://link.springer.com/article/10.1007/BF02161116

2. Hoffmann, K; Hunger, A; Rossi, A. (8 May 1960). "Patent US2935514A – Benzimidazoles." https://patents.google.com/patent/US2935514A/en

3. Vandeputte, MM; Van Uytfanghe, K; Layle, NK; St. Germaine, DM; Iula, DM; Stove, CP. Synthesis, Chemical Characterization, and μ-Opioid Receptor Activity Assessment of the Emerging Group of "Nitazene" 2-Benzylbenzimidazole Synthetic Opioids. *ACS Chem. Neurosci.* 2021, 12, 1, 28–38. https://pubs.acs.org/doi/10.1021/acschemneuro.1c00664

https://www.caymanchem.com/product/33_69/n-pyrrolidino-etonitazene

6. QUALITATIVE DATA

6.1 GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS)

Testing Performed At: The Center for Forensic Science Research and Education at the Fredric Rieders Family Foundation (Willow Grove, PA)

Sample Preparation: Standard diluted in methanol

Instrument: Agilent 5975 Series GC/MSD System

Standard: Reference material for *N*-pyrrolidino etonitazene (Batch: 0607145-2) was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Analysis of this standard resulted in positive identification of the analyte in the extract by *N*-pyrrolidino etonitazene, based on retention time (6.48 min) and mass spectral data. (https://www.caymanchem.com/product/33_69/n-pyrrolidino-etonitazene)

Injection Parameters: Column Oven: 50 °C; Source Heater: 600 °C; Injection Volume: 10 µL

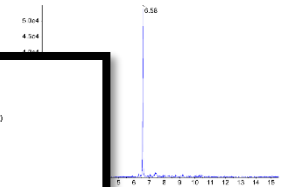
QTOF Parameters: TOF MS Scan Range: 100-510 Da; Precursor Isolation: SWATH acquisition (27 windows); Fragmentation: Collision Energy Spread (35±15 eV)

MS/MS Scan Range: 50-510 Da

Retention Time: 6.48 min

Standard Comparison: Reference material for *N*-pyrrolidino etonitazene (Batch: 0607145-2) was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Analysis of this standard resulted in positive identification of the analyte in the extract by *N*-pyrrolidino etonitazene, based on retention time (6.48 min) and mass spectral data. (https://www.caymanchem.com/product/33_69/n-pyrrolidino-etonitazene)

Extracted Ion Chromatogram: *N*-Pyrrolidino Etonitazene (Biological Sample)



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2. CHEMICAL DATA

Analyte	Chemical Formula	Molecular Weight	Molecular Ion [M+]	Exact Mass [M+H]+
N-Pyrrolidino Etonitazene	$C_{22}H_{26}N_4O_3$	394.5	394	395.2078

3. SAMPLE HISTORY

N-Pyrrolidino Etonitazene has been identified in one case in May 2021. The geographical and demographical breakdown is below:

Geographical Location: West Virginia (n=1)

Biological Sample: Blood (n=1)

Date of First Receipt: March 2021

Other Notable Findings: Hualprazolam (n=1), Fentanyl (n=1), Methamphetamine (n=1)

4. BRIEF DESCRIPTION

N-Pyrrolidino Etonitazene is classified as a novel opioid of the benzimidazole sub-class and is structurally dissimilar from fentanyl. Novel opioids have been reported to cause psychoactive effects similar to heroin, fentanyl, and other opioids. Novel opioids have also caused adverse events, including death, as described in the literature. Structurally similar compounds include etonitazene, metonitazene, and isotonitazene. Etonitazene and its analogue synthetic opioids were first synthesized and reported in the literature in the 1950s.[1] Data suggest that this group of analogues can have potency similar to or greater than fentanyl.[2,3] Recent *in vitro* data suggest that *N*-pyrrolidino etonitazene is similar in potency to etonitazene (unpublished data from M. Vandeputte and C. Stove). *N*-Pyrrolidino Etonitazene is not explicitly scheduled in the United States; however, etonitazene and isotonitazene are Schedule I substances.

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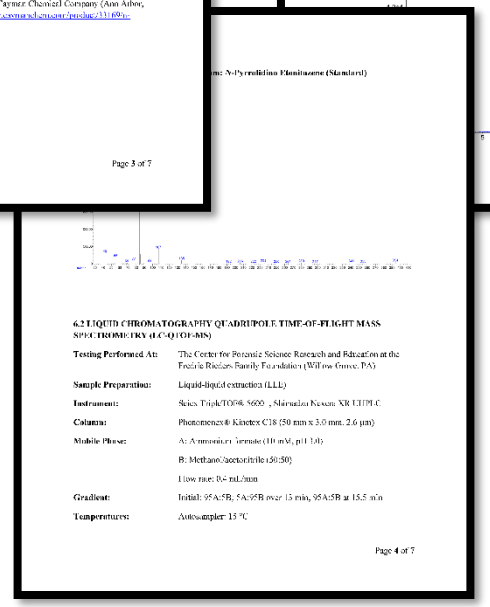

6.2 LIQUID CHROMATOGRAPHY QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY (LC-QTOF-MS)

Testing Performed At: The Center for Forensic Science Research and Education at the Fredric Rieders Family Foundation (Willow Grove, PA)

Sample Preparation: Liquid-liquid extraction (LLE)

Instrument: Sciex TripleTOF® 5600 + Shimadzu Nexera XR LC-20AD

Column: Phenomenex® Kinetex C18 (50 mm x 3.0 mm, 2.6 µm)

Mobile Phase: A: Ammonium formate (10 mM, pH 3.0); B: Methanol/acetonitrile (50:50); Flow rate: 0.5 mL/min

Gradient: Initial: 95:5 B; 5:95 B over 15 min, 95:5 B at 15.5 min

Temperatures: Autosampler: 15 °C

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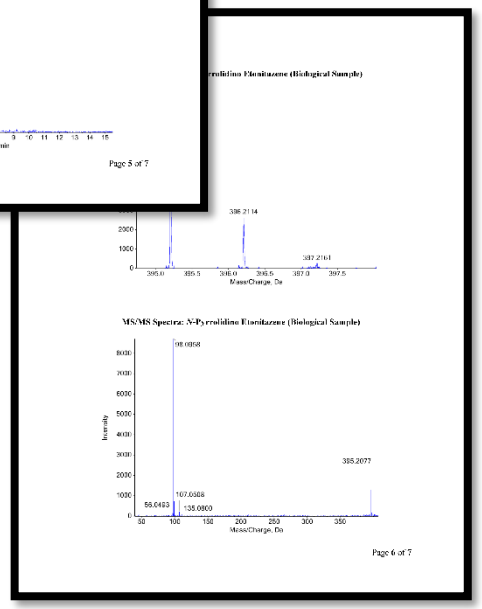

MS/MS Spectra: *N*-Pyrrolidino Etonitazene (Biological Sample)

