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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}

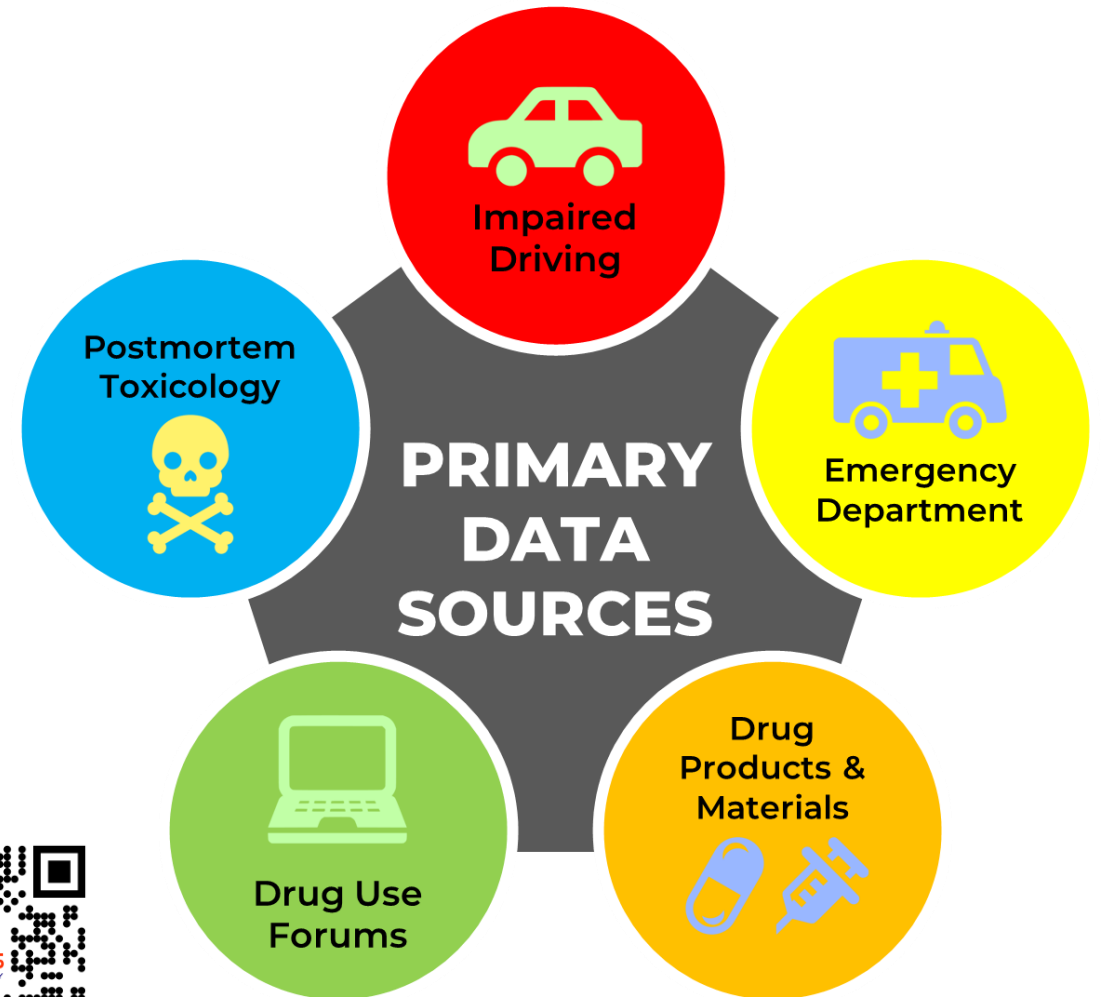
*¹Center for Forensic Science Research and Education, Fredric Rieders Family Foundation, Willow Grove, PA, ²NMS Labs, Horsham, PA
The VIII International Conference on Novel Psychoactive Substances (NPS) – Wednesday November 17, 2021 (Virtual)*

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

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



Timeline of Our NPS Discovery Program

2017

- Development of LC-QTOF-MS assay for >400 drugs (including many NPS)
- Began charactering NPS using GC-MS, LC-QTOF-MS, and/or NMR workflows



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2018

- Development and dissemination of first new drug monograph for NPS
- **Formally launched our NPS Discovery program**



NMS
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

HUPAC Name: 2-[[2-(4-ethyl-2,5-dimethoxy-phenyl)ethylamino]methyl]phenol
InChI String: InChI=1SC1=CC=C(C=C1)C=C(C=C1)N(CCC2=CC=C(C=C2)OC)C3=CC=C(C=C3)OC

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 ₁₈ H ₂₁ 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. Krasinski, M.S., Melissa F. Figgery, M.S., and Barry K. Logan, PhD, F-ABFT

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CFR: Not Scheduled (02/2018)
CAS#: Not available
Synonyms: NBOW, 25-E
Source: Department of Homeland Security
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 Prepared By: Alex J. Kozicki, M.S., Melissa F. Fogarty, M.S., and Barry K. Logan, Ph.D., ABFT

January 2019
New Synthetic Cannabinoid: 4F-MDMB-BINACA

Summary: 4F-MDMB-BINACA, first identified as a novel drug compound 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 (DUI/DWI) investigations. 4F-MDMB-BINACA is a very similar in structure to the synthetic cannabinoid 4F-ADB (4F-MDMB-PMAC), differing by the removal of one methoxy (-OCH₃) bridge from the carbon chain at the para position of the molecule. 4F-ADB has been associated with a high number of adverse events, including death. The pharmacology and toxicity of 4F-MDMB-BINACA has not been explicitly studied, but in relation to 4F-ADB and associated with drug use, acute local professional to believe the new synthetic cannabinoid could be an active novel psychoactive substance (NPS) and cause the potential to cause adverse events.

Background: Synthetic cannabinoids ("Spice" or "K2") are chemically synthesized drugs, often associated with unknown biological effects and health risks, a dangerous combination for any recreational drug use. Synthetic cannabinoids are produced as a final material product and packaged for sale and packaging for a variety of uses. Recently, synthetic cannabinoids have been identified in combination with more traditional drug supplies, including the heroin supply in Philadelphia. This is a concern that has to move the 100 drug monitoring to its only use not isolated from the drug combination 4F-ADB, heroin, and heroin. Adverse effects reported in association with synthetic cannabinoids are: tachycardia, hypertension, anxiety, panic, psychosis, aggression, irritability, paranoia, confusion, anxiety, and a proclivity to overdose (e.g., hallucinations, delirium, self-harm, etc.). Other physical effects include: tachycardia, hypertension, arrhythmia, chest pain, sedation, gastrointestinal distress, acute kidney injury, nausea, vomiting, blood hypertension/hypotension, etc. and death.

Recommendations for Public Health:

- Enforce controls for rapidly identifying drug
- Issue with evidence of potential adverse events
- Issue with evidence of potential adverse events
- Issue with evidence of potential adverse events
- Issue with evidence of potential adverse events
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Recommendations for Clinicians:

- Be aware that 4F-MDMB-BINACA has been identified in combination with more traditional drug supplies, including the heroin supply in Philadelphia.
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Recommendations for NPS & Cappers:

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Recommendations for Laboratories:

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- 2020**
 - Expanded trend reporting to include all five major subclasses of NPS
 - Continued data collections through onset of COVID-19 pandemic



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Background: Synthetic cannabinoids ("Spice" or "K2") are chemically synthesized drugs, often associated with unknown biological effects and health risks, a dangerous combination for any recreational drug use. Synthetic cannabinoids are produced as a solid material, packaged and sold in packaging for a variety of uses. Recently, synthetic cannabinoids have been identified in combination with more traditional drug supplies, including the heroin supply in Philadelphia. This is a concern that has led to more than 100 drug seizures in the city over one week from the drug combination 4F-AOH, heroin, and heroin. Adverse effects reported in association with synthetic cannabinoids are: tachycardia, hypertension, hyperlocomotion (e.g., parkinson, agitation, instability, paranoia, confusion, anxiety, etc.), psychosis (paranoia, e.g., hallucinations, delusions, self-harm, etc.), other physical symptoms (e.g., tachycardia, hypertension, arrhythmia, chest pain, sweating, gastrointestinal distress, acute kidney injury, tremor, vomiting, acute hypoglycemia, hypotension, etc.) and death.

Recommendations for Public Health:

- Enforce methods for rapidly identifying drug and monitor substances.
- Limit distribution of packaged substances.
- Block all sources of packaged drug supply.
- Track distribution and source risk factors.
- Be flexible and adaptable.
- Be aware that drug supply has changed rapidly and continuously.
- Be aware that drug supply has changed rapidly and continuously.
- Develop public health messaging about synthetic cannabinoids.

Recommendations for Clinicians:

- Be aware that drug supply has changed rapidly and continuously.
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Recommendations for NPS & Customs:

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Recommendations for Laboratories:

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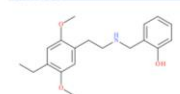
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Background: Synthetic cannabinoids ("Spice" or "K2") are chemically synthesized drugs, often associated with unknown biological effects and health risks. A dangerous combination for any recreational drug use: Synthetic cannabinoids are produced as a solid material, produced and packaged for a variety of uses. Recently, synthetic cannabinoids have been identified in combination with more traditional drug supplies, including the heroin supply in Philadelphia. This is a concern that led to more of the 100 drug seizures in the city over one weekend from the drug combination 4F-ADB, heroin, and heroin. Adverse effects reported in association with synthetic cannabinoids are: tachycardia, hypertension (e.g., tachycardia, agitation, irritability, paranoia, confusion, anxiety, or a protracted episode (e.g., hallucinations, delirium, self-harm, or a other physical symptoms (e.g., tachycardia, hypertension, arrhythmia, chest pain, seizures, gastrointestinal distress, acute kidney injury, severe vomiting, acute hypoxemic hypoxemia, etc.) and death.

Recommendations for Public Health

- Enforce methods for rapidly identifying drug and monitor substances.
- Limit distribution of seized substances.
- Block all routes of acquisition of drug supply.
- Track distribution and source risk factors for distribution and supply points.
- Take measures about the risks and dangers associated with synthetic cannabinoids.
- Develop public health messaging about synthetic cannabinoids.

Recommendations for Clinicians


- Be aware that this drug has been linked to acute cardiac symptoms, including tachycardia, hypertension, and other symptoms that may be associated with synthetic cannabinoids.
- Consider about the dangers of synthetic cannabinoids and other drugs.

Recommendations for NPS & Customs

- Use the new synthetic cannabinoids and their precursors as reported synthetic cannabinoids.
- Consider testing for synthetic cannabinoids if substances are not reported drug supply.
- Be aware that this drug has been linked to acute cardiac symptoms, including tachycardia, hypertension, and other symptoms that may be associated with synthetic cannabinoids.
- Develop analytical testing of seized drug supply.
- Be aware that this drug has been linked to acute cardiac symptoms, including tachycardia, hypertension, and other symptoms that may be associated with synthetic cannabinoids.

Recommendations for Laboratories

- Verify analytical data results specific to the identification of 4F-MDMB-BINACA and other synthetic cannabinoids.
- Consider testing for synthetic cannabinoids if substances are not reported drug supply.
- Be aware that this drug has been linked to acute cardiac symptoms, including tachycardia, hypertension, and other symptoms that may be associated with synthetic cannabinoids.



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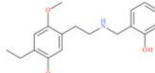
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- 2022**
 - Many more opportunities to come !!!



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Prepared By: Alex J. Kozicki, MPh, Melissa F. Fogarty, MPh, and Barry K. Logan, PhD, F-RFT

January 2019
New Synthetic Cannabinoid: 4F-MDMB-BINACA

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

Summary: 4F-MDMB-BINACA, first identified as a novel drug compound 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 drug (DUI/DWI) investigations. 4F-MDMB-BINACA is a very similar in structure to the synthetic cannabinoid 4F-ACB (4F-MDMB-Phacnac), differing by the presence of one carbon (C1) bridge from the carbon chain at the para position of the molecule. 4F-MDMB has been associated with a high number of adverse events, including death. The pharmacology and toxicity of 4F-MDMB-BINACA has not been explicitly studied, but in relation to 4F-MDMB and associated with drug use, acute local professionals to be alert for new synthetic cannabinoid users to be active and proactive substances (NPS) and assess the potential to cause adverse events.

Background: Synthetic cannabinoids ("Spice" or "K2") are chemically synthesized drugs, often associated with unknown biological effects and health risks, a dangerous combination for any recreational drug user. Synthetic cannabinoids are produced as a final material, packaged and sold in a variety of forms. Recently, synthetic cannabinoids have been identified in combination with more traditional drug supplies, including the heroin supply in Philadelphia. This is a concern that led to more of the 100 drug seizures in the city over one week from the drug combination 4F-ACB, Heroin, and heroin. Adverse effects reported in association with synthetic cannabinoids are: tachycardia, hypertension (e.g., tachycardia, agitation, irritability, paranoia, confusion, anxiety, or a profuse dry mouth (e.g., hallucinations, delusions, self-harm, or a) other physical effects (e.g., tachycardia, hypertension, arrhythmia, chest pain, seizures, gastrointestinal distress, acute kidney injury, severe vomiting, acute hypoglycemia/hyperkalemia, etc.) and death.

Recommendations for Public Health

- Explain methods for rapidly identifying drug and/or synthetic substances
- Limit use of synthetic cannabinoids
- Avoid all contact of synthetic drug users
- Track, document, and report risk factors
- Educate and monitor patients
- Make awareness about the risks and dangers associated with synthetic cannabinoids
- Develop public health messaging about synthetic cannabinoids

Recommendations for Clinicians

- Be aware of the risks and dangers associated with synthetic cannabinoids
- Consider abuse by danger of synthetic cannabinoids and other drugs
- Be aware of the risks and dangers associated with synthetic cannabinoids
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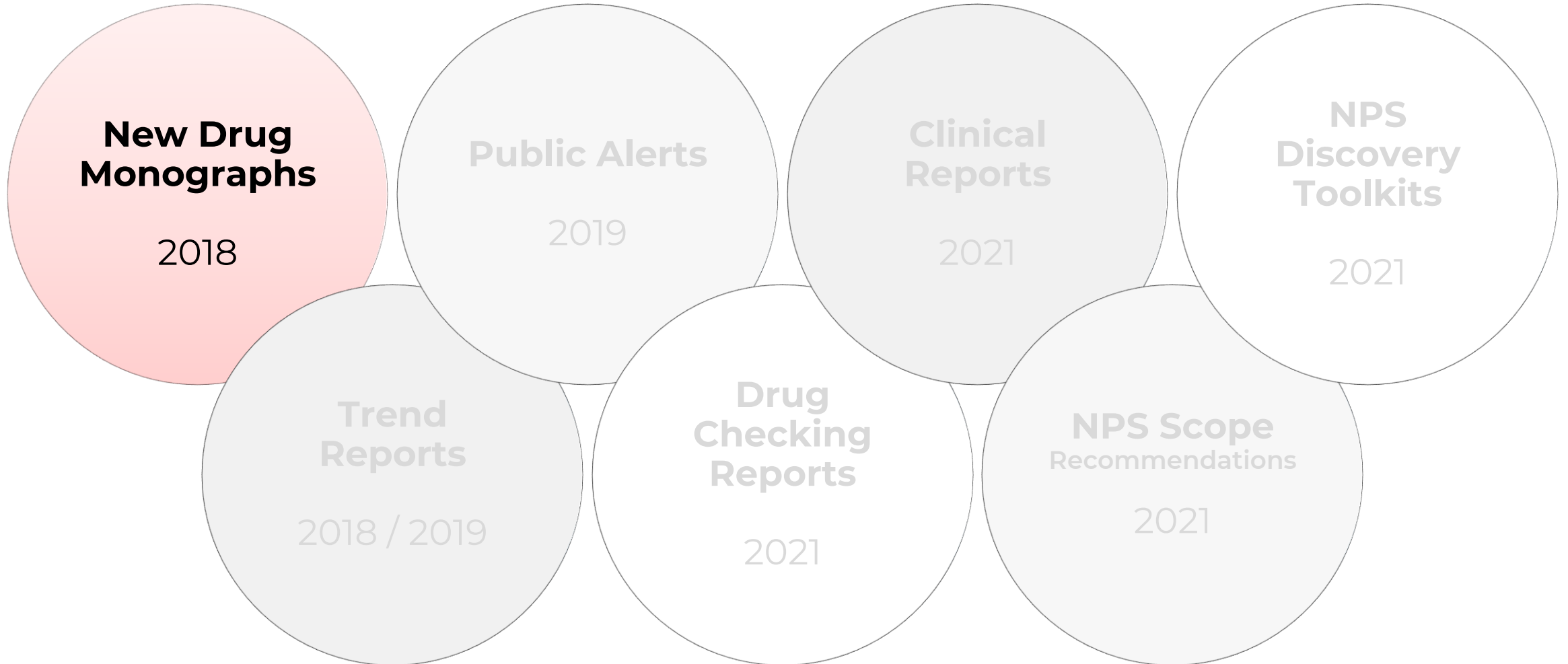
Recommendations for Law Enforcement