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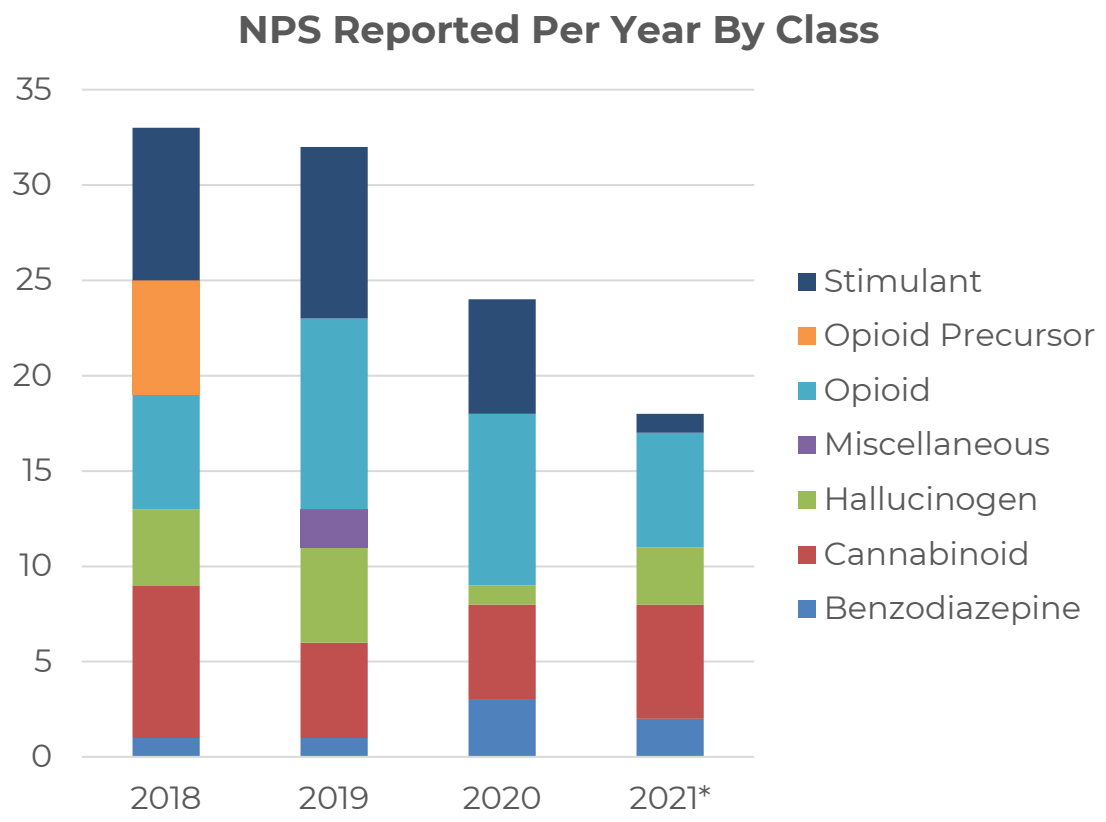
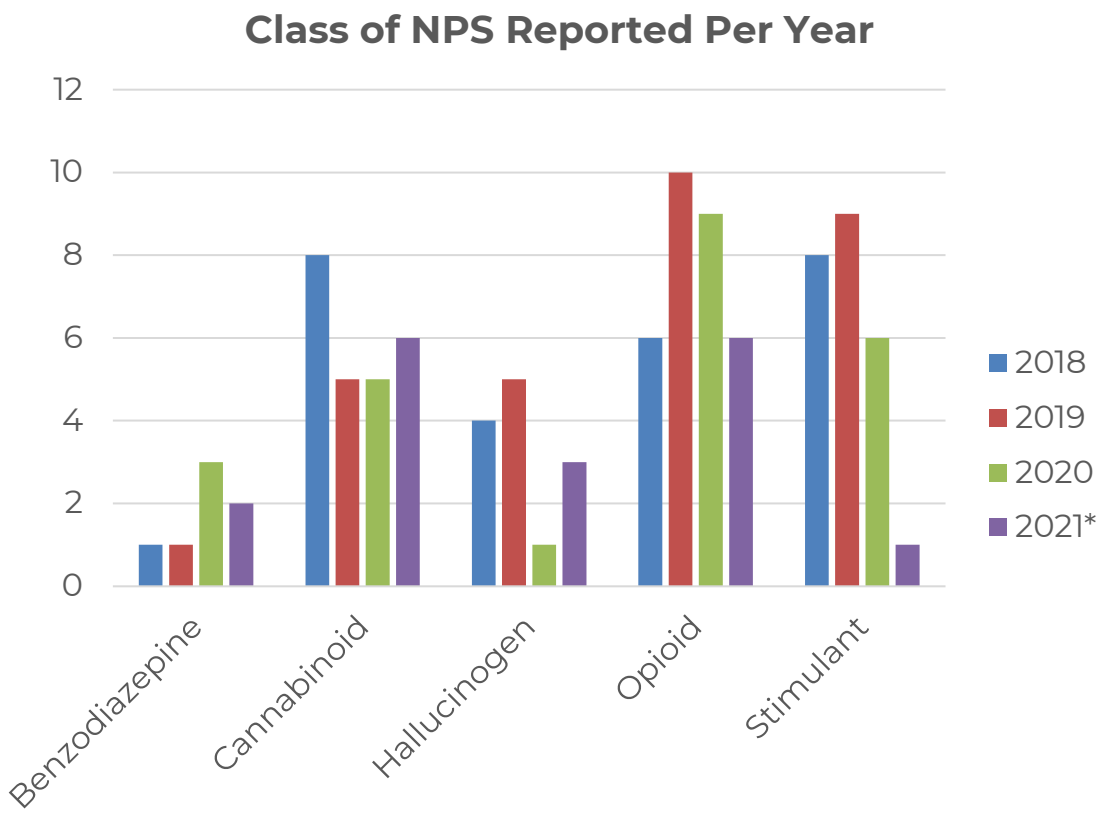
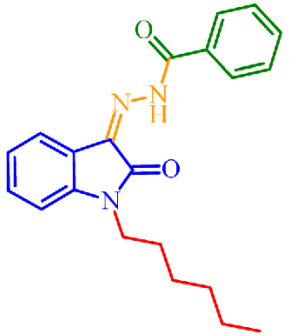
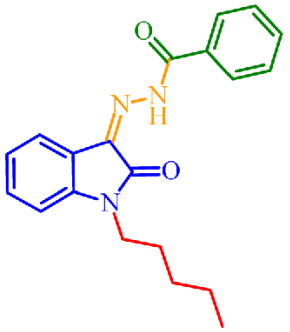
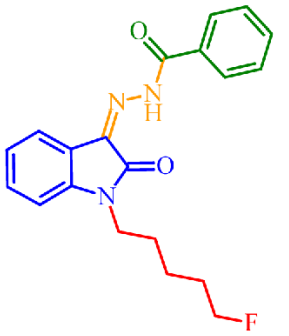
Identifications of New NPS in the United States





Newest NPS to Appear in the United States

- Metodesnitazene (9/2021)
- **5F-BZO-POXIZID, BZO-POXIZID, and BZO-HEXOXIZID (10/2021)**
- Fluclotizolam (11/2021)
- *Coming Soon:*
  - *N-Piperidinyl Etonitazene*
  - *ADB-FUBIATA*
  - *Tenocyclidine*

BZO-HEXOXIZID	BZO-POXIZID	5F-BZO-POXIZID
		
(*Z*)-*N'*-(1-**HEX**yl-2-**OX**o**I**ndolin-3-ylidene)**B**en**ZO**hydra**ZID**e	(*Z*)-*N'*-(1-**P**entyl-2-**OX**o**I**ndolin-3-ylidene)**B**en**ZO**hydra**ZID**e	(*Z*)-*N'*-(1-(**5**-**F**luoro**P**entyl-2-**OX**o**I**ndolin-3-ylidene) **B**en**ZO**hydra**ZID**e
Name: **BZO-HEXOXIZID**	*Name:* **BZO-POXIZID**	*Name:* **5F-BZO-POXIZID**
Synonyms: **MDA-19, MDA19, MDA 19**	*Synonyms:* **5C-MDA-19, MDA-19 pentyl analogue**	*Synonyms:* **5F-MDA-19, MDA-19 5-fluoropentyl analogue**

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“Next Generation Opioids”

2018

- MD-U-47700
- Phenylfentanyl
- U-47931E

2019

- Fluorofuranylfentanyl
- p-MeO-Fu-Fentanyl
- 2’,5’-DiMeO-Fentanyl
- 2-Methyl AP-237
- AP-237
- Piperidylthiambutene
- 2F-Viminol
- **Isotonitazene**
- N-Methyl U-47931E
- p-Me-Cpr-Fentanyl

2020

- 3,4-Difluoro-U-47700
- N-Ethyl-U-47700
- para-Methyl AP-237
- Brorphine
- **Metonitazene**
- AP-238
- Fluorofentanyl
- Chlorofentanyl
- Bromofentanyl

2021

- **Butonitazene**
- **Etodesnitazene**
- **Flunitazene**
- **N-Pyrrolidino Etonitazene**
- **Protonitazene**
- **Metodesnitazene**
- ***N-Piperidinyl Etonitazene***