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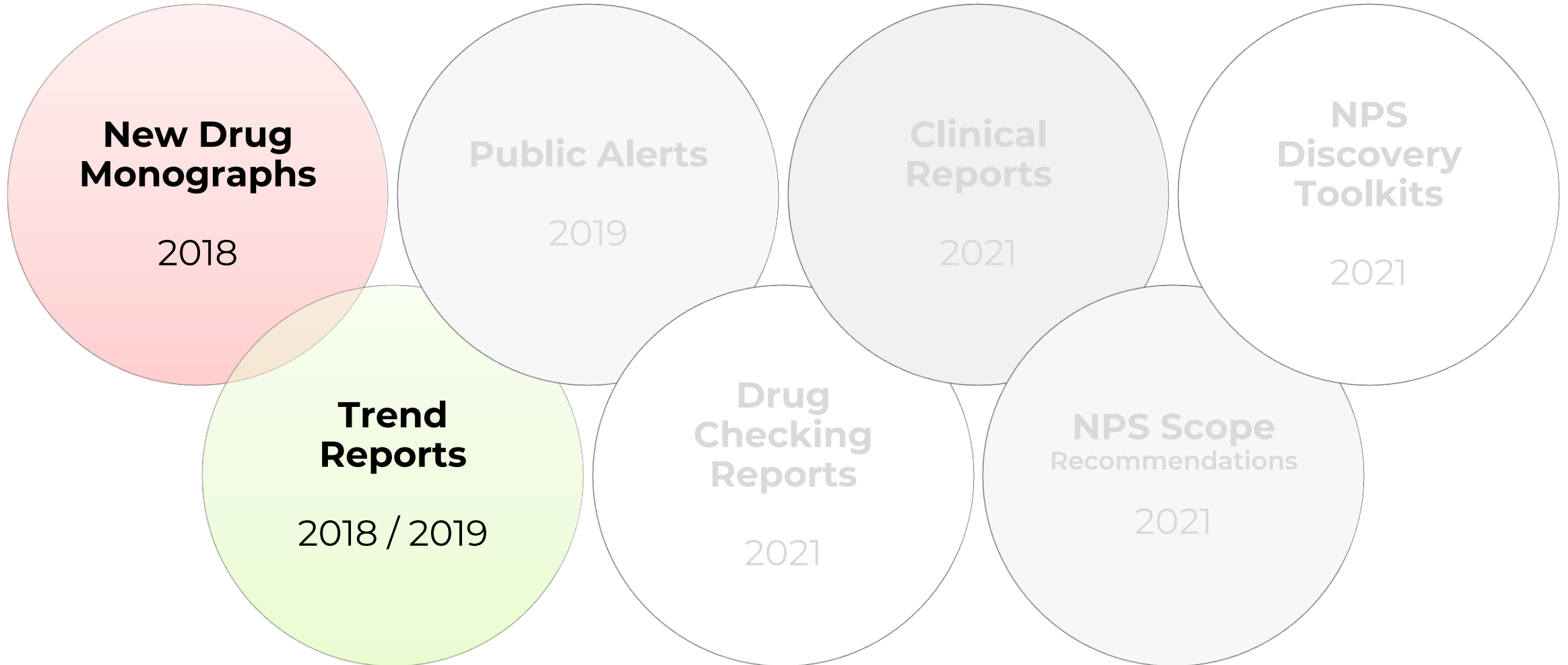
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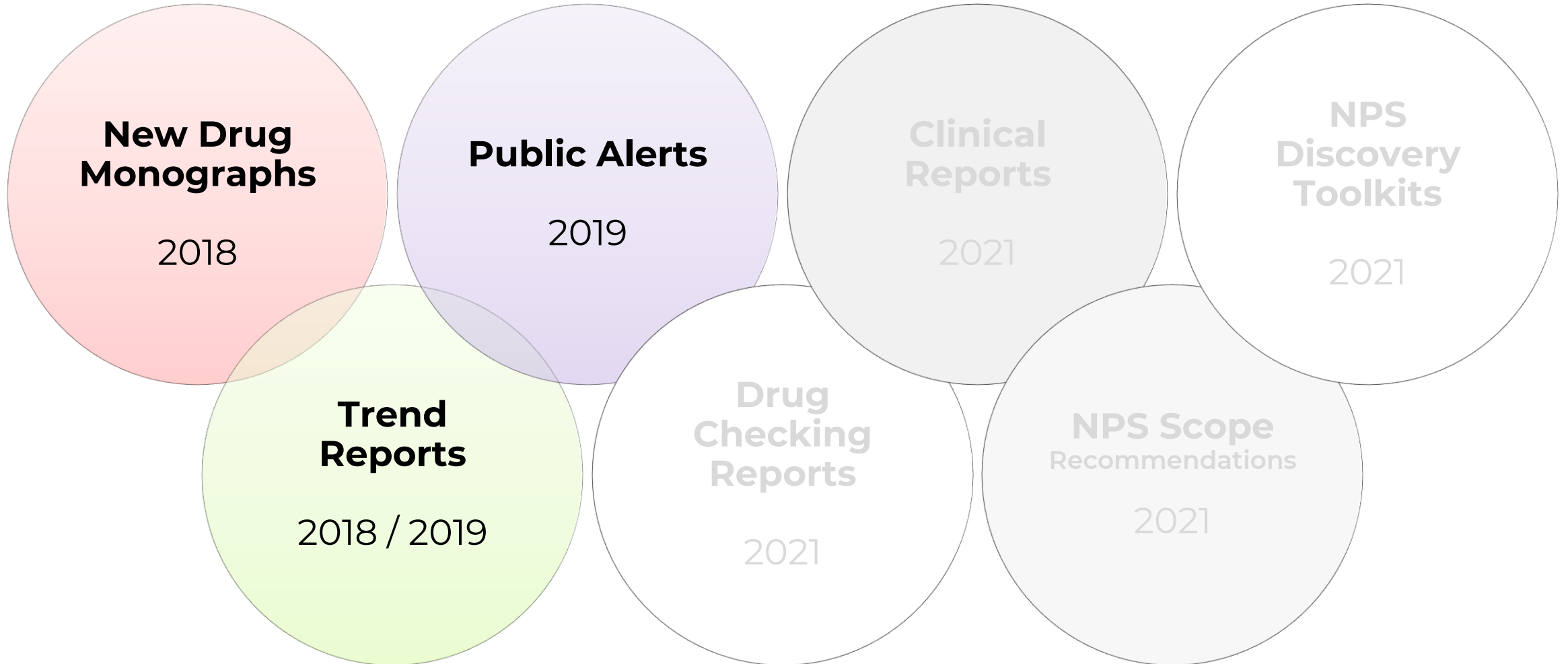
Evolution of NPS Discovery Reports



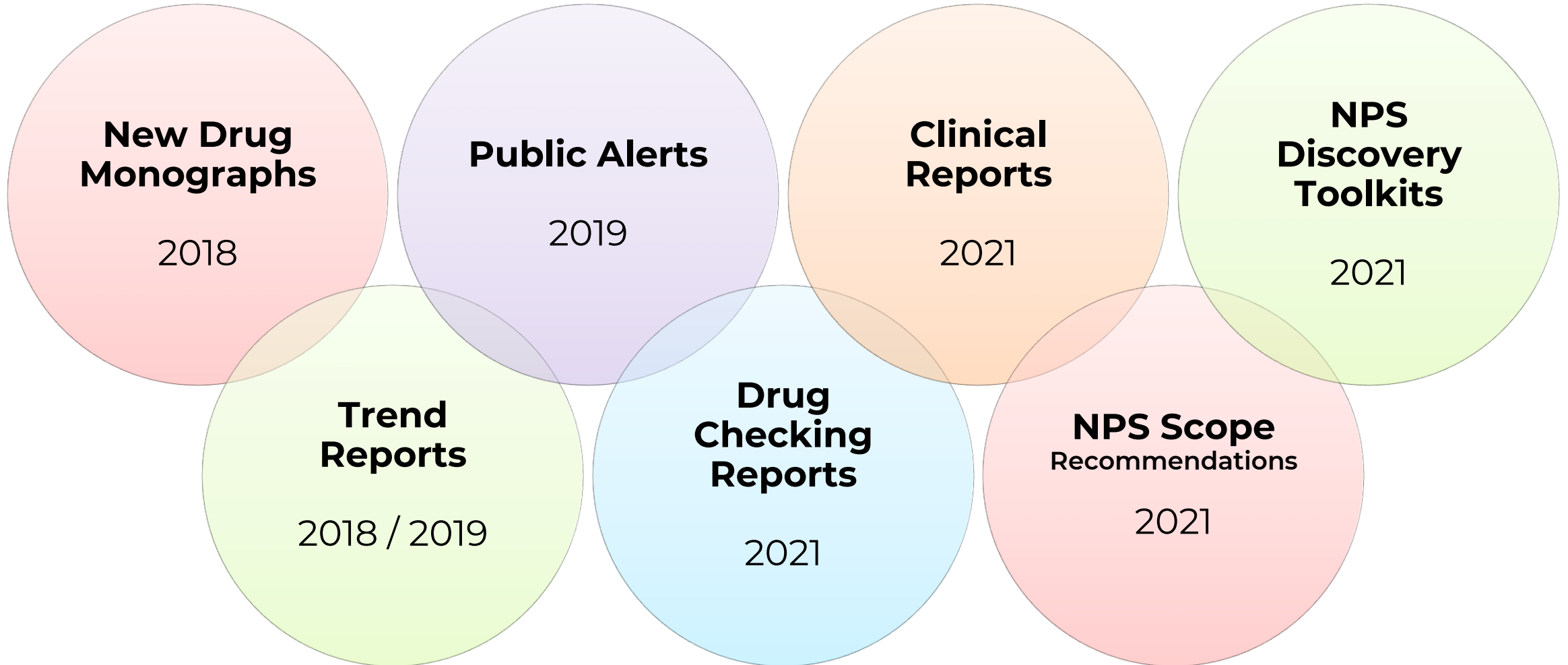
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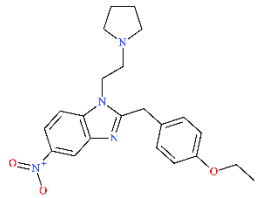
RESULTS & OUTCOMES



New Drug Monographs



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/h5-10,16,11,2-4,11-15H2,11H3

CFR: Not Scheduled (05/2021)

CAS# Not Available

Synonyms: Etonitazepine

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. Papsan, MS; D-ABFT-FT: Melissa F. Fogarty, MSFS; D-ABFT-FT; and Barry K. Logan, PhD, F-ABFT

2. CHEMICAL DATA

Analyte	Chemical Formula	Molecular Weight	Molecular Ion [M] ⁺	Exact Mass [M+H] ⁺
N-Pyrrolidino Etonitazene	C ₂₂ H ₂₆ N ₄ O 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: Flualprazolam (n=1), Fentanyl (n=1), Methamphetamine (n=1)

4. BRIEF DESCRIPTION

N-Pyrrolidino Etonitazene is classified as a novel opioid of the benzimidazole subclass and is structurally dissimilar from fentanyl. Novel opioids have been reported to cause psychotropic 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 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.

5. ADDITIONAL RESOURCES

- Heringer, A; Kehlitz, J; Rossi, A; Hoffmann, K. (1957) Synthesis of analgetically active benzimidazole derivatives with basic substituents. *Experimentia*, 13, 400-401. <https://doi.org/10.1007/BF02951436>
- Hoffmann, K; Hager, A; Rossi, A. 13 May 1959. "Patent US2951144 - Barbitindazole." <https://www.uspto.gov/patent/publications/US2951144>
- Vandeputte, MM; Van Uytendaele, K; Luyckx, ML; St-Germaine, DM; Jaha, DM; Stove, CP. Synthetic Chemical Characterization and μ -Opioid Receptor Activity Assessment of the Emerging Group of "Bitazans": 2-Benzoylbenzimidazole, Synthetic Opioids. *ACS Class. Account.* 2019. <https://pubs.acs.org/doi/10.1021/acscchem.3c00464>
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6711062/>

6. QUALITATIVE DATA

6.1 GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS)

Testing Performed At: The Center for Forensic Science Research and Education at the Forensic Toxicology Facility (WVU-GC-MS)

Sample Preparation: Standard diluted in acetonitrile

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). <https://www.caymanchem.com/product/23189>

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 Forensic Toxicology Facility (WVU-GC-MS)

Sample Preparation: Liquid-liquid extraction (LLE)

Instrument: Sciex TripleTOF 5600

Column: Phenomenex Kinetex C18 (50 mm x 3.0 mm, 2.6 μ m)

Mobile Phase: A: Ammonium formate (10 mM, pH 1.0); B: Methanol/acetonitrile (50:50)

Gradient: 15 min: 95%A/5%B; 5A/95B over 15 min; 95A/5B at 15.5 min

Temperatures: Autosampler: 15 °C

Column Oven: 50 °C

Source Heater: 500 °C

Injection Volume: 10 μ l

TOF MS Scan Range: 100-510 Da

Pre-scan Isolation: SW-11.0P (acquisition 127 windows)

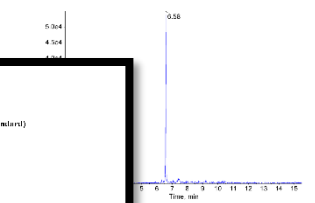
Fragmentation Collision Energy (Scan): 350.15 eV

MS/MS Scan Range: 50-510 Da

Retention Time: 6.58 min

Standard Comparison: Reference material for N-pyrrolidino etonitazene (Batch: 0607145-2) was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Analysis of the sample in acetonitrile for precise identification of the analyte in the extract by N-pyrrolidino etonitazene, based on retention time (6.48 min) and mass spectrum data. <https://www.caymanchem.com/product/23189>

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



Reference Material: N-Pyrrolidino Etonitazene (Standard)



Biological Sample: N-Pyrrolidino Etonitazene (Biological Sample)



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 Forensic Toxicology Facility (WVU-GC-MS)

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TOF MS Scan Range: 100-510 Da

Pre-scan Isolation: SW-11.0P (acquisition 127 windows)

Fragmentation Collision Energy (Scan): 350.15 eV

MS/MS Scan Range: 50-510 Da

Retention Time: 6.58 min

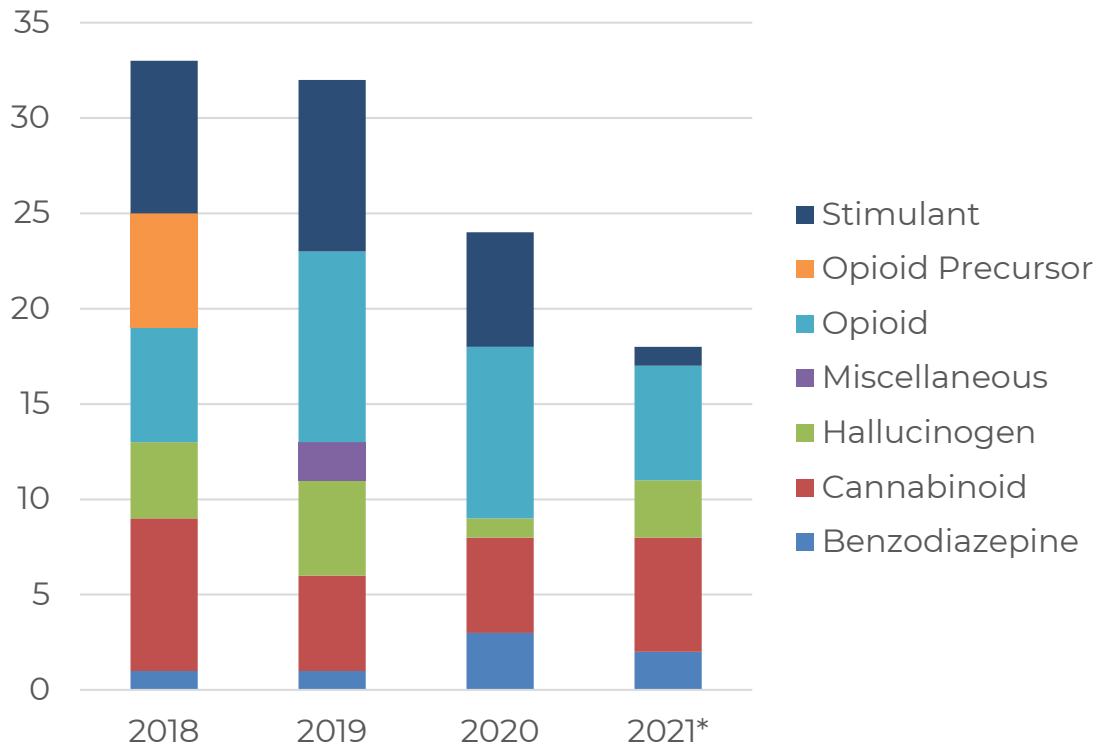
Standard Comparison: Reference material for N-pyrrolidino etonitazene (Batch: 0607145-2) was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Analysis of the sample in acetonitrile for precise identification of the analyte in the extract by N-pyrrolidino etonitazene, based on retention time (6.48 min) and mass spectrum data. <https://www.caymanchem.com/product/23189>

MS/MS Spectrum: N-Pyrrolidino Etonitazene (Biological Sample)

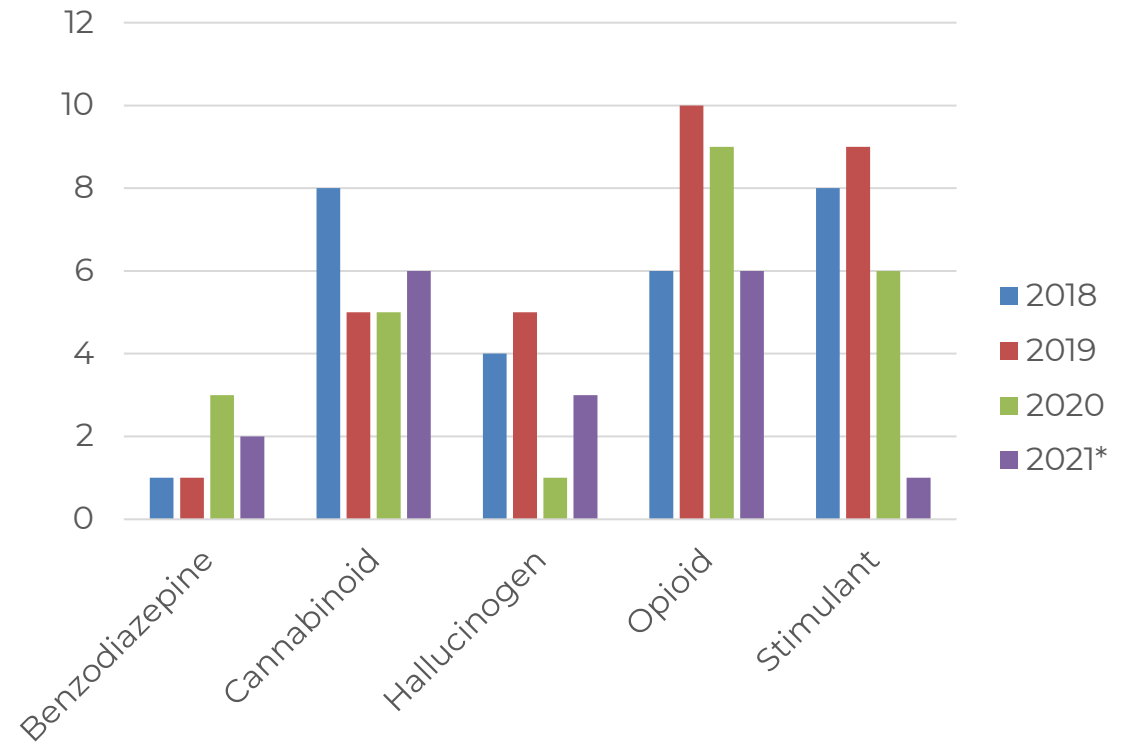


Identifications of New NPS in the United States

NPS Reported Per Year By Class

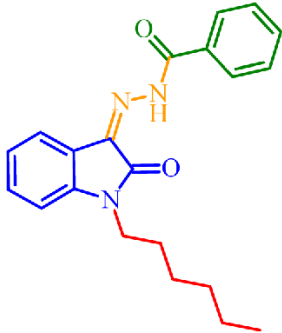
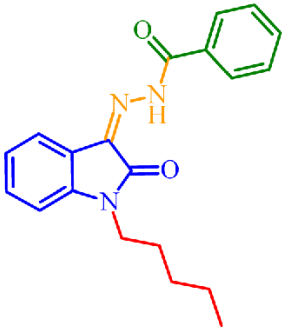
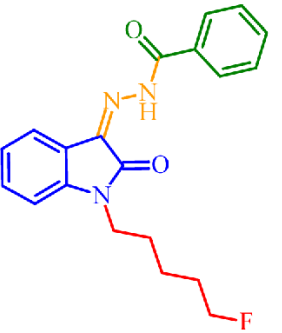


Class of NPS Reported Per Year



Newest NPS to Appear in the United States

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

BZO-HEXOXIZID	BZO-POXIZID	5F-BZO-POXIZID
		
(Z)-N'-(1- HEX yl-2- OX oIndolin-3-ylidene) BenZO hydra ZID e	(Z)-N'-(1- P entyl-2- OX oIndolin-3-ylidene) BenZO hydra ZID e	(Z)-N'-(1-(5-F luoro P entyl-2- OX oIndolin-3-ylidene) BenZO 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

“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**

Trend Reports

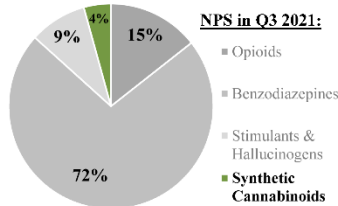
Trend Report: Q3 2021

Synthetic Cannabinoids in the United States

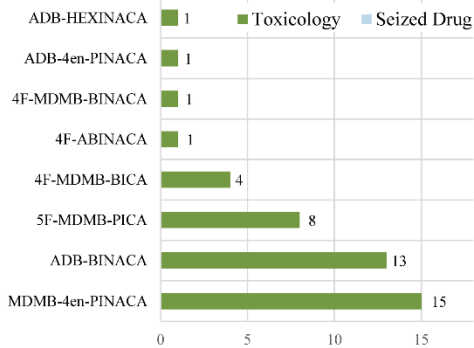
Purpose: This report provides up-to-date information regarding the status of synthetic cannabinoid prevalence and positivity within the United States.

Overview: Novel psychoactive substances (NPS), including synthetic cannabinoids, continue to pose great challenges for forensic scientists, clinicians, and public health and safety personnel. NPS substances have been implicated in an increasing number of emergency room admissions, death investigations, and intoxication events in corrections populations. Maintaining a current scope of analysis can be challenging, requiring comprehensive analytical methodologies and reference materials for identification(s).