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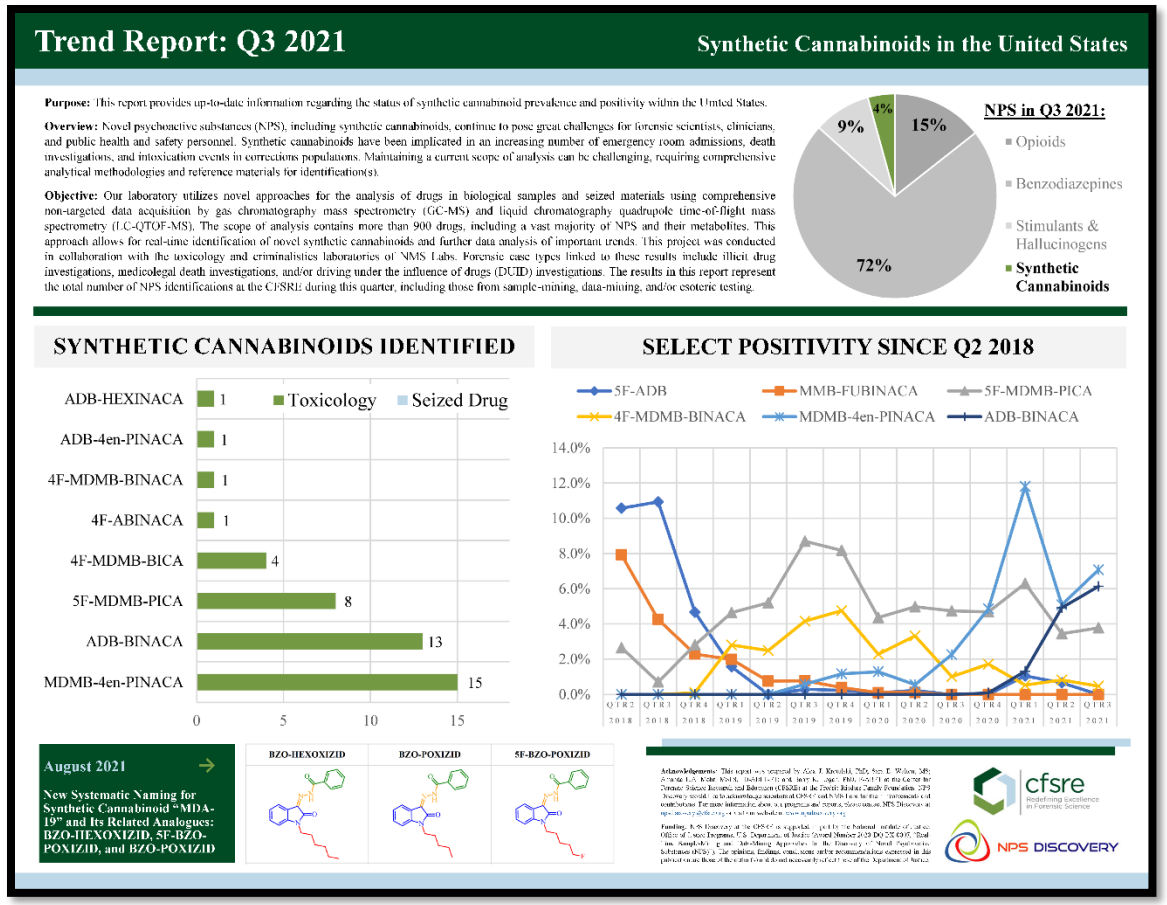
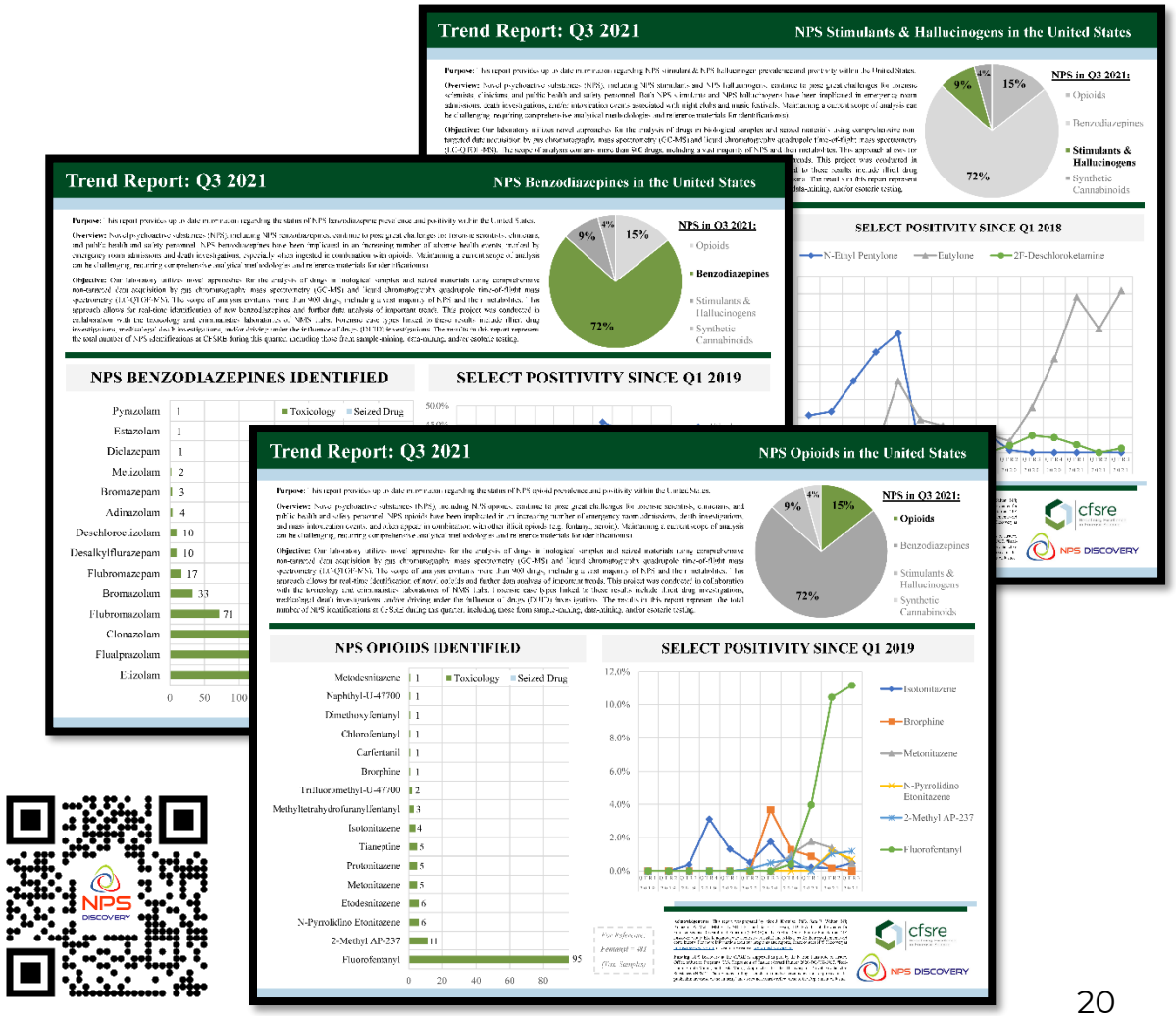
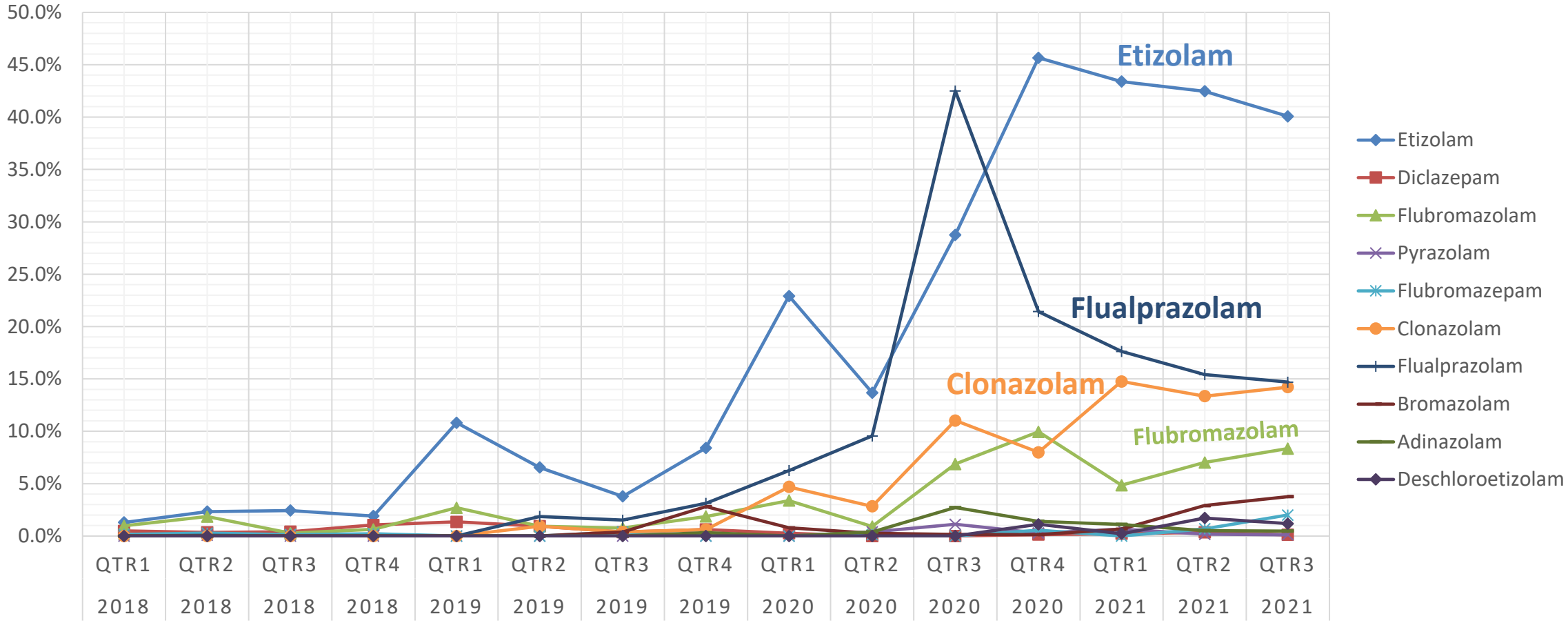
**New NPS Opioids Reported by NPS Discovery*