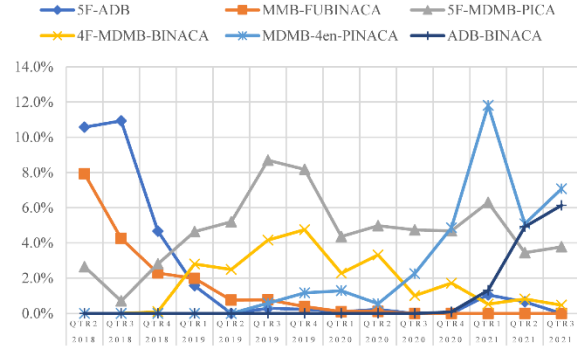
Objective: Our laboratory utilizes novel approaches for the analysis of drugs in biological samples and seized materials using comprehensive non-targeted data acquisition by gas chromatography mass spectrometry (GC-MS) and liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). The scope of analysis contains more than 900 drugs, including a vast majority of NPS and their metabolites. This approach allows for real-time identification of novel synthetic cannabinoids and further data analysis of important trends. This project was conducted in collaboration with the toxicology and criminalistics laboratories of NMS Labs. Forensic case types linked to these results include illicit drug investigations, medicolegal death investigations, and/or driving under the influence of drugs (DUID) investigations. The results in this report represent the total number of NPS identifications at the CFSRE during this quarter, including those from sample-mining, data-mining, and/or esoteric testing.



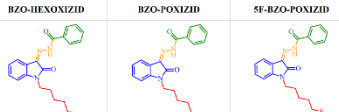
SYNTHETIC CANNABINOIDS IDENTIFIED



SELECT POSITIVITY SINCE Q2 2018



August 2021 →
New Systematic Naming for Synthetic Cannabinoid "MDA-19" and Its Related Analogues: BZO-HEXOXIZID, 5F-BZO-POXIZID, and BZO-POXIZID



Abstract: This report is written by Agnieszka Kowalska, PhD, and E. William SAE, PhD, at the NMS Labs, under the supervision of Dr. Joseph P. ...
Keywords: ADB-BINACA, 4F-ABINACA, 4F-MDMB-BINACA, 4F-MDMB-BICA, 5F-MDMB-PICA, MDMB-4en-PINACA, MMB-FUBINACA, NPS, Synthetic Cannabinoids, Toxicology, Seized Drug.



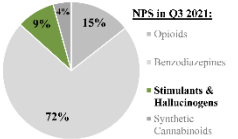
Trend Report: Q3 2021

NPS Stimulants & Hallucinogens in the United States

Purpose: This report provides up-to-date information regarding the status of NPS stimulants and hallucinogens prevalence and positivity within the United States.

Overview: Novel psychoactive substances (NPS), including NPS stimulants and NPS hallucinogens, continue to pose great challenges for forensic scientists, clinicians, and public health and safety personnel. NPS substances have been implicated in an increasing number of emergency room admissions, death investigations, and intoxication events in corrections populations. Maintaining a current scope of analysis can be challenging, requiring comprehensive analytical methodologies and reference materials for identification(s).

Objective: Our laboratory utilizes novel approaches for the analysis of drugs in biological samples and seized materials using comprehensive non-targeted data acquisition by gas chromatography mass spectrometry (GC-MS) and liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). The scope of analysis contains more than 900 drugs, including a vast majority of NPS and their metabolites. This approach allows for real-time identification of novel synthetic cannabinoids and further data analysis of important trends. This project was conducted in collaboration with the toxicology and criminalistics laboratories of NMS Labs. Forensic case types linked to these results include illicit drug investigations, medicolegal death investigations, and/or driving under the influence of drugs (DUID) investigations. The results in this report represent the total number of NPS identifications at the CFSRE during this quarter, including those from sample-mining, data-mining, and/or esoteric testing.



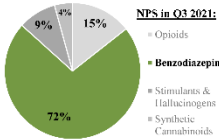
Trend Report: Q3 2021

NPS Benzodiazepines in the United States

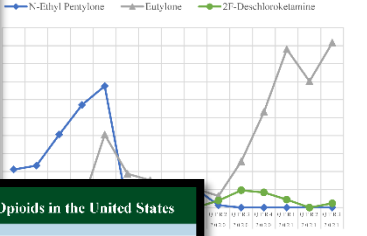
Purpose: This report provides up-to-date information regarding the status of NPS benzodiazepines prevalence and positivity within the United States.

Overview: Novel psychoactive substances (NPS), including NPS benzodiazepines, continue to pose great challenges for forensic scientists, clinicians, and public health and safety personnel. NPS substances have been implicated in an increasing number of emergency room admissions, death investigations, and intoxication events in corrections populations. Maintaining a current scope of analysis can be challenging, requiring comprehensive analytical methodologies and reference materials for identification(s).

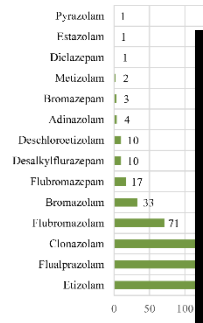
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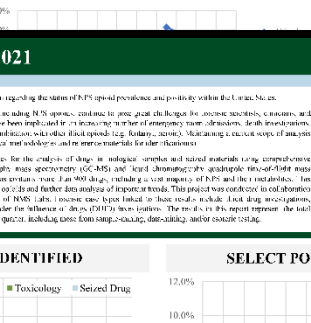
SELECT POSITIVITY SINCE Q1 2018



NPS BENZODIAZEPINES IDENTIFIED



SELECT POSITIVITY SINCE Q1 2019



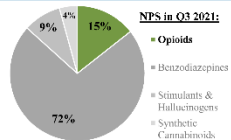
Trend Report: Q3 2021

NPS Opioids in the United States

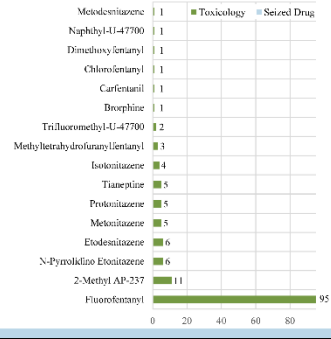
Purpose: This report provides up-to-date information regarding the status of NPS opioid prevalence and positivity within the United States.

Overview: Novel psychoactive substances (NPS), including NPS opioids, continue to pose great challenges for forensic scientists, clinicians, and public health and safety personnel. NPS substances have been implicated in an increasing number of emergency room admissions, death investigations, and intoxication events in corrections populations. Maintaining a current scope of analysis can be challenging, requiring comprehensive analytical methodologies and reference materials for identification(s).

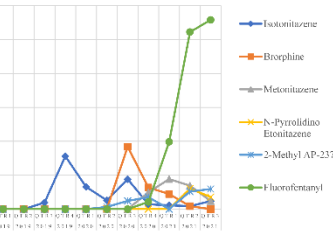
Objective: Our laboratory utilizes novel approaches for the analysis of drugs in biological samples and seized materials using comprehensive non-targeted data acquisition by gas chromatography mass spectrometry (GC-MS) and liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). The scope of analysis contains more than 900 drugs, including a vast majority of NPS and their metabolites. This approach allows for real-time identification of novel synthetic cannabinoids and further data analysis of important trends. This project was conducted in collaboration with the toxicology and criminalistics laboratories of NMS Labs. Forensic case types linked to these results include illicit drug investigations, medicolegal death investigations, and/or driving under the influence of drugs (DUID) investigations. The results in this report represent the total number of NPS identifications at the CFSRE during this quarter, including those from sample-mining, data-mining, and/or esoteric testing.



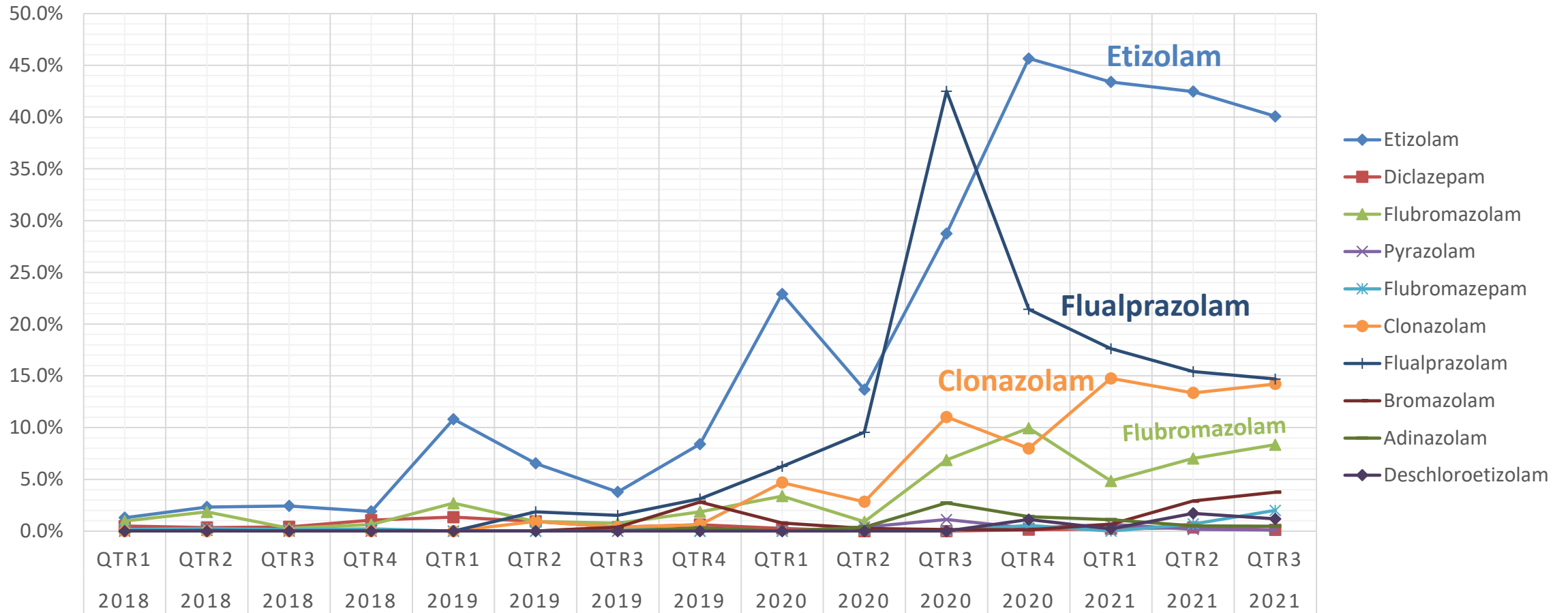
NPS OPIOIDS IDENTIFIED



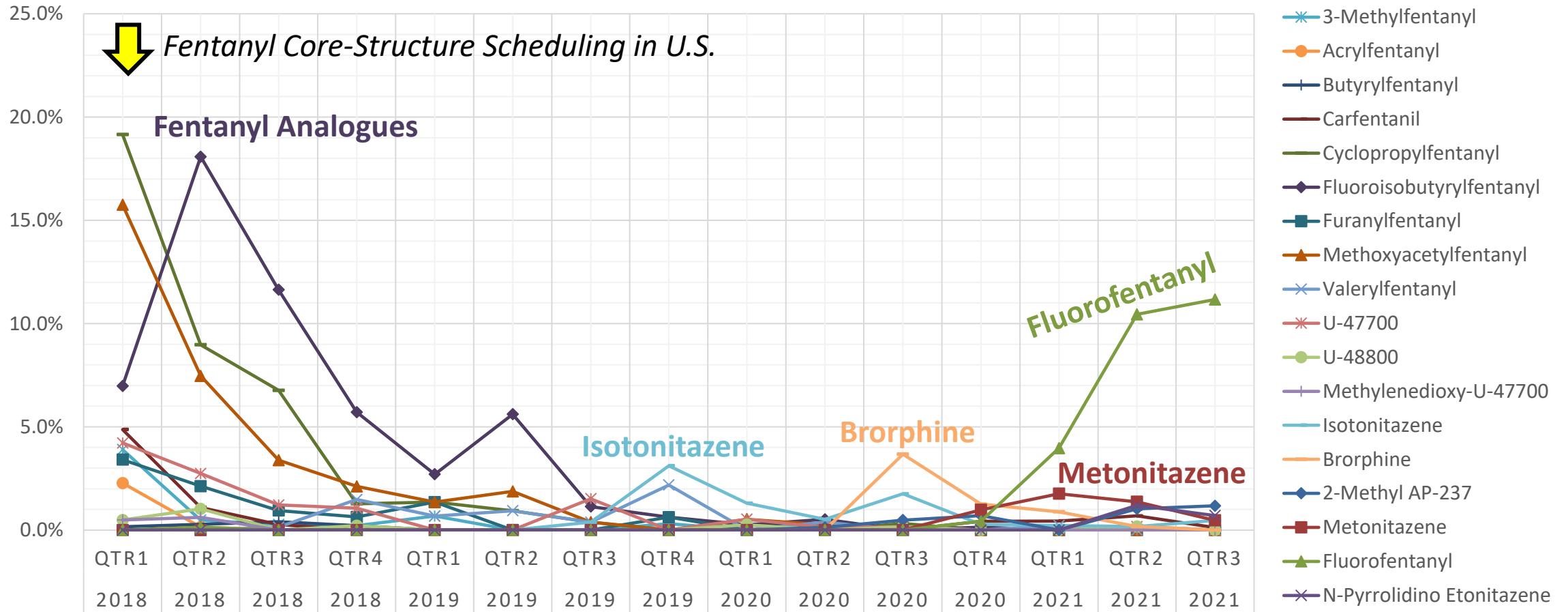
SELECT POSITIVITY SINCE Q1 2019



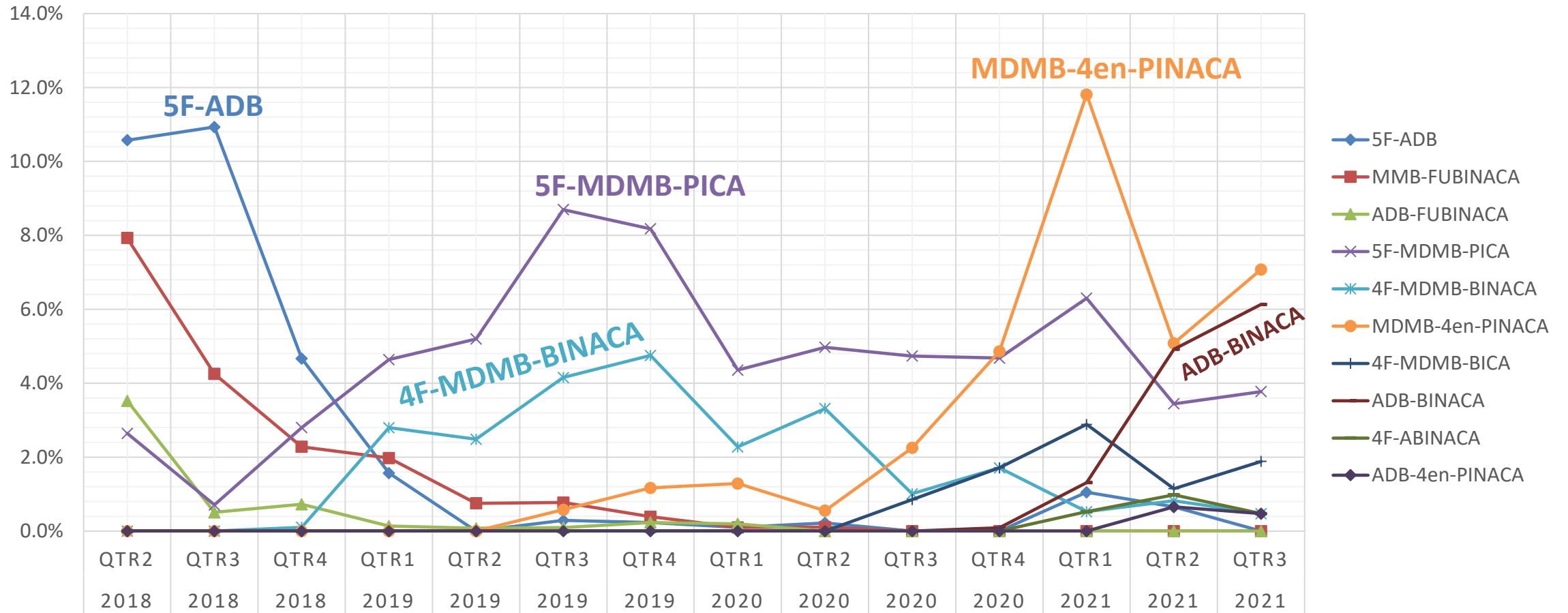
Positivity: NPS Benzodiazepines



Positivity: NPS Opioids



Positivity: Synthetic Cannabinoids



Public Alerts



June 2021 New High Potency Synthetic Opioid N-Pyrrolidino Etonitazene (Etonitazepine) Linked to Overdoses Across United States



Purpose: The objective of this announcement is to notify public health and public 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 on 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 (etonitazepine) is a new high potency synthetic opioid bearing structural resemblance to etonitazene, a synthetic opioid that is regionally 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-benzylpiperidine analogues that were first synthesized and reported in the literature in the 1950s (e.g., meperidine, 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: 20x to 50x
Date of Collection:	• January to April 2021
Other Notable Findings:	• NPS Hazards/Findings (n=7) • Fentanyl (n=1) • Methamphetamine (n=4) • Only Drug of Interest (n=1)
[N-Pyrrolidino Etonitazene]	
Case A	8.3 µg/mL*
Case B	2.4 µg/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

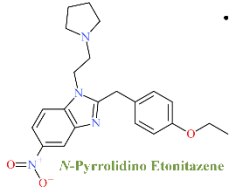


Recommendations for Laboratories

- 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 appropriate 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.

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.



Acknowledgments: This report was prepared by Alex J. Kozlowski, PhD, Dawn M. Pappas, MS, PhD, Robert S. Walker, MS, and Bruce A. Loman, PhD, C-DEET. Funding was provided from the National Institute of Justice (NIJ) of the U.S. Department of Justice (DOJ) Award # 2019-DN-00033-0120. The authors thank the following individuals for their assistance in the preparation of this report: Dr. Robert S. Walker, MS, PhD, C-DEET, NPS Discovery; Dr. Robert S. Walker, MS, PhD, C-DEET, NPS Discovery; Dr. Robert S. Walker, MS, PhD, C-DEET, NPS Discovery; Dr. Robert S. Walker, MS, PhD, C-DEET, NPS Discovery; Dr. Robert S. Walker, MS, PhD, C-DEET, NPS Discovery.

Rapid NPS Testing Now Available:

If your agency requires rapid analysis, you can now use a portable version of our testing protocol on-site or in a laboratory setting. This protocol is available for testing of synthetic opioids and is available for testing of synthetic opioids. For more information, contact NPS Discovery at 815-244-1100 or visit our website at www.npsdiscovery.org.

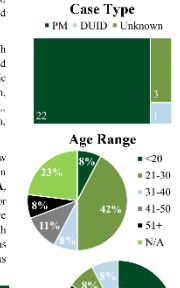
February 2021 Positivity of New Synthetic Cannabinoid 4F-MDMB-BICA Increasing in U.S. as Prevalence of 5F-MDMB-PICA Wanes



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.) and 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.

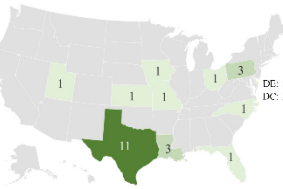
Summary: 4F-MDMB-BICA was first identified in the United States (U.S.) in plant-like material seized by law enforcement in May 2020 and soon after in toxicology casework in July 2020, with concurrent emergence in European countries. 4F-MDMB-BICA is structurally similar to the synthetic cannabinoid 4F-MDMB-BINACA, 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 mediotated 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 (DU/DI) 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.



Recommendations for Public Health

- Implement surveillance for rapid identification of drug overdose outbreaks.
- Track and monitor geographical drug distribution and trends for emerging drugs.
- Share awareness about the risks and dangers associated with synthetic cannabinoids use.

4F-MDMB-BICA Geographical Distribution



Recommendations for Clinicians

- Become familiar with the signs and symptoms associated with synthetic cannabinoids use (e.g. sedation, respiratory depression, tachycardia, hypertension, arrhythmia, chest pain, tachypnea, gastrointestinal distress, acute kidney injury, nausea, vomiting, fever, hyperglycemia, hypokalemia, sedation, etc.) and death.
- Be mindful that these drugs may contain undetected and/or adulterating substances that impact the expected clinical effects or findings.