Trend Reports

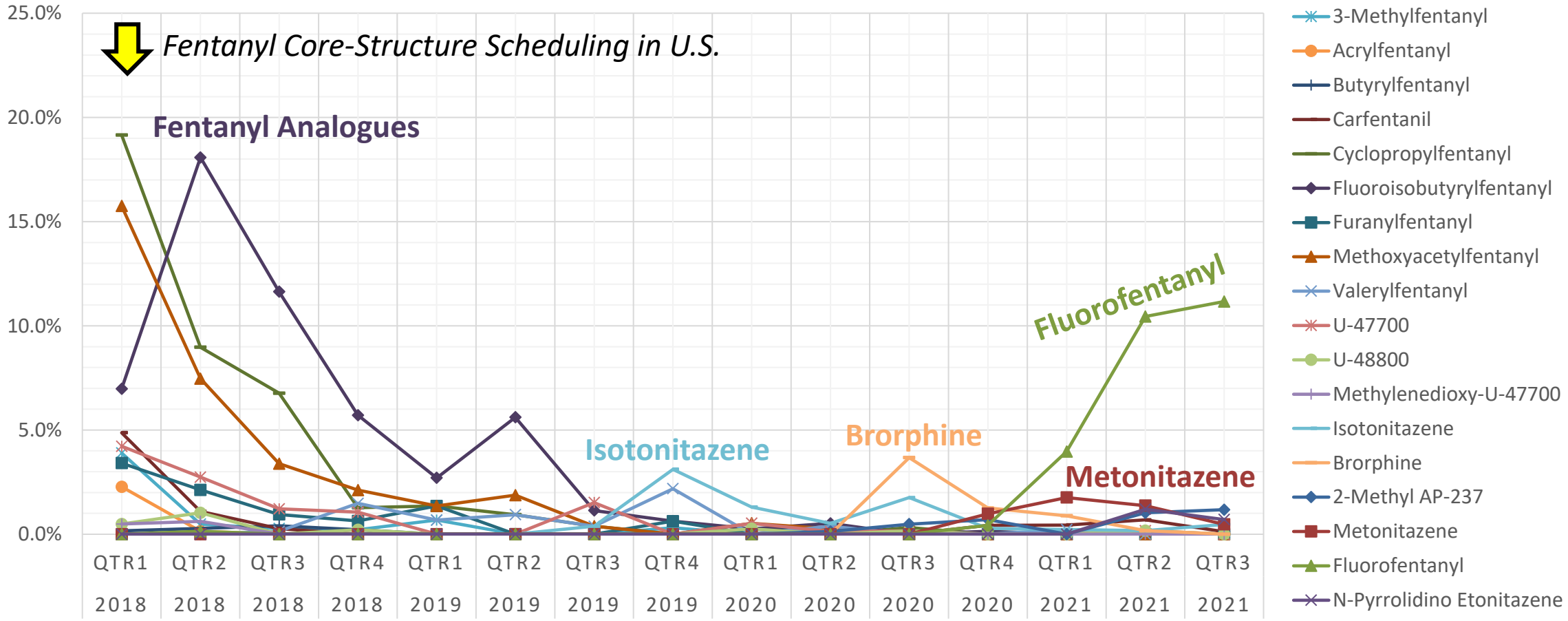




Positivity: NPS Benzodiazepines



Positivity: NPS Opioids



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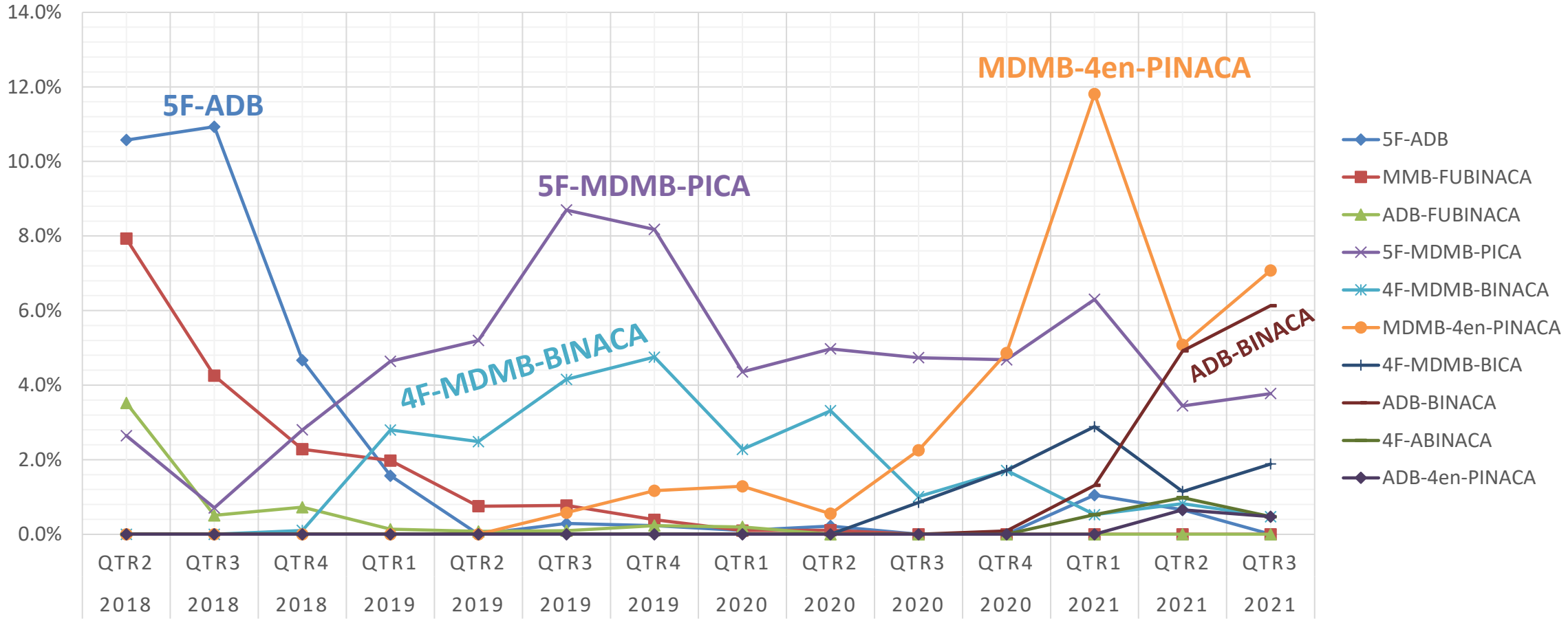
Positivity: Synthetic Cannabinoids



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Public Alerts

June 2021

New High Potency Synthetic Opioid *N*-Pyrrolidino Etonitazene (Etonitazepyne) Linked to Overdoses Across United States

Purpose: The objective of this announcement is to notify public health and safety, law enforcement, first responders, clinicians, medical examiners and coroners, forensic and clinical laboratory personnel, and all other related communities about new information surrounding the emergent synthetic opioid ***N*-pyrrolidino etonitazene**.

Background: Synthetic opioids are chemically manufactured drugs, often accompanied with unknown potency and adverse effects or health risks. New synthetic opioids may be mixed with more traditional opioids, creating additional risk and danger for recreational drug users. Synthetic opioids may be distributed in powder or tablet form. In the United States (U.S.), an alarming increase in the number of deaths linked to synthetic opioid use has been reported. The primary adverse effect associated with synthetic opioid use is respiratory depression, often leading to death.

Summary: *N*-Pyrrolidino etonitazene (etonitazepyne) is a new high potency synthetic opioid bearing structural resemblance to etonitazene, a synthetic opioid that is nationally and internationally controlled. *N*-Pyrrolidino etonitazene is dissimilar in structure to other synthetic opioids typically encountered in forensic casework (e.g., fentanyl). Unlike the 2-benzylbenzimidazole analogues that were first synthesized and reported in the literature in the 1950s (e.g., metonitazene, isotonitazene), *N*-pyrrolidino etonitazene does not appear in prior literature or patents. Recent *in vitro* pharmacological data suggest that this new opioid exhibits potency similar to etonitazene (~20x more potent than fentanyl). *N*-Pyrrolidino etonitazene was first reported by **NPS Discovery** in May 2021 following initial detection in a toxicology case. To date, eight blood specimens associated with postmortem death investigations in the U.S. have contained *N*-pyrrolidino etonitazene; additional confirmations are pending. The toxicity of *N*-pyrrolidino etonitazene has not been examined or reported but recent association with death among people who use drugs leads professionals to believe this synthetic opioid retains the potential to cause widespread harm and is of public health concern. Identifications of *N*-pyrrolidino etonitazene have also been reported recently from agencies in Europe.

Demographics

Case Type:
- Postmortem (n=8)

Age:
- Range: 20s to 50s

Date of Collection:
- January to April 2021

Other Notable Findings:
- NPS Benzodiazepines (n=7)
- Fentanyl (n=1)
- Methamphetamine (n=1)
- *Only Drug of Interest (n=1)

[N-Pyrrolidino Etonitazene]	
Case A	8.3 ng/mL*
Case B	2.4 ng/mL

Recommendations for Public Health
- Implement surveillance for rapid identification of drug overdose outbreaks.
- Engage local poison centers and clinicians to assist with treatment of affected patients.
- Track and monitor geographical drug distribution and trends.
- Track demographics and known risk factors for decedents and overdose patients.
- Raise awareness about the risks and dangers associated with opioid use.
- Make naloxone available to recreational drug users.

Recommendations for Clinicians
- Become familiar with the signs and symptoms associated with synthetic opioid use (e.g. sedation, respiratory depression).
- Naloxone should be administered to reverse critical respiratory depression and repeated naloxone administration may be necessary. Be aware that clinical conditions may change rapidly and unpredictably after naloxone administration due to precipitation of withdrawal.
- Be mindful that illicit drugs have limited quality control, containing undeclared substances that impact the expected clinical effects or findings.
- Counsel about the dangers of synthetic opioid products and other drugs.

Geographical Distribution of N-Pyrrolidino Etonitazene

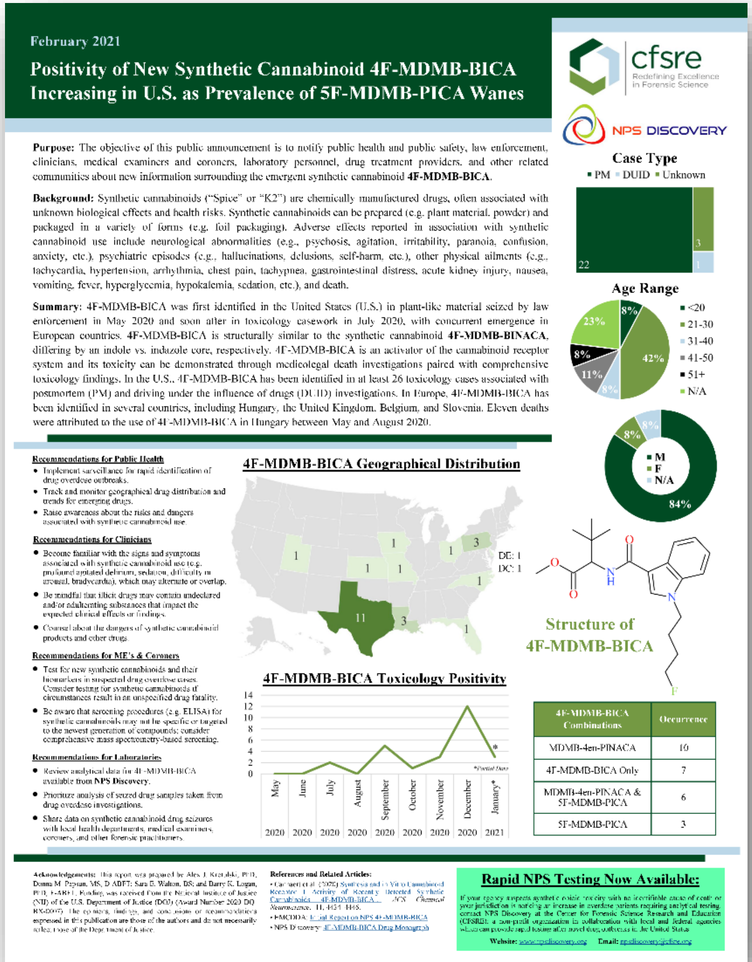
CO (n=1), IL (n=1), NY (n=1), PA (n=1), WV (n=4)

N-Pyrrolidino Etonitazene

Recommendations for MEs & Coroners
- Test for new synthetic opioids and their biomarkers in suspected opioid overdose cases.
- Be aware that ELISA screening for synthetic opioids may not be specific or specialized for the newest generation of compounds; consider mass spectrometry-based screening.
- Be aware that concentrations of synthetic opioids in biological specimens can vary and GC-MS sensitivity may not be adequate.

Recommendations for Laboratories
- Utilize analytical data available publicly for the identification of *N*-pyrrolidino etonitazene if a reference standard is not immediately available.
- Utilize previously developed non-targeted testing protocols or develop sensitive and up-to-date testing procedures for synthetic opioids.
- Prioritize analytical testing of seized drug samples obtained from drug overdose scenes during death investigations.
- Share data on synthetic opioid drug seizures with local health departments, medical examiners and coroners, and related communities.

Rapid NPS Testing Now Available:



February 2021

Positivity of New Synthetic Cannabinoid 4F-MDMB-BICA Increasing in U.S. as Prevalence of 5F-MDMB-PICA Wanes

Purpose: The objective of this public announcement is to notify public health and public safety, law enforcement, clinicians, medical examiners and coroners, laboratory personnel, drug treatment providers, and other related communities about new information surrounding the emergent synthetic cannabinoid **4F-MDMB-BICA**.

Background: Synthetic cannabinoids ("Spice" or "K2") are chemically manufactured drugs, often associated with unknown biological effects and health risks. Synthetic cannabinoids can be prepared (e.g. plant material, powder) and packaged in a variety of forms (e.g. foil packaging). Adverse effects reported in association with synthetic cannabinoid use include neurological abnormalities (e.g., psychosis, agitation, irritability, paranoia, confusion, anxiety, etc.), psychiatric episodes (e.g., hallucinations, delusions, self-harm, etc.), other physical ailments (e.g., tachycardia, hypertension, arrhythmia, chest pain, tachypnea, gastrointestinal distress, acute kidney injury, nausea, vomiting, fever, hyperglycemia, hypokalemia, sedation, etc.), and death.