4F-MDMB-BICA Toxicology Positivity

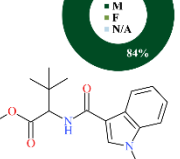


Recommendations for MEs & Coroners

- Test for new synthetic cannabinoids and their biomarkers in suspected drug overdose cases. Consider testing for synthetic cannabinoids if circumstances result in an unexplained drug fatality.
- Be aware that screening procedures (e.g. ELISA) for synthetic cannabinoids may not be specific or targeted to the newest generation of compounds; consider mass spectrometry-based screening.
- Share data on synthetic cannabinoid drug seizures with local health departments, medical examiners, coroners, and other forensic practitioners.

Recommendations for Laboratories

- Utilize analytical data available publicly for the identification of 4F-MDMB-BICA.
- Utilize previously developed non-targeted testing protocols or develop sensitive and appropriate testing procedures for synthetic opioids.
- Prioritize analytical testing of seized drug samples obtained from drug overdose scenes during death investigations.
- Share data on synthetic cannabinoid drug seizures with local health departments, medical examiners and coroners, and related communities.



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

November 2020 Updated Trend Reporting for the NPS Benzodiazepine Clonazepam Based on Data-Mining for 8-Amino-clonazepam



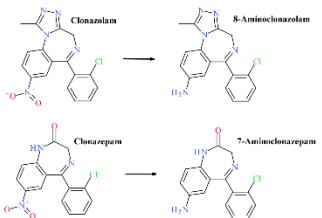
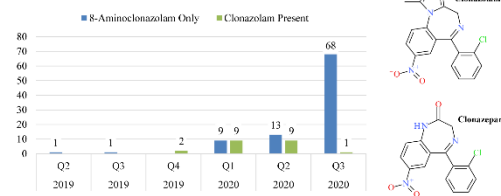
Purpose: The objective of this public announcement is to provide updated information on drug testing trends to laboratory personnel, medical examiners and coroners, clinicians, public health and public safety, law enforcement, and other related communities regarding the NPS benzodiazepine **clonazepam**.

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 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.

Clonazepam (also called clonazepam) first emerged in the recreational drug supply in 2014 (Europe) and 2016 (United States). Like many NPS, clonazepam was first synthesized during drug development in 1971 but was never approved for therapeutic use. Clonazepam is the triazole counterpart to clonazepam (Klonopin, Rivotril). Clonazepam appears in various drug preparations, including powders, tablets (e.g. counterfeit pharmaceuticals), liquids, and blotters. Clonazepam 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 clonazepam positivity in Q3 2020. However, recent developments show that the positivity of clonazepam 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. clonazepam, clonazepam) are metabolized in the body to amino counterparts (e.g. 8-amino-clonazepam, 7-amino-clonazepam). Additionally, instability of these drugs can lead to the production of these same amino-species. Therefore, data-mining for 8-amino-clonazepam was conducted on all samples analyzed in 2019 and 2020 to reevaluate the positivity of clonazepam in our sample populations. The results indicate that the positivity of clonazepam was previously underreported when targeting only the parent drug and 8-amino-clonazepam appears to be a more appropriate biomarker for accurate determination of clonazepam use.

Identifications by Analyte in Toxicology Cases



Recommendations for Public Health

- Implement surveillance for rapid identification of drug overdose outbreaks.
- Track and monitor geographical drug distribution and trends for emerging drugs, including substances identified in suspected counterfeit and/or illicit preparations.
- Raise awareness about the risks and dangers associated with benzodiazepine use, especially in combination with opioids and other depressants.

Recommendations for Clinicians

- Become familiar with the signs and symptoms associated with benzodiazepine use (e.g. sedation, respiratory depression, tachycardia, hypertension, arrhythmia, chest pain, tachypnea, gastrointestinal distress, acute kidney injury, nausea, vomiting, fever, hyperglycemia, hypokalemia, sedation, etc.) and death.
- Be mindful that counterfeit drug products often contain unknown and/or adulterating substances that may differ in expected clinical effects of traditional pharmaceutical preparations.
- Counsel about the dangers of NPS, benzodiazepine products and other drugs.

Recommendations for MEs & Coroners

- Test for NPS benzodiazepines and their biomarkers in suspected drug overdose cases.
- Consider testing for NPS benzodiazepines and their metabolites if circumstances result in an unexpected drug fatality.
- Be aware that screening procedures (e.g. immunoassays) for benzodiazepines may not be sufficiently cross-reactive or targeted to the newest NPS and/or their metabolites; consider comprehensive mass spectrometry-based screening.

Recommendations for Laboratories

- Review analytical data for Clonazepam and 8-Amino-clonazepam.
- Provide the address of NPS biomarkers for drugs that exhibit rapid metabolism or instability.
- Develop sensitive drug testing methods.
- Share data on NPS benzodiazepine drug seizures with local health departments, medical examiners, coroners, and other forensic practitioners.

Acknowledgments: This report was prepared by Alex J. Kozlowski, PhD, Dawn M. Pappas, MS, PhD, Robert S. Walker, MS, and Bruce A. Loman, PhD, C-DEET. Funding was provided from the National Institute of Justice (NIJ) of the U.S. Department of Justice (DOJ) Award # 2019-DN-00033-0120. The authors thank the following individuals for their assistance in the preparation of this report: Dr. Robert S. Walker, MS, PhD, C-DEET, NPS Discovery; Dr. Robert S. Walker, MS, PhD, C-DEET, NPS Discovery; Dr. Robert S. Walker, MS, PhD, C-DEET, NPS Discovery; Dr. Robert S. Walker, MS, PhD, C-DEET, NPS Discovery; Dr. Robert S. Walker, MS, PhD, C-DEET, NPS Discovery.

Rapid NPS Testing Now Available:

If your agency requires rapid analysis, you can now use a portable version of our testing protocol on-site or in a laboratory setting. This protocol is available for testing of synthetic opioids and is available for testing of synthetic opioids. For more information, contact NPS Discovery at 815-244-1100 or visit our website at www.npsdiscovery.org.

Clinical Reports



Opioid Overdoses from the Toxicology Investigators Consortium (ToxIC) Fentalog Study Group

Q2 2021

Purpose: This report provides new information regarding comprehensive drug testing of clinical biological specimens collected after suspected opioid overdoses in various cities across the United States.

Overview: Drug use can lead to adverse events and overdose scenarios where individuals present to emergency departments for clinical evaluation and/or treatment. The culprit can be traditional drugs (e.g., heroin, fentanyl, cocaine, methamphetamine) or novel psychoactive substances (NPS); however, proper drug testing methodologies must be employed for accurate identification and characterization. Street-level drug preparations can contain undeclared or unwanted substances, such as toxic adulterants or NPS, which can potentiate effects or lead to adverse reactions. Understanding emerging drug trends and drug testing results can help direct new or revised approaches to clinical treatment and harm reduction efforts.

Objective: A partnership between the American College of Medical Toxicology (ACMT) and the Center for Forensic Science Research and Education (CFSRE) was established to comprehensively assess the role and prevalence of synthetic opioids and other drugs among suspected overdose events in the United States.

Sample Source: Patients presented to emergency departments within ACMT's Toxicology Investigators Consortium (ToxIC) experiencing a suspected opioid overdose. Residual, discarded biological samples were obtained for testing against an expansive library of drugs and other substances. Our findings provide a near real-time assessment of the drug market and allude to resulting implications on clinical institutions.

Testing: Analysis was performed via liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). The scope of testing targeted more than 900 drugs, including a vast majority of NPS and metabolites. Drug classes included opioids, stimulants, cannabinoids, and benzodiazepines, among others.

Acknowledgments: This report was prepared by Alex Manini, MD, Alex J. Krolchick, PhD, Sara E. Walton, BS, Paul Wax, MD, Jeffrey Brent, MD, PhD, Kim Aldy, DO, Alexandra Amadio, DO, Diane Cello, MD, Adrienne Hughes, MD, Evan Schwarz, MD, and Barry K. Logan, PhD, F-ABFT. The authors acknowledge ACMT personnel, ToxIC investigators, and CFSRE staff for their contributions. Funding was received from the National Institute on Drug Abuse (NIDA) from the National Institute of Health (NIH), Award Number: R01DA048009. The opinions, findings, conclusions and/or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of NIDA, NIH, or other agencies. For more information about NPS Discovery and its programs, contact npsdiscovery@cfre.org or visit www.npsdiscovery.org.

Opioid Overdoses from the Toxicology Investigators Consortium (ToxIC) Fentalog Study Group

Q3 2021

Purpose: This report provides new information regarding comprehensive drug testing of clinical biological specimens collected after suspected opioid overdoses in various cities across the United States.

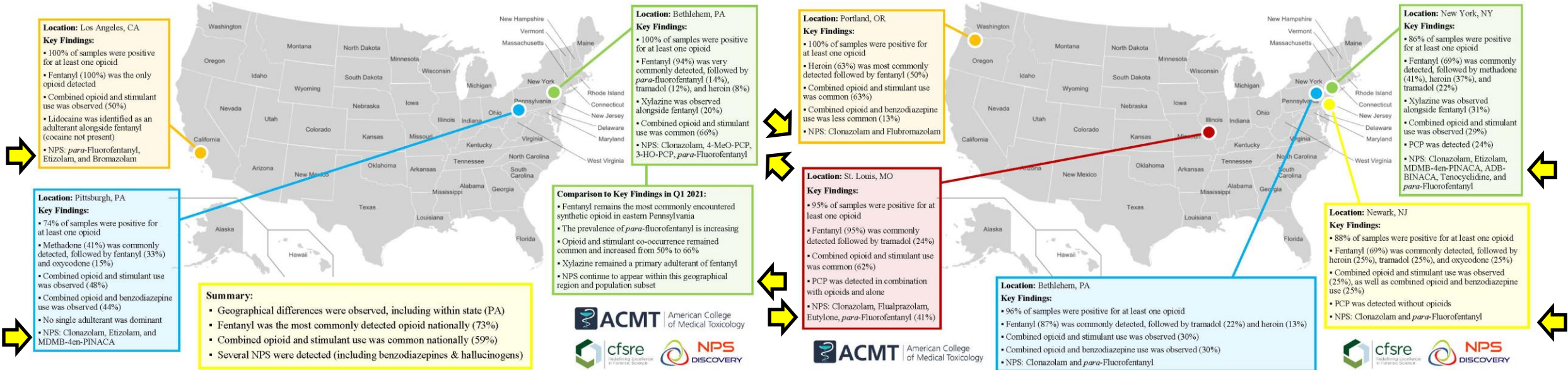
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Drug Checking Reports

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.