Summary: 4F-MDMB-BICA was first identified in the United States (U.S.) in plant-like material seized by law enforcement in May 2020 and soon after in toxicology casework in July 2020, with concurrent emergence in European countries. 4F-MDMB-BICA is structurally similar to the synthetic cannabinoid **4F-MDMB-BINACA**, differing by an indole vs. indazole core, respectively. 4F-MDMB-BICA is an activator of the cannabinoid receptor system and its toxicity can be demonstrated through medicolegal death investigations paired with comprehensive toxicology findings. In the U.S., 4F-MDMB-BICA has been identified in at least 26 toxicology cases associated with postmortem (PM) and driving under the influence of drugs (DUID) investigations. In Europe, 4F-MDMB-BICA has been identified in several countries, including Hungary, the United Kingdom, Belgium, and Slovenia. Eleven deaths were attributed to the use of 4F-MDMB-BICA in Hungary between May and August 2020.

Case Type (PM, DUID, Unknown): 22, 3, 1

Age Range: <20, 21-30, 31-40, 41-50, 51+, N/A

Sex: M, F, N/A — 84%, 8%, 8%

4F-MDMB-BICA Geographical Distribution

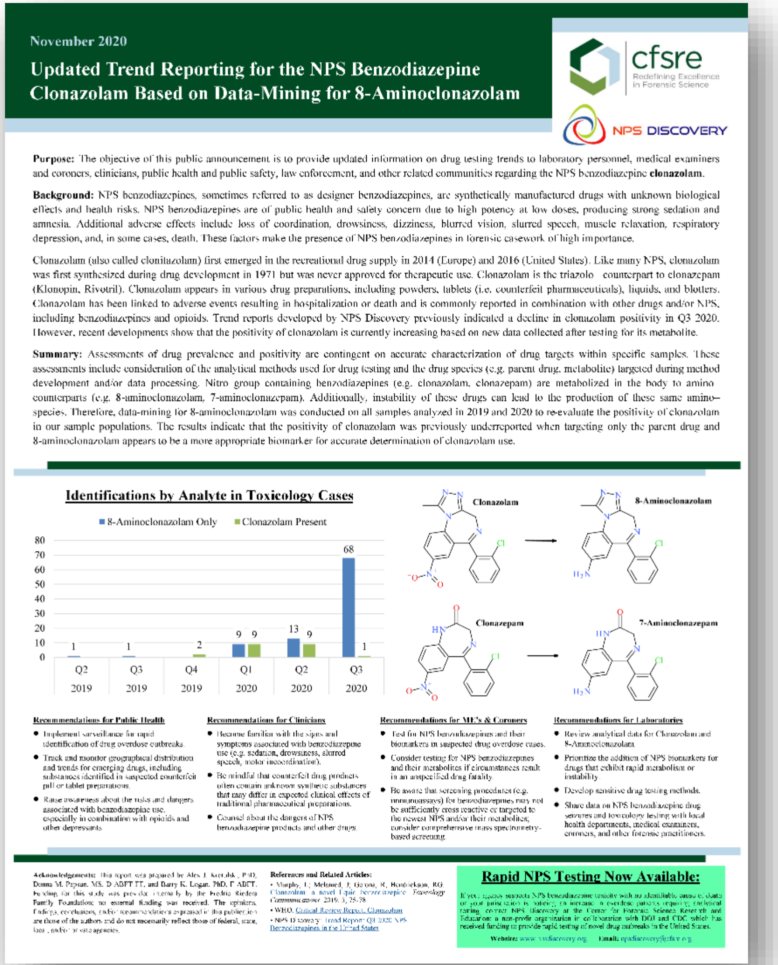
DE: 1, DC: 1

Structure of 4F-MDMB-BICA

4F-MDMB-BICA Toxicology Positivity

4F-MDMB-BICA Combinations	Occurrence
MDMB-4en-PINACA	10
4F-MDMB-BICA Only	7
MDMB-4en-PINACA & 5F-MDMB-PICA	6
5F-MDMB-PICA	3

Rapid NPS Testing Now Available:



November 2020

Updated Trend Reporting for the NPS Benzodiazepine Clonazolam Based on Data-Mining for 8-Aminoclonazolam

Purpose: The objective of this public announcement is to provide updated information on drug testing trends to laboratory personnel, medical examiners and coroners, clinicians, public health and public safety, law enforcement, and other related communities regarding the NPS benzodiazepine **clonazolam**.

Background: NPS benzodiazepines, sometimes referred to as designer benzodiazepines, are synthetically manufactured drugs with unknown biological effects and health risks. NPS benzodiazepines are of public health and public safety concern due to high potency at low doses, producing strong sedation and amnesia. Additional adverse effects include loss of coordination, drowsiness, dizziness, blurred vision, slurred speech, muscle relaxation, respiratory depression, and, in some cases, death. These factors make the presence of NPS benzodiazepines in forensic casework of high importance.

Clonazolam (also called clonitrazolam) first emerged in the recreational drug supply in 2014 (Europe) and 2016 (United States). Like many NPS, clonazolam was first synthesized during drug development in 1971 but was never approved for therapeutic use. Clonazolam is the triazolo counterpart to clonazepam (Klonopin, Rivotril). Clonazolam appears in various drug preparations, including powders, tablets (i.e. counterfeit pharmaceuticals), liquids, and blotters. Clonazolam has been linked to adverse events resulting in hospitalization or death and is commonly reported in combination with other drugs and/or NPS, including benzodiazepines and opioids. Trend reports developed by NPS Discovery previously indicated a decline in clonazolam positivity in Q3 2020. However, recent developments show that the positivity of clonazolam is currently increasing based on new data collected after testing for its metabolite.

Summary: Assessments of drug prevalence and positivity are contingent on accurate characterization of drug targets within specific samples. These assessments include consideration of the analytical methods used for drug testing and the drug species (e.g. parent drug, metabolite) targeted during method development and/or data processing. Nitro group containing benzodiazepines (e.g. clonazolam, clonazepam) are metabolized in the body to amino counterparts (e.g. 8-aminoclonazolam, 7-aminoclonazepam). Additionally, instability of these drugs can lead to the production of these same amino-species. Therefore, data-mining for 8-aminoclonazolam was conducted on all samples analyzed in 2019 and 2020 to re-evaluate the positivity of clonazolam in our sample populations. The results indicate that the positivity of clonazolam was previously underreported when targeting only the parent drug and 8-aminoclonazolam appears to be a more appropriate biomarker for accurate determination of clonazolam use.