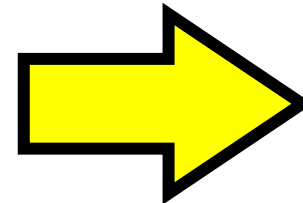
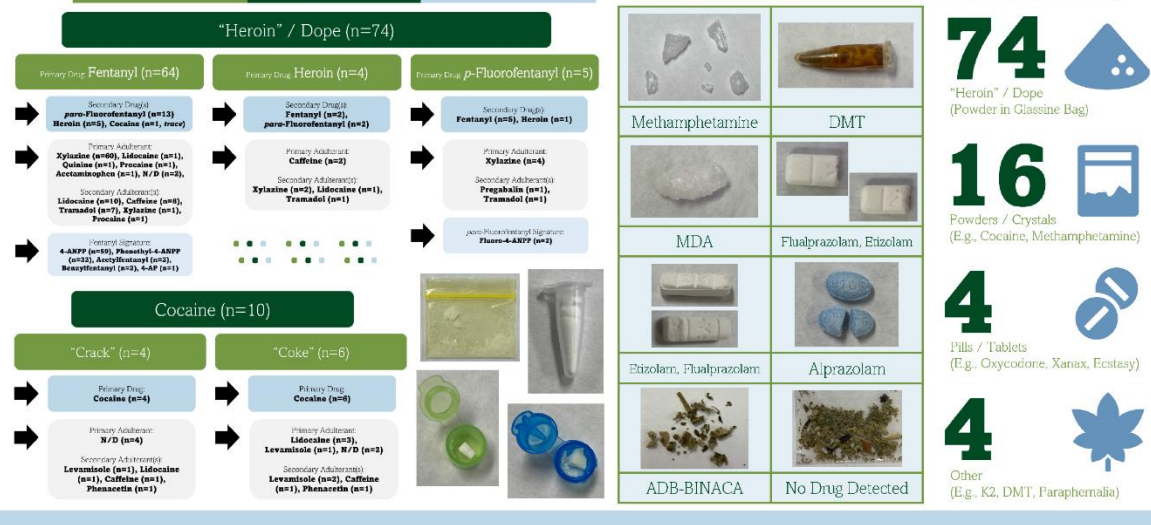
Acknowledgements: This report was prepared by Alex J. Krutolski, PhD; Jen Shinefeld, MS; Kendra Viner, PhD, MPH; Jeffrey Hom, MD, MPH; Sara E. Walton, MS; and Barry K. Logan, PhD, P-ABFT. The authors acknowledge CFSRE and PDPH personnel for their involvements. Funding for this study was provided internally by the Treckie Bledsoe 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.



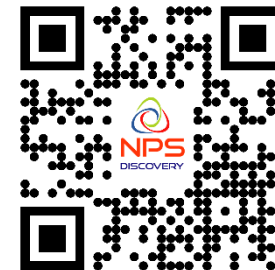
Summary and Key Findings:

- 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.



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



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 must evaluate 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 SDOIT 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.



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

Learn More About NPS Discovery:
NPS Discovery at the CFSRE is working to establish the NPS Discovery public, local, and public safety agencies to create a national network of experts in the field of NPS. We are currently accepting applications for individuals who are interested in the field of NPS. For more information, please visit our website at www.npsdiscovery.org.

Benzodiazepines	Opioids	Stimulants & Hallucinogens	Synthetic Cannabinoids
TIER ONE (STRONGLY RECOMMEND)			
Etizolam [†]	1-10	2-Methyl AP-237 >10	Eutylone >10
Clonazepam	<1	N-Pyrrolidino Etonitazene <1	↑N,N-Dimethyl Pentylone >10
8-Aminoclonazepam	1-10	Metonitazene <1	α-PHP / α-PiHP >10
Flualprazolam	1-10	↑Protonitazene <1	↑2F-Deschloroketamine <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	↓3-Cl-PCP / 4-Cl-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	↓Bupropione <1	↑Pentylone <1
↑Metizolam	1-10	↑Butonitazene 1-10	↑Methylenedioxy-PV8 <1
↓Pyrazolam	1-10	↑Metodesnitazene 1-10	↑4-HO-DiPT <1
			4F-ABINACA <1
			ADB-HEXINACA <1

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.

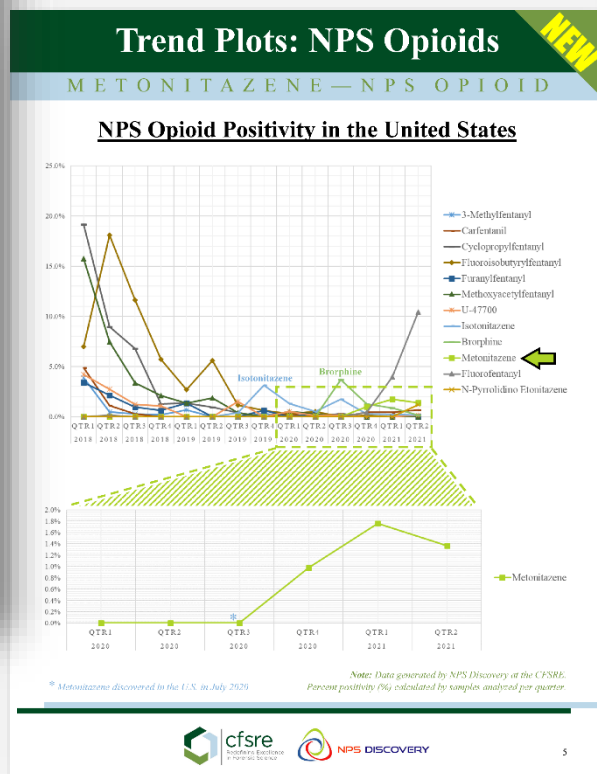
NPS Discovery Toolkits

NPS DISCOVERY

NPS Discovery Toolkit
» Metonitazene

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Analytical Methods

METONITAZENE — NPS OPIOID

Purpose: This section provides example analytical methods for the analysis of metonitazene. These two instrumental approaches provide a starting point for laboratories looking to develop methods for this new drug, ultimately saving valuable time and resources. In addition, mass spectrometer equipment could be used to initiate ion monitoring in new surveys/trace prior to availability of reference material in the laboratory.

Agilent Technologies (Santa Clara, CA)		Waters™ Corporation (Milford, MA)	
Liquid Chromatograph	1260 HPLC	Liquid Chromatograph	ACQUITY UPLC I-Class
Mass Spectrometer	6495 QQQ-MS	Mass Spectrometer	Neo TG-S micro QQQ-MS

Liquid Chromatograph Parameters		Liquid Chromatograph Parameters	
Column	Agilent Zorbax Lab Pursuit 120 TC-C18 (3.0 x 100 mm, 2.7 µm)	Column	Agilent Zorbax Lab Pursuit 120 TC-C18 (3.0 x 100 mm, 2.7 µm)
Column Temp.	50 °C	Column Temp.	30 °C
Mobile Phase A	0.1% Formic Acid in Water	Mobile Phase A	0.1% Formic Acid in Water
Mobile Phase B	0.1% Formic Acid in Acetonitrile	Mobile Phase B	0.1% Formic Acid in Methanol
Flow Rate	0.4 mL/min	Flow Rate	0.4 mL/min
Gradient	Initial: 90:10 A:B 1 min: 85:15 A:B 4 min: 95:5 A:B 5 min: 95:5 A:B 5.1 min: 50:50 A:B 6 min: 50:50 A:B	Gradient	Initial: 60:40 A:B 1 min: 60:40 A:B 3 min: 70:30 A:B 7.5 min: 40:60 A:B 6 min: 60:40 A:B 7 min: 60:40 A:B

Mass Spectrometer Parameters		Mass Spectrometer Parameters	
Gas Temp.	250 °C	Gas Temp.	250 °C
Gas Flow	16 L/min	Capillary	2.2 V
Nebulizer	40 psi	Desolvation Temp.	600 °C
Capillary	3,000 V	Desolvation Flow	800 L/hr
Nozzle	1,500 V	Cone Flow	69 L/hr
Sheath Gas Temp.	400 °C	Source Temp.	150 °C
Sheath Gas Flow	12 L/min		

Multiple Reaction Monitoring (MRM) Transitions		Multiple Reaction Monitoring (MRM) Transitions	
Fragmenter	MS1	MS2	Collision
385	383.2	72.1	20
		100.1*	30
		221.1	30

Metabolism

METONITAZENE — NPS OPIOID

Purpose: The primary metabolites of metonitazene were investigated through *in vivo* experiments. Authentic biological specimens collected after confirmed metonitazene ingestion were examined. Analysis was performed using a SCIEX TripleTOF® 5600+ LC-QTOF-MS (Framingham, MA).

Reference: Kirovski A.J., Papan D.M., Walsh S.E., Logan B.K. Metonitazene in the United States – Forensic Toxicology Assessment of a Potent New Synthetic Opioid using Liquid Chromatography-Mass Spectrometry. *Drug Test. Anal.* 2021. <https://doi.org/10.1039/d1ob00031a>

ID	Biotransformation	Formula	RT (min)	Exact [M-H] ⁺	Measured [M-H] ⁺	Mass Error (ppm)	Diagnostic Product Ions
P.0	Metonitazene	C ₂₁ H ₂₈ N ₂ O	6.12	383.2078	383.2077	-0.2	100, 1121, 72, 0808
M.1	N-Dealkylation	C ₁₉ H ₂₂ N ₂ O	6.00	355.1765	355.1765	0.2	284, 1030, 72, 0808
M.2	N,N-Didealkylation	C ₁₇ H ₁₆ N ₂ O	5.84	327.1452	327.1447	-1.5	284, 1030, 44, 0500
M.3	O-Dealkylation	C ₂₀ H ₂₄ N ₂ O	5.21	369.1921	369.1925	1.0	100, 1121, 107, 0497
M.4	Nitro Reduction	C ₂₁ H ₂₈ N ₂ O	3.53	353.2336	353.2336	-0.7	100, 1121, 72, 0808



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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- 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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