Identifications by Analyte in Toxicology Cases

8-Aminoclonazolam Only; Clonazolam Present

Q2 2019, Q3 2019, Q4 2019, Q1 2020, Q2 2020, Q3 2020

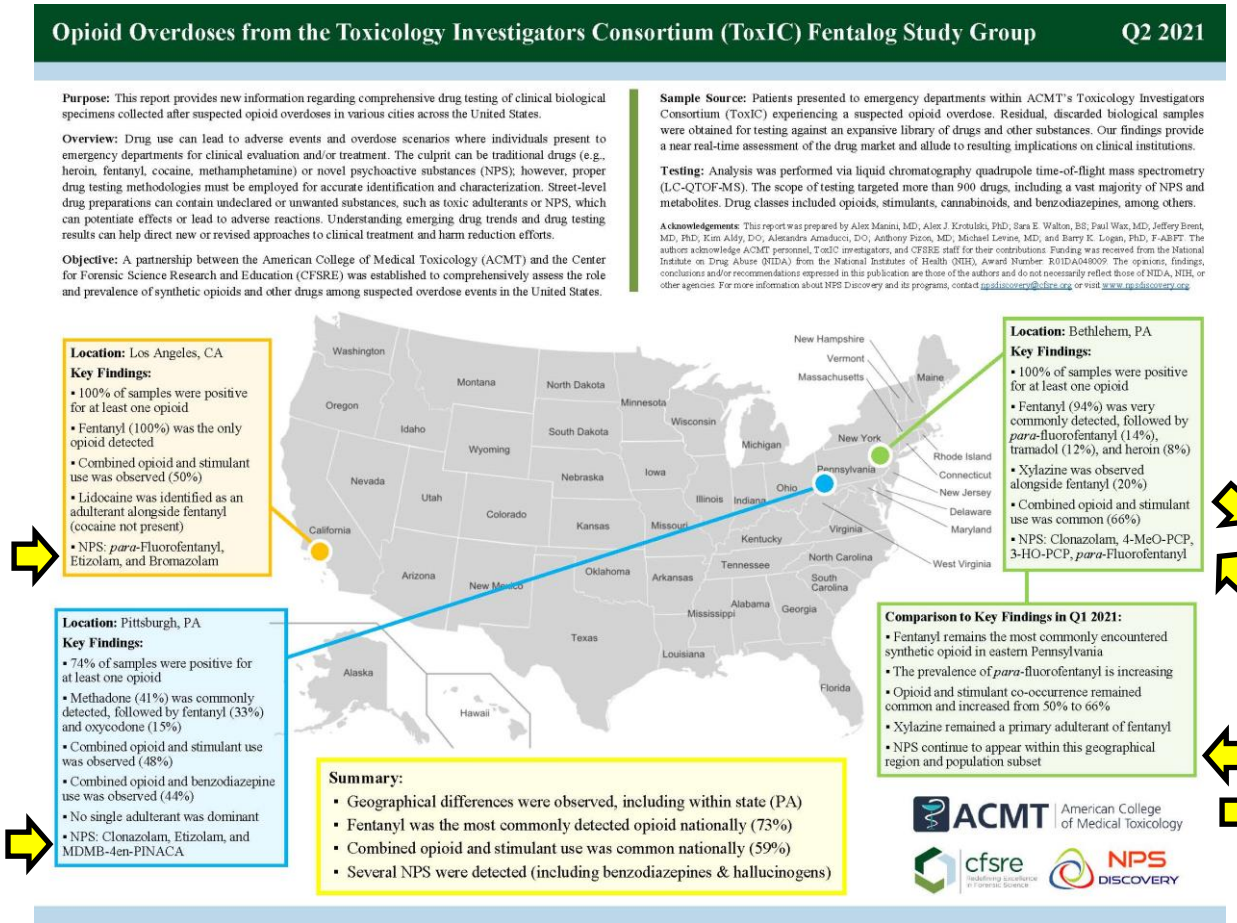
Clonazolam → 8-Aminoclonazolam

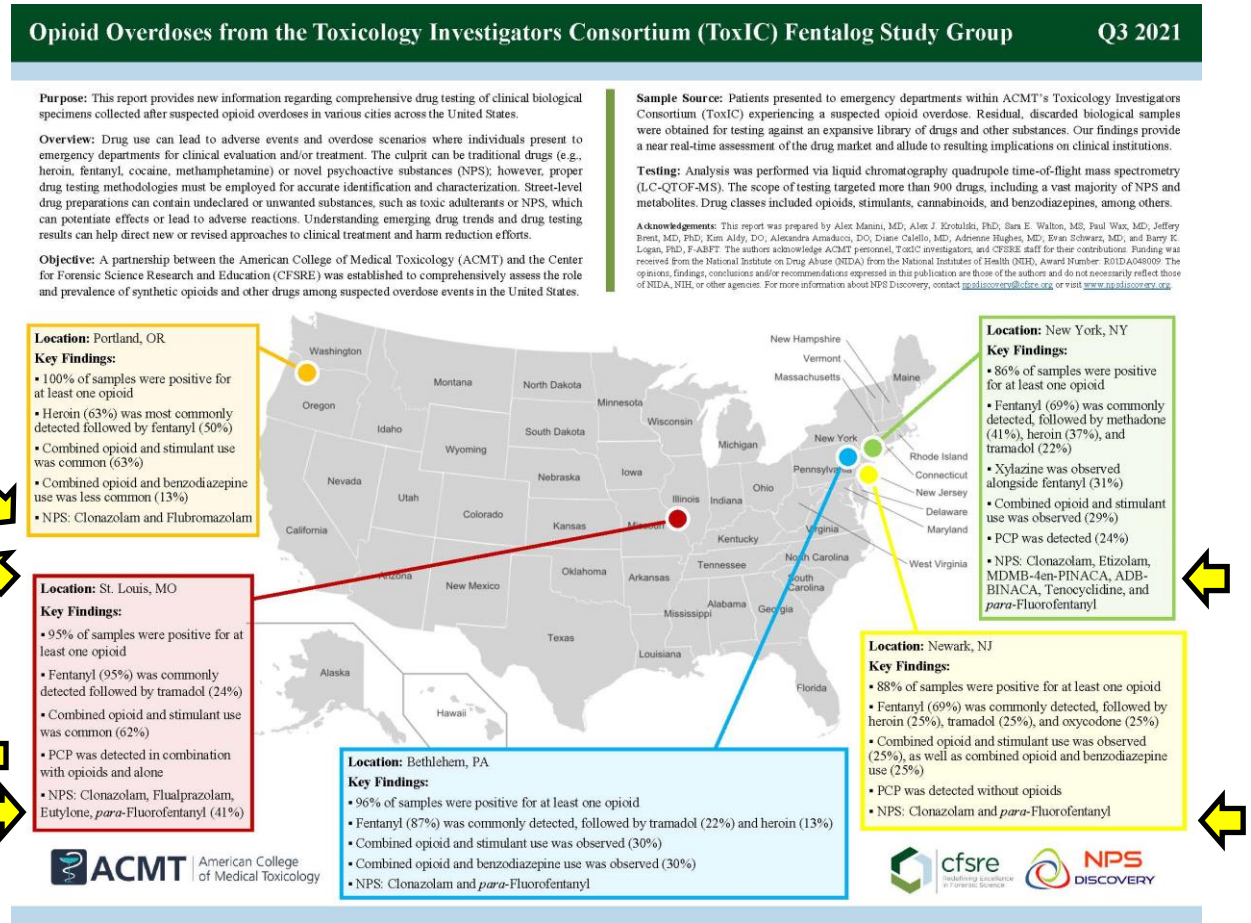
Clonazepam → 7-Aminoclonazepam

Rapid NPS Testing Now Available:

Coming soon: Protonitazene, Etodesnitazene, Bromazolam, & ADB-BINACA

Clinical Reports





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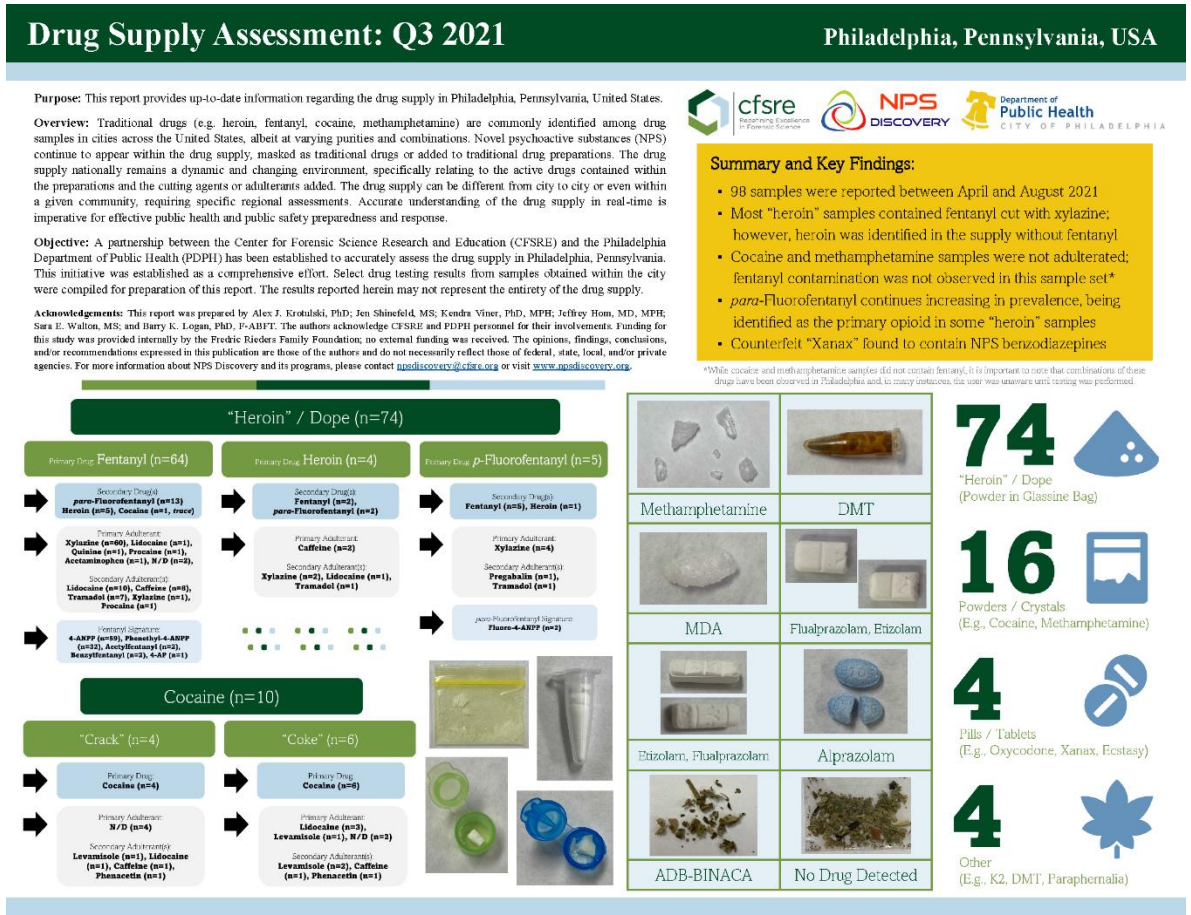
Drug Checking Reports



Drug Supply Assessment: Q3 2021 — **Philadelphia, Pennsylvania, USA**

Purpose: This report provides up-to-date information regarding the drug supply in Philadelphia, Pennsylvania, United States.

Overview: Traditional drugs (e.g. heroin, fentanyl, cocaine, methamphetamine) are commonly identified among drug samples in cities across the United States, albeit at varying purities and combinations. Novel psychoactive substances (NPS) continue to appear within the drug supply, masked as traditional drugs or added to traditional drug preparations. The drug supply nationally remains a dynamic and changing environment, specifically relating to the active drugs contained within the preparations and the cutting agents or adulterants added. The drug supply can be different from city to city or even within a given community, requiring specific regional assessments. Accurate understanding of the drug supply in real-time is imperative for effective public health and public safety preparedness and response.

Objective: A partnership between the Center for Forensic Science Research and Education (CFSRE) and the Philadelphia Department of Public Health (PDPH) has been established to accurately assess the drug supply in Philadelphia, Pennsylvania. This initiative was established as a comprehensive effort. Select drug testing results from samples obtained within the city were compiled for preparation of this report. The results reported herein may not represent the entirety of the drug supply.

Acknowledgements: This report was prepared by Alex J. Krotulski, PhD; Jen Shinefeld, MS; Kendra Viner, PhD, MPH; Jeffrey Hom, MD, MPH; Sara E. Walton, MS; and Barry K. Logan, PhD, F-ABFT. The authors acknowledge CFSRE and PDPH personnel for their involvements. Funding for this study was provided internally by the Fredric Rieders Family Foundation; no external funding was received. The opinions, findings, conclusions, and/or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of federal, state, local, and/or private agencies. For more information about NPS Discovery and its programs, please contact npsdiscovery@cfsre.org or visit www.npsdiscovery.org.

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Summary and Key Findings:

- 98 samples were reported between April and August 2021
- Most "heroin" samples contained fentanyl cut with xylazine; however, heroin was identified in the supply without fentanyl
- Cocaine and methamphetamine samples were not adulterated; fentanyl contamination was not observed in this sample set*
- *para*-Fluorofentanyl continues increasing in prevalence, being identified as the primary opioid in some "heroin" samples
- Counterfeit "Xanax" found to contain NPS benzodiazepines

*While cocaine and methamphetamine samples did not contain fentanyl, it is important to note that combinations of these drugs have been observed in Philadelphia and, in many instances, the user was unaware until testing was performed.

"Heroin" / Dope (n=74)

Primary Drug: Fentanyl (n=64) — Secondary Drug(s): *para*-Fluorofentanyl (n=13), Heroin (n=5), Cocaine (n=1, *trace*); Primary Adulterant: Xylazine (n=60), Lidocaine (n=1), Quinine (n=1), Procaine (n=1), Acetaminophen (n=1), N/D (n=2); Secondary Adulterant(s): Lidocaine (n=10), Caffeine (n=8), Tramadol (n=7), Xylazine (n=1), Procaine (n=1); Fentanyl Signature: 4-ANPP (n=59), Phenethyl-4-ANPP (n=32), Acetylfentanyl (n=2), Benzylfentanyl (n=2), 4-AP (n=1)

Primary Drug: Heroin (n=4) — Secondary Drug(s): Fentanyl (n=2), *para*-Fluorofentanyl (n=2); Primary Adulterant: Caffeine (n=2); Secondary Adulterant(s): Xylazine (n=2), Lidocaine (n=1), Tramadol (n=1)

Primary Drug: *p*-Fluorofentanyl (n=5) — Secondary Drug(s): Fentanyl (n=5), Heroin (n=1); Primary Adulterant: Xylazine (n=4); Secondary Adulterant(s): Pregabalin (n=1), Tramadol (n=1); *para*-Fluorofentanyl Signature: Fluoro-4-ANPP (n=2)

Cocaine (n=10)

"Crack" (n=4) — Primary Drug: Cocaine (n=4); Primary Adulterant: N/D (n=4); Secondary Adulterant(s): Levamisole (n=1), Lidocaine (n=1), Caffeine (n=1), Phenacetin (n=1)

"Coke" (n=6) — Primary Drug: Cocaine (n=6); Primary Adulterant: Lidocaine (n=3), Levamisole (n=1), N/D (n=2); Secondary Adulterant(s): Levamisole (n=2), Caffeine (n=1), Phenacetin (n=1)

Methamphetamine	DMT
MDA	Flualprazolam, Etizolam
Etizolam, Flualprazolam	Alprazolam
ADB-BINACA	No Drug Detected

74 "Heroin" / Dope (Powder in Glassine Bag)

16 Powders / Crystals (E.g., Cocaine, Methamphetamine)

4 Pills / Tablets (E.g., Oxycodone, Xanax, Ecstasy)

4 Other (E.g., K2, DMT, Paraphernalia)

MDA	Flualprazolam, Etizolam
Etizolam, Flualprazolam	Alprazolam
ADB-BINACA	No Drug Detected

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NPS Scope Recommendations

Recommended Scope for NPS Testing in the United States Q3 2021

Purpose: The objective of this report is to provide updated guidance in developing an appropriate analytical scope of testing for novel psychoactive substances (NPS) in the United States based on current trends and intelligence. *This report is based on information available in Q3 2021 and is subject to change along with the drug market.*

Summary: The NPS landscape is changing rapidly, requiring laboratories to constantly remain abreast of new and emerging drugs locally, nationally, and internationally. To meet individualized needs, laboratories amend existing methods or develop new ones for detection and confirmation. This can be challenging for scientists as information about NPS detections can be regionalized and/or out-of-date, making it difficult to determine which drugs should be prioritized at a given time. **NPS Discovery** and the **SOFT Designer Drugs Committee** have established the below recommendations for NPS scope based on information from extensive collaborations, partnerships, and initiatives which yield national perspectives. Suggested cut-off concentrations or reporting limits (in ng/mL) are listed for each NPS. These values were categorized (i.e., <1, 1-10, and >10 ng/mL) and determined based on currently available quantitative data and/or comparison to structurally similar NPS within the given sub-class.

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NPS DISCOVERY

Benzodiazepines		Opioids		Stimulants & Hallucinogens		Synthetic Cannabinoids	
TIER ONE *(STRONGLY RECOMMEND)*							
Etizolam†	1-10	2-Methyl AP-237	>10	Eutylone	>10	MDMB-4en-PINACA	<1
Clonazolam	<1	*N*-Pyrrolidino Etonitazene	<1	↑*N,N*-Dimethyl Pentylone	>10	ADB-BINACA (-BUTINACA)	<1
8-Aminoclonazolam	1-10	Metonitazene	<1	alpha-PHP / alpha-PiHP	>10	4F-MDMB-BICA	<1
Flualprazolam	1-10	↑Protonitazene	<1	↑2F-Deschloroketamine	<1	5F-MDMB-PICA	<1
Flubromazolam	1-10	*o/m/p*-Fluorofentanyl	1-10	3-HO-PCP / 4-HO-PCP	<1		
TIER TWO *(RECOMMEND)*							
Bromazolam	1-10	↑Etodesnitazene	1-10	↓3Cl-PCP / 4Cl-PCP	<1		
↑Flubromazepam	1-10	↓Carfentanil	<1	3-MeO-PCP / 4-MeO-PCP	<1		
N-Desalkylflurazepam†	1-10	Isotonitazene	<1	3-HO-PCE / 4-HO-PCE	<1		
Deschloroetizolam	1-10	↑*o/m/p*-Chlorofentanyl	1-10	3-MeO-PCE / 4-MeO-PCE	<1		
TIER THREE *(CONSIDER)*							
Meclonazepam	1-10	↓AP-238	>10	Deschloroketamine	<1		
Adinazolam†	1-10	↓Brorphine	<1	↑Pentylone	<1		
↑Metizolam	1-10	↑Butonitazene	1-10	↑Methylenedioxy-PV8	<1		
↓Pyrazolam	1-10	↑Metodesnitazene	1-10				

Note: This may not be an all-inclusive list. Laboratories should consider additional NPS for inclusion (or exclusion) based on local, national, and/or international trends.

Learn More About NPS Discovery:

Benzodiazepines		Opioids		Stimulants & Hallucinogens		Synthetic Cannabinoids	
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TIER TWO *(RECOMMEND)*							
Bromazolam	1-10	↑Etodesnitazene	1-10	↓3Cl-PCP / 4Cl-PCP	<1	↑5F-BZO-POXIZID	<1
↑Flubromazepam	1-10	↓Carfentanil	<1	3-MeO-PCP / 4-MeO-PCP	<1	↑BZO-POXIZID	<1
N-Desalkylflurazepam†	1-10	Isotonitazene	<1	3-HO-PCE / 4-HO-PCE	<1	↑BZO-HEXOXIZID	<1
Deschloroetizolam	1-10	↑*o/m/p*-Chlorofentanyl	1-10	3-MeO-PCE / 4-MeO-PCE	<1	↑5F-AB-PFUPPYCA	<1
TIER THREE *(CONSIDER)*							
Meclonazepam	1-10	↓AP-238	>10	Deschloroketamine	<1	↑5F-EDMB-PINACA	<1
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↑Metizolam	1-10	↑Butonitazene	1-10	↑Methylenedioxy-PV8	<1	↓4F-ABINACA	<1
↓Pyrazolam	1-10	↑Metodesnitazene	1-10	↑4-HO-DiPT	<1	ADB-HEXINACA	<1

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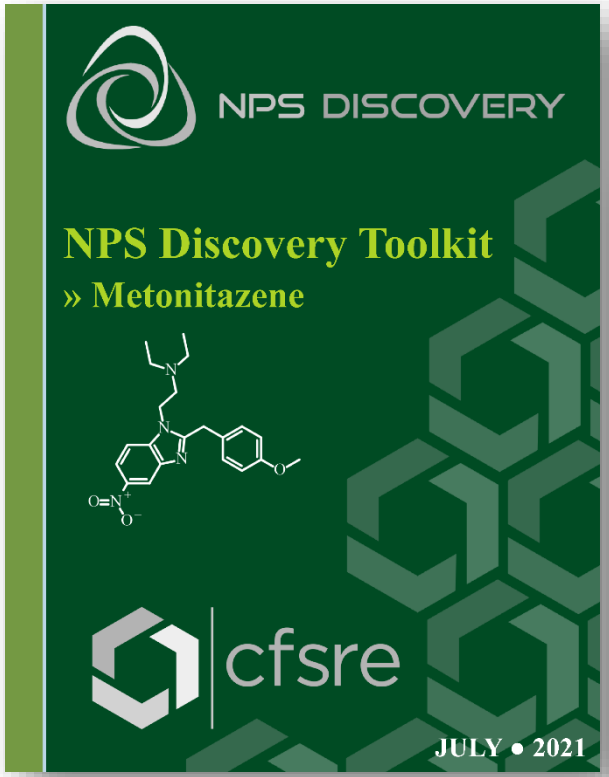
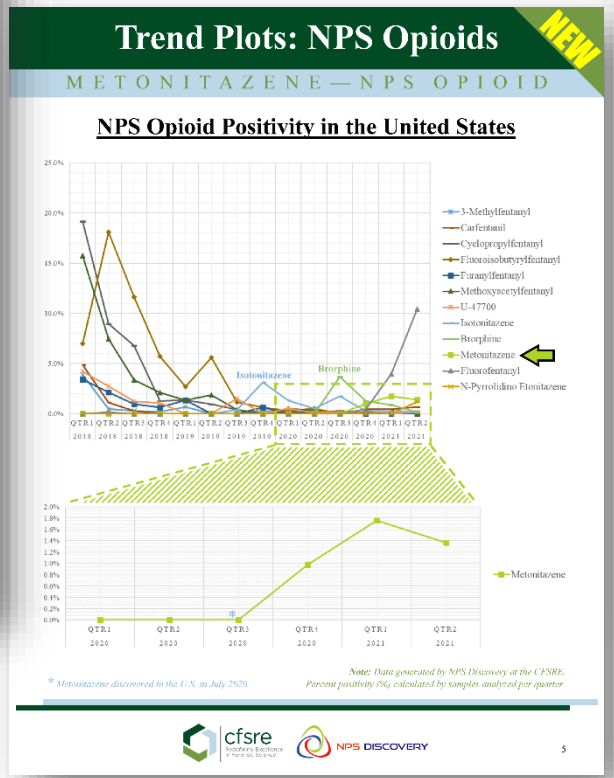
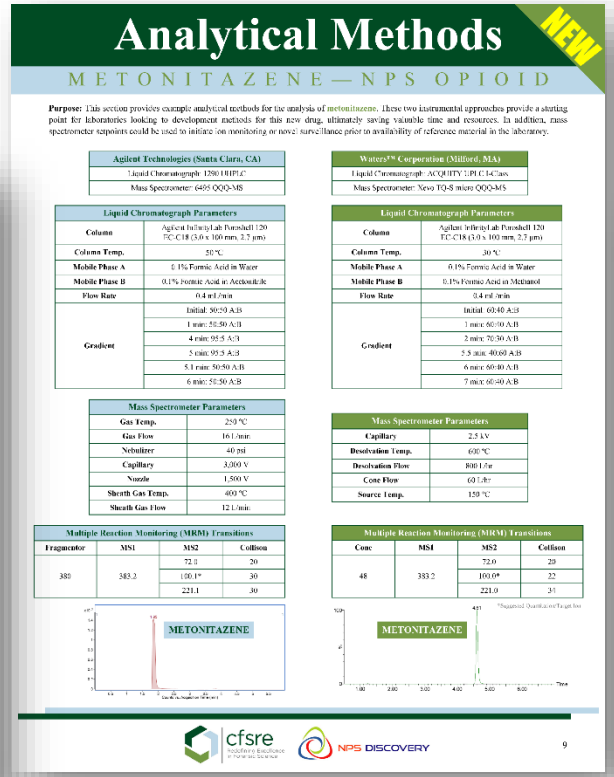
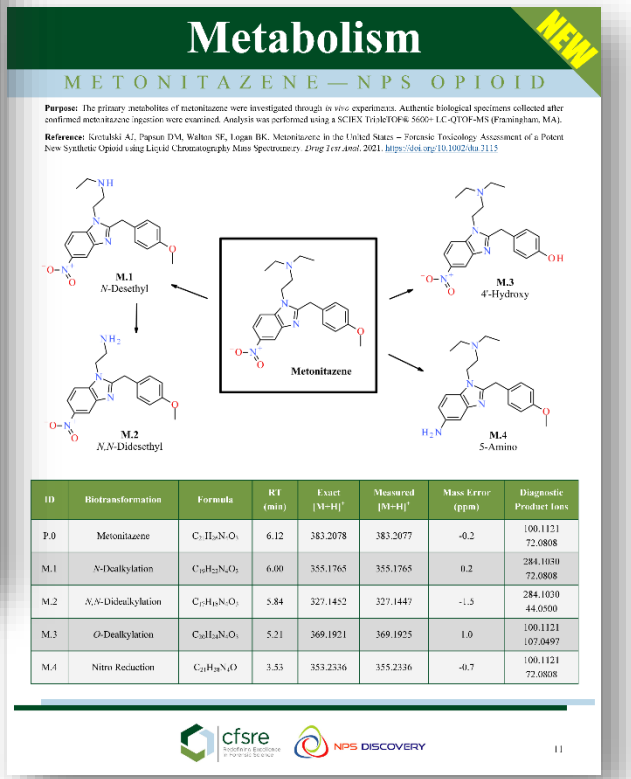
NPS Discovery Toolkits









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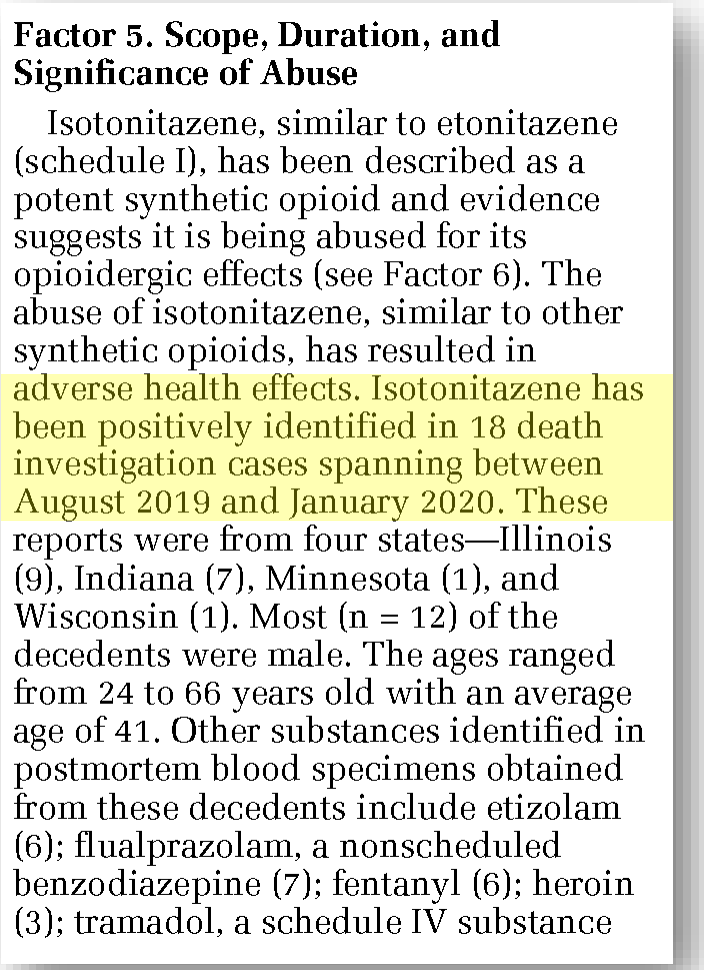
Value of Open Access Drug Early Warning System

- Readily available information can:
  - Build greater understanding of **drug markets, drug trends, and use patterns**, etc.
  - Assist medical examiners and coroners (and toxicologists) determining **cause and manner of death**
  - Assist clinicians in understanding **sign, symptoms, and care**
  - Allow for **scheduling / control** of new synthetic drugs →
  - Allow people who use drugs to **make more informed decisions** and promote harm reduction
  - Steer future **NPS, scientific, and medical research**
  - *And so much more …*



Factor 5. Scope, Duration, and Significance of Abuse

Isotonitazene, similar to etonitazene (schedule I), has been described as a potent synthetic opioid and evidence suggests it is being abused for its opioidergic effects (see Factor 6). The abuse of isotonitazene, similar to other synthetic opioids, has resulted in adverse health effects. Isotonitazene has been positively identified in 18 death investigation cases spanning between August 2019 and January 2020. These reports were from four states—Illinois (9), Indiana (7), Minnesota (1), and Wisconsin (1). Most (n = 12) of the decedents were male. The ages ranged from 24 to 66 years old with an average age of 41. Other substances identified in postmortem blood specimens obtained from these decedents include etizolam (6); flualprazolam, a nonscheduled benzodiazepine (7); fentanyl (6); heroin (3); tramadol, a schedule IV substance

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  - Sherri Kacinko
  - Carolina Noble
- **Medical Examiner and Coroner Partners**
- **Public Health Departments**
- **ACMT and Clinical Partners**
- **National Institute of Justice (NIJ/DOJ)**
- **National Institute on Drug Abuse (NIH)**

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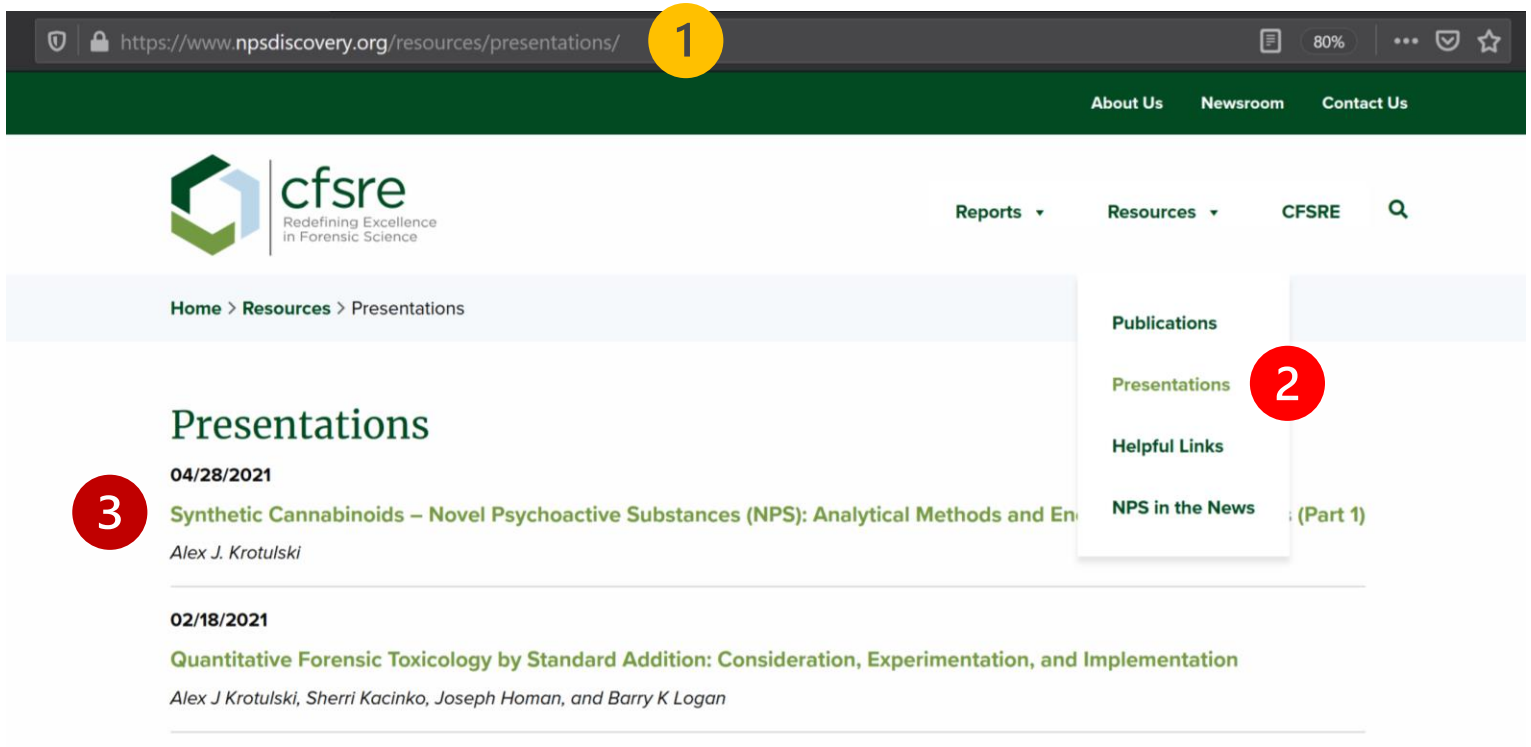

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